ABSTRACT

Title of Document: RELEASE, SURVIVAL, AND REMOVAL OF BOVINE MANURE-BORNE INDICATOR BACTERIA UNDER SIMULATED RAINFALL

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The effects of simulated rainfall intensities and its interactions with manure consistency and weathering on the release, survival, and removal of fecal indicator bacteria, *Escherichia coli* and enterococci, from land-applied dairy manure were evaluated. Rainfall intensity had significant effects on the number of bacteria in the soil following rainfall. Bacteria concentrations in soil decreased with increased soil depths and the topmost centimeter of soil accounted for the greatest proportion of bacteria. *Escherichia coli* persisted longer than enterococci once removed from manure. Manure consistency was not a significant factor in the removal of bacteria when manure was fresh, but as manure weathering progressed, consistency became a significant factor. The Vadas-Kleinman-Sharpley model was preferred over the exponential model for simulating the removal of manure-borne bacteria. Results of this work will be useful for

improving predictions of the human health risks associated with manure-borne pathogenic microorganisms.

RELEASE, SURVIVAL, AND REMOVAL OF BOVINE MANURE-BORNE INDICATOR BACTERIA UNDER SIMULATED RAINFALL

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master in Science
2017

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DEDICATION

I dedicate this thesis to my mother, Joyce Stocker, who successfully raised two young children as a single parent. It is also dedicated to my father, Daniel Stocker, who left us too soon, but was able to make enough of an impact in the short time I had with him that resulted in me pursuing education and employment within the field of environmental science. Additionally, this thesis is dedicated to Dr. Yakov Pachepsky who on one day in late December of 2012 took a chance and extended me a position within his lab group. I also dedicate this thesis to other family and friends who supported me throughout my graduate program. Finally, to Ms. Megan Silk who has been with me for the entirety of my program and has helped me to prepare for the presentation of my findings.

ACKNOWLEDGEMENTS

I recognize and thank the following individuals and organizations for their assistance over the past two years.

I thank the USDA-ARS Environmental Microbial Food Safety Laboratory (EMFSL) and the University of Maryland, College Park: Department of Environmental Science and Technology for providing the financial support for this research.

To Dr. Robert Hill, who served as the Major Professor in my Master of Science program, who has spent a considerable amount of time and effort in helping to prepare my thesis as well as working with me to practice and streamline the information in my exit seminar presentation. I am also grateful for your patience with me as a student in general and for teaching me the basics of soil physics. I would also like to thank my co-advisor Dr. Yakov Pachepsky for his years of training me to be a competent scientific researcher and for always showing me the best ways to analyze any dataset. I am also grateful for Dr. Stephanie Yarwood's input on my thesis and for lending her microbiological ear to the work, who also taught me the basics of soil microbial ecology as my very first class in my program.

I would very much like to acknowledge Lynda Kiefer for very patiently and eagerly mentoring me in the laboratory when I first started at the USDA. I am glad we were able to work side-by-side on projects towards the end of your time at EMFSL.

Additional thanks to my coworkers at the USDA: Mr. Ryan Blaustein, Mrs. Billie Jean Griffith, Mr. Cary Coppock, Ms. Rachel Kierzewski, Dr. Eunmi Hong, Dr. Yonguen Park, for their technical assistance, guidance, and help with experimentation. With their

help, we were able to plan and run experiments that we otherwise would not have had the manpower to perform.

Thank you to other members of the EMFSL. To Dr. Shelton, the research leader of EMFSL during my masters. To Doctors Jeffrey Karns and Jo Anne van Kessel for answering many of my microbiological questions and allowing us the use of their equipment and lab space, and technicians. To Ms. Laura Del Collo and Jackie Sonnier who very willingly helped in the lab when staffing was short and for generally being extremely helpful during my time at EMFSL.

Thank you also to other members of the USDA staff. To Mr. Guy Stone who prepared the synthetic rainfall mix. To Mr. Dave Swain who acquired the soil for the laboratory studies and helped organize field operations. To Mr. George Meyers and Mr. Ted Currier, and their work crews who were invaluable to field operations. To Mr. Jared Bouma for allowing me the use of a greenhouse space to house my soil boxes. To Dr. Jack Meisinger who helped coordinate and maintain the spaces we used for rainfall simulations.

Thank you also to the faculty, staff, and graduate students of the Environmental Science and Technology Department at the University of Maryland. I will always be grateful for the magnitude of science I learned in the Soil and Watershed Sciences concentration. Finally, thank you to Dr. Martin Rabenhorst for his help as graduate coordinator of the program, and for answering numerous questions I had regarding the program and for the critical, but friendly, evaluation he provided me for my departmental poster and oral presentations.

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Chapter 1 – Introduction

The spreading of manures on fields is an efficient method of animal waste disposal that also improves soil health and provides essential plant nutrients to agricultural fields. During rainfall on lands that have received manures, large numbers of fecal bacteria are released from manure and are subsequently transported to surface waters. This transport and deposition compromises the microbial quality of surface waters and poses risks to human health via contact with contaminated water typically as a result of irrigation and/or recreational activities. The organization of this thesis presents a literature review on microbial fate and transport processes (Chapter 2), and the results from three original experiments on the release, survival, and removal of fecal indicator bacteria – *Escherichia coli* and enterococci (*Enterococcus* spp.) – from dairy cattle manure and their removal within runoff and/or infiltration (Chapter 3, 4, 6).

Chapter 2 is a review of our current knowledge concerning the fate of manure-borne microbial contaminants in the environment as affected by chemical, physical, and biological factors. Transport processes, both above and below ground, are also discussed at length. Knowledge gaps are identified and avenues for future research are discussed which leads into the following chapters that describe the three original studies performed to address these knowledge gaps.

Chapter 3 describes the first research study which focuses on rainfall intensity as a factor influencing the number of indicator bacteria infiltrated into the soil following

manure application and rainfall. Depth-dependent observations on the abundance of *E. coli* and enterococci within specific soil depth ranges are also reported.

Chapter 4 describes the second research study and focuses on the effects of manure consistency and manure weathering as factors influencing the removal of fecal indicator bacteria from vegetated soils. In this chapter, total microbial release is considered as the sum of the total number of *E. coli* and enterococci bacteria that were recovered within runoff, infiltration, and the soil following different rainfall events. This chapter statistically analyzes differences in the removal and survival of manure-borne bacteria as affected by manure consistency and manure weathering. The results presented in this chapter are also used for modeling purposes in later chapters.

Chapter 5 focuses on modeling the removal of *E. coli* and enterococci in both runoff and infiltration. Two different bacterial release / removal equations were compared and recommendations were made on which model was the most appropriate for simulating the release / removal processes. The fitting parameters generated from both models were reported and statistically analyzed. An additional goal of the research described in this chapter was to quantify the contribution of fecal bacteria within the soil to the microbial populations of the indicator bacteria measured in the runoff.

Chapter 6 is a summary of the conclusions, analyses, and discussion of the three studies presented in this thesis.

Chapter 2 - Literature Review

2.1. Animal waste and manures in environment

2.1.1. Animal agriculture as a source of animal waste

In the United States, an estimated 2.2 billion livestock generate 1.1 billion tons of waste annually that are primarily classified as animal manures (USEPA, 2013). The application of animal manures to agricultural fields is a common practice that aims to utilize the plant nutrients within the manures to improve soil health and fertility for increased yields in crop production (Sharma and Reynnells, 2016). Using animal manures as a soil amendment provides an important avenue of waste disposal and has become a tenant of sustainable and organic farming practices (Sheldrick, 2003; USDA, 2015). In 2006, 15.8 million acres of cropland in the US received manure amendments which served as an economically viable way to dispose of the waste while also recycling nutrients back into the soils used for plant production (Mishra, 2003; USDA, 2009). These amendments are a source of nitrogen, phosphorous, potassium, and a slew of other macro and micro nutrients required for plant growth. Other benefits of manure applications are the improvement of soil quality by neutralizing acidity, increasing organic matter, decreasing compaction, increasing water holding capacity, and the enhancement of beneficial microorganisms in the soil (USDA, 2009; NSAC 2014). The use manure amendments are also a cost-saving practice as commercial fertilizer costs have increased sharply in recent years (Huang, 2009). Nutrients from manure applications are also slowly released and do not have as large a potential for leaching into groundwater that exists with commercial inorganic fertilizers (Schmidt et al., 2014).

Within US agriculture in the last 50-60 years, there has been a shift away from small family farmers to larger commercial operations. Livestock production during this time period has more than doubled while the number of livestock operations has declined by 80%, resulting in more than 43% of all beef cattle, dairy cattle, swine, and poultry being raised by the largest two percent of operations (Graham and Nachman, 2010; Goellehon et al., 2001; Rodgers and Haines, 2005). The consolidation of animal operations in the US poses many challenges including the safe disposal of animal wastes that may present substantial human and environmental health risks. This situation becomes more of a problem if the ratio of waste generated to land area is large, as seen with concentration animal feeding operations (CAFOs), such that the available lands are oversaturated with waste which may increase the likelihood of the waste entering surface water or groundwater (USEPA, 2013). The increased size of livestock operations and the subsequent manure production often exceed the holding and disposal capabilities of available agricultural land (Edrington et al., 2009). Because of this, CAFOs with excess manure will often sell and transport their manures to farmers elsewhere (UDSA, 2009) or some producers may apply manure at rates exceeding the agronomic nutrient needs of crops (USDA-ERS, 2009).

2.1.2. Animal waste storage and composting.

Animal waste is usually stored prior to land application so that farmers can make sure its eventual application follows their nutrient management plans and / or so that it may be used during appropriate times of year in terms of crop production (USDA, 2013). The storage method is usually dictated by the water content of the waste or waste mixture. For fresh manure, water contents of 88 to 92% and 73 to 75% are consistently

observed for non-poultry and poultry wastes, respectively, assuming the animal to be healthy (James et al., 2006). The former water content range is handled as a liquid while the latter as solid (Figure 2.1).

While liquid manure is stored, it can be physically, chemically, or biologically treated. Liquid or slurries are stored in pits, lagoons, and slurry tanks, which greatly dilute the waste so that it can be easily spread with irrigation sprinklers or mobile equipment (USDA, 2009). In these storage systems, solids are either mechanically separated from the liquid waste or are allowed to settle to the bottom. If liquid manure is kept in a tank or digester, it is maintained anaerobically which helps to reduce pathogen concentrations, break down the waste products, reduce the overall volume of manure, and produce valuable gas by-products such as methane that can be sold by farm operations (USEPA, 2013). Earthen or concrete holding ponds may also be employed to contain, but not necessarily treat, liquid and slurry manures so that surface and groundwater contamination potential is reduced (James et al., 2006). While in holding, liquid or slurry manures may have lime added (CaO or CaOH) to reduce pathogens or have coagulants added that help separate liquids from any remaining solids (USEPA, 2013).

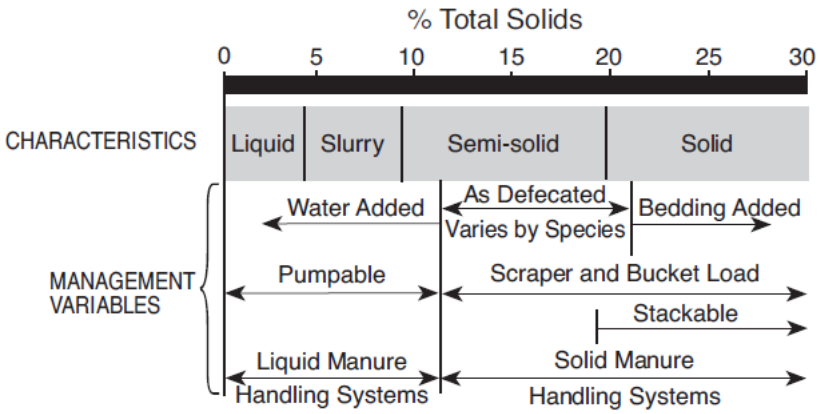

Figure 2.1. The consistency and management practices associated with animal waste at various solid contents (%) (James et al., 2006)

Solid and semi-solid manures often contain bedding materials such as straw or sawdust and may have water intentionally withheld which keeps the manure aerated and allows for stacking (Hutchison et al. 2005; James et al., 2006). Solid manures may be composted for weeks to months with the goal of achieving high internal temperatures which promotes pathogen inactivation. Composting manures also reduces their volume and increases their quality due to nitrogen compounds becoming more stable which in turn means once applied, the nitrogen compounds are released more slowly as compared with raw manures (USEPA, 2013). Due to the scale of some farming operations as well as the time and space needed to compost manure, composting may not always be a practical approach to the storage of solid and semi-solid manures (Kudva et al., 1998).

2.1.3. Manure application

Manures are spread on fields in various consistencies. In the order of increasing solid contents these consistencies include liquid, slurry, semi-solid, and solid (Hamilton, 2011). Liquid manure application methods include drag-hose, dragline or umbilical applicators, drop tube/drop hose applicators, injection, manifold, opener, or splash plate methods. Solid manures are typically surface broadcasted either with or without incorporation into the soil depending on the crop. By and large, surface broadcasting of liquid and solid manures is the most prevalent application method (USDA, 2009).

Federal, state, and local governments may require farmers to develop and implement nutrient management plans (NMPs) which are based on the nutrient contents in manure and appropriate agronomic application rates (USDA-ERS, 2009). The rates of manure application vary by the nutrient content of manure type used, the nutrient requirement of specific crops, the area receiving manure, and the anticipated crop yields. The nutrient requirements for different crops vary considerably. Corn has greater nutrient requirements than crops like peanuts, wheat, or sorghum and because of this, corn accounts for over half of all acreage spread with manure (USDA-ERS, 2009). Application rates are primarily based on the nutrient requirement of the planted crop and the farmers anticipated yield. The application rate is calculated to meet this requirement and is a function of the nutrient content of the manure itself. Typically, the limiting nutrient (phosphorus, nitrogen, or potassium) within a manure will determine the amount of manure applied (James et al., 2006). Manure nutrient concentrations vary considerably by animal source and consistency and thus application rates will increase or decrease to meet the nutrient requirements of a crop and the total anticipated yield (USEPA, 2012). Figure 2.2 shows the amount of liquid or solid manure required to fertilize a corn field at

various anticipated yields. The values were generated using a manure application calculator developed by South Dakota Department of Environment and Natural Resources (SD-DENR, 2016) and assumes a residual nitrate-nitrogen content in the soil of 23 lbs/acre.

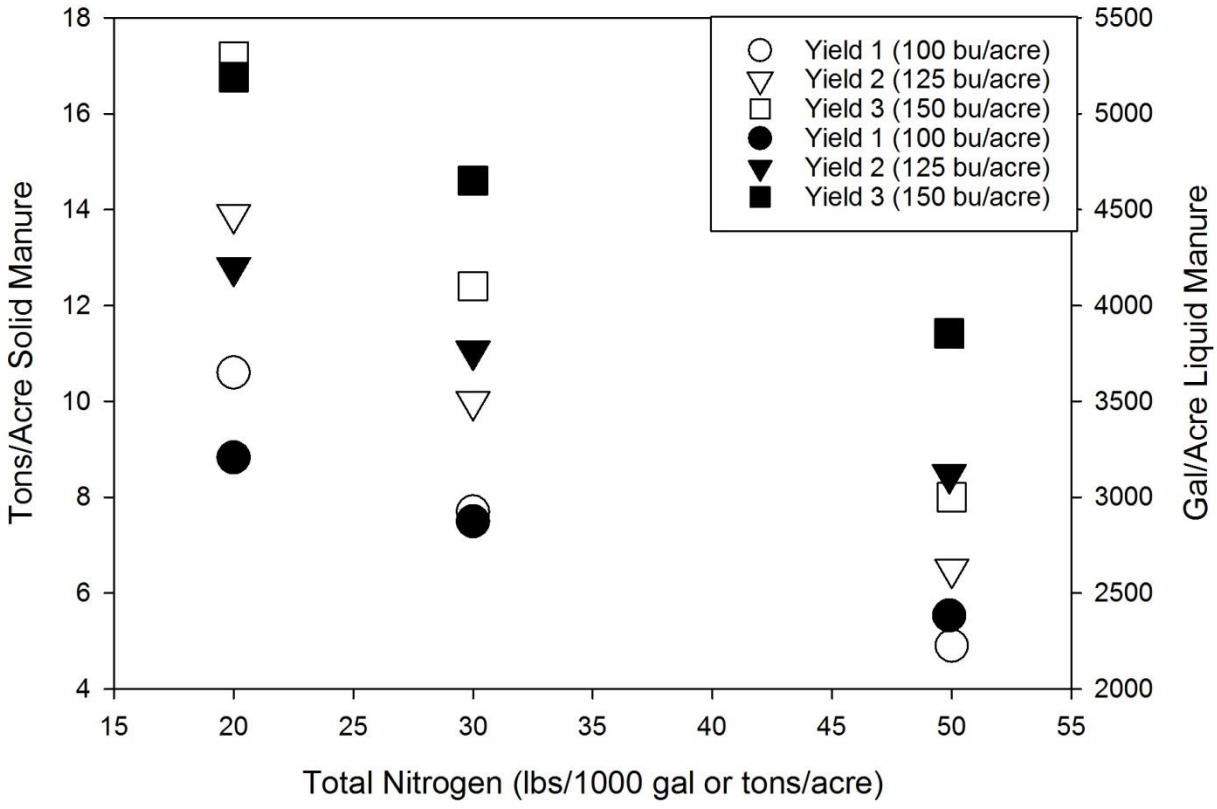

Figure 2.2. Application rates of solid (hollow symbols) and liquid (filled symbols) manures as a function of expected corn yields.

Liquid manure applications are oftentimes limited by the water holding capacity of the soil receiving the amendment whereby no more than a half inch or 13,500 gallons per acre are allowed to be applied (Arnold and Elder, 2016; James et al., 2006).

Exceeding this application rate would likely result in the substantial runoff of liquid manure.

2.2. Manure borne-pathogenic and indicator organisms

2.2.1. Pathogen-related health issues

The handling and disposal of animal wastes can present a significant problem for human and environmental health. Potentially harmful fecal bacteria are also deposited onto fields and pastures when manures are spread and/or defecating livestock graze. Consequently, the microbial quality of surface and groundwater sources may be compromised when nutrients and bacteria are transported during rainfall and/or irrigation events. Infections can occur when these pathogens are introduced to waters used for recreation or irrigation. Every year, 1 in 6 humans will contract a foodborne illness associated with fecal contamination (CDC, 2011). The associated cost-of-illness nationally has been estimated at between $4.4 to $152 billion dollars annually (Hoffman et al., 2012; Scharff, 2012). The USEPA currently lists the leading probable source of the impairment of rivers and streams as being due to agricultural practices and lists the number one and three causes for impairment as pathogens and nutrients, respectively (USEPA, 2016).

2.2.2. Manure-borne pathogens

Manure-borne microbial pathogens may include bacteria (*Escherichia coli* O:157H7, *campylobacter spp., Salmonella spp.*, and *Yersinia enterocolitica*, viruses (rotavirus, norovirus, and hepatitis A & E), and protozoan parasites (*Cryptosporidium* and *Giardia*) (Sobsey et al. 2006; Ziemer et al., 2010; Wei et al, 2010; Meng et al. 1998;

Bradford and Schijven, 2002). Manure-borne pathogens can exceed millions to billions per gram of feces and can infect humans through routes such as contaminated air, interaction with livestock animals and/or their waste products, swimming in contaminated waters, exposure to potential vectors (mosquitoes, flies, rodents), or consumption of food or water contaminated by animal wastes (Rodgers and Haines, 2005). Once contracted, the typical symptoms of pathogen exposure include abdominal pain, nausea, vomiting, cramping, fever, headache, and gastrointestinal bleeding resulting in an estimated 128,000 hospitalizations and 3,000 deaths annually (CDC, 2016).

The majority of microbes contained in manure are not pathogenic to humans. (Zeimer et al. 2010). The actual prevalence (%) of pathogens in manure varies widely depending on pathogen and host animal type and age. Hutchison et al., (2004) examined the prevalence of zoonotic organisms in British livestock manures and found that 30% of manures contained at least one enteric pathogen. Another study found 78% of 67 farms examined in Switzerland had at least one manure sample positive for pathogenic *E. coli*. Table 2.1 shows the prevalence of select pathogens found in animal wastes in the United States.

Table 2.1. Examples of the prevalence of select pathogens within domestic animal feces.

Pathogen	Source	Location	Prevalence (%)	Sample #	Reference
E. coli O157:H7	ruminants, domestic	California	6.63	3,964	Cooley et al 2013
E. coli O157:H7	cattle, feedlot feces	Kansas	9.2	891	Alam and Zurek 2006
E. coli O157:H7	Cattle, dairy	TN, NC, AL, WA, CA	3.9	408	Doane et al. 2007
E. coli O157:H7	Cattle, beef	TN, NC, AL, WA, CA	4.7	408	Doane et al. 2007
E. coli O157:H7	chicken and turkey	TN, NC, AL, WA, CA	2.7	444	Doane et al. 2007
E. coli O157:H7	Swine	TN, NC, AL, WA, CA	8.8	426	Doane et al. 2007
Salmonella	swine feces		7.3	600	Callaway et al. 2010
Salmonella	Chicken and Turkey	TN, NC, AL, WA, CA	0.2	480	Rodriguez et al 2006
Salmonella	Swine, rectal sample	TN, NC, AL, WA, CA	6	480	Rodriguez et al 2006
Salmonella	Beef cattle	TN, NC, AL, WA, CA	0.2	480	Rodriguez et al 2006
Salmonella	Dairy cattle	TN, NC, AL, WA, CA	0.4	480	Rodriguez et al 2006
Giardia	cattle feces	California	34.3	201	Oates et al 2012
Cryptosporidium	cattle feces	California	6.5	201	Oates et al 2012
norovirus	swine feces	U.S.	20	61	Constantini et al. 2007
rotavirus A,B,C	swine feces	U.S	67, 0, 44	61	Constantini et al. 2007

A seasonality effect on the prevalence of certain pathogens like *E. coli* O157:H7 and Listeria was documented by Hutchison et al. (2005) in which prevalence increased during summer months.

Recreation activities in fresh waters create a pathway for water-borne pathogens to reach humans. Another pathway of water-borne pathogens to humans is present in irrigated agriculture, predominantly in vegetable and fruit production. More than 40% of food poisoning in USA is currently caused by produce consumption (Painter et al., 2013). Manure is recognized as a source of pathogens in produce, and the FDA regulations regarding food safety include the manure safety rules which prescribes manure handling, application, and composting practices (HHS and FDA, 2015). Pathogens from agricultural raw manure applications have also been found to make their way into public drinking water during flooding and storm events (Health Canada, 2000).

2.2.3. Indicators of fecal contamination

Within fecal and environmental samples, many microbial pathogens are difficult to detect and quantify especially for their infectivity. Processing samples for pathogens may also be impractical and unfeasible at times due to the inability of some methods to detect infectious microbes while more sensitive methods are lengthy and come at a higher cost of analysis (Sobsey et al., 2006). Because of these difficulties with measurement, researchers often study fecal indicator bacteria (FIB) to infer the presence of fecal pathogens. FIB are fecal bacteria that are nonpathogenic and serve as surrogates for fecal

pathogens, but are easier to detect and enumerate (Indest 2003). The presence of FIB in an environment indicates fecal contamination and, therefore, the possible presence of fecal pathogens and the associated risk of illness. FIB are useful in studying the release, fate, and transport of fecal bacteria in the environment. The properties of FIB include the following: 1) the bacteria should be present wherever enteric pathogens are present; 2) the bacteria should be useful for all types of water; 3) the bacteria should survive longer than hardy enteric pathogens; 4) the bacteria should not grow in water; 5) the bacteria should be found in warm-blooded animal intestines; 6) the bacteria should be detected and quantified by easy, rapid, and inexpensive methods; and 7) the bacteria should be nonpathogenic (Bitton, 2005). The most common fecal indicator bacteria are *Escherichia coli* (*E. coli*) and *Enterococcus sp*. (enterococci) which are primarily used for studies within freshwater and marine water environments, but these bacteria are often used in conjunction for many water, soil, and sediment studies (Indest 2003; USEPA 1986; Cools et al., 2001; Bai et al., 2005, Bradshaw et al., 2013; Haller et al., 2009).

The United States Environmental Protection Agency (USEPA) sets the guidelines for the microbiological quality of surface waters based on the levels of FIB contained within the surface waters. Guidelines are decided as a compromise between financial costs and the practicality of achieving certain minimal levels and the estimated illness rate. The most recent recreational water quality criteria mandated by the USEPA contained two recommendations for state adoption. Recommendation 1 requires a geometric mean (GM) and statistical threshold value (STV) not to exceed 35 CFU/100 mL and 130 CFU/100 mL, respectively, for enterococci and 126 CFU/100 mL and 410 CFU/100 mL, respectively, for *E. coli.* Recommendation 2 requires a GM and STV of 30

CFU/100 mL and 110 CFU/100 mL, respectively, for enterococci, and 100 CFU/100 mL and 320 CFU/100 mL, respectively, for *E. coli*. These standards are typically set for a specific waterbody within a 30-day interval. The estimated illness rates associated with recommendations 1 and 2 are 36 illnesses in 1,000 and 32 illnesses in 1,000 water users, respectively, which are determined by the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) (USEPA, 2012).

There exists a tremendous amount of variation in the concentrations of a given fecal indicators and pathogens in manure. As presented in Table 2.2, the prevalence of actual pathogens is relatively low and when coupled with their transient presence means organisms such as *Salmonella, cryptosporidium,* and norovirus are typically not present in detectable amounts or at all in manures. However, bacteria such as *E. coli* or enterococci are present in the feces of all warm-blooded mammals and as such the relative concentrations of these bacteria are a useful metric in comparing one source to another. While concentrations of *E. coli* and enterococci vary wildly in manures, initial concentrations of between 10^5 and 10^7 cells per gram of manure seems to be more or less common across various indicator release and survival studies (Blaustein et al. 2015; Guber et al., 2007; Brooks et al., 2009; Thurston-Enriquez et al., 2005; Lau and Ingham, 2001). It is important to note that while the population of fecal indicators such as *E. coli* and enterococci in manure are high, they represent only a small fraction of the total bacterial population within a fecal source (Brooks et al., 2009).

Other fecal microbes used as indicators of contamination include fecal anaerobes *Clostridium* (Thurston-Enriquez et al., 2005) and staphylococci (Brooks et al., 2007),

fecal anaerobes *Bacteroides* spp. and *Bifidobacterium* spp. and fecal phages *Coliphage* and *Bacteriophage* (Savichtcheva et al., 2006).

Additionally, chemical tracers such as chloride or bromide ions (Guber et al., 2006; Blaustein et al., 2016) have been successfully used as surrogates for the release of fecal bacteria from manure under rainfall and their subsequent removal from the manure application area. Similarities between fecal microbes and phosphorous transport have also been documented. Stout et al. (2005) found that the transport of fecal coliform released from manure was highly correlated with the transport of total phosphorous. Dao et al. (2008) found strong correlations between organic phosphorous and *E. coli* and enterococci populations released from grass-applied manure and suggested the use of organic P as a surrogate for fecal contamination. Other studies found stronger correlations of fecal microbe movement in soils with ammonia and nitrate suggesting the chemotactic movement of bacteria toward essential nutrients had occurred (Gagliardi and Karns, 2000).

2.1.4. Wildlife as a source of animal waste

While animal wastes and manure from agriculture presents the greatest risk to water quality, wildlife wastes can also substantially affect the quality of water. In fragmented agricultural and forested areas, wildlife can represent a large portion of fecal pollution (Guber et al., 2015). Graves et al. (2002) performed genetic analysis of fecal bacteria from a stream that drains a watershed and found that wildlife accounted for 40% of the bacteria while agriculture and human sources made up the remaining 50% and

10%, respectively. Similarly, Burnes (2003) found that during baseflow in Big Creek, a mixed-use watershed, most fecal bacteria originated from human sources, but during hydrologic events, wildlife and livestock sources dominated. Waterborne infections stemming from wildlife feces have been documented, but are often difficult to differentiate from infections stemming from manure-borne pathogens without performing genetic fingerprinting (Guselle and Olson, 2004).

2.3. Release of microorganisms from manures and animal waste

Pathogens and other fecal bacteria from animal manures are released by interactions with water and are then transported by surface runoff and subsurface infiltration (Jamieson et al. 2002). The movement of fecal bacteria in runoff and infiltration has the potential to contaminate surface and groundwater sources (Unc and Goss, 2003).

2.3.1. Release modes

Solid manure is a heterogeneous matrix of macro- and microscopic dietary fibers, microbial colloids, biopolymers, and soluble components (Guber et al. 2006). When water is applied to manure, either through rainfall and/or irrigation events, some portion of the applied water will travel through the manure's complex matrix before exiting (Blaustein et al., 2015). The close proximity that water has within the manure matrix as well as simply its contact with the surface of manure results in the enrichment of water with nutrients and fecal microorganisms. In addition to the travel through manure,

raindrop action on the surface of the manure will detach and release microbes (Tyrell and Quinton, 2003). As water application continues, fecal microorganisms that are in the liquid fraction of manure are readily displaced by water while other microbes may exhibit slower release if contained in less readily available, possibly solid, components (Guber et al., 2007; Kouznetsov et al., 2007). During the course of a rainfall event, the concentrations of microbes residing near the solid fecal surfaces may become exhausted due to a prolonged washing-out thus slowing microbial release until new manure surfaces are exposed by the destruction of the fecal matrix via rainfall action (Schijven e al., 2004).

2.3.2. Factors affecting release

In general, the extent and rate of the release of fecal microorganisms from manure is dependent on numerous factors including manure composition and age, precipitation type and intensity, volume of water applied, concentration of a given microorganism, and water solution chemistry (Goss et al., 2002; Blaustein et al. 2016; Guber et al 2015; Bradford et al. 2013). The release of fecal pathogens also varies by organism type (e.g., bacteria vs. parasite) and can further vary between specific species of the same organisms (Brooks et al., 2009; Guber et al. 2007; Blaustein et al., 2015)

2.3.2.1. Microorganism morphology and physiology

Manure-borne microorganisms have unique release patterns dependent on cell physio-morphology. The chemical and physical properties of bacteria affect their

likelihood of becoming dislodged from micro-habitats (Lombard et al., 2011). Positive or negative surface charges as well as whether a cell is hydrophilic or hydrophobic will impact the attachment of that cell and its likelihood to enter into solution (Doyle, 2000). The same microorganisms may also experience different release responses under different environmental conditions. For example, viruses are generally negatively charged and are thus attracted to and entrapped by positively charged materials, but in neutral and alkaline conditions viruses will move freely (Bosch et al., 2006). This finding indicates that the release of viruses from manure may be greater in manures having a higher pH and may be lower in acidic manures. The bacteria may be gram-positive or gram-negative which may partially explain the differences in their release from the manure and entrance to water. The shape of the microorganisms may also influence their movement. The bacteria exist in rod, ellipsoidal, and spiral shapes and can have varying degrees of extra-cellular molecules attached which has led some researchers to question whether these shapes have an impact on the attachment/detachment process and subsequent release and transport (Bradford et al., 2013).

Within manure, the variability of the same organism type within its population may result in differential release from the manure during rainfall. DeFlaun et al., (1997) observed different transport properties in two different sub-populations of the same strain of bacteria and Bolster et al. (2009) observed diversity in transport behavior among 12 different strains of *E. coli* from the manures of 6 different animal sources.

2.3.2.2. Rainfall properties

The physical and chemical properties of precipitation can influence the release of microbes from fecal material. Bradford and Schijven (2002) simulated precipitation events on dairy calf manure using water of varying salt contents and found an increase in the amounts of *Cryptosporidium* and *Giardia* released with decreasing rainfall salinity. The authors attributed this finding to the stabilization of the manure through the compression of the double layer thickness between negatively charged components of the manure.

Rainfall intensity can affect the rate and, therefore, the quantity of fecal microbes released during a given time period. In one study comparing the effects of using a low and high intensity rainfall, it was found that a 3.5 times greater intensity resulted in five-, three-, and five-times-higher cumulative release of manure solids, protozoa oocysts, and cysts, respectively, from cattle manure undergoing simulated rainfall (Schijven e al., 2004). The higher intensity rainfalls imparted more energy to release fecal microbes from manure or soils than lower intensity events (Ling et al., 2009). Blaustein et al., (2016) examined the effects of rainfall intensity on the release of *E. coli* and enterococci from solid dairy manure. The authors found greater total release of *E. coli* at a 9 cm/hr rainfall intensity than for a 6 cm/hr or 3 cm/hr rainfall intensity. Enterococci release was similar for the 9 cm/hr rainfall intensity and the 6 cm/hr rainfall intensity with both being greater than the 3 cm/hr rainfall intensity.

The type of rainfall that interacts with manure or soil has been shown to influence the release of bacteria. In one study, precipitation in the form of rain showers was shown to release seven times more fecal protozoa than when precipitation was applied in the

form of a mist or a light drizzle (Schijven e al., 2004). The authors attributed this difference to the greater mechanical forces (raindrop impact energy) acting on the manure source during the rain shower.

Very little is known on the role of temperature in the release of microorganisms from manure. One such study compared the release of *Cryptosporidium oocysts and* giardia from cow manure at 5°C and 23°C and found no statistical differences in release rates, but surmised that the role of temperature probably increases with time due to the degradation of the manure and the oocysts (Schijven e al., 2004). Farghangi et al. (2012) observed reduced bacterial release from cow manure interacting with unsaturated soil water flow when temperatures were lower and attributed this difference to the increased viscosity of the flowing water.

The volume of water applied or the duration of rainfall will also impact the release of the fecal microbes from manure. The release curves for the fecal bacteria eluted from manure typically demonstrates a two-stage dynamic whereby release is initially rapid, but then slows as the microbial stocks are depleted within the fecal matrix. Guber et al. (2007) simulated rainfall on bovine-amended soil plots and described the bacteria release using first-order release kinetics for the first 40-minutes of rainfall and zero order kinetics for the bacteria release for the additional rainfall after this time. These same dynamics have been documented in a number of other release studies (Schijven e al., 2004; Guber et al., 2006; Blaustein et al., 2015).

2.3.2.3. Manure age

As manure age increases, the weathering processes oftentimes results in the formation of a surface crust on the manure. Surface crusts on manure act to retard the release of microorganisms during water application (Muirhead et al., 2005; Hodgson et al., 2009; Kim et al., 2016). Ferguson et al. (2007) found that the cumulative release of *Cryptosporidium oocysts*, *E. coli*, and PRD1 bacteriophages released from artificial fecal pats under simulated rainfall was greater for fresh manure pats when compared to aged pats. Thurston-Enriquez et al., (2005) reported larger concentrations of fecal bacteria and protozoa in runoff from fresh cattle manure than in aged manure treatments likely due to the inaccessibility of microbes contained in crusted manure and because of the ability of dry, aged manure to absorb more water which keeps it from running off. Aging may greatly reduce the amount of fecal microbes that leach out of manure largely because of the reductions in fecal loads within the manure over time (NRCS/USDA 2012). Hodgson et al. (2009) observed reductions in fecal microbial release with the increasing age of manure despite the concentrations of fecal microbes within the manures remaining high. The authors attributed the reductions to the likelihood of bacteria becoming encapsulated within fecal matrices and thus less likely to be transported during subsequent precipitation events.

2.3.2.4. Manure consistency effect on FIB release

The internal structure and the high surface area of solid manures relative to liquid manures will likely aid in the retention of microorganisms within the solid manures. Liquid manures do not contain this internal structure that prevents water from interacting

with the microbes and may consequently result in higher release potentials from the liquid manures (Kim et al., 2016). To our knowledge, there has been no systematic research that has quantified the effects of manure consistency on the FIB release rates.

2.3.2.5. Models of bacteria release

Considerable effort has gone into the development of predictive models that describe the release of fecal microorganisms from manure sources (Guber et al., 2006; Blaustein et al., 2015;, Schijven et al., 2004). The extent of microbial release following a precipitation event is an important question in terms of water quality forecasting. Through our understanding of the microbial release process, we are better able to gauge their removal and transport in surface water and groundwater. The microbial release rates also facilitate the estimations of the cumulative loading of fecal pathogens in waters by which contamination potentials may be assessed.

Predictive models are often used to simulate the ratio of the concentration of bacteria released, C, to the initial concentration of bacteria in the manure source, C_0. The use of these predictive models is an extension of our previous study of solute transport using predictive equations. Concentrations of microorganisms are typically presented per g^{-1} dry weight, g^{-1} wet weight of manure, ml^{-1} total volume of manure, ml^{-1} liquid content of manure, or ml^{-1} of runoff water (Blaustein et al. 2016). As release occurs, the relative release (C/C_0) is initially rapid, but slows overtime. Guber et al. (2007) described the FC release from manure under a simulated rainfall event as having first order kinetics for the first 40 minutes of rainfall and then zero order kinetics for the remainder of the release

event. Similarly, Schijven et al. (2004) observed decreased release rates of *Cryptosporidium* from manure over time which they attributed to the depletion of the manure components that had been exposed to rainfall.

Sometimes instead of using microbial concentrations (C/C_0), the microbial transport is described using the basis of the number of microbes released as a fraction of the total number applied (N/N_0). There are three such predictive models that have been widely employed among fecal bacteria release studies for a single event (Blaustein et al. 2016). These models include 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).

1. The one-parametric release model was described in Guber et al. (2006) as:

$$C_{t=}C_0[1-\exp(-kqt)] \quad (1)$$

where C_t is the amount of bacteria in CFU released from the manure in time t, C_0 is the initial amount of bacteria in the manure that can be released, k is the rate constant for the exponential model, and q is the precipitation rate. Interchanging N and N_0 for C and C_0, respectively, and rearranging the equation for relative release results in the following expression:

$$\frac{N}{N_0} = 1 - e^{(-k_e w)} \quad (2)$$

where N is the total number of bacteria released from the manure at time t, N_0 is the initial number of bacteria in manure before the rainfall event, k_e is the rate constant for the exponential model, and w is the runoff depth.

2. The two-parametric Bradford-Schijven model was presented in Guber et al. (2006) as:

$$C(t) = \frac{dM_w}{Qdt} = \frac{C_0 \alpha V m}{Q}(1 + \alpha\beta t)^{(1+1/\beta)} \quad (3)$$

where M_w is the cumulative manure mass released into the aqueous phase (g), Q is the aqueous phase flow rate ($cm^3\ h^{-1}$), V_m is the manure volume (cm^3), and $\alpha(h^{-1})$ and β are the fitting parameters defining the shape of the release curve.

3. The Vadas et al. (2004) equation was originally developed to describe organic phosphorous loss in runoff from surface-applied animal manures, but has since been adapted to predict bacterial release (Guber et al., 2006; Blaustein et al., 2016).

$$\frac{N}{N_0} = AW^n \quad (4)$$

where N is the total number of bacteria removed per unit area of manure with runoff, [N] = CFU m^{-2}; N_0 is the initial total number of bacteria in the applied manure, [N_0] = CFU; A is the rate constant parameter, [A] = cm^{-n} (for removal dependency on rainfall depth); W is the rainfall depth, [W] cm rainfall; and n is a dimensionless parameter.

Table 2.2. Summary of representative release studies demonstrating the percent of each organism released from the manure source under simulated rainfall.

Organism	Factor	Rain intensity (mm/hr)	Duration (min)	Animal	Consistency	Manure Age	Removed %	Remaining %	Reference
Crpytosporidium	Aging	55	30	Cattle	Cow pat	Fresh	0.7 [a]	99.30	Ferguson et al. 2007
E. coli	Aging	55	30	Cattle	Cow pat	Fresh	1.35 [a]	98.65	Ferguson et al. 2007
Bacteriophage	Aging	55	30	Cattle	Cow pat	Fresh	0.045 [a]	99.96	Ferguson et al. 2007
Crpytosporidium	Aging	55	30	Cattle	Cow pat	1 Week	0.035 [a]	99.97	Ferguson et al. 2007
E. coli	Aging	55	30	Cattle	Cow pat	1 Week	7.52 [a]	92.48	Ferguson et al. 2007
Bacteriophage	Aging	55	30	Cattle	Cow pat	1 Week	0.0303 [a]	99.97	Ferguson et al. 2007
Total Coliforms	Diet (Corn)	70	30	Cattle	Solid	Fresh	13	87.00	Durso et al. 2011
Total Coliforms	Diet (grain)	70	30	Cattle	Solid	Fresh	1	99.00	Durso et al. 2011
E. coli	Diet (Corn)	70	30	Cattle	Solid	Fresh	9	91.00	Durso et al. 2011
E. coli	Diet (grain)	70	30	Cattle	Solid	Fresh	0.8	99.20	Durso et al. 2011
Enterococcus	Diet (corn)	70	30	Cattle	Solid	Fresh	7	93.00	Durso et al. 2011
Enterococcus	Diet (grain)	70	30	Cattle	Solid	Fresh	1	99.00	Durso et al. 2011
E. coli	Aging	70	30	Cattle	Solid	Fresh	0.01	99.99	Thurston-Enriquez 2005
E. coli	Aging	70	30	Cattle	Solid	Aged	2.56	97.44	Thurston Enriquez 2005
E. coli	Aging	70	30	Swine	Slurry	Fresh	6.99	93.01	Thurston Enriquez 2005
Enterococci	Aging	70	30	Cattle	Solid	Fresh	0.07	99.93	Thurston Enriquez 2005
Enterococci	Aging	70	30	Cattle	Solid	Aged	4.65	95.35	Thurston Enriquez 2005
Enterococci	Aging	70	30	Swine	Slurry	Fresh	0.52	99.48	Thurston Enriquez 2005
Clostridium	Aging	70	30	Cattle	Solid	Fresh	0.38	99.62	Thurston Enriquez 2005
Clostridium	Aging	70	30	Cattle	Solid	Aged	0.12	99.88	Thurston Enriquez 2005
Clostridium	Aging	70	30	Swine	Slurry	Fresh	3	97.00	Thurston Enriquez 2005
Coliphage	Aging	70	30	Cattle	Solid	Fresh	0.07	99.93	Thurston Enriquez 2005
Coliphage	Aging	70	30	Cattle	Solid	Aged	2.36	97.64	Thurston Enriquez 2005
Coliphage	Aging	70	30	Swine	Slurry	Fresh	0.64	99.36	Thurston Enriquez 2005
E. coli	Consistency	62	42	Swine	Slurry	Fresh	3.0 [a]	97.00	Jaffrezic et al., 2011
Enterococci	Consistency	62	42	Swine	Slurry	Fresh	2.4 [a]	97.63	Jaffrezic et al., 2011
Coliphages	Consistency	62	42	Swine	Slurry	Fresh	9.2 [a]	90.80	Jaffrezic et al., 2011
E. coli	Consistency	69	85	Cattle	Solid	Fresh	13.0 [a]	86.97	Jaffrezic et al., 2011
Enterococci	Consistency	69	85	Cattle	Solid	Fresh	4.1 [a]	95.87	Jaffrezic et al., 2011

Coliphages	Consistency	69	85	Cattle	Solid	Fresh	2.0[a]	97.97	Jaffrezic et al., 2011
E. coli	Consistency	45	30	Cattle	Liquid	NA	0.1	99.90	Soupir et al., 2003
Fecal Coliform	Consistency	45	30	Cattle	Cowpie	3-6 Weeks	0.3	99.70	Soupir et al., 2003
Enterococcus	Consistency	45	30	Turkey	Solid	Fresh	0	100.00	Soupir et al., 2003
E. coli	Consistency	45	30	Cattle	Liquid	NA	0.5	99.50	Soupir et al., 2003
Fecal Coliform	Consistency	45	30	Cattle	Cowpie	3-6 Weeks	0.6	99.40	Soupir et al., 2003
Enterococcus	Consistency	45	30	Turkey	Solid	Fresh	0.3	99.70	Soupir et al., 2003
E. coli	Consistency	45	30	Cattle	Liquid	NA	5.7	94.30	Soupir et al., 2003
Fecal Coliform	Consistency	45	30	Cattle	Cowpie	3-6 Weeks	14.5	85.50	Soupir et al., 2003
Enterococcus	Consistency	45	30	Turkey	Solid	Fresh	1.3	98.70	Soupir et al., 2003

a = Average release of replicates

2.4. FIB removal

The literature cited in Table 2.2 seems to make a poor distinction between FIB release and removal. The true release studies focus on the detachment of microorganisms from the manure matrix. Removal, which is dependent on release, is the transport of microorganisms from the manure source in runoff and/or infiltration.

In the case of liquid manure or slurry, removal is the export from the source area, in case of solid manure, removal is more complex as it includes the transport among the manure clumps, cowpats, or similar spots of complete soil coverage, and the subsequent export from the application area.

Once released from manure, the fecal microorganisms are transported with water and may either infiltrate through the soil profile or move with overland flow depending on site specific hydrologic conditions (e.g. storm intensity and duration) and soil hydraulic properties (e.g. permeability, antecedent moisture content and temperature) (Bradford et al., 2013). They can also get trapped in soil pore spaces along with manure particles.

2.4.1. Amounts of bacteria removed during precipitation events

The amount of manure applied impacts the total number of microorganisms available for transport as does the method of manure application and the area of the land receiving the manure application (Oliver et al. 2005; Durso et al. 2011). The greater the concentration of a microorganism within feces results in an increased likelihood that specific microorganisms will be transported (Goss et al., 2002; Muirhead et al., 2005).

The type and number of microorganisms in manure can vary by the animal species, age of animals, types of bedding used, the methods of storage, and the storage periods (Unc and Goss, 2004). The abundance of factors that influence the numbers of bacteria transported during precipitation events makes a prediction of bacteria transport very difficult.

The majority of fecal microorganisms in feces are retained within the fecal matrix after precipitation events. Table 2.2 presents several studies that have reported the percentage of microorganisms released and retained by feces after simulated rainfall. Greater than 90% of fecal organisms in manure are typically retained within the manure matrix. The retention within the fecal matrix is the result of rainfall not fully eroding the fecal structure and/or because concentrations within the surface of fecal deposits are exhausted quickly while interior concentrations that are not subjected to water remain high (NRCS-USDA, 2012; Schijven e al., 2004). Thurston-Enriquez et al. (2005) reported retention of 93.01 to 99.99% of fecal indicators within manure after simulated rainfall. A number of authors have hypothesized additional explanations for the commonly observed low quantities of release including adsorption to soil particles too large to be carried in runoff, microbial die-off, predation, or infiltration into the soil (Thurston-Enriquez et al., 2005; Ling et al., 2009; Cardoso et al., 2012).

The population of retained organisms may exhibit increased concentrations within manure after rainfall that are released during subsequent rainfall events.

2.4.2. FIB transport modes

During field applications, portions of liquid manure may quickly infiltrate into soils or travel with overland water while the majority of solid manure constituents stay at the surface and are more slowly eluted by water (Mishra et al., 2008; Vadas et al. 2004; Thurston et al., 2005).

Within waters of either transport method, microbes may move as single or clumped cells or attached to soil or fecal particles (Muirdhead et al., 2005; Tyrell and Quinton, 2003; Pachepsky 2006; Soupir et al. 2010s; Dao et al., 2008). Following precipitation events, the microbial cells carried in the overland flow are deposited on the soil surface layer and infiltrate the soil where the cells either remain within the soil water or become adsorbed to soil surfaces (USEPA, 2013). Additional future precipitation events may cause additional microbial release with each subsequent precipitation event.

2.4.3. Transport of released bacteria in runoff

When rainfall exceeds the capacity of soil to infiltrate water then overland flow will occur. Overland flow transport has been identified as the largest and quickest transport route of fecal microbes (Muirhead et al., 2009; Tyrrel and Quinton, 2003; Jamieson et al., 2004) and occurs to a greater extent in soils with low permeability, moderate to high antecedent water conditions, steep slopes, and during intense rainfall events (USEPA, 2013). Because of the greater partitioning of fecal microbes to the runoff rather than the infiltration, the runoff is often the main focus of field release and transport studies. The rate and volume of overland flow is directly proportional to inflow and

water depth and indirectly proportional to water infiltration, depression storage (ponding), and surface flow resistance from obstructions such as vegetation (Bradford et al., 2013).

When released from a manure source, the microorganisms may be found as single cells, flocculated cells, or attached to manure particulates (Blaustein et al., 2015). Muirhead et al. (2005a) reported that only 8% of *E. coli* released from dairy cowpats under rainfall were attached to particles whereas the vast majority were transported as single cells. The authors stated that the impact energy of raindrops may have been sufficient to break up flocs of cells prior to transport. The authors concluded that during rainfall events < 25% of the cells released from the manure will be attached to particles. The same researchers also found no evidence of flocculated cells in the runoff (Muirhead et al., 2005b). Soupir et al. (2010) reported that 28 to 49% of *E. coli* and enterococci released from cowpats under simulated rainfall were attached to particles. The authors believed this attachment occurred within the manure prior to the rainfall-runoff events. Due to the small size and low density of planktonic microbial cells, once entrained in suspension, the microbial cells will remain in suspension and tend to not easily settle out (Tyrrel and Quinton, 2003).

Microorganisms attached to particles or flocculated with other cells are not transported as easily as planktonic cells. Particle-associated bacteria are generally less mobile in the environment and sink faster when in suspension (Fries et al., 2006). Using data on soil particle transport, Muirhead et al. (2006) calculated that bacteria attached to soil particles > 63 um in diameter would settle out of overland flow (Muirhead et al., 2006; Fiener and Auserwald, 2003). They also calculated that flocs of cells > 500 um in

diameter might be filtered out by grasses. Soupir et al. (2010) reported that a minimum of 60% of *E. coli* and enterococci were associated with particles within the 8- to 62-um particle size category in their release study. Pachepsky et al. (2008) found that 90% of particles released from manure under rainfall had mean diameters between 0.6 and 17.8 um. This information paired with the calculation by Muirhead et al. (2006) suggests that the majority of fecal bacteria will remain suspended in runoff whether they are planktonic or attached to particles.

Tyrrel and Quinton (2003) described the entrainment of microorganisms in overland flow as being subject to the same processes as soil particles: detachment, transport, and deposition. These three processes will occur as a result of the action of raindrops or the action of flowing water or some combination of both. Tyrell and Quinton (2003) believed that the concentration of microorganisms on the soil surface and the kinetic energy of rainfall were the two dominant factors affecting the quantity of microorganism detached from the soil during rainfall. In addition to rainfall kinetics, the attachment/detachment of cells in soil depends on the clay content, the abundance of manure particulates, cellular morphology and chemical properties, and the energy of overland flow among a number of other factors (Guber et al., 2007b; Ling et al., 2009; Soupir et al., 2010; Bradford et al., 2013).

Most agricultural fields and pastures are covered by vegetation and, thus, it would be prudent to examine the contribution of soil-borne fecal bacteria under these settings so that predictions of microbial loading to surface waters may be improved. Watershed models software such as SWAT take into account only a partial mixing of the top 1 cm of soil when calculating bacterial contribution to runoff from soil (Nietch et al., 2011). It is

unclear how accurate this calculation is and whether it would greatly benefit from site- and/or situation-specific information on what affects the release of bacteria from soil to runoff.

2.4.4. Bacteria transport with the infiltration flow

Once infiltrated, the fecal microorganisms undergo a number of processes within the soil including straining/filtering and adsorption/desorption as well as differential flow conditions depending on soil hydraulic properties (USEPA, 2013). Pathogen movement within the soil is generally several orders of magnitude slower than overland movement (Bradford et al., 2013). Unc and Goss (2003) summarized the four major factors that influence microbial movement within soil as i) the flow characteristics, which depend on the grain size of the porous medium and on the soil structure which controls the active porosity; ii) the filtration effects due to soil micropores, clogging in macropores necks, and filtration pads formed by solid components from applied manure as a function of the size of microbial cells; iii) the straining within organic material pads formed on the soil surfaces; and iv) the retention of bacterial cells by adsorption and adhesion on the surfaces of soil mineral and organic particles.

Roodsari et al. (2005) observed a major shift in the dominant transport route of fecal coliforms (FC) from runoff to infiltration in a manure-amended soil covered with grassy vegetation. The major transport route shifted from infiltration to runoff for the same manure-amended soil that had a bare surface with no vegetative cover. In the same study, the authors detected FC only within the upper 10 cm of the clay soil, but detected

FC up to a 60-cm depth in the sandy. This result demonstrated the impact texture can have on FC adsorption and, therefore, retention in soils. In both soil types, the authors found the highest concentrations of FC in the top centimeter with continued reductions with as soil depth increased. Ramos et al., (2006) found that the application of liquid swine manure to bare soil reduced soil erosion by 30%, but resulted in much higher surface runoff in comparison to untreated plots. In general, surface vegetation enhances infiltration rates by (1) intercepting and dissipating the energy delivered by raindrops, thereby minimizing surface sealing; (2) reducing overland flow velocity; (3) increasing soil hydraulic conductivity attributable to the plant root systems; (4) increasing the number of macropores by macro-invertebrate activity; and (5) enhancing the permeability of soil surfaces through the accumulation of organic matter residues (Cardoso et al., 2012). Solid manure applied to soil surfaces has also been noted to have a mulching effect, thereby, increasing hydraulic resistivity and helping to prevent the formation of surface seals (Bruggeman and Mostaghimi 1993; Mishra et al., 2008).

Liquid manures are typically homogenous solutions of urine, feces, and water whereas solid manures are heterogeneous mixtures of cowpat and bedding. As such, liquid manures are easily carried away in runoff or rapidly infiltrate soil (Thurston et al., 2005; Smith et al. 2007; Vadas et al., 2004; Kleinman et al., 2004), whereas solid wastes primarily remain on the soil surface and may enhance the retention of microorganisms by the soil surface (Chetochine et al., 2006; Gottschall et al., 2009; Horswell et al., 2010).

If soils are well structured, coarsely textured, or have large distributions of pore networks, then liquid manures may infiltrate the soils leading to reduced removal via runoff. Jaffrezeic et al., (2012) reported smaller percentages of fecal bacteria and viruses

recovered in runoff from plots amended with liquid swine slurry than in those amended with solid cow manure despite roughly equivalent numbers of each organism applied per manure type. The infiltration of the fecal organisms likely occurred within the 2-hr time period the researchers waited after manure application, but before the initiation of simulated rainfall. Vinten et al. (2002) applied cattle slurry to three lysimeter plots and measured *E. coli* in runoff and infiltration under natural rainfall conditions throughout the 80-day experiment. They estimated that approximately 0.003 % of applied *E. coli* was removed from the plots as runoff whereas around 20% of the applied *E. coli* was removed via infiltration. In their experiment, they reported times when the concentrations of *E. coli* were considered to be high in the drainage water despite relatively low concentrations in the soil. This result was attributed to: i) the mobilization of slurry colloids by impacting raindrops, and ii) the transport of these colloids from the soil surface to drains by bypass flow. This mobilization would only be expected to occur when the slurry is still present at the soil surface which they hypothesized would promote the transport of colloids through the soil profile. It may be extrapolated that if rainfall is absent around the time of liquid manure application, most of the applied microorganisms would be expected to infiltrate and remain in the soil, but the manure particle-associated bacteria applied to the soil surface may still substantially contaminate infiltrated water. In a lysimeter study using cattle slurry, Fenlon et al. (2000), determined that 80-90% of the total *E. coli* applied was retained in the soil after rainfall. Some of these bacteria were then slowly eluted in the subsequent rainfall events. Thus, after such rainfall events on manured areas, it is possible that, like the fecal matrix in the solid manures, the soil may act as a new matrix in which enteric bacteria can reside and be subject to release.

2.4.5. Application method and FIB removal rates

Incorporating the manures into soil may enhance pathogen survival, but in general the incorporation removes microorganisms from interaction with surface runoff (Meals and Braun, 2006). Quinton et al., (2003) found consistently a higher release of microbes from surface applied manures than those incorporated into the soil. They determined the greater release from surface applied manures was a result of erosive effects of raindrop splash and overland flow. Mishra et al., (2008) found greater mean concentrations of *E. coli* and enterococci in runoff from surface applied dairy manure than in incorporated manure, but found the opposite was true for fecal coliforms. Meals and Braun (2006) observed higher mean *E. coli* concentrations in runoff when manure was not incorporated into the soil versus when it was incorporated, however; their finding was not statistically significant. Likewise, Durso et al., (2011) observed no significant effects of till verse no-till on the transport of Phages, Total coliforms, *E. coli*, and enterococci from manure-amended fields after simulated rainfall.

The application method is a factor of the transport to groundwater and drainage**.** Samarajeewa et al., (2012) studied the effects of three different application methods on the release of fecal bacteria into groundwater and tile drainage water from liquid swine manure applied to fields. The authors found that surface application lead to the highest concentrations of *E. coli* in tile drainage water and shallow groundwater. They also determined that pre-tillage and post-application incorporation methods yielded significantly lower concentrations of *E. coli* removed from the fields.

2.4.6, Weather and bacteria removal

The weather patterns surrounding the manure application date can impact the number of fecal microorganisms removed. The increased removal of fecal bacteria can occur when manures are applied on frozen or snow-covered grounds or in early spring runoff when the soils are saturated (USEPA, 2013). Storm events have generally been reported to mobilize large numbers of fecal bacteria from grazed and slurry amended soils (Oliver et al., 2003). The loading of salmonella to surface waters has been shown to increase during months of the year with the highest precipitation, thus there is a seasonality aspect to the removal of fecal bacteria from manure sources (Haley et al., 2009).

If manures are applied prior to heavy precipitation events then it is likely that increased removal will occur (Olson, 2001), especially during the first stages of the rainfall event (Ramos and Quinton 2006; Mishra et al., 2008 Samarajeewa et al., 2012).

Meals and Braun (2006) observed a significant effect of the time period between manure application and the occurrence of rainfall on the levels of *E. coli* in the runoff from agricultural fields. These researchers found that runoff contained ~50% less *E. coli* when manures were applied 3 days prior to a rainfall event than when applied 1 day prior to the event. They attributed these differences to bacterial die-off, the immobilization of the bacteria through adsorption-fixation to the surface soils and vegetation, and the exposure of the manure to UV radiation and subsequent desiccation. Heinonen-Tanaksi and Uusi-Kampaa (2001) found that greater concentrations of fecal microorganisms in

runoff from plots that received autumn slurry application than those that received applications in the early summer when bacterial die-off inducing processes would be the greater. Vadas et al. (2011) performed a meta-analysis of nine studies involving the release of phosphorous from manures under simulated rainfall and found that in 5 of the 9 studies, the increase in the time period between manure application and a rainfall event led to reductions in the P concentrations within the runoff.

2.4.7. Runoff delay time and FIB removal

The initiation time of the runoff following the start of a rainfall event as well as the ratio of runoff-to-rainfall can be important in determining the concentrations of fecal microbes removed from manure-amended fields and pastures. If soils are initially wet then the onset of runoff following the start of a rain event will be relatively short compared to the onset if the soils are initially dry. Likewise, the ratio of runoff-to-rainfall will be larger under wet initial soil conditions than under dry initial soil conditions. In a study involving dissolved phosphorous, Vadas et al., (2004) surmised that the greater rainfall-to-runoff ratios resulted in more runoff being produced earlier during the rain events that the runoff had higher concentrations of dissolved phosphorus. In a later study, Vadas et al. (2011) proposed that if the runoff-to-rain ratio is relatively low then runoff will occur later during a precipitation event and the runoff should have lower solute concentrations. The authors believed this result was attributable to a longer time period for the initiation of runoff and that the rainfall would have a larger amount of time to interact with manure and, subsequently, infiltrate solutes. This occurrence will be enhanced if the soils are initially dry which will result in greater water absorption relative

to wet soils. Cardoso et al. (2012) compared the effects of wet and dry antecedent soil moisture conditions on the release of *E. coli* and salmonella from liquid dairy manure applied to vegetated plots. They observed much longer delays to the initiation of runoff in the dry plots and attributed these delays to the higher rates of infiltration that occurred in the soil plots. Consequently, the recovery of both bacteria in the dry plots was 5-6% of the amounts of bacteria that wre applied versus a 33% recovery in the wet plots. They explained that the majority of bacteria release occurred during the first 10 minutes after rainfall initiation. In this time period, the bacteria in the dry plot infiltrated the soil and thus was not recovered. Similarly, Kim et al. (2016) observed greater delays before the initiation of runoff in dryer field plots compared to wet ones. The recovery of *E. coli* and enterococci were the lowest in the dry plots.

In addition to initial soil conditions, the nature of the manure spread will have an influence on the initiation of runoff. Solid manures can affect the runoff start time by acting as sponges for water and once the manure is saturated, the enriched water drains from the manure and becomes runoff. Thelin and Giford (1983) theorized that the time taken to hydrate aged manure deposits delays the time to reach equilibrium in the concentration of the fecal coliforms being removed. Conversely, the application of liquid manures will likely result in increased soil moisture and, therefore, a shorter onset for the initiation of runoff (Vadas et al., 2004; Soupir et al., 2003).

Assuming equal rainfall intensities and rainfall volumes applied, the delay prior to the initiation of runoff can be reliant on the moisture content of the manure with wetter manures resulting in shorter release times than when drier manures are used. No data on this effect are available in literature.

2.4.8. Bacterial exchange between soil and runoff

Fecal microbes released and transported from manure typically remain in relatively large numbers at the soil surface relative to the rest of the soil profile (Faust, 1982; Ling et al., 2009). Once the rainfall declines or ceases, the cells that are carried in the overland flow will return to the soil surface under the effects of gravity.

A review by Bradford et al., (2013) reported that at the soil surface microbial release increases as a function of increasing raindrop impact, surface water hydrodynamics (e.g. precipitation intensity, water velocity and depth), surface vegetation cover, and the biological materials present. Mishra et al., (2008) found that during repeated rainfall simulations, reduced infiltration rates were observed due to surface sealing caused by raindrop impact.

The early research into assessing which soil depths contribute to the solute concentrations in runoff determined that soil-runoff interactions occur primarily within the upper 2 cm of the soil surface with an exponential decrease in the interactions with increased soil depths (Ahuja and Lehman, 1983). This depth of interaction (EDI) was found to increase with land slope and rainfall intensity and to decrease with increased surface coverage and to also vary with the soil texture (Sharpley, 1985). Zhang et al., (1997) used gypsum as a tracer to study the EDI and detected a mixing zone under the soil surface where the rainwater, the soil solution, and the osmotic water were instantaneously mixed and that no chemical transport was determined to have occurred below this mixing zone. These researchers later determined the mixing depth to be < 5

mm (Zhang et al., 1999). More recently, Tian et al., (2012) described the mixing zone process as complicated due to being a function of: (i) the convection with the vertical hydraulic gradient, (ii) the convection via the surface flow or the Bernoulli effects, (iii) diffusion, and (iv) the soil loss. The initial moisture conditions have also been found to play an import role in EDI because the variations in initial water content influence soil infiltrability and detachability (Yang et al., 2015). All studies performed with the intent to determine the EDI have been done using chemical tracers and either poorly aggregated or highly artificial soils. The effects of living surface vegetation on EDI have not been determined; rather the effects have only been estimated by the use of placing mesh screens or crop residues atop soils under simulated rainfall (Ahuja et al., 1982; Sharpley et al., 1985).

While there has been considerable research into determining an EDI for solute transport, research to determine an EDI for microorganisms in soil has not been done. Commonly, a depth of 10 mm is assumed in models (Muirhead, 2009; Muirhead and Monaghan, 2012; Cho et al., 2016; Neitsch et al., 2011; Benham et al., 2006). This value originates from both the aforementioned studies of EDI using chemical tracers or nutrients and from a practicality standpoint which acknowledges the difficulty in sampling soil cores less than 10 mm in height. No support for this value exists in the scientific literature.

2.5. FIB survival in manure and soil

Fecal pathogens can survive in extraenteric environments such as in or on plants, deposited feces and manure, soils, and waters for a wide range of times depending on environmental conditions and the specific pathogen. Survival outside of the host means facing new stresses including limited nutrient availability, osmotic stress, variations in temperature and pH, and predation (Winfield and Groisman, 2003). Generally the conditions for pathogen survival outside a host are less favorable than survival in the intestinal system (Tauxe et al., 1997). Exponential decline in pathogen concentrations in non-host environments has long been assumed, however, growth and even naturalization of fecal bacteria in these environments is becoming more prominently documented (Ishii and Sadowsky 2008).

2.5.1. Survival in manure

Survival of fecal pathogens in manure is dependent on manure type, application method, type of soil receiving treatment, how the manure was stored prior to application, microbial diversity in the manure, the nutrient ratios of the manure, pH, temperature, season, dry matter content, age, and weathering (Hirneisen et al. 2012, Gueselle and Olson, 2004; Muirhead and Littlejohn, 2009). Kudva et al. (1998) observed *E. coli* O157:H7 survival for greater than 1 year in a non-aerated ovine manure pile that was left open to environmental conditions. The same study documented survival in bovine manure piles for a period of 47 days. Other studies have also found fecal organisms in

manure and feces to persist for a matter of weeks to months through a range of storage and consistency types (Wang et al., 1996; Nicholson et al., 2004; Hutchison et al. 2004; Rodgers and Haines 2005; Muirhead and Littlejohn, 2009). Himathongkham et al. (1999) observed smaller decimal reduction times for both *E. coli* O157:H7 and *Salmonella enterica* in liquid manure than in solid manure held at 20 and 37°C indicating a more rapid die-off rate in liquid manures, however this finding was reversed at 4°C. The same finding was also reported by Kudva et al., (1998) for *E. coli* O157:H7 in bovine feces held at the same temperatures in non-aerated conditions. These researchers also compared aerated versus non-aerated conditions and concluded aeration of manure increased pathogen die-off rates. Conversely, Nicholson et al., (2005) observed the greatest survival times of *E. coli* O157:H7, Salmonella, Listeria, and Campylobacter in dairy slurries rather solid farm yard manures, however, in their study they maintained the solid manure at consistently higher temperatures than the slurry. In general, increased aeration, temperature and holding times (age) lead to more rapid die-off rate of pathogens in manure (Rodgers and Haines, 2005).

As previously mentioned, temperature plays an important role in pathogen survival in all mediums. The greatest pathogen survival times in manure have been documented at temperatures below 23°C and temperatures exceeding this value have resulted in a more rapid die-off rate (Wang et al., 1996; Kudva et al., 1998; Nicholson et al., 2004). Parasitic protozoan are not as susceptible to temperature extremes compared to bacterial pathogens likely due to the ability of protozoans to form cysts and oocysts for protection from environmental stresses (Rodgers and Haines, 2005). When manure pH is strongly basic or acidic, certain bacterial pathogens like *E. coli* O157:H7 and

campylobacter as well as viruses cannot replicate and the population begins to decline (van Elsas et al., 2011; Sobsey et al., 2006).

No studies have focused on pathogen survival in liquid manures after application due to the transient nature of the liquid manure and the lack of material to sample due to low solids content. Rather, the soil that received the application is sampled for pathogen content so comparison of liquid vs solid pathogen concentrations in manure after application is difficult or impossible. Additionally, survival of pathogens in animal manures and manure slurries is typically studied in laboratory settings and field data is sparse (Rodgers and Haines, 2005). From the field studies currently available, it seems pathogens in field-applied solid manures seem to die-off at more rapid rates than in storage settings primarily due to newly introduced stresses such as UV light, desiccation, and temperature fluctuations (USEPA 2013; Maeys et al. 2005; Hodgson et al., 2016). However, fecal bacteria within manure have demonstrated extended persistence in the environment when crusts form on the surface of manure which seem to provide protection from the elements (Muirhead et al. 2005; Soupir et al. 2008). Muirhead and Littlejohn (2009) reported fecal bacteria survival of 5-6 months in intact cowpats left on pasture land and attributed this survival largely to manure crust formation. Sinton et al. (2007) observed fecal bacteria survival in pasture applied cowpats for up to 150 days and concluded that when moisture in cowpats falls below 70 to 75% the counts of enteric bacteria are likely to decline. They also describe the role of sunlight as both aiding and hindering pathogen survival in that sunlight initially helps with crust formation, but later acts to desiccate pats by providing unfavorable heat. This result supports the finding of

van Kessel et al. (2007) and Maeys et al. (2005) who found greater fecal bacteria persistence in cowpats under shaded conditions than those left open to sunlight.

In both stored and field-applied manures, pathogens typically exhibit initial growth and increased concentrations, but also may exhibit later growth phases depending on the environmental conditions. Wang et al. (1996) performed a laboratory experiment using fresh bovine feces placed in open stomacher bags in temperature-controlled settings. These researchers found that *E. coli* O157:H7 experienced initial growth phases in the 23°C and 37°C climates, but declined initially at 5°C. Growth of fecal bacteria within manure has also been reported in numerous other studies (Muirhead et al. 2005; Sinton et al. 2007; van Kessel et al. 2007; Soupir et al. 2008). As previously stated, this growth is typically observed at the onset of the experimental studies. A potential explanation for this initial growth may be that within the intestines of mammals, fecal microorganisms are constantly provided nutrients and are thus in a perpetual exponential growth phase and when they are deposited, they remain in this phase until environmental conditions dictate they shift to a more conservative survival pattern (Thelin and Gifford, 1983).

It has been noted that the most productive metabolic mode for certain pathogens like *E. coli* O157:H7 occurs in the presence of oxygen (Partridge et al. 2006) and thus once expelled from the host, the initial placement in an oxygen-rich environment spurs exponential growth as oxygen replaces other elements as the primary electron acceptor in microbial respiration. Eventually this growth subsides, and long term storage without regular additions of fresh manure is highly effective in reducing counts (Meals and Braun, 2006; Patni et al., 1985)

2.5.2. Survival in soils

Survival is the critical factor when using the fecal organisms as indicators of fecal contamination. Population growth or loss in applied manure and soil controls the total FIO numbers that are released to runoff and exported from manured fields or pastures to surface water sources. The effects of management practices on FIO concentrations in surface waters may be miscalculated if the survival is not properly estimated.

A large number of survival experiments with various *E. coli* strains have been done in batch setups where soil was inoculated with suspensions of bacteria (Park et al., 2016). These experiments were important in determining the role of survival factors such as soil texture, initial concentration in suspension, temperature, and organic matter content. It has typically been found that the rates of decline fall as the least hardy organisms are removed from the population and leave behind only those organisms that are better equipped for survival. Since decline is not a linear process, it is not possible to extrapolate pathogen survival from the "initial levels of zoonotic agents" (Hutchison et al., 2005). Given the focus of this work on the export of FIO from manured fields, this section reviews only works which studied survival of generic *E. coli* introduced in soil with manure or manure leachates. We have focused on survival within two to four weeks after manure application since this is the period during which either plowing, and/or export with runoff or irrigation takes place.

Table 2.3 presents results of representative field studies in which the *E. coli* survival in soil was monitored within the first several weeks after application. The most common feature is that either a very slow die-off or a growth increase occurs during the first one to two weeks after manure application. The die-off rate increases two to three

weeks after application. In the case of liquid swine waste being land applied, the *E. coli* almost always exhibited die-off after the liquid swine waste was applied (e.g., Avery et al., 2004, Cools et al., 2001). When solid bovine waster was applied, the *E. coli* tended to grow, in some cases at rates up to two orders of magnitude (Saunders et al., 2012).

Several mechanisms were previously mentioned that are detrimental for *E. coli* survival. Solar radiation and especially its UV component were shown to impede survival (Yaun et al., 2003). Microbes have been shown to preferentially associate with particulates that offer some protection from sunlight (Thurston-Enriquez et al., 2005). Low soil water contents have been shown to cause a rapid decline of *E. coli* populations (Berry and Miller, 2005; USEPA 2013; Olson 2001; Rodgers and Haines, 2005; Bowman, 2009; Bosch et al., 2006). Fecal bacteria have also been shown to better rates of survival in neutral soils (Unc and Goss, 2004). It has been suggested that the competition for nitrogen, glucose, lactose, and organic acids with other soil microorganisms, as well as soil conditions such as a lack of oxygen, a lower soil pH, and temperature, may account for the observed reductions in the number of *E. coli* bacteria within soils (Park et al., 2016). Soil type has been found to play a major role in pathogen survival. The greater persistence in soils with a higher clay content has been observed for multiple pathogens and has been largely attributed to a higher organic matter and nutrient content, moisture capacity, and proportion of micropores that provide physical protection from predation (Nicholson et al., 2002; Holley et al., 2005; Rodgers and Haines, 2005; Park et al., 2016). Some have argued that native microbial communities predominately affect pathogen survival in soils (Moynihan et al., 2015). Several studies have compared pathogen survival in autoclaved versus non-autoclaved soils have shown lower rates of

survival in the presence of native microflora (Jiang et al., 2002; You et al., 2006). Klein and Casida (1967) concluded that a major factor affecting *E. coli* die-out within "natural soil" is the inability of *E. coli* to reduce its metabolic rate in response to the low availability of usable organic carbon within soil.

During the first weeks after bovine manure application, the effects of the survival impeding processes appear to be mitigated or overridden by mechanisms supporting *E. coli* survival. There is currently no commonly accepted explanations for the slow die-off or growth increase of *E. coli* populations after manure applications. One possibility is that after the manure application, the *E. coli* population remains within the manure matrix which is expected to be conducive for *E. coli* survival. The formation of crusts on the manure clods may thwart the negative action of dessication and UV radiation (Kress and Gifford, 1984) as well as complicate the predation by other soil organisms. In such cases, the survival of *E. coli* within solid manure should be better than the survival of *E. coli* contained within liquid manure or slurry. Evaluations of such comparisons are currently absent in the literature.

The FIB populations may begin to grow in response to environmental events, namely precipitation. Sharples et al. (2004) documented that following the surface broadcasting of dairy manure to an agricultural field, *E. coli* populations increased during the first week within both the 0-5 cm and 5- 10 cm soil depths during the summer months. Lau and Ingham (2001) observed a 1-2 Log10 CFU/g^{-1} growth of *E. coli* and enterococci during the first week after bovine manure incorporation into a loamy sand soil. Zaleski et al. (2005) observed the growth of total and fecal bacteria groups in biosolids-amended soils following rewetting events that occurred between 3 to 5 weeks

after incorporation. Byappanahalli et al., (2004) inoculated soil with *E. coli* and *E. faecalis* and let the soil desiccate over four days before rewetting. The authors observed an 6.5 Log10 CFU/g^{-1} increase in *E. coli* concentrations and about a 0.5 log10 increase in *E. faecalis* Concentrations. Hodgson et al., (2016) reported numerous instances of growth for *E. coli* and enterococci within slurry-amended agricultural soils over 50-day periods during the summer months. The authors credited the rehydration of soils during precipitation events as the likely reason for observing this growth.

It appears to be important to understand not only the growth of manure-borne *E. coli* within soils, but to also understand the soil depths within the soil profile in which the surviving *E. coli* reside. The field data on the survival of manure-borne organisms within soils have typically been collected from soil core samples encompassing a range of depths that are much deeper than the estimations of the mixing layer thickness (Table 2.3). Microbial survival, growth, and decline have been studied in soil samples that were taken from the surface to depths of 1 cm (Muirhead, 2009.), 2.6 cm (Islam et al., 2004.), 3 cm (Chandler and Craven, 1980), 4 cm (wood etal., 2010), 5 cm (Avery etal., 2004, Nyberg et al., 2010; Entry JA, 2000; Crane et al., 1980)..), 7.5 cm (Nicholson et al., 2004), 10 cm (Saunders et al., 2011; Ohtomo et al., 2004; Entry et al.,2010.), 15 cm (32. Stoddard et al., 1998;. Hutchison et al., 2005), and 20 cm (34–36). There have been a very limited number of studies that have examined the depth-dependent survival of the fecal microbes within soil. Sharples et al., (2005) applied liquid dairy manure to an agricultural field using broadcast and incorporation techniques. The authors found similar survival patterns between *E. coli* in the 0 – 5 cm and 5 – 10 cm soil depth range, but noted longer survival times within the upper soil ranges for both years that their

experiment was performed. Fenlon et al. (2001) studied the survival of generic *E. coli* and *E. coli* O157:H7 within soil after the application of cattle slurry. These authors partitioned their survival analysis by the depth ranges of 0 to 2.5 cm, 2.5 to 5 cm, and 5 to 20 cm. Between the 1st and 3rd day following slurry application, *E. coli* grew within the top 2.5 cm soil depths before beginning to decline on the 7th sampling day. Throughout the remaining 29 days of their experiment, the top 2.5 cm retained the highest *E. coli* concentrations which typically decreased with depth and time. It is imperative to know whether the surviving FIO population is located within the topmost part of the soil profile that is accessible for mass exchange with runoff water.

The comparative data on the short-term survival for two of the most common FIO – *E. coli* and enterococci – are contradictory. Chandler et al. (1981) noted that fecal streptococci exhibited similar survival rates within soil and vegetation during the 14 days following manure application and seemed slightly better suited for survival in the environment than did fecal coliforms. The short-term survival of *E. coli* and enterococci was found to be similar in incubation experiments (Cools et al.,2001). Conversely, fecal streptococci exhibited more rapid rates of decline in a study when poultry manure was applied (Crane et al. 1980). In another study, the enterococci numbers increased at a slower rate and then declined at a more rapid rate when compared to *E. coli* populations within mixtures of soil with fresh bovine manure (Lau and Ingham, 2001)., From the prospective of the FIO export from manured fields, it is beneficial to know the survival of enterococci within the thin mixing layer of soil that is in contact with runoff. This information is unfortunately currently absent in the literature.

Table 2.3. Representative studies of the short-term Survival of *Escherichia coli* and fecal coliforms in soil after manure applications

Table Authors	Animal	Consistency	Application method	Lab/Field; soil type	Sampling Depth (cm)	Kinetics in soil
Avery et al., 2004	Bovine, ovine, swine	Slurry	Grazing	Field, ND	NA	No significant changes in concentrations during the first week, 1/2 to 1 order of magnitude decrease on the second week
Barry and Miller, 2005	Bovine	Slurry	Incorporated	Lab, Silt Loam	NA	Observed growth of the generic *E. coli* concentrations during first 2 to 6 days in soil with added bovine manure. The growth was two orders of magnitude within first 4 days when 5 % of manure was added, and reached four orders of magnitude when 75% of manure was added. The water content controlled growth in these experiments. Low water contents led to the die-off rather than growth, more water was needed for growth as the manure content increased.
Chandler et al., 1981	Swine	Effluent	Flooding	Field, Fine sand	0 - 3	90% decrease in FC population within a period from one to the three weeks irrespective of the fecal coliform count in the applied effluent. Faster die-off on vegetation than in soil for *E. coli*, Fecal streptococci survived similarly on soil and vegetation (T90 ca. 2 weeks).
Cools et al., 2001	Swine	Effluent	Incorporated	Lab, Sand, Loam, Loamy Sand	NA	*E. coli* concentrations in soil mixed with pig manure did not change for about a week at temperatures 5 and 25 C. No short-term effect of sol texture was found. After 3 weeks, however 2 orders of magnitude loss was encountered. No enterococci decrease was observed in first 10 days and only about ½ order of magnitude decrease was seen after 20 days
Entry et al., 2000	Swine	Wastewater	Overland flow	Field, Loamy Sand	0 - 5, 5 - 15	Fecal coliform numbers in the 0 to 5, 5 to 15, and 15 to 30 cm depths declined approximately 10-fold every 7 to 14 d after waste application in all seasons of the year
Entry et al., 2005	Bovine	Solid	Broadcast	Field, Sand	0 - 10	solid dairy manure application to soil at rates to meet crop phosphorus uptake did not consistently increase Enterococcus spp. and fecal coliform numbers in bulk soil.
Entry et al., 2010	Bovine	Solid	Incorporated	Field, Fine sand	0 - 10	*E. coli* in soil was falling 40 % per week for seven weeks. Enterococci were substantially growing.
Fenlon et al., 2000	Bovine	Slurry	Broadcast	Field, Sandy, Loam, and Clay	0 - 2.5, 2.5 - 5, 5 - 20	Recovery from soil was nearly 100% for the first 3 days in 0-2.5 cm layer and 1 log decrease occurred on day 14 thereafter declined to less than 1% after 29 days. Practically everything was retained on grass and in the top 0 - 2.5 cm. There was significant transport to deeper layers, but this never exceeded 2% of applied numbers.

Ogden et al., 2001	Bovine, ovine, swine	Slurry	Broadcast	Field, Sand, Silt Loam, Silty Clay Loam	0 - 15	After the first week, no significant changes were found in silty clay loam and clay loam, whereas more than 1 order of magnitude decrease was documented in sandy loam
Ohtomo et al., 2004	Bovine	Slurry	Broadcast	Field, NR	0 - 5	No changes in soil during the first week in summer, and the decrease about of half order of magnitude was observed in fall and in winter
Saunders et al., 2012	Bovine	Slurry	Broadcast	Field, Silty Clay Loam	0 - 10	Observed 0 to 2 orders of magnitude increase in 1 to 3 days after application.
Sharples et al., 2004	Bovine	Slurry	Broadcast	Field, Sandy Loam	0 - 5, 5 - 10	No changes in *E. coli* concentrations in soil at the depth 0-5 cm were found for the first three weeks. Slight, less than ½ order decline was found at the depth 5-10 cm. First three days did not show any decline in 2002. Slight decrease about 0.5 order of magnitude occurred after the first week and no significant change was found in the next three weeks.
Trevisan et al., 2002	Bovine	Slurry	Broadcast	Field, Sandy	0 - 30, 30 - 60	No change for days 15 to 45

NA = Not available.

2.6. Research needs

A substantial volume of research has been previously conducted to study the release, survival, and removal of manure-borne FIB. The fate and transport mechanisms were thoroughly studied, predictive models were developed, and regulatory actions were taken to improve microbial water quality for recreation, irrigation, and other purposes. However, several issues that are critical for successful predictive modeling at different measurement scales remain unexplored, thus hampering site specific-informed decision making regarding microbial water quality.

This literature review demonstrated that useful and accurate information is extremely scarce on the survival of FIB near the soil surface at depths where the FIB are available for loss within runoff. It is not known whether the same distribution and availability are applicable for the two major FIB – *E. coli* and enterococci. Given the need for this information to accurately estimate the FIB removal at the field scale, the topical research directions are (i) to document the temporal changes in the profile distributions of manure-borne *E. coli* and enterococci in the near-surface soil layers after simulated rainfalls and (ii) to examine the differences in the survival characteristics of the two fecal indicator bacteria.

The differences in consistency of manures have not so far been factored into the predictive models related to microbial water quality even though such differences are strongly expected to exist. It would be advantageous to use the same fate and transport parameters for liquid manures, slurries, and solid manures, or to develop conversions for the fate and transport parameters dependent on the manure consistency. Therefore,

examining the effects of manure consistency on FIB release and removal presents a topic of interest for both major FIB – *E. coli* and enterococci.

It is not known how manure-borne FIB release and removal kinetics are changing as the applied manure is exposed to weathering in the field prior to the initiation of rainfall. Studies of the differences in phosphorus release and removal suggest the possibility of temporal changes not only of bacteria concentrations in manure, but also of the access of rainfall or irrigation water to elute bacterial cells. Research that documents the effects of the delay between manure application and rainfall on the FIB release kinetics will improve the predictability of the pathogen impairment of water sources caused by the FIB removal from manured fields. Improving the accuracy of existing predictive models of FIB fate and transport requires research focused on the explanation and estimation of model parameter variation.

Chapter 3 - Depth-dependent inactivation of *Escherichia coli* and enterococci in soil after manure application and simulated rainfall

3.1. Introduction

Fecal contamination of soil and water presents a major worldwide health risk. When pathogenic microorganisms enter soil or water, they can infect humans via drinking or recreational water and through consumption or handling of contaminated produce. The Centers for Disease Control and Prevention (CDC, 2011) estimate that each year nearly one in six Americans (or 46 million people) get sick and roughly 3,000 die from foodborne diseases. Another study performed in the same year estimated that foodborne pathogens resulted in medical care costs in the range from $4.4 billion to $33.0 billion (Hoffman et al., 2012). Contamination of soil and water leading to foodborne illness can originate from numerous sources such as direct deposition by animals, by overflow or leakage of faulty septic and sewage systems, or by manure application to agricultural fields and pastures (Van Donsel et al., 1967; Zhang et al., 2013; Sjorgen, 1993). The potential presence of pathogens that cause foodborne illness can be inferred via the monitoring of fecal indicator bacteria (FIB) such as *E. coli* and enterococci (USEPA, 1986).

It has been found that both *E. coli* and enterococci can survive in the soil for several weeks after deposition, if not longer (Rodgers et al., 2011; Sharples et al., 2004). Some studies have found that populations of these organisms can experience initial growth phases in the soil which can extend their prevalence for longer than previously

thought. For example, Lau and Ingham (2001) reported that *E. coli* and enterococci survived in soil following manure incorporation for more than 19 weeks. Avery et al. (2004) observed *E. coli* persistence in soils amended with cattle, sheep, and swine manure for up to 19 weeks, while Entry et al. (2005) observed prolonged survival of fecal coliforms and enterococci (at least 42 weeks) in soils amended with dairy manure. Such extended survival in soils can result in the occurrence of indicator organisms in runoff and infiltration waters from sites that have not had prior manure applications for weeks, months, or even years (Brennan et al., 2010; Muirhead, 2009)

Suspended, dissolved, and adsorbed substances and organisms are known to be released to runoff from a thin near-surface layer of soil. Ahuja et al. (1981) proposed the term "effective depth of interaction" (EDI), defined as the thickness surface soil in which the degree of interaction is equal to that at the soil surface. The soil layer between the surface and EDI is commonly called the mixing layer. Sharpley (1985) estimated the EDI to be three to five mm for a 5 deg slope in experiments with clay loam and sandy loam soils under 50 mm per hour of simulated rainfall. Yang et al. (2015) found EDI values in the range from 1 to 11 mm such that the value increased with increased rainfall intensity and slope. Sanchez and Boll (Sanchez and Boll, 2005) experimented with the 5 mm-thick mixing layer enriched with phosphorus. Tong et al. (2010) estimated a mixing layer thickness of 1.5 cm for unsaturated soil and noted the decrease of the EDI with increasing saturation. Mamo et al. (2005) and Owens et al. (2008) indicated the need to account for profile distributions of P in topsoil to correctly model the phosphorus losses from land to water.

Field data on survival of manure borne organisms in soils have typically been collected from core samples encompassing a range of depths much deeper than the mixing layer thickness estimates. For example, in experiments with surface applied manures, samples were taken from the surface to the depth of 2.6 cm (Islam et al., 2004), 3 cm (Chandler et al., 1980), 4 cm (Wood et al., 2010), 5 cm (Nyberg et al., 2010; Entry et al., 2000; Crane et al., 1980; Avery et al., 2004), 7.5 cm (Nicholson et al., 2004), 10 cm (Sanders et al., 2011; Ohtomo et al., 2004; Entry et al., 2010; Entry et al., 2005), 15 cm (Stoddard et al., 1998; Hutchison et al., 2004), and 20 cm (Trevisan et al., 2001; Scott et al., 2006; Cote and Quessy, 2005). In only one study (Fenlon et al., 2000) were concentrations of *E. coli* reported for several depth ranges: 0 to 2.5 cm, 2.5 to 5 cm, and 5 to 25 cm. They applied dairy cattle slurry on surfaces of grassed lysimeters and found only 1% of the applied manure in the 2.5- to 5-cm layer while the remainder of the bacteria were in the 0- to 2.5-cm layer.

Given the scarcity of the information on survival of bacteria near the soil surface at depths comparable with the depth of mixing layer estimates, the objectives of this study were to (1) document temporal changes in profile distributions of manure-borne *E. coli* and enterococci in the near-surface soil layers after simulated rainfalls, and (2) examine differences in survival characteristics of the two fecal indicator bacteria.

3.2. Materials and Methods

Experiments were performed at the Beltsville Agricultural Research Center (BARC). A variable controlled-intensity rainfall simulator (Meyer and Harmon, 1979) was used to

apply rainfall for the release manure-borne *E. coli* and enterococci from soil-applied dairy cattle manure to runoff and infiltration. The rainfall simulator sprinkler nozzles (Veejet 80150; Spraying Systems Co., Wheaton, IL) were positioned 3 m above the soil boxes which allowed rain drops to reach near terminal velocity upon landing with an energy impact of approximately 275 kJ/ha-mm, which is similar to natural rainfall events greater than 25 mm hr^{-1}. The rainfall simulator was calibrated to deliver a relatively uniform rainfall distribution for a central 1-m^2 area with a Christiansen coefficient of uniformity in the range from 84% to 86 %.

The 100-cm x 26.5-cm x 15-cm experimental boxes, described by Isensee and Sadeghi (1999) and Faucette et al. (2009) were filled with a sandy loam soil. A 2-cm thick sand layer was evenly placed over the bottom of the box to facilitate infiltration release through the three mesh-covered drains. On top of this initial sand layer, six additional layers of an air-dried sandy loam soil (a mixture of various USDA-ARS Beltsville A horizons of no single soil series) that had been screened prior to placement in the soil boxes were added. Each layer was evenly spread throughout the box, packed flat with a plywood board, and then scored at the surface prior to placement of additional layers atop it. This packing procedure was performed to create a uniform bulk density throughout the box. The final bulk density of the soil boxes was 1.34 ± 0.07 g cm^{-3}. Soil textural composition was 63.8 % sand, 24.8 % silt, and 11.4 % clay, chemical properties were pH=7.0, electrical conductivity in 1:2 paste of 0.36 (mmhos cm^{-1}), and an average total C of 2.23 %.

Packed soil boxes were placed in a temperature controlled hoop house set to operate at 18° C located at the USDA-ARS BARC North Farm in Beltsville, MD. The

soil in each box was watered and cross-scored at the surface before Kentucky 31 tall fescue grass seed was applied at a rate of 49 g m^{-2}. Boxes were watered twice daily until germination and then once a day following germination. After 20 days of grass growth, the soil boxes were over-seeded with additional grass seed at the same rate as previously indicated to fill in spots of uneven growth in the boxes. An additional 2 kg of topsoil was added to cover the newly added seed. Daily watering continued until the newly added seeds germinated and then watering was reduced to once every 2-3 days.

Manure was prepared by mixing fresh dairy cattle excreta collected at the USDA-ARS Dairy Research Facility, with sawdust bedding to reach a 30% dry solid content. The manure application rate of each soil box was 60 ton ha^{-1} (2.1kg box^{-1}). Manure properties as determined in the Penn State Agricultural Analytical Services Laboratory were pH=8.25 ± 0.16, carbon content of 14.9 ± 1.0 %, and C:N ratio of 40.9 ± 5.6. Contents of *E. coli* and enterococci were (5.30 ± 4.24) x 10^5 CFU gdw^{-1} and (3.81 ± 1.64) x 10^6 CFU gdw^{-1}, respectively, the '±' separates average and standard error.

The synthetic rainwater was prepared by adding reagent-grade chemicals to deionized water to obtain a rainfall composition typical for the Maryland, Pennsylvania, and Delaware region, which consists of 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). Rainfall water pH was adjusted to 4.5 just prior to the experiments. Boxes were placed within the central 1-m^2 area and adjusted to a 5% slope steepness. Antecedent water contents in each soil box were made uniform by applying a pre-wetting rainfall simulation event for 30 minutes at an intensity 3 cm hr^{-1} 24 hours prior to the actual experiment. Rainfall in the amounts of 30 mm, 60 mm, and 90 mm was

applied for one hour in triplicate for each rainfall intensity according to the randomized design for the sequence of the irrigations.

Each box was transported to the hoop house shortly after the simulated rainfall. Soil was sampled in triplicate from four depths and at three locations from each box weekly for one month. Specifically, soil samples were taken with a sterilized handheld soil core probe to a depth of 10 cm in triplicate at 30-cm, 50-cm, and 70-cm length marks along the box. The 10-cm length cores were then subdivided into 0-1 cm, 1-2 cm, 2-5 cm and 5-10 cm sections. This sampling was performed at 30-cm, 50-cm, and 70-cm length marks in the box to see if any effect of location existed. All subsamples were immediately placed in sterile bottles and placed on ice until processing.

Hoop house air temperature was recorded with a HOBO Pendant Temperature/Light Data Logger (Onset Computer Corporation, Bourne, MA). Grass in each box was sprinkler-watered with approximately 7 mm of water once a week immediately after soil sampling.

Microbiological analyses were done with an approximately 2-gram subsample of each soil sample. Subsamples were placed into sterile blenders where they were ground with 200 mL of sterile D.I. water for 2 minutes to create an initial dilution factor of 10^{-2}. Samples were then poured into sterile beakers and left to settle for 1 hour. After settling the supernatant was diluted and *E. coli* contents were determined using a Colilert 18 and a QuantiTray 2000 (IDEXX Laboratories, Inc, Westbrook, MA). From the initial blended sample, 250 uL of supernatant was pipetted onto m-Enterococcus agar (Neogen Corporation, Lansing, MI) and then spread plated to enumerate enterococci. Plates were

then incubated for 48 hours at 35±0.5 °C. A portion of the unblended original sample was weighed and placed in a drying oven for at least 24 hours to determine water content. Results of microbial analysis were presented as most probable number (MPN) per g of dry weight and colony forming unit (CFU) per g of dry weight for *E. coli* and enterococci, respectively. Total numbers of bacteria in soil were computed for columns having the 1-cm^2 cross-section and height equal to the soil layer thickness.

Decimal reduction times were calculated by fitting the data in coordinates of time vs. total organism number to the exponential decay equation (1):

$$\frac{c}{c_0} = 10^{-\frac{t}{a}} \quad (1)$$

with two estimated parameters: a - the decimal reduction time, i.e. time when the fitted concentration is ten times less than the initial concentration, and c_0 – the effective concentration at time zero. Value of a and c_0 were estimated from concentrations observed on days 7 to 21 when equation (1) was deemed to be valid; therefore c_0 did not represent the actual initial concentration whereas a represented the inactivation rate.

The total numbers of organisms in soil N_T were obtained as the results of summation by layers:

$$N_T = \sum_{i=1}^{4} c_i h_i \rho_i \quad (2)$$

Where N_T is the total number of organisms within the 1-cm thick soil layer per 1-cm^2 surface of soil, c_i, i=1,2,3,4, is the concentration of microorganisms in layer "*i*" in CFU

(g dry soil)$^{-1}$, h_i, i-1,2,3,4, are thicknesses of layers "0-1 cm", '1-2 cm", "2-5 cm", and "5-10 cm", i.e. 1, 1, 3, and 5 cm, respectively, ρ_i , i=1,2,3,4, are soil bulk densities in layers.

3.3 Data analysis

All experiments were performed in triplicate. Tray and plate counts for *E. coli* and enterococci, respectively, were converted to dry weight equivalents and subjected to a multi-factorial analysis of variance (Sigmaplot 12.5, Systat Software, Inc. San Jose, CA). Statistical significance was evaluated at the 0.05 probability level.

3.4 Results

Soil water content was not significantly different between any of the rainfall intensities immediately after rainfall application or at any point during the experiment (P = 0.934). Soil moisture significantly declined from week to week in all instances (P <0.001). Soil water storage changed over time are shown in Fig. 3.3.1a. Bars represent the mean soil moisture of all depths.

Average daily air temperatures are shown in Fig. 3.3.1b. Temperature regimes were slightly different among the treatments due to the randomized experimental design that

resulted in boxes arriving within the hoop house at slightly different times.

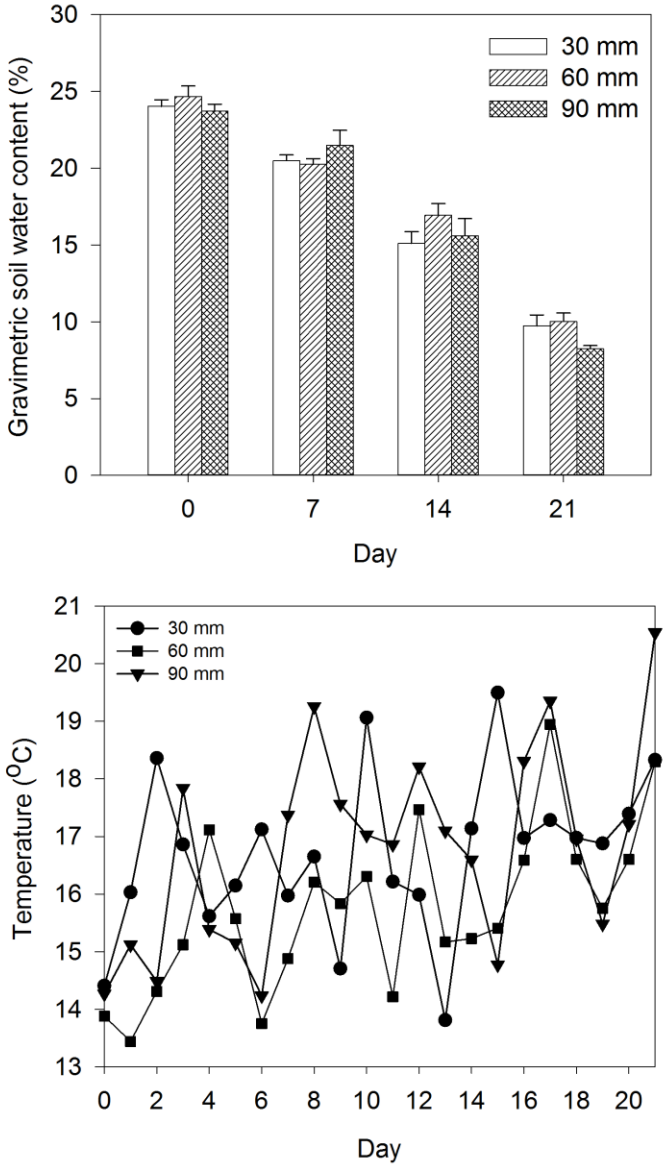

Figure 3.1. Mean soil water contents in the beginning of each week (top) and mean daily air temperatures (bottom). The plotted values are averages across three replications. Error bars show standard errors.

Bacteria survival by layers is shown in Fig. 3.3.2. The pattern of the initial increase of bacteria content with following decrease was observed at all depths for *E. coli*. Enterococci contents steadily decreased at all depths. The decimation times could be approximately estimated for *E. coli*, but not for enterococci. The latter organism had survival dynamics that did not follow Eq. (1). Decimal reduction times for concentrations of *E. coli* at the four examined depths and across the three rainfall intensities are shown in Table 3.1. The estimated decimation times decreased with depth in the 0- to 5-cm layer, but were very large in 5- to 10-cm layer.

Table 3.1. Average decimal reduction time (days) for *E.coli* for the period of day 7 to day 21 of the experiment.

Depth (cm)	30 mm	60 mm	90 mm
0 – 1	18.0	12.7	15.7
1 – 2	11.7	9.4	7.1
2 – 5	6.0	4.8	15.7
5 - 10	130.8	ND	150.6

ND: Not Determined.

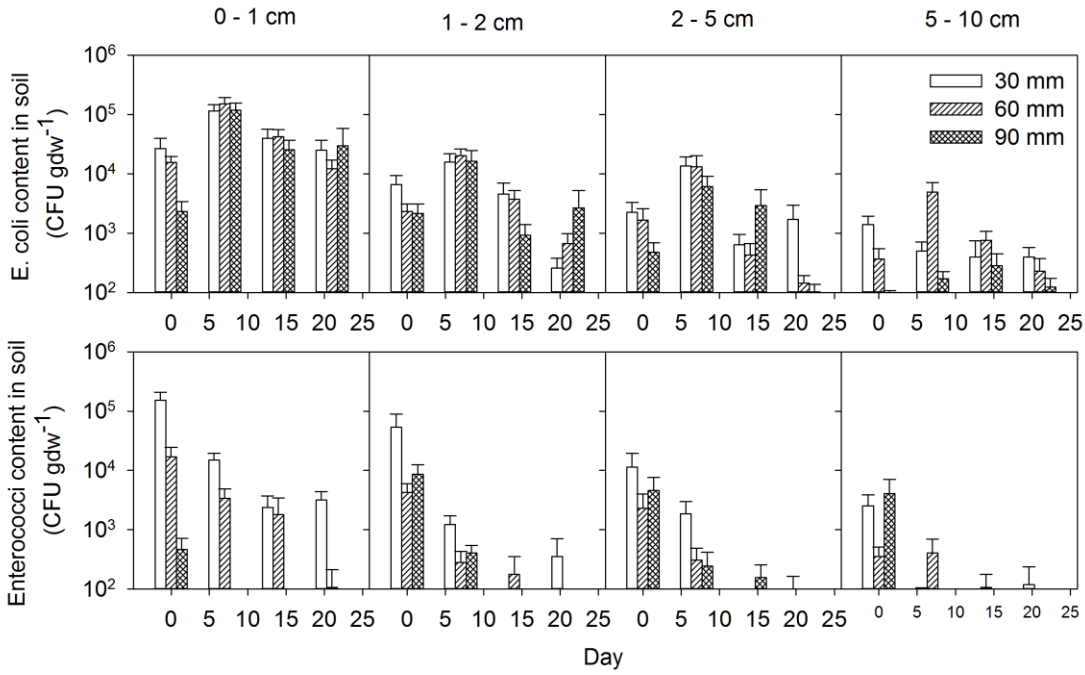

Figure 3.2. Changes in *E. coli* and enterococci contents in soil layers over time. Error bars show standard errors of concentrations.

Total initial numbers of bacteria in the soil boxes following simulated rainfall varied with the amounts of water applied (Fig. 3.3). Total *E. coli* numbers significantly differed between 30-mm and 90-mm treatments ($P < 0.05$) and between 60-mm and 90-mm treatments ($P<0.05$), but did not significantly differ between 30 mm and 60 mm. Total enterococci numbers varied significantly between 30-mm and 60-mm treatments ($P<0.05$) as well as between 30-mm and 90-mm treatments ($P<0.05$), but were not significantly different for 60-mm and 90-mm treatments. No significant trends were observed between total number of organisms and sampling location along the box (data not shown). *E. coli* and enterococci populations exhibited very different survival

dynamics (Fig 3). *E. coli* population at all three rainfall intensities exhibited initial growth phases during the week following the application of simulated rainfall. After the initial growth phases, *E. coli* numbers began to decline, but remained relatively similar to initial concentrations by the end of the 21-day long experiment. Initial total numbers of *E. coli* in the soil were not significantly different from the final total numbers in the 30-, 60-, and 90-mm/hour treatments ($P = 0.418$, $P=0.122$, and $P=0.808$, respectively). Concentrations of *E. coli* in the 90-mm treatment boxes were higher than initial concentrations at the end of the experiment. The total numbers of enterococci in the soil boxes consistently declined throughout the duration of the experiment with concentrations in the 60-mm and 90-mm treatment boxes being undetectable by the end of the experiment.

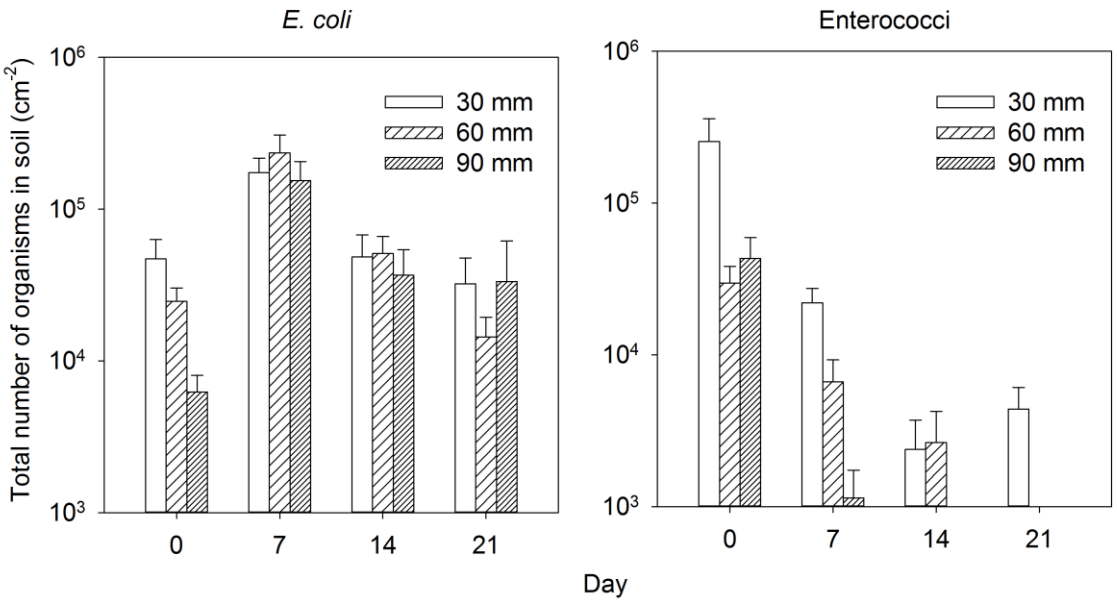

Figure 3.3 Total number of *E. coli* cells (MPN per square centimeter) and enterococci (CFU per square centimeter) in soil over time following the application of 30-, 60-, or 90-mm h^{-1} rainfalls. Error bars show standard errors of concentrations.

E. coli populations in soil did not significantly differ between rainfall treatments one week after the application of simulated rainfall (P = 0.587). Conversely, enterococci populations were significantly different between the three rainfall intensities one week after the application of simulated rainfall (P <0.001).

Figure 3.4 shows the relative contribution of each soil layer to the vertical distribution of total number of organisms within the soil profile. Both indicator organisms were overwhelmingly concentrated in the surface layer of 0 – 1 cm. In almost all instances the number of bacteria in the surface layer accounted for a greater proportion of the overall population than the next three depths combined. Additionally in most cases,

the overall proportion of bacteria in the surface layer increased over time as concentrations in the lower depths declined.

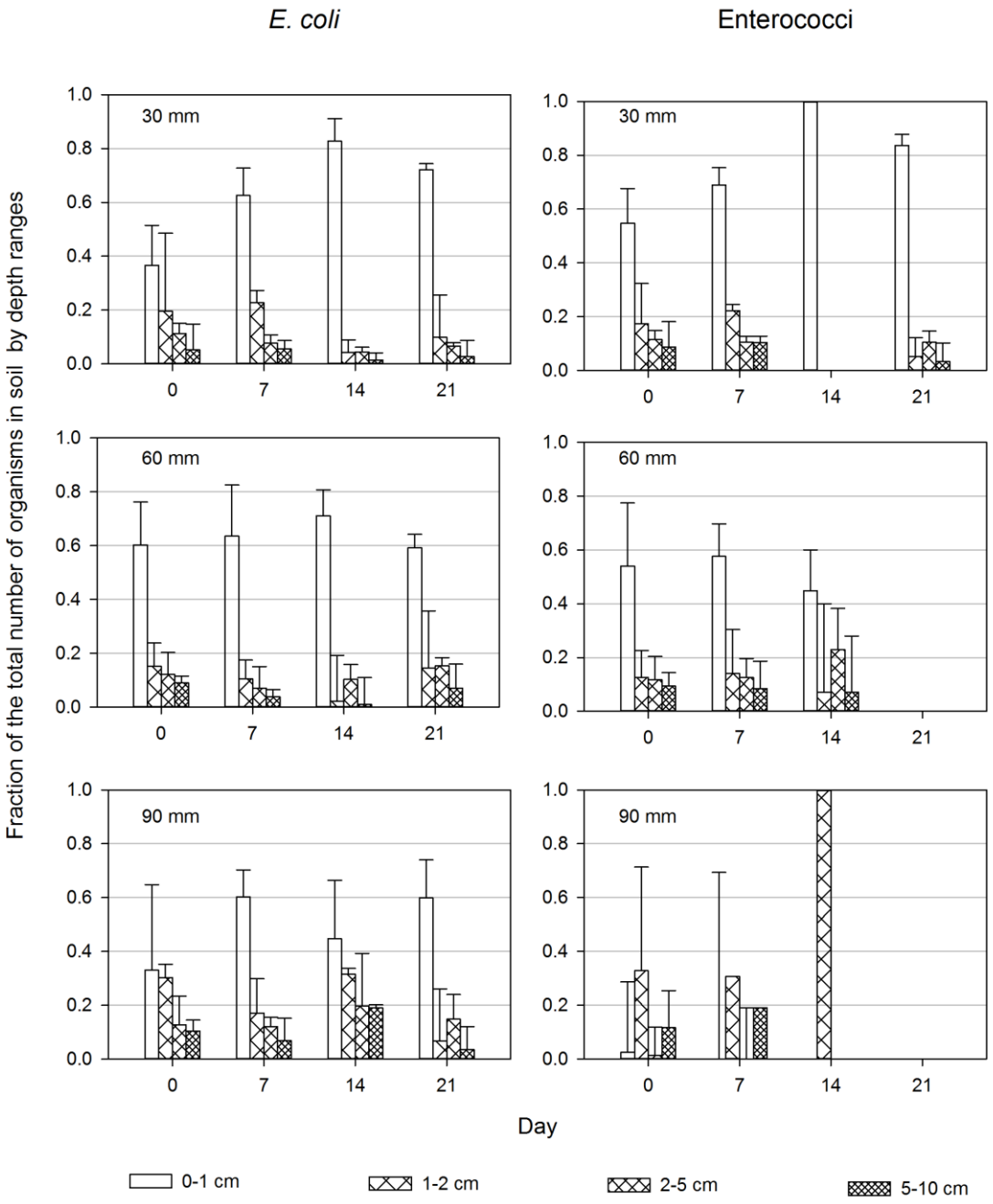

Figure 3.4. Distribution of indicator organisms in soil for three rainfall intensities on observation times. Error bars show standard errors.

3.5 Discussion

The initial populations of fecal indicators in soil after rainfall simulation showed a dependence on the amount of simulated rainfall applied (Fig. 3.3.2). The total numbers were generally lower as the amount of rain water increased. Possible reasons for this observation include the increased leaching of bacteria through macropores in the soil with an increase in rainfall intensity (Guber et al., 2005), formation of a layer of the dispersed manure material on the soil surface that would prevent bacteria accumulation within soil layer (Burkhardt et al., 2005), and/or the slowdown of manure bacteria release with the increased amount of applied water (Dao et al., 2008) that would lead to a larger dilution of released bacteria with rainfall water within the manure layer before it reaches soil.

All treatments in this work resulted in qualitatively similar microbial population dynamics within each microorganism group. *E. coli* concentrations initially increased and then slowly decreased, whereas populations of enterococci began to decrease from the start and were not detectable after four weeks in all, but for treatments that received the least intense rainfall application where they remained at a total number that was 1.3% of the initial population. The initial growth of *E. coli* populations was observed in the top 1-cm layer where large manure particles were strained and provided a nutrient-rich environment. Some of the dissolved manure nutrient material may have moved to deeper layers with the infiltrating water. Soil water contents also changed with depth such that greater water contents were observed close to surface where the conditions were more favorable for *E. coli* survival. Additionally, the presence of oxygen results in aerobic respiration which is the most productive metabolic mode for *E. coli* (Partridge et al., 2006). Increased enterococci survival in top layers may also be explained by this as

enterococci are facultative anaerobes as well. The loss of some water from the soil occurred due to plant transpiration and evaporation from the soil surface; dissolved nutrients moved to the surface to replace the evapo-transpired water and accumulated there. *E. coli* may have responded to the increasing concentrations of nutrients near the surface via chemotaxis (Duffy et al., 1997).

E. coli concentrations in soil were not significantly different among the treatments one week after simulated rainfall, whereas initial concentrations of indicator organisms in the soil decreased significantly with increasing amounts of water applied. One possible explanation is that *E. coli* growth was limited by competition or predation such that *E. coli* populations above a certain level did not depend on the differences in initial concentrations within the observed range.

Decimal reduction times for *E. coli* demonstrated a trend of decreasing reduction time with increased depth. This observation held true in most cases, but *E. coli* survival data for the deepest depth was not able to be adequately fitted with the Eq. (1); hence, why the 5 – 10 cm values are many times higher than values for shallower depths and/or are not determinable. These values, however, cannot be considered as reliable since they are outside of the duration of the experiment.

Numerous factors could be responsible for the difference in survival dynamics of *E. coli* and enterococci. *E. coli* populations have been shown to experience initial growth phases in soil following rainfall events and rises in water tables (Tate, 1978; Hagedorn et al., 1978). Sinton et al. (2007) found that during flooding events enterococci did not show growth in deposited cow feces following flooding events and that the organism was

quickly inactivated in all seasons. These conditions are comparable to the conditions in the soil environment following the simulated rainfall events performed in this study. Wang et al. (2004) observed the initial growth phases of both organisms in cow manure, but found a survival enhancing moisture effect only for fecal streptococci which may indicate that enterococci is sensitive to low moisture conditions. Another study that looked at survival in cow pats found that when water content falls between 70 and 75% fecal streptococcus exhibit slower decay than *E. coli*, however, in dry conditions *E. coli* exhibited slower decay (Sinton et al., 2007). This finding is consistent with the decrease of soil water contents in the soil boxes throughout the experiment with each organism's unique inactivation. Solo-Gabriele et al. (2000) found that *E. coli* was able to multiply several orders of magnitude as soil was drying. The authors hypothesized that this was due to the capability of *E. coli* to survive under dry conditions with limited competitor/predator involvement. Howell et al. (1996) observed that fecal coliforms experienced greater growth in warm conditions than fecal streptococcus. This study found that fecal coliforms often experienced regrowth, while fecal streptococci did not, which is similar to what was observed for *E. coli* vs. enterococci in the present study. Kibbey et al. (1978) posited that increased soil temperatures increased microflora activity within soils of which *E. faecalis* may suffer from more than *E. coli*. Byappanahalli et al., (2012) also reported that enterococci growth is hampered by competition from native soil biota.

Yet another possible explanation for different survival dynamics is the availability of nutrients within the soil. Both *E. coli* and enterococci are facultative anaerobic bacteria and can thus survive in both oxygen rich and poor environments. However *E. coli* is

versatile in its ability to obtain energy and only requires simple carbon and nitrogen sources (Ishii and Sadowsky, 2008), whereas enterococci have more complex nutrient requirements (Byappanahalli et al., 2012). The soil boxes did not receive any nutrient addition after initial application of manure and this lack of nutrient inputs may have had detrimental effects on both organisms of which enterococci was more susceptible because of its complex nutrient requirements. Both organisms were less persistent at lower depths and it has been has speculated that this may be partially due to low nitrogen availability (Zhai et al., 1995).

We realize that the weekly refreshing irrigation, albeit in small amounts, may have influenced the results. Lau and Ingham (2001) found no significant difference in population decline between *E. coli* and enterococci when biweekly watering was implemented, but when watered once a week *E. faecalis* declined significantly faster than *E. coli*. We also note that the observed differences in survival patterns may be specific for the sandy loam texture of the soil used in this study. Cools et al. (2001) found that Enterococcus spp. out survived *E. coli* in fine textured soils, whereas in sandy soil *E. coli* thrived and Enterococcus spp. showed the least survival.

The initial growth of *E. coli* and enterococci populations in soils after their release from manure can undoubtedly complicate the development of regulatory guidance. The extended persistence of indicator bacteria can lead to false indications of recent fecal contamination where there has been none. On the other hand, the common assumption that once deposited into extra-enteric environments *E. coli* experiences immediate exponential decay (Winfield and Groisman, 2003) and is obviously not valid in the present study. More information on the survival patterns of manure-borne indicator

microorganisms after their release in soil needs to be collected to make best management practices more efficient.

Results on retention, growth, and survival of indicator microorganisms in the thin top layer of soil present a substantial interest for microbial water quality modeling. Popular watershed models such as SWAT and KINEROS/STWIR account for bacterial contributions from the top one centimeter of soil where mass exchange between the soil and runoff is assumed to occur. In the present study, 40 to 100% of all detected bacteria occurred within the top 1 cm. Results of this work show that much higher concentrations of bacteria are available to be released from soil to runoff when concentrations of bacteria in the mass exchange layer are computed from sampling of several centimeter-thick surface soil layers. To be used in modeling with the mass exchange layer, or mixing zone, such data on bacteria concentrations have to be adjusted to reflect realistic profile distributions. Another consequence of the observations in this work is the limited validity of the model assumption of the immediate exponential decay of microorganisms excreted by livestock or wildlife. In this study, it was found that *E. coli* released from manure with other components of the manure matrix can experience initial growth in soils. Therefore, the assumption of immediate exponential decay for this indicator organism may also introduce errors in modeling which need to be evaluated to decide on the need for model modifications.

3.6 Conclusions

Rainfall intensity displayed significant impacts on initial concentrations of indicator bacteria deposited from manure to soil. Increasing rainfall intensity resulted in reduced amounts of deposited bacteria, most likely due to the combination of surface sealing and

dilution effects. Concentrations of indicator bacteria decreased with increasing depth. *E. coli* and enterococci were both found to persist in soil layers up to 10 cm deep. Total numbers of *E. coli* increased at all rainfall intensities between simulated rainfall and one week afterwards, whereas enterococci declined in each treatment and at each depth. Different survival dynamics were observed for each rainfall intensity which demonstrated rainfall intensity as a factor in survival, although this relationship varied by organism and by depth. Findings of this work can be useful in manure application guidance as it remains to be investigated whether current microbial water quality models need to be amended to account for the initial bacteria growth phase.

3.7 Acknowledgements

We thank Ryan Blaustein and Billie Griffith for their technical and laboratory assistance.

Chapter 4 – Influence of manure consistency and weathering on the release, survival, and removal of fecal bacteria from vegetated soils under simulated rainfall.

4.1 Introduction

Manures are applied to fields in a number of different consistencies including liquid, slurry, semi-solid, and solid. These designations are determined based on the solid content of the manure. When applied, liquid manures may partially infiltrate into soil, whereas solid manure mostly remains on the surface. This difference does not matter if the manure is incorporated into the soil and with it the associated fecal bacteria are distributed over the soil plow layer. However, if a rainfall event occurs before incorporation, some manure constituents may be removed during rainfall. The difference between liquid and solid manure in terms of the rainfall-induced release of fecal microorganisms may be critically important for bacteria indicators like *E. coli* and enterococci since the presence of these bacteria are used to evaluate water quality.

When lands that have been treated with liquid manure undergo rainfall, the components of the liquid manure enter runoff mainly via interaction with the soil solution. Little to no remaining solids from the liquid manure may remain on the surface after rainfall depending on hydrological conditions and the extent of the straining of solids at the surface. For solid manures which contain a greater structure due to higher solid contents, the release process is much more complicated. For instance, one or more of the following occurs during release: (i) incoming rain suspends manure constituents

and then releases them by sloughing of manure under rainfall, (ii) an internal mixing process occurs during the manure water absorption-event followed by release of a diluted manure solution upon saturation (iii) and/or the initial manure solution is pressed out of the manure-matrix and then the release of diluted and mixed components occurs (Blaustein et al., 2015). Microorganisms released due to these processes may then be transported with surface runoff or infiltrate the soil.

After some fraction of the manure material is lost to runoff, the remainder of the manure on the field may be subjected to subsequent rainfall events. Since the manure frequently remains on the soil surface, the manure undergoes environmental weathering that includes changes in its physical structure, chemical composition, and microbiological communities (Muirhead and Littlejohn, 2009). The population of bacterial indicators may experience growth increases and/or declines in different parts of the manure matrix as changes in structure may can change the accessibility of the manure matrix to rainfall water. It is possible that the fraction of manure-borne indicator bacteria that is removed from a manure-covered land area mays depend on the weathering processes or on the duration of weathering. Results of the review in Chapter 2 show that this hypothesis has also not been previously tested in a controlled experiment.

The objectives of the work described in this chapter were to test the two stated hypotheses using bovine liquid and solid manure that had been exposed to different durations of manure weathering. Similar soil, vegetation, and simulated rainfall intensities were used with the wooden boxes, described in Chapter 3 that were designed to monitor compositions of runoff and infiltration waters. The first rainfall – week 0 event - allowed us to test the first hypothesis regarding the effects of manure consistency

on manure-borne indicator bacteria removal in runoff and infiltration. Two other simulated rainfall events – after one week and after two weeks of weathering – allowed us to test the second hypothesis regarding the effects of the weathering duration on the manure-borne indicator removal. All hypotheses were tested separately for *E. coli* and enterococci.

4.2 Materials and Methods

The study was conducted at the Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland. A variable controlled-intensity rainfall simulator (Meyer and Harmon, 1978) was used to apply rainfall for the release of manure-borne *Escherichia coli* and enterococci to runoff and infiltration from soil-applied dairy cattle manure.

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). Twelve soil boxes were packed with soil and seeded with fescue for use in the study. Each box was equipped with a height-adjustable runoff drain (10 mm diam.) positioned at the front of the box and three infiltration drains (6 mm diam.) positioned inside at the center of the base at 1 cm, 34 cm, and 67 cm from the front of the box. A mesh screen (1-mm^2 openings) was set over each infiltration drain to act as a filter and prevent clogging during the release of infiltration. 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 infiltration from each section of a box.

Soil boxes were packed with a loamy sand soil, the physical and chemical composition of which can be seen in Table 4.1. A 2-cm thick sand layer was evenly placed over the bottom of the box to facilitate infiltration release through the three mesh-covered drains. On top of this initial sand layer, six additional layers of the air-dried, 2-mm screened loamy sand soil were added to a precise given height within each box. Each layer was evenly spread throughout the box, packed flat with a plywood board, and then scored at the surface prior to placement of additional layers. The packing procedure was performed to create uniform bulk density conditions throughout the box. There was 59 kg of soil added to each box that resulted in an estimated bulk density of 1.33 ± 0.02 g cm^3.

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

Soil Component	*Value*
Sand	83.9 %
Silt	8.9 %
Clay	7.1 %
[1]pH	7.1
[2]Organic Matter	1.10
[3]Soluble Salts	0.09 (mmhos cm^{-1})
[4]Total Carbon	0.78 %
[4]Total Nitrogen	0.08 %
[4]Total Phosphorous	86 ppm
[5]Ammonium-N	2.03 ppm
[5]Nitrate-N	27.1 ppm

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

The packed soil boxes were transported to a BARC greenhouse that was programmed to maintain a temperature of 80 F° (26.7 C°). Temperature data can be seen in Fig. 4.2. The soil in each box was watered and cross-scored at the surface; then,

Kentucky 31 tall fescue was seeded 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 (Blaustein et al., 2016; Edwards et al. 2000; Sistani et al., 2009). Each seeded box was appropriately watered twice a day until germination and once a day following germination. After 30 days of grass growth, the soil boxes were over-seeded at the same rate as the initial seeding rate with an emphasis to establish uniform vegetation, and then 2 kg of topsoil was added to cover the new seeds. A final over-seeding occurred two months after the initial seed application and was accompanied with a 1 kg soil cover to aid germination. Daily watering occurred up until germination of the over-seeded grass, after which time the watering frequency was reduced to once every 2-3 days. The grass blades were trimmed to a height of 7 cm on a biweekly basis and on the day before each rainfall simulation event, and the excess trimmings were removed.

Manure and rainwater composition

The manure used in this study was obtained from dairy cattle at a concentrated animal feeding operation (CAFO) located at the USDA-ARS Dairy Research Facility, in Beltsville, MD. At these 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), a synthetic manure was prepared to represent the farmyard manure that is typically produced at dairy CAFOs and applied to cropland as fertilizer. The manure collection occurred on the day before the manure was to be applied to the surface of the fescue-vegetated wood boxes. The cattle feces and urine were sampled from 10 and 5 different cows, respectively, in

disinfected 5 gallon buckets. Feces and urine were then mixed together at a 6:1 ratio of feces/urine (volumetric) to prepare the synthetic manure. This manure mixture was stored at 4°C until usage. On the morning of each rainfall simulation event, the synthetic manure was mixed with either sawdust bedding to obtain a dry solid content of approximately 30% or was diluted with deionized water to obtain a dry solid content of approximately 5% for solid and liquid manures, respectively. Composite manure samples were collected just before each rainfall simulation to obtain the average physical, chemical, and microbial contents of the manure that was used on that day of study (Table 4.1).

Table 4.2. Mean values of the chemical properties and microbial contents of the dairy cattle manure measured from composite manure samples that were collected on each morning of manure application. The "±" separates average and standard error.

	Solid Manure	*Liquid Manure*
Solid Mass	26.9 ± 0.5 %	4.5 ± 0.8 %
Wet Mass	73.1 ± 0.5 %	95.5 ± 0.8 %
[1]Ph	8.3 ± 0.31	7.8 ± 0.08
[1]Carbon	11.62 ± 0.14 %	1.83 ± 0.38 %
[1]C:N ratio	32.06 ± 4.3	10.66 ± 1.92
Escherichia coli	$8.58 \pm 1.89 \times 10^5$ CFU g^{-1}	$3.18 \pm 0.68 \times 10^5$ CFU g^{-1}
Enterococci	$5.47 \pm 1.01 \times 10^4$ CFU g^{-1}	$1.09 \pm 0.13 \times 10^5$ CFU g^{-1}

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

Rainwater was prepared to mimic the ion content and pH for rainfall in the Maryland, Pennsylvania, and Delaware region. The synthetic rainwater mix was made by adding reagent-grade chemicals to reverse-osmosis deionized water to obtain 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 a 100-gal tank that was connected to the rainfall

simulator. Just prior to rainfall, the pH of the rainwater solution in the 100 gal tank was adjusted to 4.5 using additions of HCL and/or NaOH.

Experimentation

The treatments followed a 2 x 2 factor design with two manure types (liquid and solid) and two manure weathering regimes (1 week and 2 week). The four treatment combinations included liquid manure consistency with a 1-week weathering duration between simulated rainfalls events (LM 1), liquid manure consistency with a 2-week weathering duration between simulated rainfall events (LM 2), solid manure consistency with a 1-week weathering duration between rainfall events (SM 1), and solid manure consistency with a 2-week weathering duration between rainfall events (SM2). The effects of weathering was not assessed in the initial rainfall event since the fresh manure had not experienced weathering. The boxes of similar consistency that were assigned different weathering treatments were treated as replicates until just before the second rainfall event (i.e. LM 1 and LM 2, and SM 1 and SM 2), hereafter treatments were analyzed individually.

The liquid and solid manure treatments were each applied to 6 of the wooden boxes containing soil and fescue grass. Simulated rainfall was applied to all the boxes to obtain the initial week 0 results. The 12 boxes were divided into two groups with 3 boxes of each manure treatment in each group. Each group was respectively used to evaluate the 1- and 2 - week weathering treatments. While the first group was evaluated at 1 week, the other group remained in the greenhouse. The situation was reversed when the 2 week group was evaluated.

A rainfall intensity of 6 cm h^{-1} was chosen based on its correspondence to precipitation events in the mid-Atlantic and to previous research on microbial release from soil boxes (Blaustein et al., 2016). Soil boxes were elevated on the back end to mimic a 5% land slope. The rainfall simulator sprinkler nozzles (Veejet 80510; Spraying Systems Co., Wheaton, IL) were positioned 3-m above the soil surface, which allowed rain drops to reach near-terminal velocity upon landing, with an energy impact of approximately 275 kJ ha^{-1} mm^{-1}, which is similar to natural rainfall events greater than 25 mm h^{-1}. The rainfall simulator was calibrated to deliver a relatively uniform rainfall distribution for a central 1-m^2 area with a Christiansen coefficient of uniformity in the range of 87% to 89%.

Pre-soil samples were taken prior to manure application in order to assess background concentrations of *Escherichia coli* and enterococci in the soil boxes. Manure was applied to the soil boxes at a rate of 60 ton ha^{-1} wet weight (i.e., 2.94 kg box^{-1}). Three manure samples were collected from each box just prior to the start of rainfall for initial manure analysis. During rainfall, runoff and infiltration were collected from their respective troughs in sterile 100-ml bottles upon their initial release (time 0) and then subsequently at 3, 5, 10, 15, 20, 25, 30, 40, 50, and 60 minutes. All collection times and the duration for each collection were recorded. Samples were kept on ice and in the dark until processing occurred within 2 hours of collection.

The boxes were allowed to drain for approximately 30 minutes prior to the start of post-soil sampling to improve soil core integrity during extraction. Both pre-soil and post-soil core sampling locations were randomly assigned prior to the start of the experiment. Upon extraction, post-soil samples were carefully subdivided into 0-1 cm, 1-

2 cm, 2-5 cm, and 5-10 cm portions using sterile tongue depressors. Sample subsections were carefully placed into sterile 100 mL bottles and immediately stored on ice. Three post manure samples were also taken from random locations within the box and placed in the same 100 mL bottles and were stored on ice. Post soil and post manure sample processing occurred within one hour of collection.

After sample collection, soil boxes were transported back to the greenhouse where they remained until the second rainfall event. After the second rainfall event, the soil boxes were stored in the greenhouse for the remainder of the study.

4.3 Microbiological Analyses

The manure and soil samples were thoroughly homogenized using sterile tongue depressors prior to blending. A 2-g sub-sample of each soil and manure sample was then used for microbiological processing. Sub-samples were placed into sterile blenders where each sub-sample was ground with 200 ml of sterile D.I. water for 2 minutes to create an initial dilution factor of 10^{-2}. The resulting manure and soil solutions were poured into sterile beakers and allowed to sit for 15 minutes and 1 hour, respectively, to allow for larger particles to settle. The soil solution supernatants were pipetted onto TBX and mEnterococcus agars in 100ul and 250 ul aliquots for the enumeration of *E. coli* and enterococci, respectively. The manure solutions were serially diluted and pipetted onto the same agars in 100 ul aliquots. The samples plated onto TBX agar plates were incubated at 37 °C for 2 hours followed by 22 hours at 44.5 °C. The samples plated on mEnterococcus agar were incubated for 24-48 hours at 37 °C. After the incubation period, the green colonies on TBX plates and the red colonies on mEnterococcus agars were counted as presumptive *E. coli* and enterococci cells, respectively.

The runoff and infiltration samples were serially diluted and pipetted onto the same agars. All samples were plated in duplicate. The volume of each sample was determined just prior to microbiological analysis.

4.4 Data Analysis

For each rainfall simulation event, the concentrations of *E. coli* and enterococci in collected runoff and infiltration samples were multiplied by the water flux, based on the effluent flow rate, and the products were integrated to quantify the cumulative numbers of bacteria released within each effluent type. These cumulative numbers were converted into relative ratio values N_{Runoff}, or simply N to N_0 by dividing the quantity in the runoff by the initial total quantity of bacteria applied in the manure for each bacteria group.

The quantities of bacteria in the soil at the 0 – 1 cm, 1 -2 cm, 2 – 5 cm, and 5 – 10 cm soil depth ranges after rainfall were 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 (1, 1, 3, 5 cm) x box length (100 cm) x box width (35 cm) x average soil bulk density (g cm^{-1})). The total number of bacteria by depth ranges were summed to estimate the total number within the soil profile. Fractions of bacteria by soil depth ranges and by the entire profile were obtained by dividing these numbers by the number of bacteria applied to the box with the manure treatment (N_0).

The Paleontological Statistics Software (PAST) version 3.0 (Hammer et al., 2001) was used for statistical comparisons of data. In the initial rainfall event, a Student's t-test was used to compare the effects of the manure and weathering treatments on the relative fractions (N/N_0) of bacteria recovered in runoff, infiltration, and within the soil. For the

same rainfall event, a Student's t-test was also used to determine the effects of manure consistency on gravimetric soil moisture contents (%), partitioning of cumulative water among runoff, infiltration, and soil water; flow rates of water leaving the box as either runoff or infiltration; and the time to the initiation of runoff and infiltration. In the 1- and 2-week rainfall events, a two-factor ANOVA was used to assess the effects of consistency and weathering on the release, survival, and removal of indicators from the soil boxes for the dependent variables previously stated. . A one-factor ANOVA was used to assess differences in the fractions of bacteria indicators by the soil depth ranges.

Temperature regimes were slightly different among the treatments due to the randomized experimental design that resulted in boxes arriving within the green house at different times. As such, the mean daily air temperature for when each set of treatment replicates were in the greenhouse was determined and a one-factor ANOVA was used to compare statistical difference between the 28-day mean daily temperature series between treatments. For all hypothesis testing, significance was assessed at the 0.05 and 0.01 probability levels, and will be stated as significant and highly significant, respectively, in the discussion of the results. Figures were created using Sigmaplot 13.0 (Systat Software)

4.5. Results

3.1 Soil water content

Throughout the experiment, the gravimetric water contents were generally slightly higher in the solid manure (SM) treatments than in the liquid manure (LM) treatments (Fig. 4.1). After the final rainfall event for each treatment, the soil water content

gradually declined, with the absence of any additional simulated rain water, and eventually reached approximately 3-6% by the termination of the study. The final soil water contents did not significantly differ in response to any treatment combination by the termination of the study.

3.1.1. Soil water content before and after first rainfall event

Prior to the initial rainfall event, the mean gravimetric soil water contents within the boxes of all treatments ranged between 19.3% and 22.2%, but did not significantly differ. Following the initial event, the soil water contents increased on average by 1.5%, but still were not significantly different between the soil boxes that received LM or SM treatments. Soil moisture contents significantly differed by depth in the LM treatments after the initial rainfall event, with mean moisture contents of 21%, 22%, 23.2%, and 20.3% in the 0 – 1 cm, 1 – 2 cm, 2 – 5 cm, and 5 – 10 cm depth ranges, respectively. A Tukey multiple means comparison test indicated a significant difference existed between the 2 – 5 cm and 5 – 10 cm depth layers. In the SM treatments, soil moisture contents did not vary significantly by depths after the initial rainfall event.

3.1.2 Soil and manure water content prior to and after second rainfalls

Just prior to the 1 week rainfall event, there were no statistical differences detected between the soil water content levels at the different soil depth ranges within the soil boxes of either LM or SM treatments. A two-way ANOVA for comparisons between pre-rainfall soil water contents of the 1- and 2-week rainfall boxes determined both weathering and consistency to be significant factors with solid treatments having statistically greater soil water contents than liquid treatments, and 1-week soil water

content levels having significantly larger soil water contents than the 2-week pre-rainfall water content levels (Table 4.3). In the LM treatments, the 1-week pre-rainfall soil water contents were just over two times as great as the 2-week pre-rainfall soil water contents (15.6% and 7.6%, respectively). In the SM treatments, these levels were 17.8 ± 0.6 and 10.8 ± 0.9% for the 1- and 2-week water contents prior to rainfall, respectively. Prior to the second rainfall event, the manure's gravimetric water content atop the SM treatments did not statistically differ between the 1- and 2-week weathering levels as the 2-week water content levels were only 2% less than the 1-week levels.

Table 4.3. Results of two-factor ANOVA on the effects of consistency and weathering on soil moisture contents before and after the second simulated rainfall event. Statistical comparisons were made between soil moisture contents measured in 1- and 2-week weathering treatments between manure consistencies.

Effect	Pre-rainfall	Post-rainfall
Consistency	**	**
Weathering	**	**
Consistency × Weathering	NS	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.
NS = nonsignificant at the 0.05 probability level.

The soil depth ranges did not significantly differ in the soil water contents (%) immediately after the 1- week rainfall event or 2-week rainfall event in either the LM or SM treatments, respectively.

The mean gravimetric soil water contents at the post-rainfall were significantly different between the consistencies and the levels of weathering (Figure 4.1). The measured soil water contents were 22.2 ± 0.13 and 23.4 ± 0.58 for LM and SM treatments, respectively, after the 1-week rainfall and 20.9 ± 0.6 and 22.2 ± 0.3 for the LM and SM treatments, respectively, after the 2-week rainfall.

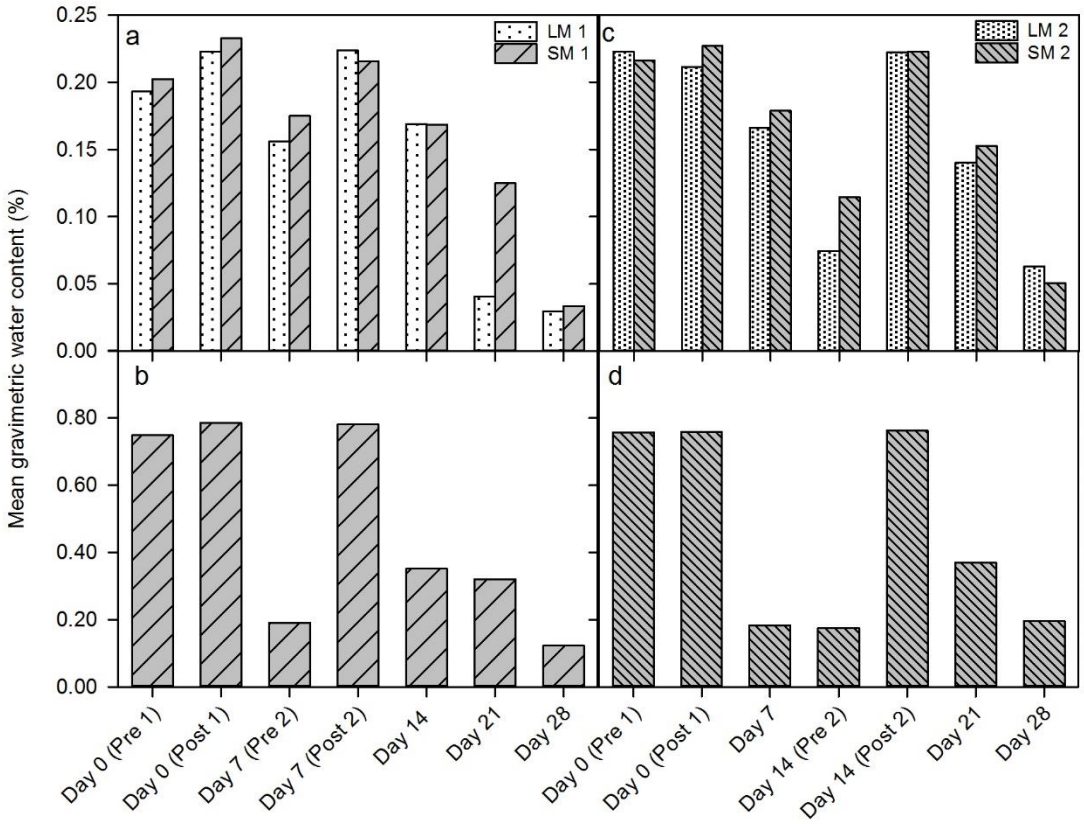

Figure 4.1. Mean gravimetric soil water contents (%) throughout the experiment. Graphs a, b, c, and d are soil water contents in the 1 – week weathering liquid and solid manure treatments (LM 1 and SM 1), manure water content in the SM 1 treatment, soil water content in the 2 – week liquid and solid manure treatments (LM 1 and SM 1), and manure water content in the SM 2 treatment, respectively.

3.2 Temperature in the greenhouse

The mean daily temperatures for the greenhouse room used to store the soil boxes is shown in Fig. 4.2. Generally, the soil boxes in the (LM 1), the (SM 1), and the (SM 2) treatments experienced similar temperatures throughout the experiment; however, soil

boxes in treatment the (LM 2) treatment experienced significantly lower temperatures namely between the 5-10 day and 20-30 day periods. The averages of the mean daily temperatures were 25.3, 25.3, 25.3, and 23.9 ºC for the LM 1, LM 2, SM 1, and SM 2 treatments, respectively.

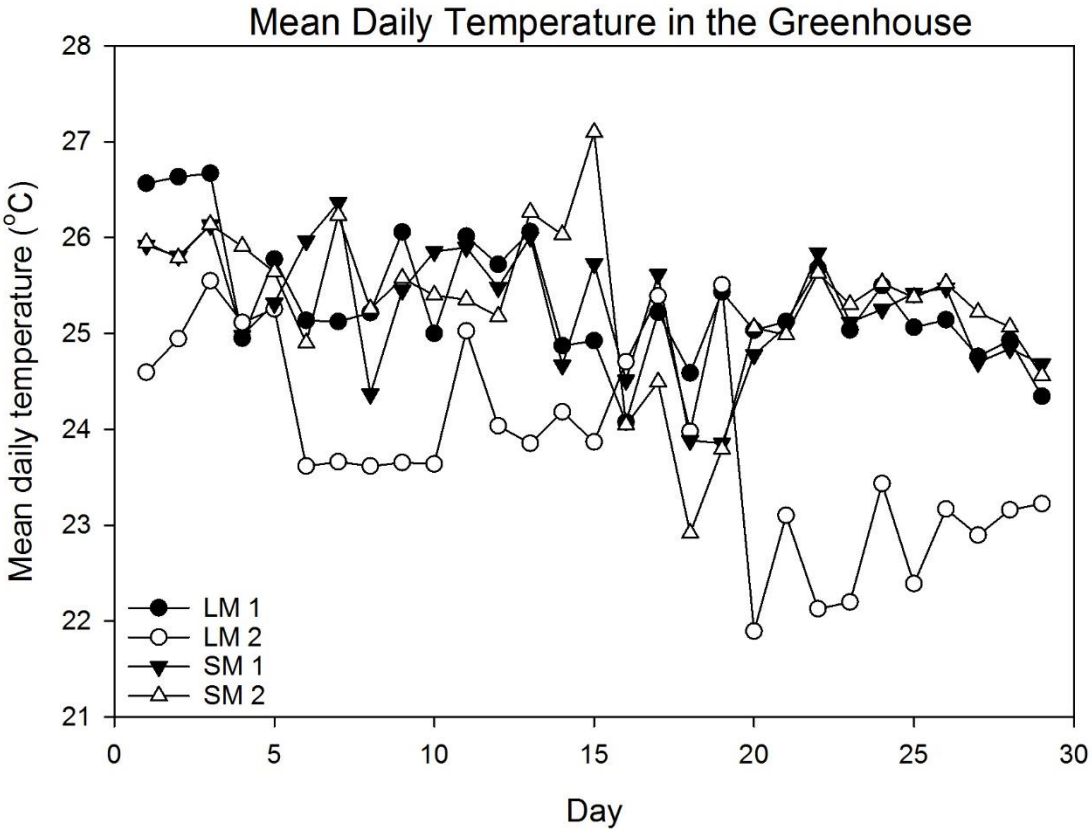

Figure 4.2. Mean daily air temperature (°C) in the greenhouse throughout the experiment. The plotted values are averages across three replications.

3.3. Water partitioning during the first rainfall event

The runoff in the initial rainfall events for both LM and SM treatments accounted for the greatest proportion of water at 58.7% and 62.2% of the total amount applied for

LM and SM treatments, respectively (Fig. 4.3). The infiltration accounted for 38.4% and 32.5% for the LM and SM treatments, respectively. The amount of water retained in the soil was estimated by the difference in the initial and final soil gravimetric water contents and accounted for 2.9% and 5.3% for the LM and SM treatments, respectively. The amounts of water recovered as runoff, infiltration, or in changing the soil water contents did not significantly differ between the LM and SM treatments in response to the first rainfall event.

Water partitioning during the second rainfall event

The dominant mode of water transport changed from runoff to infiltration between week 0 and week 1 in the LM treatments (Fig. 4.3). While the leachate volumes increased in the SM treatments, the volumes of water removed were still less that the volumes removed in runoff. In both consistency treatments, the amounts of runoff decreased over time and the amounts of water in leachate and remaining in the soil increased. The degree of weathering was not found to significantly affect the volume of water partitioned as runoff or infiltration, but did significantly increase the amounts of water retained in the soil after rainfall for the 2-week versus the 1-week rainfall events (Table 4.4). The amount of water leaving the boxes as infiltration was significantly greater for the LM treatments than for the SM treatments. The interactions between factors of weathering and consistency were not significant for runoff, infiltration, or soil water volumes.

Table 4.4. Results from the two-factor ANOVA on the effects of weathering and consistency on the partitioning of runoff, infiltration, and soil water between the 1- and 2-week rainfall events. Statistical comparisons were made using cumulative volumes of runoff and infiltration for the 1- and 2-week rainfall events and the estimated volume of water retained in the soil after the rainfall event.

Effect	Runoff	Infiltration	Soil Water
Consistency	NS	*	NS
Weathering	NS	NS	**
Consistency × Weathering	NS	NS	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.
NS = nonsignificant at the 0.05 probability level.

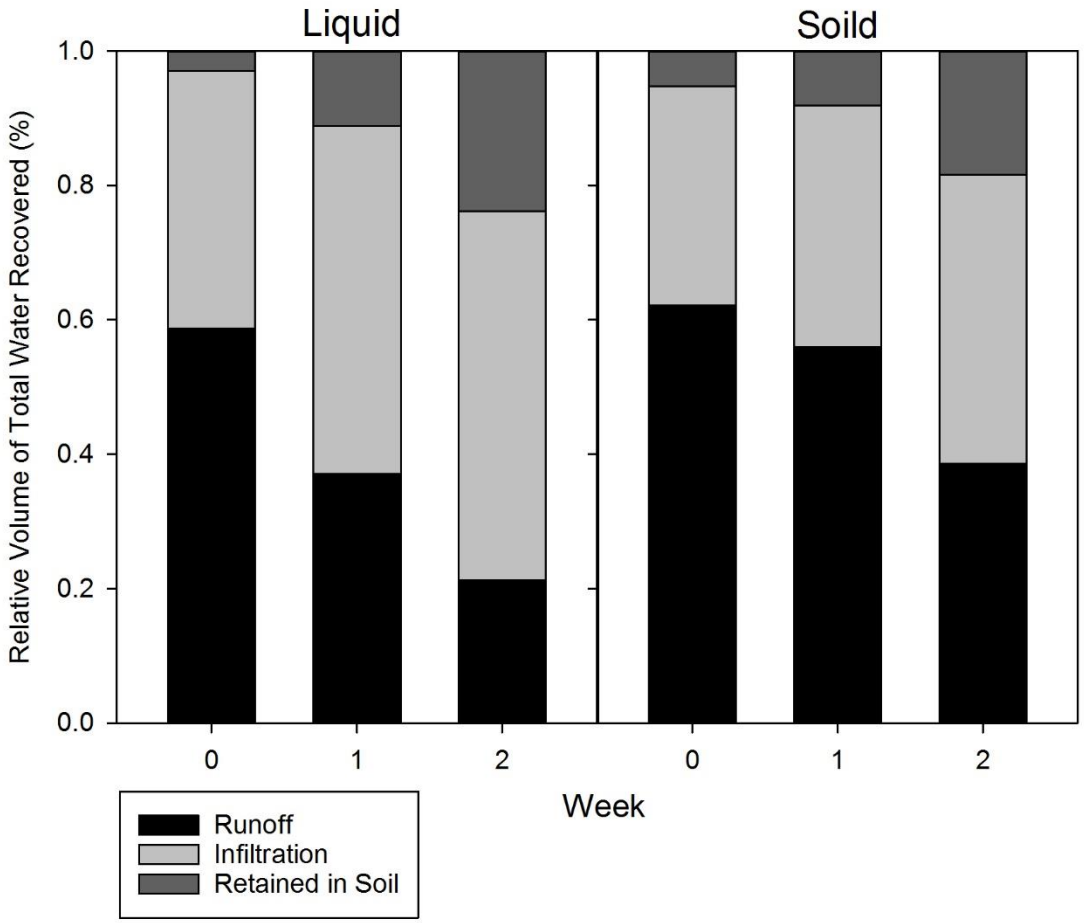

Figure 4.3. The fractions of water removed as runoff, infiltration, or retained in the soil following each rainfall event for the liquid and solid manure treatments.

The runoff and infiltration rates are shown in Figure 4.4. The rates of both effluents increased greatly within the first 1-cm rainfall depth after initiation and then began to stabilize. The water removal rates were more or less consistent for the 1 – 6 cm rainfall depths after initiation. During the first rainfall event, the mean runoff rates were significantly greater than the infiltration rates for all treatments. The manure consistency did not have significant impacts on the mean runoff and infiltration rates during the first

rainfall event. During the 1- and 2-week rainfall events, the dominance of either runoff or infiltration was less clear: in the LM 1 treatment, there were no statistically significant differences between the infiltration and the runoff; in the LM 2 treatment, the infiltration was highly significantly greater than the runoff ($P < 0.01$); in the SM 1 treatment, runoff was highly significantly greater than infiltration ($P < 0.01$); and in the SM 2 treatment, there were no statistically significant differences between the infiltration and the runoff.

Both consistency and weathering were found to significantly impact the mean runoff flow rates and the interaction between these factors was found to be significant (Table 4.5). The average runoff flow rates were greater during the 1- week treatments with rates of 2.63 and 2.65 ml s^{-1} from the LM and the SM treatments, respectively, which were greater than the respective values of 1.19 and 2.053 ml s^{-1} during the 2-week rainfall event. The infiltration was significantly affected by the level of weathering, but there were no significant differences between the LM and the SM treatments and the interaction between the factors also was not significant (Table 4.5). The average infiltration rates were 2.87 and 1.58 ml s^{-1} in the LM and the SM treatments during the 1-week rainfall event, respectively, and 2.80 and 1.89 in the same respective treatments during the 2-week rainfall event.

Table 4.5. Results from the two-factor ANOVA evaluating the effects of weathering and consistency on the mean flow rates of runoff and infiltration. Statistical comparisons were made between mean runoff and infiltration flow rates between the week 1- and 2 liquid and solid manure treatments.

Effect	Runoff	Infiltration
Consistency	**	NS
Weathering	**	**
Consistency × Weathering	*	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.

NS = nonsignificant at the 0.05 probability level.

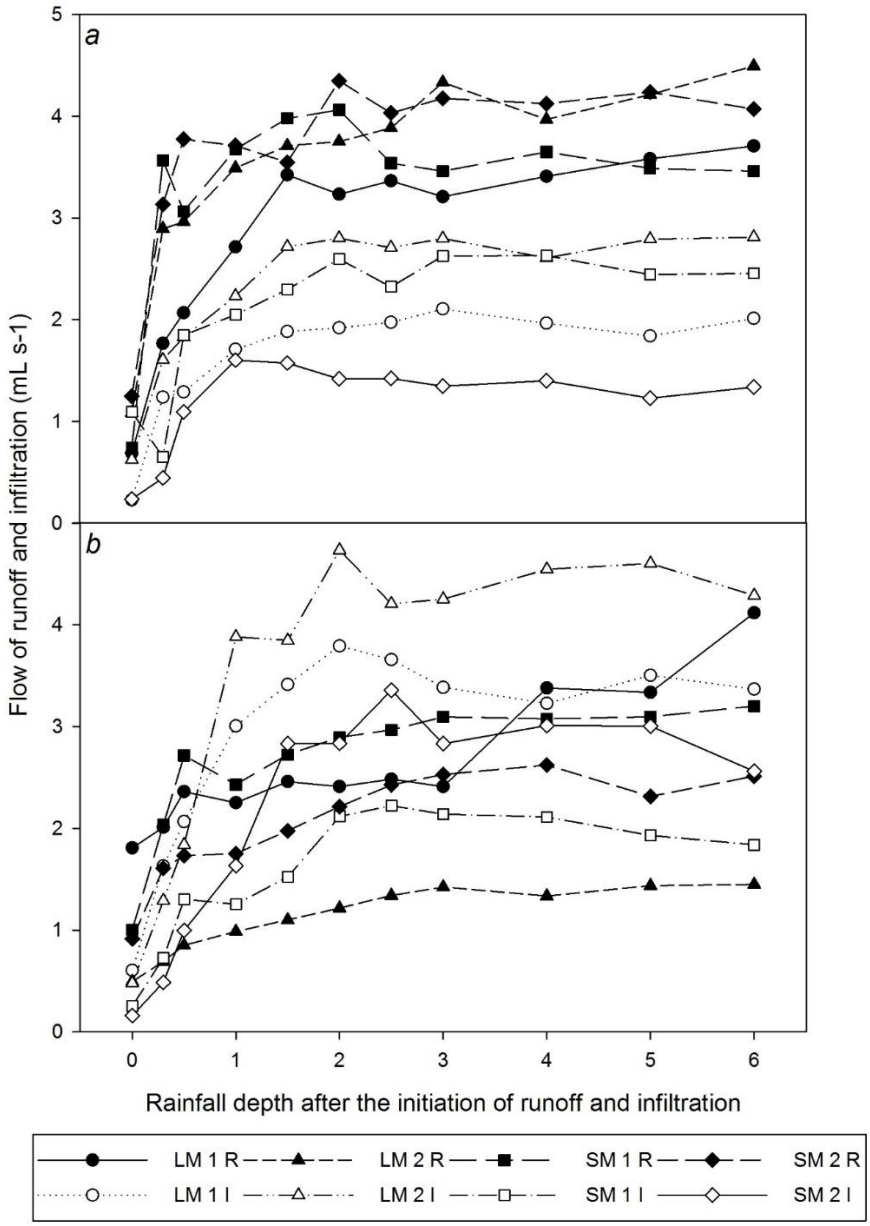

Figure 4.4. Flow rates of water leaving the boxes as runoff and infiltration during the rainfall events. The upper panel a displays the results of the initial rainfall event and the lower panel b displays the results of the 1- week and 2-week rainfall events. An R or I following the treatment designation indicates the symbols for the runoff and the infiltration, respectively. All flow rates are reported from the beginning of runoff and infiltration.

3.3. Time before initiation of runoff or infiltration

The time until the initiation of runoff and infiltration for liquid and solid manure treatments was similar in the first week although both started slightly earlier in the liquid treatments (Table 4.6). The differences between time of initiation of runoff and infiltration was not significant for the LM treatments, but for the SM treatments, the time to the initiation of runoff was significantly longer than for infiltration.

In the initial rainfall event, the time it took for runoff to begin was not statistically different between the manure consistencies. However, the time for infiltration to begin was significantly longer for the solid manure than for the liquid manure treatments.

With increasing weeks since the initial rainfall, the time until the initiation of runoff and infiltration increased. This time increase was more pronounced for the liquid treatment boxes than the solid treatment boxes. In the 2-week weathering rainfall event, there was a later initiation of runoff compared to the infiltration in both the manure consistency treatment types. A trend of increasing time to the initiation of either the runoff or the infiltration with decreasing soil and manure water contents was also observed.

Table 4.6 Average time (minutes) required for the initiation of runoff or infiltration for the different consistency and weathering treatments.

	Liquid Manure		Solid Manure	
Weathering	Runoff	Infiltration	Runoff	Infiltration
Fresh	6 ± 2.8	3 ± 0.5	10 ± 0.4	5 ± 0.7
1 Week	35 ± 11.2	22 ± 4.3	18 ± 1.8	11 ± 2.2
2 Week	52 ± 4.5	28 ± 1.2	38 ± 2.3	20 ± 2.7

Plus/minus separates the mean from the standard error of the mean.

A two-way ANOVA used to compare the times to the initiation of runoff or infiltration in the 1- and 2- week rainfall events revealed that runoff in the LM treatments took significantly longer to begin than in the SM treatments (Table 4.7). The runoff delays were also significantly longer in the 2-week than the 1-week weathering treatments. The interaction between the consistency and weathering factors was not significant.

Table 4.7. Results from two-factor ANOVA (p-values are displayed) on the effect of weathering and consistency on the delay in initiation of runoff and infiltration between the 1- and 2-week rainfall events. Statistical comparisons for runoff and infiltration delay times were made between times for the initiation of runoff and infiltration in the 1- and 2-week rainfall events and for both manure consistencies.

Effect	Runoff	Infiltration
Consistency	*	**
Weathering	*	*
Consistency × Weathering	NS	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.
NS = nonsignificant at the 0.05 probability level.

The delay before the start of infiltration was significantly longer for the LM treatments than the SM treatments (Table 4.7). The 2-week weathering treatments also took significantly longer to begin than the 1-week weathering treatment. The effects of

weathering on the initiation of infiltration were not different for the two consistency types. Pairwise testing showed the effects of the LM and the SM treatments on the initiation of infiltration significantly differed in the 1-week rainfall event, but were not statistically significant in the 2-week rainfall event.

3.4. Indicator removal in runoff and infiltration

3.4.1 Removal in runoff

Because of the lag period between the start of rainfall and the generation of runoff, the kinetics for indicator bacterial removal with the runoff and the infiltration were related to the rainfall depth after runoff began rather than on total rainfall.

Both *E. coli* and enterococci were eluted in high concentrations within the runoff. The mean concentrations in the initial runoff during the first week were $4.89 \pm 1.22 \cdot 10^4$ and $8.67 \pm 2.22 \cdot 10^4$ for *E. coli* and $1.85 \pm 6.03 \cdot 10^3$ and $9.80 \pm 3.20 \cdot 10^3$ for enterococci for the LM and SM treatments, respectively. The average concentrations in the runoff throughout the 1-hour collection period were $1.38 \pm 0.33 \cdot 10^4$ and $3.79 \pm 0.68 \cdot 10^4$ *E. coli* CFU ml^{-1} and $4.58 \pm 1.28 \cdot 10^3$ and $4.75 \pm 0.90 \cdot 10^3$ enterococci CFU ml^{-1} for the LM and SM treatments, respectively.

After the initiation of runoff, the removal curves displayed two-stage dynamics in which the majority of release occurred in the first 1-cm of rainfall for both organisms (Fig. 4.6). Within this first centimeter of rainfall, 54% and 36% of *E. coli* were removed in the runoff from the LM and SM treatments. With the same rainfall depth, 37% and 30% of enterococci were removed in the runoff from the LM and SM treatments,

respectively. Following these initial losses in runoff, the rainfall depths of 2 to 6 cm contained much less bacteria relative to what was removed in the first centimeter of rainfall. This trend persisted in subsequent rainfall events across both liquid and solid manure consistency treatments.

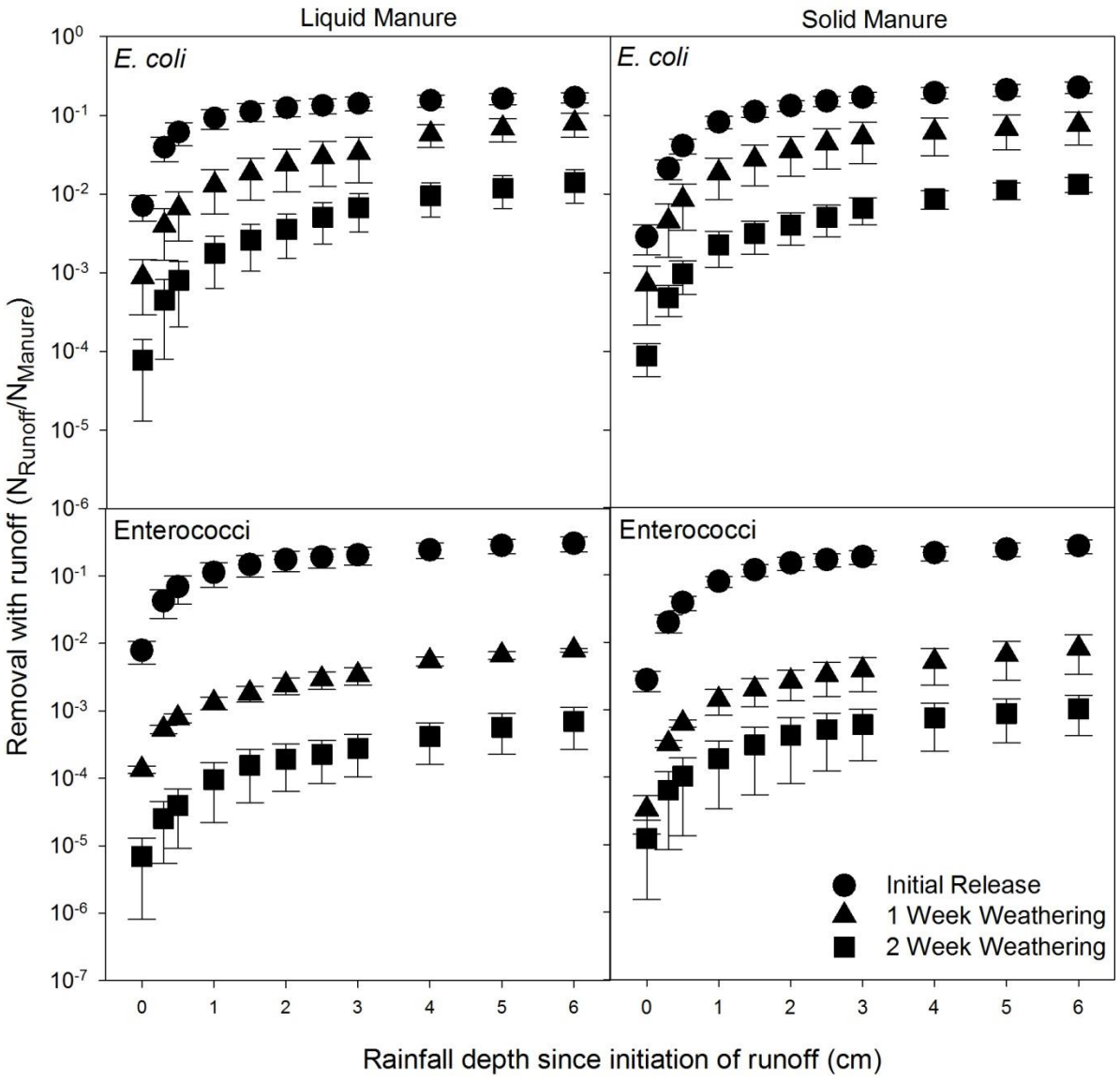

Figure 4.6. The cumulative amounts of *E. coli* and enterococci removed with runoff (N_{Runoff}/N_{Manure}) as dependent on the rainfall depth since the initiation of runoff. Error bars represent the standard error of the mean.

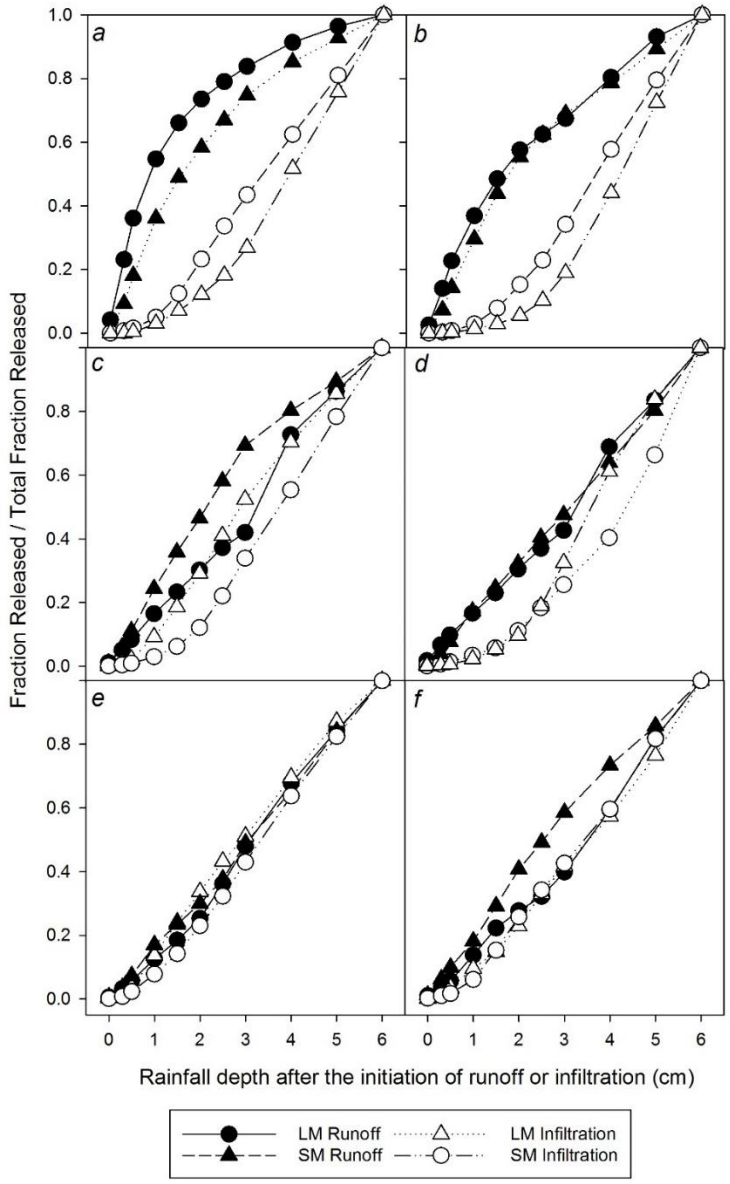

Figure 4.7. The ratio of the fraction of each indicator released at each sampling depth to the total fraction removed in that rainfall event. Panels a, c, and e show *E. coli* removal with runoff and infiltration in the initial, 1-week, and 2-week rainfalls events, respectively. Panels b, d, f show enterococci removal with runoff and infiltration in the initial, 1-week, and 2-week rainfall events, respectively.

In the first rainfall event, the removal of both indicators in runoff occurred much earlier than removal with infiltration (Fig 4.7). The removal with infiltration was slow in the first 0 – 3 cm rainfall depths after the initiation of infiltration, but after 2 cm had been applied, the rate of removal greatly increased (panels a and b). In the second rainfall event, the rates of removal between runoff and infiltration are more similar, but the infiltration continues to show a trend of the greatest bacteria removal in the latter half of the rainfall event than the first half.

Table 4.8. The mean fractions of *E. coli* and enterococci removed in the runoff compared to the number of cells applied in each manure treatment ($100*(N_{Runoff}/N_{Manure})$).

	Liquid		Solid	
Rainfall	EC	EN	EC	EN
Week 0	16.92 ± 2.61	30.49 ± 7.64	22.75 ± 3.82	27.10 ± 6.29
Week 1	5.35 ± 2.69	0.80 ± 0.05	7.63 ± 3.44	0.82 ± 0.48
Week 2	1.40 ± 0.64	0.07 ± 0.04	1.34 ± 0.29	0.10 ± 0.06

In the initial rainfall event, the fractions of *E. coli* released did not significantly differ between the LM and SM treatments. Table 4.8 shows the fraction of *E. coli* and enterococci removed with runoff by the consistency and weathering treatments. A greater fraction of *E. coli* was removed from soil boxes amended with solid manure than those with liquid manure in the initial and 1-week rainfall events, but this fraction was nearly equal in the 2- week rainfall event. Compared to the initial rainfall event, the fractions of *E. coli* removed in the 1- and 2-week rainfall events were on average 3.16 and 12.1 times less, respectively in the LM treatments, and 2.98 and 5.7 times less, respectively in the SM treatments. In the 2-week rainfall event 3.8 and 5.7 times less *E.*

coli were removed than in the 1-week rainfall for the LM and SM treatments, respectively. The mean concentrations of *E. coli* in the 1-week runoff event were (4.10 ± 0.47) · 10^3 and (1.21 ± 0.09) · 10^4 CFU ml^{-1} in the LM and SM treatments, respectively. In the 2-week rainfall, the mean concentrations of *E. coli* were (9.87 ± 1.12) · 10^2 and (2.41 ± 0.25) · 10^3 CFU ml^{-1} for the LM and SM treatments, respectively.

The two-factor ANOVA showed that the fractions of *E. coli* removed in the 1- and 2-week events did not statistically differ between the consistencies or by the level of weathering (Table 4.9). The interaction of these factors on fractions of both indicators removed in runoff were not significant.

Table 4.9. Results from two-factor ANOVA on the effects of weathering and consistency on the fraction of *E. coli* and enterococci removed in runoff in the 1- and 2-week rainfall events.

Effect	*E. coli*	Enterococci
Consistency	NS	NS
Weathering	NS	*
Consistency × Weathering	NS	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.
NS = nonsignificant at the 0.05 probability level.

The fractions of enterococci removed did not significantly differ between the manure consistencies in the initial rainfall event. Unlike *E. coli*, a greater fraction of enterococci were released from the LM treatments in the initial rainfall event (Table 4.9). The fractions of enterococci removed in the runoff for the 1- and 2-week rainfall events were approximately the same. Larger reductions in the fraction of enterococci compared to *E. coli* were removed in the runoff in the subsequent rainfalls. Compared to the initial event, the enterococci removed in the runoff in the 1- and 2 week rainfalls events was

38.2 and 438.3 times less, respectively, for the LM treatments and 33.1 and 263.0 times less, respectively, for the SM treatments. The difference in the fraction of enterococci removed between the 1- and 2- week rainfalls was 11.5 and 8.0 times for the LM and the SM treatments, respectively. The mean fractions of enterococci in runoff were $7.32 \pm 1.10 \cdot 10^1$ and $7.36 \pm 1.16 \cdot 10^1$ CFU ml^{-1} in the 1-week rainfall and $1.56 \pm 0.21 \cdot 10^1$ and $2.67 \pm 0.40 \cdot 10^1$ CFU ml^{-1} in the 2-week rainfall in the LM and SM treatments, respectively.

The consistency was not found to significantly impact the fraction of enterococci removed in the runoff during the 1- and 2 – week rainfall events, but there was a significantly smaller fraction of enterococci removed in the 2-week rainfall event than in the 1-week rainfall event (Table 4.9).

In the initial week, the ratio of *E. coli* to enterococci removed in runoff is less than one, however, in subsequent weeks this ratio becomes increasingly large (Table 4.10). Runoff indicator ratios increased by 3.0 and 1.4 times between the 1- and 2- week rainfall events for the LM and the SM treatments, respectively.

Table. 4.10. The ratio of fractions of *E. coli* to the fractions of enterococci removed in runoff.

	Liquid		Solid	
Rainfall	Runoff	Infiltration	Runoff	Infiltration
Week 0	0.55	0.80	0.84	0.44
Week 1	6.70	3.98	9.32	9.66
Week 2	20.17	10.03	12.97	18.61

3.4.2. Removal in Infiltration

The initial concentrations of indicators in the infiltration were considerably lower than in the initial runoff water. The mean concentrations in the initial infiltration were $3.58 \pm 1.22 \cdot 10^2$ and $2.33 \pm 1.67 \cdot 10^1$ *E. coli* CFU mL^{-1} and $1.38 \pm 1.11 \cdot 10^2$ and 0 enterococci CFU ml^{-1} for the LM and SM treatments, respectively. The removal in infiltration tended to steeply increase between 0 and 2 cm of rainfall and exhibit a more gradual increase from 2 to 6 cm of rainfall (Fig. 4.8). The removal of indicators with the increasing depth of rainfall after the initiation of infiltration was in stark contrast with the removal in runoff. The largest increase in the fractions removed was in the 4 – 6 cm rainfall depth range after the initiation of infiltration and for runoff the largest quantities were generally in the first 1 cm after runoff initiation (Fig. 3.6). This trend persisted for the subsequent rainfall events for both liquid and solid manure consistency treatments. The removed fractions of both *E. coli* and enterococci steadily declined with each rainfall event (Table 4.11). The mean concentrations of *E. coli* removed with infiltration in the initial rainfall were $3.61 \pm 0.54 \cdot 10^3$ and $5.08 \pm 1.06 \cdot 10^3$ in the LM and SM treatments, respectively. These values for enterococci were $6.94 \pm 0.97 \cdot 10^2$ and $5.95 \pm 1.13 \cdot 10^2$ for the LM and SM treatments, respectively.

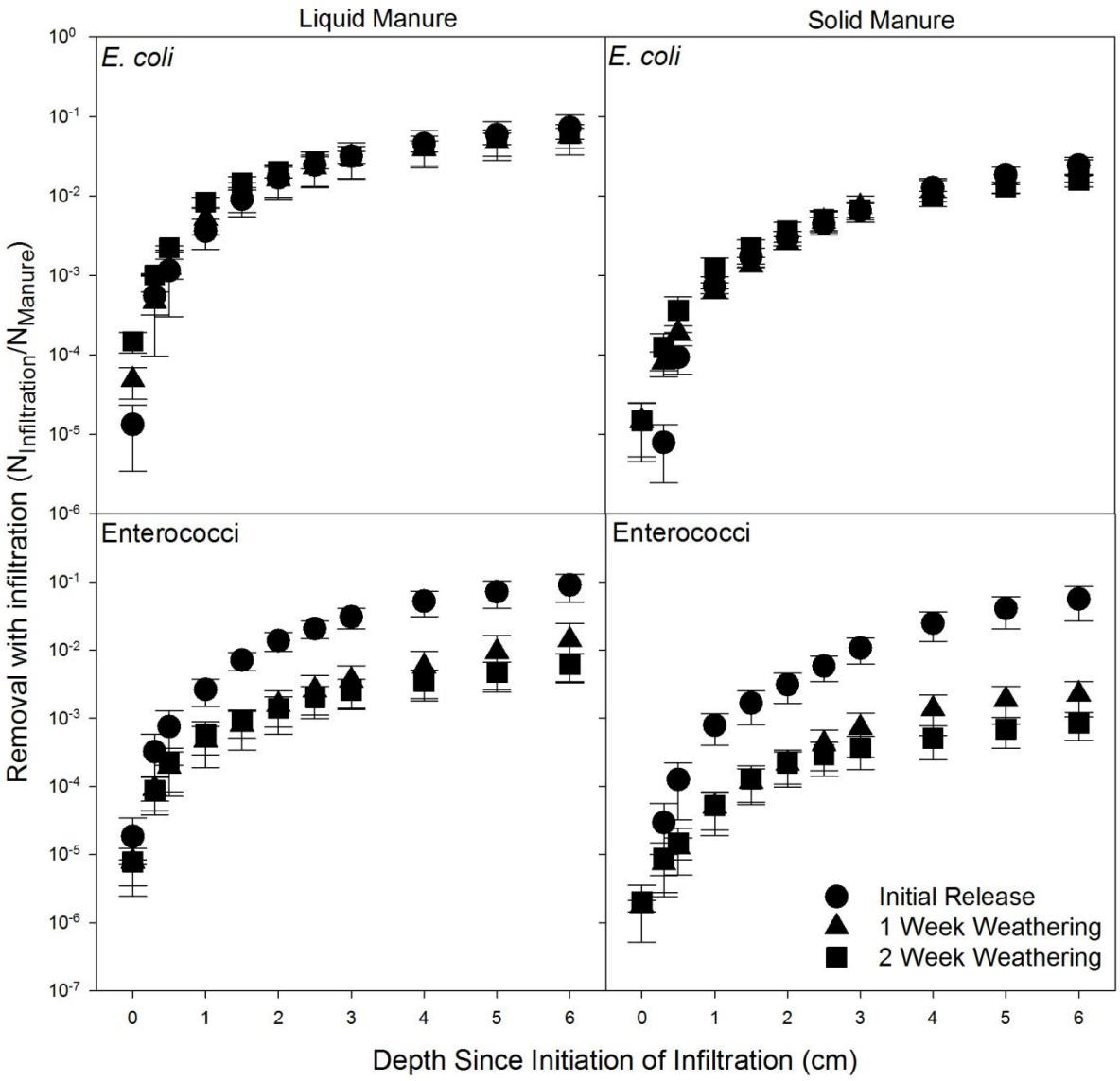

Figure 4.8. The cumulative amounts of *E. coli* and enterococci removed with infiltration (N_{Runoff}/N_{Manure}) as dependent on the rainfall depth since the initiation of infiltration. Error bars represent the standard error of the mean.

E. coli removal in infiltration in the initial rainfall event did not statistically differ between the manure consistencies. The fraction of the enterococci removed in infiltrating

water did not significantly differ by manure consistencies. For both indicators, the fractions removed were greater for the LM treatments than for the SM treatments. This trend persisted in each subsequent rainfall event. Like in runoff, the enterococci removal in the first week was greater than *E. coli* removal relative to what was applied, however; this trend was reversed for the 1- and 2- week rainfalls where enterococci removal was less than that of *E. coli* (Table 4.10).

Table 4.11. The mean fraction of *E. coli* and enterococci removed in infiltration compared to the number of cells applied in the liquid and solid manures ($100*(N_{Runoff}/N_{Manure})$).

	Liquid		Solid	
Rainfall	EC	EN	EC	EN
Week 0	7.27 ± 3.24	9.04 ± 3.95	2.45 ± 0.65	5.67 ± 2.94
Week 1	5.63 ± 2.32	1.41 ± 1.08	2.16 ± 0.68	0.22 ± 0.12
Week 2	6.14 ± 0.98	0.61 ± 0.27	1.58 ± 0.29	0.09 ± 0.04

In the 1- and 2- week rainfall events, the effects of weathering were not significant on the fractions of *E. coli* or enterococci removed in the infiltration (Table 4.12). There were significantly greater fractions of both *E. coli* and enterococci removed in the LM treatments than the SM treatments in the latter two rainfall events. The interactions between the consistency and weathering were not significant for either organism.

The fractions of *E. coli* removed in all events were similar and actually increased from the 1-week to the 2-week rainfall in the liquid manure treatments (Table 4.11). In the solid manure treatments, the *E. coli* removal steadily declined with each rainfall event. The total reductions in fractions removed for *E. coli* between the initial and final rainfalls were only 1.2 percent and 0.9 percent for the LM and SM treatments,

respectively. Compared to the initial rainfall event, the fractions of *E. coli* removed in the 1- and 2-week rainfall events were 1.3 and 1.2 times less, respectively, in the LM treatments and 1.1 and 1.6 times less, respectively, in the SM treatments. In the 2-week rainfall event, 0.9 and 1.4 times less *E. coli* were removed than in the 1-week rainfall for the LM and SM treatments, respectively.

Table 4.12. Results from the two-factor ANOVA on the effects of weathering and consistency on the fraction of *E. coli* and enterococci removed with infiltration in the 1- and 2-week rainfall events.

Effect	*E. coli*	Enterococci
Consistency	*	*
Weathering	NS	*
Consistency × Weathering	NS	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.
NS = nonsignificant at the 0.05 probability level.

Enterococci removed in the infiltration declined to a greater degree than *E.* coli with each subsequent rainfall event with the largest decline between the initial and 1-week rainfalls for both consistency types. The 1- and 2- week removal events were 6.4 and 14.8 times less, respectively, than the initial event for the LM treatments and 25.2 and 66.6 times less, respectively, in the SM treatments. The differences between the 1- and 2- week fractions removed was 2.3 times and 2.6 times less for the LM and SM treatments, respectively.

3.5 Dynamics of indicators within soil

3.5.1 Fractions in total soil profile after initial event

After the initial rainfall event, the relative fraction of *E. coli* in the total soil profile did not statistically differ between the consistency types. The mean fractions for

the LM and SM treatments were 35.4 ± 5.5 and 29 ± 4.5, respectively. The enterococci was deposited into the soil in greater initial fractions than the *E.* coli at 52.8 ± 11.8 and 51.0 ± 11.6 for LM and SM treatments, respectively (Figure 4.9). The fractions of enterococci in the soil did not statistically differ between the consistencies. The greater fractions of both indicators were found in the LM treatments rather than the SM treatments immediately after the first rainfall event. There were no statistical differences between the fractions of *E. coli* and enterococci across the treatments after the first rainfall event.

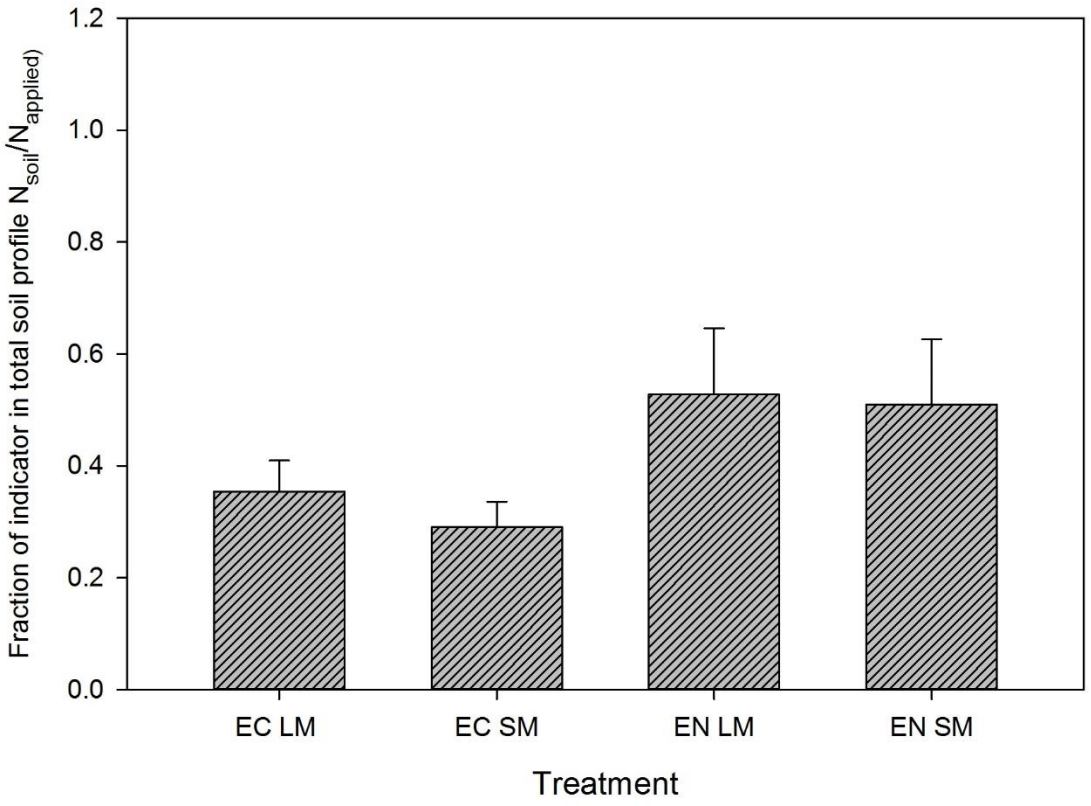

Figure 4.9. Fraction of *E. coli* (EC) and enterococci (EN) in the total soil profile in liquid (LM) and solid (SM) manure treatments immediately after the initial rainfall event. Error bars show the standard error of the mean.

3.5.2. Fractions by soil depth range after initial rainfall event

The fraction of each indicator by depth range relative to the fraction applied can be seen in Figure 4.10. The fraction of *E. coli* did not statistically differ by depth range in either the LM or SM treatments. The largest fraction of *E. coli* found was in the LM treatments at the 2 – 5 cm depth range. There were no consistent trends of a greater fraction of the indicators in any soil depth range when compared across consistencies. After the initial rainfall event, there were no statistically significant differences in the fraction of *E. coli* within any soil depth range for either manure consistency.

The fractions of enterococci were generally larger in each soil depth range than those of *E. coli*. Similar to *E. coli*, there were no statistically significant differences in the fraction of enterococci within any soil depth range for either manure consistency

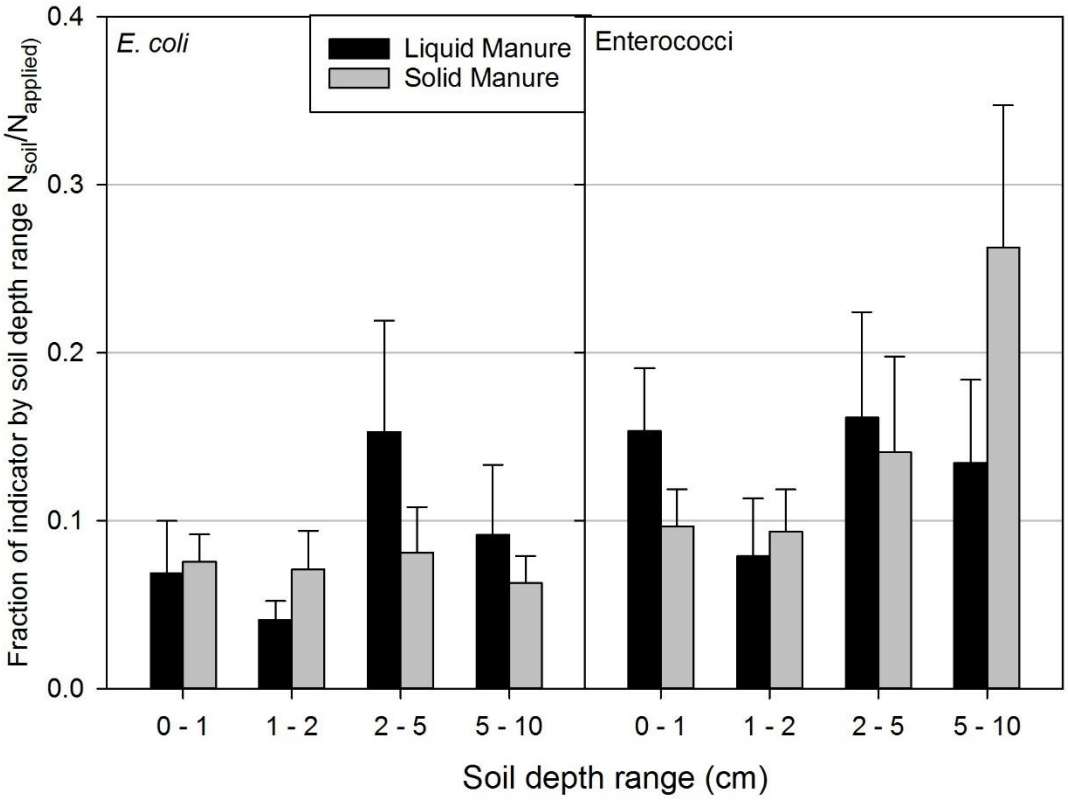

Figure 4.10. Indicator fraction relative to number applied by soil depth range. Error bars show the standard error.

3.5.3 Fractions of indicator in soil 1- week after initial rainfall

In all instances, there were greater fractions (N_{soil}/N_0) of indicator bacteria in the soil following a rainfall event than preceding it. The extent of this increase was greater for *E. coli* than for enterococci. The *E. coli* was found in higher fractions after rainfalls in the SM treatments than in the LM treatments for the same weathering durations (Figure 4.11). The enterococci fraction did not show a stable trend of increases after each rainfall.

The mean fractions of *E. coli* in the total soil profile one week after the initial rainfall event were 125.1 ± 2.7 and 39 ± 1.7 for the LM and SM treatments, respectively, signifying a major growth event for *E. coli* in the LM treatments. The fractions of *E. coli* in the soil that received the LM treatments were significantly greater than those in the SM treatments one week after the initial rainfall. Conversely, the fractions of enterococci sharply declined in all treatments over the first seven days. The mean fractions in the entire soil volume for the LM and SM treatments were 2.7 ± 2.1 and 1.7 ± 0.9, respectively. The fractions of enterococci within the soil did not statistically differ between consistencies one week after the initial rainfall event.

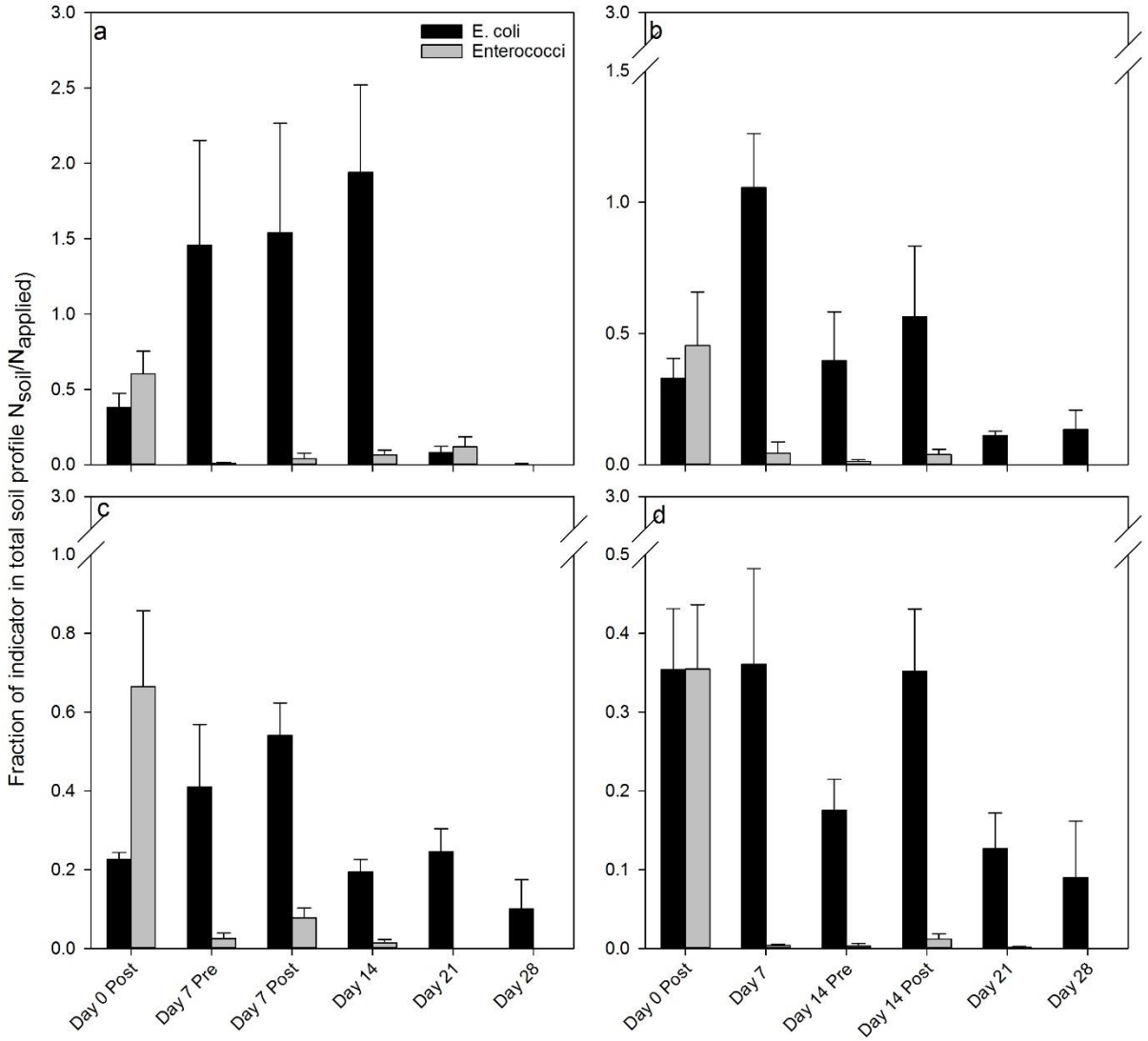

Figure 4.11. Survival dynamics of both indicators within the total soil profile throughout the study. Panels *a, b, c,* and *d* are LM 1 – week, SM 1 – week, LM 2 – week, and SM 2 – week treatments, respectively. Error bars show the standard errors.

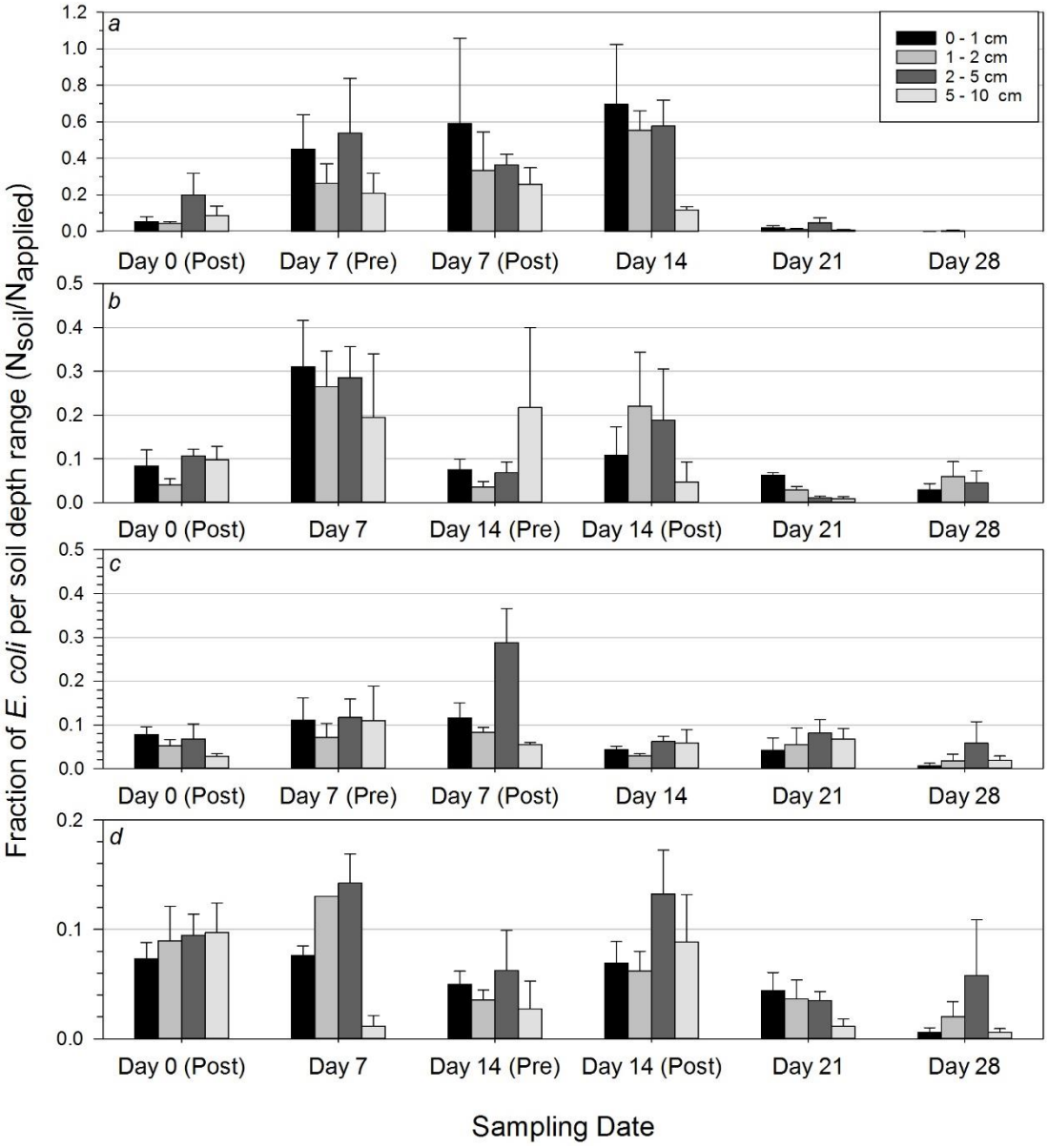

Figure 4.12. The fraction of *E. coli* by weathering and consistency treatments for different soil depth ranges over the entire study. Panels *a*, *b*, *c*, and *d* are LM 1 – week , SM 1 – week, LM 2 – week, and SM 2 – week treatments, respectively. Error bars display the standard error of the mean.

The *E. coli* fraction significantly differed with consistency in the top 0 – 1 cm and 1 – 2 cm of soil between rainfall events with a higher fraction detected in the LM treatments than the SM treatments one week after the initial rainfall event. The lower two depth ranges did not statically differ with consistency. The fractions of *E. coli* and enterococci by soil depth ranges are presented in Figures 4.12 and 4.13, respectively. The enterococci fraction did not statistically differ with consistency in any depth range one week after the initial rainfall event.

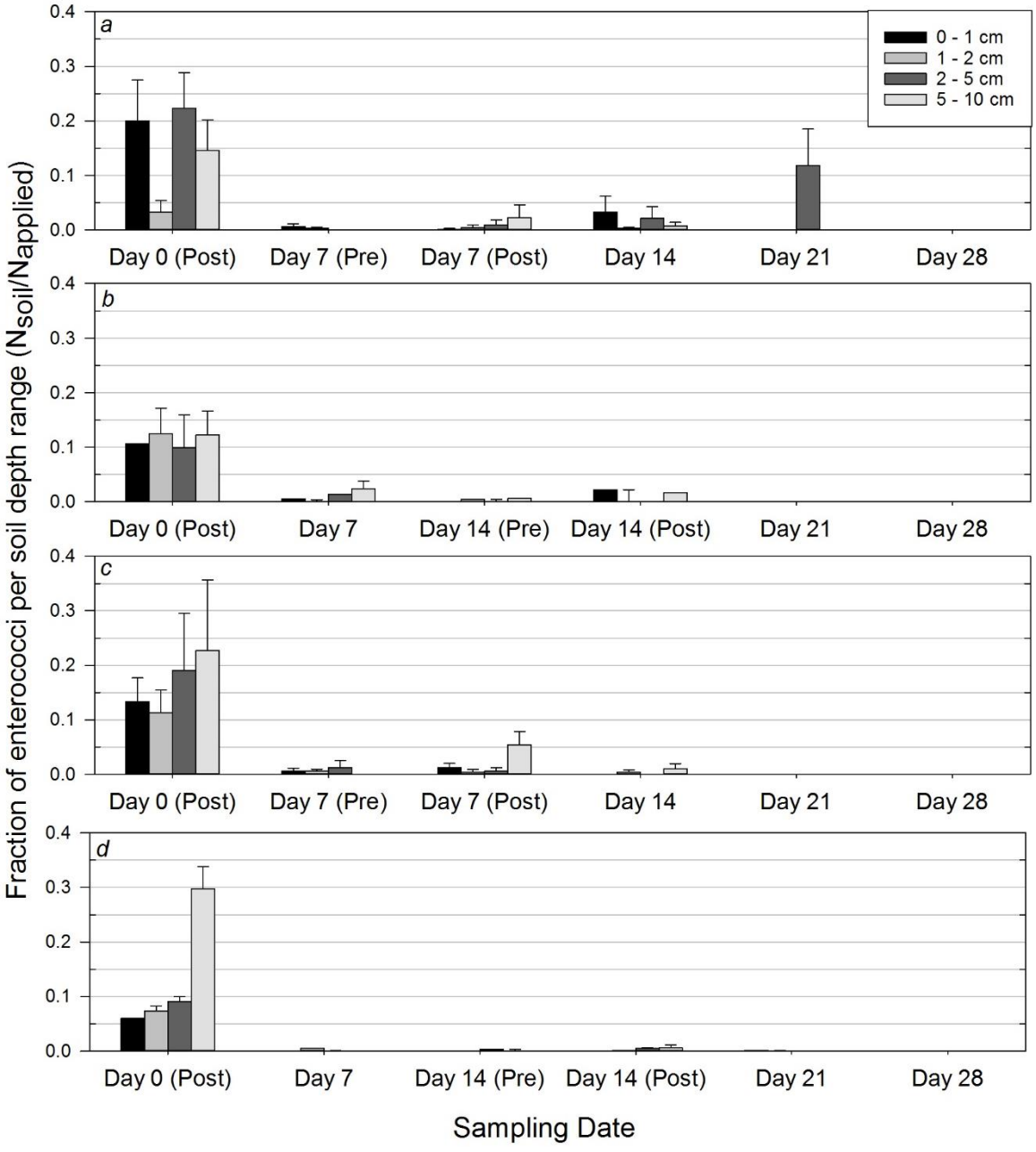

Figure 4.13. Fraction of enterococci by treatment and soil depth range over the entire study. Panels *a, b, c,* and *d* are LM 1 – week , SM 1 – week, LM 2 – week, and SM 2 – week treatments, respectively. Error bars show the standard error of the mean.

3.5.4 Fractions of indicators in soil prior to the second rainfall event

The pre-rainfall fractions of *E. coli* in the total soil profile between the 1- and 2-week rainfalls were significantly greater in the LM treatments than in the SM treatments (Table 4.13). There was a significant effect of weathering with the lower mean fraction found within the soil before the 2-week rainfall event than within the soil in the 1 – week weathering treatment. The fractions of enterococci did not statistically differ by the level of weathering or by the manure consistency type between the 1- and 2-week pre-rainfall soil depths.

Table 4.13. Results from the two-factor ANOVA on the effects of weathering and consistency the fraction of *E. coli* and enterococci in the soil prior to the second rainfall event. Statistical comparisons were made with the fractions of *E. coli* and enterococci within the soil between treatments.

Effect	*E. coli*	Enterococci
Consistency	*	NS
Weathering	**	NS
Consistency × Weathering	NS	NS

*, ** significant at the 0.05 and 0.01 probability levels, respectively.
NS = nonsignificant at the 0.05 probability level.

3.6. Indicator survival in solid manure

The relative concentrations of *E. coli* and enterococci in the solid manure after the first rainfall did not statistically differ between the SM 1 and SM 2 treatments. In the week following the initial rainfall, both treatments increased in concentrations of *E. coli* and declined in concentrations of enterococci (Figure 4.14). The extent of the growth of *E. coli* differed between solid manure treatments with SM 1 and SM 2 experiencing a near 3-fold and 2-fold increase, respectively, relative to post-rainfall concentrations after

the initial event. Conversely, enterococci experienced a near half and 6-fold reduction in each respective treatment. Statistical comparison of SM 1 and SM 2 could not be performed due to low number of replicates in SM 2 treatment 1-week pre-rainfall manure samples

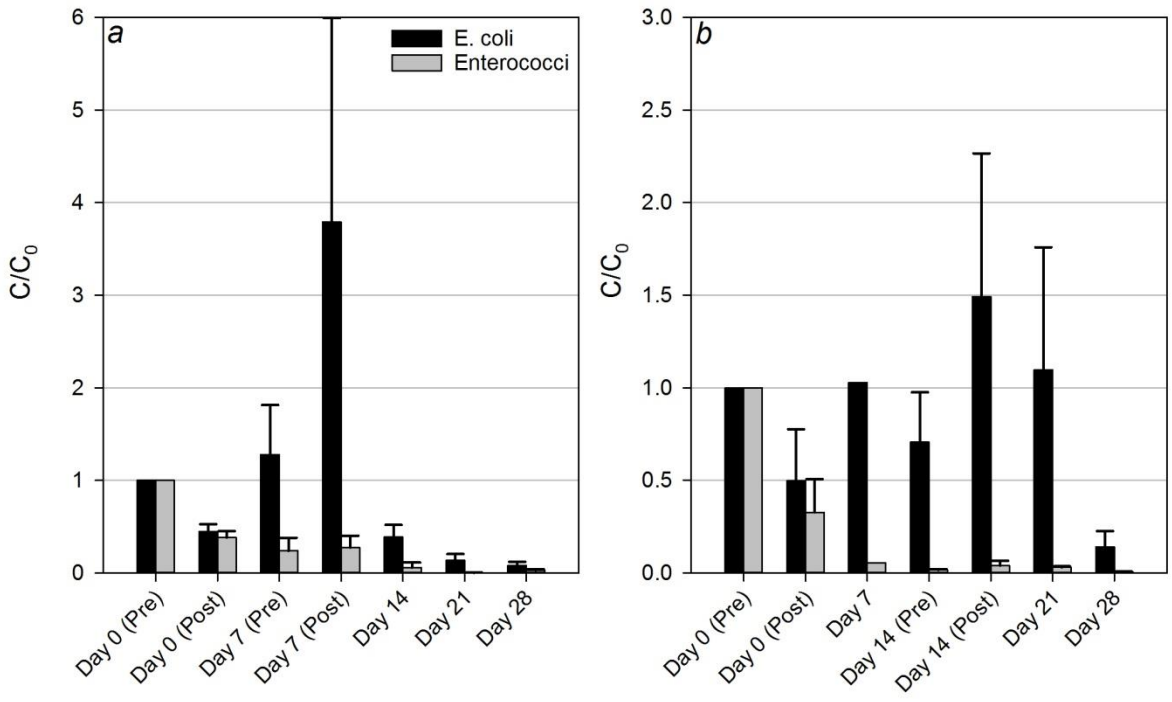

Figure 4.14. Relative concentrations (C/C_0) of *E. coli* and enterococci within solid manure atop boxes of the 1-week (panel *a*) and 2-week (panel *b*) weathering treatments. Error bars show the standard error.

4.5 Discussion

In more cases than not, the gravimetric soil water content was higher in boxes that received solid manure treatments (Fig 4.1). It is likely that the solid manure atop these

boxes aided in the retention of water by slowing atmospheric interactions with the soil whereas the boxes treated with liquid manure were more exposed to the air. The solid manure atop boxes in the SM 1 and SM 2 treatments was highly saturated after each rainfall event with gradual declines in water contents with time since the last rainfall event (Fig. 4.1). Following a rainfall event, the manure can release adsorbed water into the underlying soil thus increasing the soil water contents for some time after a precipitation event (Srinivisan et al., 2007). In the LM treatments, this wetting mechanism probably did not occur as there were only small and dispersed patches of manure solids left on the surfaces of these boxes. The organic matter within the soil would have aided infiltration and would help retain water in soils of both manure consistency treatments (Jamieson et al., 2002). There was much less organic carbon and nitrogen as well as other nutrients in the liquid manure relative to the solid manure so the water holding capacity of the SM treatments should be higher (Table 4.2). The carbon content of manure can be correlated with its dry matter content hence liquid manures contain smaller amounts of carbon per unit fresh weight than do solid manures (Unc and Goss, 2004).

Similar trends of increased soil water content following manure application have been observed by other authors. Srinivasan et al. (2007) applied fresh dairy manure to some field plots while other plots were left untreated. After applying simulated rainfall once a day for three consecutive days, such that 30 minutes of runoff was achieved, the authors reported consistently higher water content levels in the manured soils than in the un-manured soils prior to the second and third rainfalls. They found that the final water content levels were significantly greater in the manured soils than in the un-manured

soils. The flow of the retained water from the solid manure was attributed to be the major factor. The manure solids at the top of the soil also likely reduced sunlight and air interactions with the soil both of which act to reduce water content levels.

In the first rainfall event, the greatest proportion of water was eluted from boxes of both consistency treatments as runoff (Figure 4.6). Additionally, the amount of water leaving the boxes as infiltration and runoff did not vary greatly by consistency although slightly more rainfall was removed as runoff in the solid manure treatments. This behavior could be attributable to the clogging of soil pores with manure particulates as the solid manure broke down under the rainfall action. The effects of this clogging might be compounded in later weeks judging by the fact that SM boxes consistently had higher volumes of runoff than the LM boxes. Because of slightly lower gravimetric soil water contents for the LM treatments, more water might be absorbed into the soil upon re-wetting which would bolster infiltration rates until the soil approached saturation. The SM treatments would not be expected to absorb as much water and would probably reach saturation more rapidly resulting in greater runoff. In the 1- and 2-week rainfall events, some combination of differences in the soil water content and the relative degree of clogging due to manure solids likely resulted in a significantly greater amount of water being removed with infiltration (Table 4.4). Cardoso et al. (2012) applied liquid manure to a vegetated loamy soil, and reported that the majority of simulated rainfall was lost to infiltration for their dry plots whereas in water-saturated plots, runoff was the dominant mode of water transport from the plot. They concluded that infiltration rates in dry treatments exceeded rainfall rates until the soil saturated and infiltration declined resulting in runoff becoming a greater fraction of the water removed. In this study, the

liquid manure treatments absorbed and retained slightly less rain water than those treated with solid manure in the initial rainfall event, but this was reversed in both subsequent rainfall events (Fig. 4.3). The greater retention of water likely occurred for the solid manure treatments due to the presence of saturated manure on the surface after rainfall. In later weeks, the solid manure atop the soil for the SM treatments helped retain water whereas for the LM treatments the water retention within manure was much less because of the majority of the manure being washed away in the first rainfall event. For this reason, the LM treatments were able to retain more rain water because of the relatively lower antecedent water contents. The amount of water retained in the soil after the 1- and 2-week rainfalls was significantly greater in the latter event (Table 4.4). This reduction in soil water contents was due to a greater degree of soil desiccation with time since the initial rainfall event (Fig 4.3).

During the first rainfall, the delay in time before the start of runoff and infiltration was similar between both treatments; however, both transport modes took longer to initiate for the solid manure treatments (Table 4.6). Because antecedent soil water content conditions were found to be similar among all treatments prior to manure application and rainfall, it follows that the manure consistency controlled the initiation of runoff and infiltration. It is felt that the LM treatment, consisting of approximately 95% water, readily entered the soil upon application. The solids within the liquid manure likely infiltrated the soil or remained on the surface depending on the size of the solid particles within the liquid manure. Engstrom et al., (2015) reported that depth-dependent infiltration of colloids increases with colloid size whereby larger colloids undergo straining at the surface and smaller colloids are able to infiltrate to greater depths. Any

debris on the surface as well as those particles that have infiltrated into the soil would act to clog soil pores. In the SM treatments, the manure is hydrated, but not to saturation which means it acts as a porous media sponge for applied rainwater until its water content approaches saturation. This effect would be significantly compounded in later weeks as weathering of manure progresses (Table 4.7). Also, the higher degree of solids in the solid manure treatment are not available initially to clog soil pores, rather these are gradually released with time as the manure is sloughed by rainwater. Jaffrezic et al. (2011) reported that rainfall durations needed to generate the desired amount of runoff were twice as long for solid cattle manure treatments in comparison to swine slurry treatments. The authors attributed these differences to the heterogenous mixture of feces and bedding within the solid manure structure and stated runoff is greatly stimulated when the manure is in liquid form rather than solid. The results of Jaffrezic and the present study may have been explained by observations of Neave and Abrahams (2002) who reported that under rainfall, surface litter could lead to dams, blocking, and ponding of overland flow. It follows then that surface runoff took longer to initiate in the solid treatments due to a combination of absorption of applied water and/or the blockage or ponding of water that worked against overland flow. The delay to the initiation of infiltration was likely greater for the solid manure treatments due to the larger proportion of water within the liquid treatments relative to the solid treatments.

Weathering significantly delayed the initiation of both runoff and infiltration in the 1- and 2-week rainfall events (Table 4.7). During these events, the initial soil water contents were much lower than in the first week and as expected decreased with time after the initial rainfall event given that there was no rewetting of the soil between the

rainfall events (Fig 4.1) The delay in runoff initiation under dry conditions relative to wet conditions was observed by Cardoso et al. (2012). These researchers applied swine slurry to vegetated soil plots of high and low antecedent soil water contents and then simulated rainfall at the rate of 8 cm hr^{-1}. In their study, under wet conditions runoff occurred within minutes of rainfall application; however, in low antecedent water content treatments, the runoff started substantially later. The same result was found by Kim et al. (2014) who reported much greater runoff delays in field plots with low antecedent soil water contents relative to high soil water content plots. Additionally, the weathering of manure at the surface results in progressively longer delays of the initiation of runoff due primarily to the process of rehydration of manure solids (Srinivasan et al., 2007). Keeton et al. (1970) reported that dry manure could absorb a rainfall equivalent to half of the depth of the manure. This water absorption would be expected to have a large effect for the SM treatments and a smaller effect for the LM treatments.

Runoff and infiltration delays were significantly greater for the LM treatments than for the SM treatments in later weeks of the study (Table 4.7). These delays are thought to be due to the lower antecedent soil water content for the LM treatments compared to the slightly higher soil water contents for the SM treatments. It is also possible that by the second rainfall event the effects of the high manure solids content in the SM treatments resulted in more clogged surface pores than the LM treatments. Surface sealing likely occurred to some extent after the first rainfall event which would increase the time until initiation of infiltration in later events. However, as runoff initiation time was always longer than infiltration in later weeks, the effects of surface

sealing was felt to not likely be great. Any sealing was likely minimized due to the dense grass coverage and/or manure remnants on the soil surface.

The removal curves of indicators in the runoff displayed shapes similar to what has been seen in other studies (Fig. 4.6) which is defined by a log-linear increase in the initial rate of release and the removal from manure which persists for a short time before there is a drastic decrease of the removal rate (Guber et al., 2006; Cardoso et al. 2013; Bradford and Schijven, 2002; Kim et al., 2016; Ferguson et al., 2007). During this time, the majority of released bacteria were removed from the box. In the present study, the high rate of release was found for the 0 – 1 cm range of rainfall depth after the initiation of runoff (Fig. 4.6). Following this early release, the rates of removal began to decline and became more or less steady for the 2 to 6 cm rainfall depths. Interestingly, this observation was consistently accurate for both manure consistency types. Such dynamics is similar to the one observed by Blaustein et al. (2016) who applied solid manure to vegetated soil boxes under simulated rainfall and measured *E. coli*, enterococci, and total coliform concentrations. The two-stage dynamics is likely due to the quick release of bacteria that are in manure liquid phase which readily exchanges with incoming rainwater followed by the gradual release of bacteria from the manure solids as rainfall continues to slough the manure. In later weeks, the initial stage of the two-stage dynamics becomes less pronounced likely due to die-off, weathering, and large reductions of the initial liquid component of the manure as the percentage water content drops significantly (Figure 4.1).

Both *E. coli* and enterococci were removed in large percentages from the manure treatments with runoff relative to the number of indicator bacteria applied (Table 4.8).

The percentage of bacteria removed with runoff in the initial rainfall event were relatively high (16.9% and 22.7% for *E. coli* and 30.5%, and 27.1% for enterococci in the LM and SM treatments, respectively) compared to what some other studies have reported (Durso et al. 2011; Ferguson et al. 2007; Thurston-Enriquez et al., 2005; Brooks et al., 2009). These other studies often only simulate rainfall for 30-minutes total whereas in the present study rainfall was applied to achieve 1 hour of runoff which meant total rain time that exceeds the 1-hour time period due to the delay before the initiation of runoff. Guber et al. (2007) found that after 40 minutes of rainfall, the release of enterococci and *E. coli* from manure under simulated rainfall occurred with zero-order kinetics. The authors believed that 30-min rainfall simulations may not be sufficient in manure-borne bacteria release studies since manure lumps are continuously breaking apart under rainfall thus providing additional opportunities for the release of these organisms via being newly exposed to water surfaces. Even so, the majority of both organisms were removed with runoff within the first 30 minutes of rainfall in the present study.

A number of other studies have also reported large percentages (N/N_0) of released bacteria. In a similar study to the present one, Jaffrezic et al. (2012) simulated rainfall onto swine slurry and solid cow manure at a rainfall rate of 62 ± 2 and 69 ± 5 mm h^{-1} for each treatment, respectively. In their first replication for solid cow manure, these authors recovered 28.3% of *E. coli* which is very similar to what was observed in the present study. However, between two other replications performed by these authors, the average *E. coli* recovery dropped to 13%. For enterococci, these authors recovered an average of 4.1% which is substantially different than the present study. Jaffrezic et al. (2011) recovered an average of 3.0% and 2.4% of *E. coli* and enterococci from swine slurry

applied to their plots which further contradicts the present study in that percentage release was greater from liquid manure treatments than from solid manure treatments in the initial week. Two notable differences between this present study and the current study are the initial concentrations of both indicators in the manure and that these pervious authors did not measure bacteria removed with infiltration. The average concentrations of indicators in their study were $3 \cdot 10^4$ CFU g^{-1} *E. coli* and $3.5 \cdot 10^4$ CFU g^{-1} enterococci in the slurry and $5.1 \cdot 10^4$ CFU g^{-1} *E. coli* and $8.5 \cdot 10^4$ CFU g^{-1} enterococci which are all lower than the analogous values in this study (Table 4.2).

In another similar study, Blaustein et al. (2016) simulated rainfall on vegetated soil boxes amended with solid dairy manure of a near equal composition as the solid manure in the present study. In their 6 cm hr^{-1} treatment, the authors recovered on average approximately 58.1% of applied *E.* coli and 62.1% of applied enterococci, respectively, within runoff which is approximately twice what was recovered in the present study. In their previous study, the concentrations of enterococci in the applied manure were about 35 times greater than in the present study, but the concentrations of *E. coli* were similar between the two studies.

Guber et al. (2013) applied bovine slurry (~8% solids) to grass covered soil boxes and then simulated rainfall at 3.36 cm h^{-1} until 1-hour of runoff was collected. The average percent they recovered relative to what was applied was 41.9% and 21.8% for *E. coli* and enterococci, respectively, which was similar to what was recovered in the LM treatments in the present study. Additionally, Stout et al. (2005) reported recovery of fecal coliforms from solid dairy manure amended soil boxes under a rainfall intensity of 2.66 cm hr^{-1} as being in the range of 30.3 to 38.6%.

In the initial rainfall event, the enterococci were removed with runoff in higher proportions than *E. coli* in both the LM and SM treatments although not by an appreciable amount (Table 4.8). The same result was observed by Blaustein et al. (2016) who reported a 3% greater removal of enterococci with runoff than that of *E. coli* from solid dairy manure applied to vegetated soil boxes under simulated rainfall of the same intensity as the present study (58.9% and 62.1%, for EC and EN, respectively). Kim et al. (2016) consistently recovered greater percentages of applied enterococci than *E.* coli across four seasons and with each of the three manure types in their field experiments. Conversely, other authors have documented a greater removal of *E. coli* than enterococci from liquid manure (Guber et al., 2007; Guber et al., 2013; Jaffrezic et al., 2011; Thurston-Enriquez et al., 2005) and solid manures (Jaffrezic et al., 2011; Hodgson et al., 2009; Dao et al. 2008) in studies that paired these two indicators. The microbial species-specific release might be due to differences in the preferential microhabitats within the manure that would be accentuated in a comparison of liquid and solid manures. Different species will compartmentalize to different microhabitats within porous structures and their efficiency of becoming dislodged is affected by their specific physical and chemical properties (Blaustein et al., 2015; Lombard et al., 2011). Cell characteristics such as surface charge, size, hydrophobicity, and external structures such as flagella, fimbriae, and lipopolysaccharides (LPS) influence their movement in solution as well as their ability to attach/detach from surfaces (Blaustein et al., 2015; Critzer and Doyle, 2010; Foppen and Schijven, 2006; and Pachepsky et al., 2008).

The fractions of both indicators removed with runoff decreased with each subsequent rainfall event (Figure 4.8). The recurrence of rainfall was reported to decrease

the amounts of fecal bacteria released with each subsequent rainfall event (Kress and Gifford, 1984; Drapcho, 2003; Brooks et al., 2009; Sistani et al., 2009). Since *E. coli* grew in soil and manure between the initial rainfall event and the 1-week rainfall, it is likely that microbial die-off did not play a major role in the decreased fraction of *E. coli* removed in the 1-week rainfall. Rather, in the SM treatments manure weathering was likely the contributing factor. Numerous authors have documented reduced microbial release when manure is allowed to weather (Ferguson et al., 2007; Thurston-Enriquez et al., 2005; Sistani et al., 2010). Hodgson et al., (2009) attributes this reduction to the likelihood of bacteria becoming encapsulated within fecal matrixes and thus are less likely to be transported during subsequent precipitation events. Conditions such as high temperatures or temperature fluctuations, solar radiation, or declining water content within the manure as it weathers puts stress on cells which increases die-off rates of bacteria (NRCS/USDA, 2012; Van Kessel et al., 2007). The removed fraction of Enterococci was substantially reduced in subsequent rainfall events in the fraction removed with runoff probably due to some overlap of die-off and weathering. Thus, the differences between the initial fraction removed with runoff and later fractions are greater for enterococci than those of *E. coli* which experienced some degree of growth (Fig 4.11 and Fig 4.14 for soil and manure, respectively).

The differences in indicator fractions removed over time are in stark contrast to the studies performed by Kim et al. (2016) in which they observed much greater fractions of enterococci removed with subsequent rainfall events than for *E. coli*. The differences between Kim et al. (2016) and the present study serve to highlight the inherent variability of indicator release not only by manure type and/or weathering, but by differences within

the same species of bacteria. For instance, the strain-dependent attachment of *E. coli* to soil particles has been documented which has implications for the movement of bacteria within runoff (Pachepsky et al. 2008). It was additionally reported that the transport behavior differed between 12 different strains of *E. coli* from 6 different animal sources (Bolster et al., 2009) and that animal diet can impact the transport of bacteria released from manure (Durso et al., 2011).

In the LM treatments, the removal of the majority of manure solids during the first precipitation event likely led to reductions in the fractions removed in the later rainfall events. While always lower than the initial week, the fractions removed in later weeks in the LM treatments may have remained high due to interactions between soil bacteria, rainfall action on these bacteria, and the overland water movement as well as any debris of manure left at the surface, albeit in minute amounts. Thurston-Enriquez et al. (2005) applied liquid swine manure to experimental field plots and performed three rainfall simulations on consecutive days. The authors measured *E. coli*, enterococci, *clostridium*, and coliphage in runoff and found the majority of the bacteria release for the three events occurred during the initial rainfall event. The two latter rainfall events combined only contributed 44%, 32%, 10%, and 3% of the total *E. coli*, enterococci, *clostridium,* and coliphage recovered, respectively, showing that even at such a short temporal scale that the reductions in the bacteria removal from the liquid manure applications may be substantial. The authors also applied fresh cattle manure to plots that underwent simulated rainfall and reported increases in the populations of *E. coli*, enterococci, and coliphage which was subsequently removed in later rainfall events indicating the growth of these organisms after the first rainfall event. There was no

population growth reported for the swine slurry treatments likely due to the heavily degraded or non-existent nature of the fecal matrix in liquid manures.

By the 2-week rainfall event, both indicator organisms were detected in much lower fractions in the manure and soil relative to that after the first rainfall event (Fig 4.11 and Fig. 4.14). A higher level of weathering relative to the 1-week rainfall as well as the further progression of die-off were the likely reasons for the lowest fractions removed with runoff in the 2-week rainfall event.

The removal of bacteria with infiltration demonstrated a log-linear increase in the fraction removed primarily in the 0 – 2 cm rain depths after the initiation of infiltration, but before the infiltration began to decrease (Fig 4.8); however, as Fig. 4.7 demonstrates, the rate of removal with infiltration is actually the greatest towards the end of the removal event.

Both indicators were removed with infiltration in much smaller fractions than with runoff for almost all rainfall events and for both manure consistencies. The one exception is with the fraction of *E. coli* removed in the 2-week SM treatment in which runoff accounted for 1.3% and infiltration accounted for 1.6%. Runoff has been described to be the largest and quickest transport route for microorganisms eluted from manure during rainfall events (Muirhead et al., 2009; Tyrrel and Quinton, 2003; Jamieson et al., 2004). Bacteria that infiltrate into the soil are subjected to adsorptive/desorptive forces as well as straining by micropores which slow their collective movement (Bradford et al., 2013). Infiltration rates can be high in coarser soils, when dense vegetation is present, or when preferential flow pathways are present (Engstrom et al., 2015). All three items

apply to the present study as the soil was coarse, vegetation was relatively dense, and any preferential flow pathways may have existed via root lines.

The rate at which both indicators were removed with infiltration was initially slow, but began to increase substantially at a rainfall depth of 4 – 6 cm after the initiation of infiltration in the initial rainfall event (Fig. 4.7). This occurrence was in stark contrast to what was occurring in the runoff. The percentage removed in runoff increased greatly for the 0 – 1 cm rainfall depths after the initiation of runoff, but the rate of removal slowed after this initial increase. It is interesting that the large increases in the percentages of both indicators removed during the 4 – 6 cm rainfall depths after infiltration initiation was not associated with an increased rate of water removal as infiltration. Both the runoff and infiltration rates stabilized within 10 minutes after the start of either eluent (Fig. 4.5). The reasons for the large increase in the fraction of indicators removed with infiltration later in the experiment may have to do with the availability of exchange sites, the flow velocity, the spatial variability in the soil structure, or the dispersity of the indicators as they infiltrate the soil. Guber et al. (2005) documented the decreased attachment of *E. coli* in soils with increases in the manure concentration and attributed this inverse relationship to some combination of the following: (i) the modification of the soil mineral surfaces by soluble constituents, (ii) the competition of dissolved organic matter and bacteria for adsorption sites, and (iii) the modification of bacteria surfaces by dissolved organic matter. In the present study, the rate at which bacteria were removed with infiltration in the LM treatments was greater than the rate of removal in the SM treatments during the initial rainfall event (Fig 4.7). The *E. coli*, enterococci, and dissolved organic matter are postulated to have all quickly

infiltrated into the soil upon application of the liquid manure. Zandsalimi et al. (2009) found that manures containing greater concentrations of dissolved organic matter (DOM) resulted in greater transport of gram-negative bacteria relative to those with lower DOM. Bradford et al. (2013) explains that when DOM is present it may sorb to bacterial cells which alters their electrophoretic mobility and hydrophobicity leaving them with a net negative charge which tends to diminish attachment capabilities onto negatively charged soil surfaces. The SM treatment contained more organic matter (Table 4.2), but this organic matter was not as readily available as it was in the LM treatment. In the solid manure treatments, the manure is continuously being broken apart by rain action so the release of indicators and organic materials is slower. As these indicators enter the soil, they are able to attach to soil and may be adsorbed to surfaces especially in the beginning of the rainfall event when infiltration rates were relatively low. There may be a point in time, namely the 3 – 4 cm rainfall depth after the initiation of infiltration, that most available exchange sites are occupied either by previously infiltrated bacteria or organic matter such that most infiltrating bacteria in this rainfall depth range are unable to be retained in soil and are removed with infiltration. Bradford et al. (2013) describes this process as the occlusion of the collector surface which is described by interaction of incoming particles with primarily unfavorable attachment sites due the chemically favorable sites already being filled which results in failure of retention. The lower initial infiltration rates at the beginning were more favorable to bacterial attachment in the soil than the faster rates towards the end of the rainfall event due to increased hydrodynamic shearing stress caused by high rates of water removal with infiltration (Fig. 4.5). The highly-concentrated liquid portions of the LM and SM treatments likely infiltrated into

the soil soon after the application of rainfall, or immediately in the case of the LM. It may be that this concentrated "pulse" of bacteria was greatly slowed upon entering the soil and it took at least 3 – 4 cm of rainfall depth after infiltration initiation before it began to leave the boxes.

Additionally, the appearance of certain subpopulations of bacteria released from the same source has been shown to vary dependent on the cellular characteristics and capabilities of those sub-populations (Bengtsson and Lindqvist, 1995; Simoni et al., 1998; Huysman and Verstraete, 1993). It is possible then that certain *E. coli* and enterococci sub-populations moved throughout the soil much quicker than the larger population which began to exit the box around 3 – 4 cm rainfall depth after infiltration initiation.

It is interesting to note that the fraction of *E. coli* removed in each rainfall event was not much different over the three week study, whereas for enterococci, there were clear differences between the fractions removed in the initial event and those similar amounts removed in the 1- and 2- week rainfalls events. As a result, it was determined that weathering was not a significant factor for the *E. coli* removed with infiltration (Table 4.12). The stability of the *E. coli* fraction removed in infiltration in all the rainfall events was the result of a large growth of this bacteria within the soil and manure following the initial rainfall event (Fig 4.11 and 4.14). Surprisingly, the enterococci were not significantly affected by the level of weathering despite the dramatic declines in the soil and manure populations. The reductions in the soil and manure populations kept the runoff and infiltration enterococci concentrations relatively low which reduced the

variability of the enterococci removal in the subsequent rainfall events as fractions removed hovered around 0%.

The consistency was not a significant factor on the indicator fractions removed during the initial event where the fractions removed were similar between the LM and SM treatments. During the 1- and 2-week events, the consistency had significant effects on the infiltration removal of both indicator as the fractions from the LM treatments were always greater than the fractions from the SM treatments (Table 4.11).

The substantial growth of *E. coli* in later weeks may have compounded the effects of there being an insufficient number of attachment sites such that the newly infiltrated *E. coli* were unable to attach. There were also simply more *E. coli* available for detachment and subsequent removal in the infiltration because of the increased growth. The increased growth of *E. coli* may have also resulted in the diffusion of cells which in soil has been found to be a function of the density of bacteria on a solid surface as well as in the liquid phase such that when adsorbed densities are great, desorption rates are higher than adsorption rates (Bengtsson and Lindqvist, 1995). During the later weeks of the experiment, the starvation of *E. coli* cells may have resulted in greater numbers removed with infiltration. It has been shown that the starvation of *E. coli* results in poor retention and greater transport which is due to some combination of reduced bacterial size, changes in zeta-potential, and the relative lack of extracellular polymeric substances on starved cells compared to non-starved cells (Han et al., 2013; Hanznedaroglu et al., 2008). The enterococci removal with infiltration decreased with increasing time following the initial rainfall event (Table 4.11) which was most likely due to the substantial and immediate population declines in the soil and manure (Figure 4.11 and 4.14). It cannot be ruled out

that the percentage of *E. coli* removed within infiltration in the latter two rainfalls may have remained high due to the creation of preferential flow paths as the soil desiccated and shrunk somewhat in the soil boxes. Soil core locations were plugged tightly with wooden cylinders after sampling in the first week, but it is likely that some water traveled down the soil plug cavities during each subsequent rainfall. However, the increasing delays for infiltration initiation during the later weeks of the study (Table 4.6) indicates that water flow through the soil cavities likely was not a serious problem otherwise the infiltration would have started much sooner.

In their study using vegetated soil boxes amended with solid cow manure, Blaustein et al. (2016) reported the fractions of both *E. coli* and enterococci removed with infiltration accounting for 0.19% and 0.28% of the total number applied when a 6 cm hr^{-1} simulated rainfall event occurred. The authors reported the runoff fractions as 59.9% and 62.1% for *E. coli* and enterococci, respectively, which was more than 2 orders of magnitude greater removal in comparison to the infiltration removal. In the present study, the ratio of the runoff fractions to the infiltration fractions removed during the first rainfall was 2.3 and 2.5 for *E. coli* and 9.0 and 5.6 for enterococci in the LM and SM treatments, respectively. In the LM treatments for the 1- and 2-week rainfall events, this ratio for both *E. coli* and enterococci dropped below 1 indicating a shift to infiltration as the dominant mode of transport and removal of the indicator bacteria. The growth of *E. coli* within the soil after the initial rainfall event could explain the large percentages removed with infiltration during the later weeks of the study seeing as how the pre-1 and 2-week rainfall fractions of *E. coli* in both manure treatments were greater than fractions

in the soil immediately following the first rainfall event (Figure 4.11). The enterococci did not experience observable growth prior to the 1- and 2-week rainfalls events and the soil population only represented a small fraction of the amount applied. The resultant fractions removed with infiltration during the later weeks of the study were many times smaller than the initial fractions removed (Table 4.11). For both indicators, the populations within the manure solids on the surface also likely contributed bacteria to infiltration, but this surface placement did not appear to be a major factor since the fractions eluted from the LM treatments were greater than the SM treatments during the later weeks (Figure 4.8). Figure 4.14 shows that concentrations of both indicators in manure were still high in SM treatments during the later stages of the study; however, these bacteria were not easily removed during rainfall likely due to the substantial weathering of the manure which reduced the bacteria's interactions with rain water. In the SM treatments, the fraction of indicators removed with runoff remained greater than the fraction removed with infiltration by up to 4 times between indicators, except with *E. coli* in the 2-week rainfall in which infiltration accounted for 1.6% and runoff accounted for 1.3% removed (Table 4.8). These differences would indicate that the majority of released bacteria from the weathered manure were carried over the soil surface rather than through the soil profile. After the first rainfall, it is possible that manure solids from the SM treatment were removed from the manure under rainfall action and may have created a partial clogging of soil pores at the surface.

We understand the infiltration conditions in the present study are not directly applicable to field settings due to the relatively shallow depth of the soil boxes. Although the results of this study are useful for modeling the removal of fecal bacteria with

infiltration through a 10-cm thick soil profile. Depending on the soil texture, it can be expected that bacteria would continue to infiltrate downward through a soil profile. Roodsari et al., (2005) simulated rainfall atop a manure-amended grassy lysimeter plot with a sandy loam texture soil. In their study, they recorded very high infiltration rates and recovered fecal coliforms as deep as 60 cm below the soil surface. The bacteria they recovered in the 60 cm of soil only accounted for 11% of the total number applied and no bacteria was recovered in runoff and no manure was left atop the surface of their plots. Thus, 89% of bacteria likely infiltrated through the profile. Conversely, in their clay loam soil plots, Roodsari et al. (2005) recovered 90% of applied fecal coliforms within the top 10 cm. It appears then the removal of infiltrated bacteria is highly dependent on the soil texture. It would be interesting to reexamine the *E. coli* and enterococci removal in the present study had a soil with a higher clay content been used.

4.11. Soil dynamics

The majority of the manure-released bacteria were estimated to be within the soil after the initial rainfall event. The fraction in the soil was found to be greater than the combined infiltration and runoff fractions in every treatment for both organisms. Blaustein et al. (2016) also reported substantial percentages of *E. coli* and enterococci measured within soil following the solid manure application and simulated rainfall event, however, in their study they consistently found greater numbers of *E. coli* rather than enterococci within soil. In both Blaustein et al. (2016) and the present study, the manure was applied atop dense vegetative cover. Vegetation increases infiltration rates by

slowing the water movement and setting up preferential flow pathways (Cardoso et al., 2012). The fact that the number of bacteria retained within the soil was so much greater than the bacteria removed with infiltration shows that these two bacteria indicators were becoming adsorbed to soil surfaces and/or experienced straining in soil micropores. Nola et al. (2008) reported that enterococci were preferentially adsorbed to soil surfaces, and that *E. coli* and enterococci sorption appeared to be a competitive process when both bacteria were present. This competition coupled with the known physiological differences (i.e. cell charge, extracellular structure, cell size…etc.) may help explain the relative differences in the abundance of both indicators within the soil following rainfall.

The initial fractions of both indicators in the soil after rainfall (N_{soil}/N_0) did not significantly differ by manure consistency although the fractions were higher in the LM treatments than the SM treatments (Figure 4.9). These differences would be expected since the liquid manure readily infiltrates the soil upon application whereas the solid manure remains on the surface prior to rainfall. The release from solid manure is then dependent on rainfall action to deteriorate the manure structure and make the interior bacteria available for release. It is likely that more organic matter from the manure was infiltrated into the soil in the LM treatments than in the SM treatments. The presence of organic matter in the soil acts as increased surface area and, therefore, provides more sorption sites to which indicators may bind (Morita, 1997). Infiltrated organic matter would also provide nutrients and water content to infiltrated bacteria (Jamieson et al., 2002).

Fractions of both organisms did not significantly differ by depth range in either consistency immediately following the first rainfall and there were no clear trends

regarding fractions by depth ranges (Figure 4.10). However, the indicator concentrations displayed a trend of decreased concentration with increased soil depth. This trend becomes evident in Fig. 4.10 when considering the relative volumes of each soil layer. Although the top two soil ranges have a 1-cm height, these ranges still contain a large fraction of released bacteria relative to the 2 – 5 cm and 5- 10 cm depth ranges which are 3 and 5 times greater in volume, respectively.

There is a surprising lack of information on the depth-dependent concentrations of bacteria within soil after release from manure. Only one other author has performed a depth-dependent investigation of *E. coli* and enterococci in soil immediately following rainfall on solid manure amended soil boxes (Blaustein et al. 2016). They reported a clear trend of decreased concentration of both indicators with increased depth. This phenomenon has been observed more commonly in soil column experiments. Huysman and Verstraete (1993) observed that surface-inoculated bacteria decreased exponentially by depth noting a 10-fold decrease for every 1.0 – 6.0 cm of soil depth after rainfall. Roodsari et al. (2005) applied bovine manure to a lysimeter site and applied simulated rainfall to induce the release of fecal coliforms. These authors noted that fecal coliform concentrations in the top 1 – cm of soil were close to the maximum concentrations of fecal coliforms observed in runoff. They also noted consistent declines in concentration with soil depth. Possible explanations for the high concentrations of bacteria near the surface include bioclogging or cell aggregation and straining of cells by small pores (Engstrom et al., 2015). In addition, when manures are surface-applied there is oftentimes good contact between the manure and the top layer of soil. Manure solids that do not infiltrate the soil may remain on the surface due to straining which results in their

incorporation into measurements aimed to only assess soil concentrations. The depth-dependent infiltration of colloids has been reported to increase with increasing colloid size and both *E. coli* and enterococci have been reported to become associated with soil and manure colloids (Engstrom et al., 2015; Dao et al., 2008; Pachepsky et al., 2009).

The persistence of indicator bacteria in the top 1 – cm of soil was found to be greater than at other depths in Chapter 3 of this thesis. The higher concentrations in the upper layers may be related to the straining of organic matter which leads to decreased organic matter with increased soil depth. The microbial populations have been shown to decline sharply with soil depth within the vadose zone, along with the concentrations of oxygen, nitrogen, phosphorous, and organic matter (Engstrom et al., 2015). As previously mentioned, the organic matter may provide nutrients for bacteria growth and has water holding properties both of which can extend the persistence of fecal bacteria in soil (Jamieson et al., 2002). It should be noted that due to the relatively higher concentrations of nutrients in the soil depth ranges closer to the soil surface, an additional explanation for higher bacteria concentrations in this area may be due to the chemotactic movement of bacteria towards nutrients which has been documented for both *E. coli* and enterococci (Ford and Harvey, 2007; Johnson, 1994). In the same regard, since organic matter has a relatively high water holding capacity and is retained closer to the soil surface, the bacteria may migrate towards these local regions of relatively higher water content (Engstrom et al., 2015).

The survival and growth of indicators were generally better in the LM treatments than in the SM treatments (Fig. 4.11). As previously stated, more organic matter likely entered the soil in the LM treatments which provided a rich bed of nutrients and

attachment sites. Furthermore, the bacteria in terrestrial environments encounter a wide range of stresses upon leaving the host such as water content and temperature fluctuations and poor nutrient availability (Winfield and Groisman, 2003; Rodger and Haines, 2005). The stressed bacteria attach to sediments and organic particles to increase survival (Morita, 1997) which would explain the large bacteria growth in the LM treatments due to infiltrated organic matter.

In all treatments, there was a large growth of *E. coli* followed eventually by a major decline towards the end of the study (Figure 4.12). The initial growth of fecal indicators within soil after manure deposition has been well documented. Topp et al. (2003) reported large initial growth of *E. coli* in soil amended with swine manure slurry and an absence of this growth when the *E. coli* was inoculated into the soil without slurry. Growth in their study was found to be strain- and temperature-dependent with temperatures of 30 °C resulting in growth whereas bacteria die-off occurred immediately at the lower temperature treatment of 4 °C. Hodgson et al. (2016) observed the initial growth phases of both *E. coli* and enterococci within soils that received manure slurry, but only in the spring as the application in other seasons were shown to have immediate exponential decline. Similarly, Lau and Ingham (2001) observed a 1 – 2 Log CFU g^{-1} growth of *E. coli* and enterococci during the first week after bovine manure incorporation into a loamy sand soil. Berry and Miller (2005) observed growth of *E. coli* during the first 2 to 6 days in soil after bovine manure application. They observed higher growth when the manure concentration was increased and also observed that growth was dependent on water availability with low water contents causing die-off.

Fecal bacteria exist within the host in a phase of constant exponential growth (Thelin and Gilford, 1983). When released into extra-enteric environments, *E. coli* and enterococci, both facultative anaerobes, are exposed to an abundance of oxygen and switch to aerobic respiration which is the most productive metabolic mode (Partridge et al., 2006). The coupling of the exponential growth phase, a favorable change in metabolism, and abundant amounts of nutrients from manure results in large initial increases of these organisms in non-host environments. However, in the present study only *E. coli* displayed substantial population growth within the soil. The enterococci experienced rapid initial die-off within the first week after rainfall in all treatments. The only increase in fractions within the soil was after subsequent rainfalls due to newly infiltrated enterococci bacteria from weathered manure, but this growth increase was always substantially less than the growth displayed by *E. coli*. These same patterns of population growth was also observed for both indicator bacteria in the preceding study described in Chapter 3 of this thesis.

E. coli and enterococci are often paired in studies that research fecal bacteria survival in soils due to their abundance in feces and the inherent differences between their release and survival which should be considered when drafting water quality regulations or when forecasting microbial water quality. The comparative survival of the two indicators is obviously contradictory. A recent review by Byappanahalli et al. (2012) concluded that enterococci survival in the environment was superior to *E. coli* survival and, therefore, was a better indicator of fecal contamination. Similarly, Hodgson et al. (2016) found greater survival for enterococci than for *E. coli* in soils of both broadcasted and injected liquid manure treatments on farm land. The differences they observed

persisted through three seasons of study each with periodic observations that span 50+ days. A seemingly equivalent number of studies have documented better survival of *E. coli* than enterococci including the present study (Lau and Ingham, 2001; Sinton et al., 2007; Howell et al., 1996). The discrepancies between studies may be due to differences in the strains of either organism being studied. It has been shown that different strains of fecal bacteria can have significantly different survivability in soil environments (Topp et al., 2003). The explanations for the stark differences in indicator survival dynamics may also be attributable to differences in water content tolerance (Wang et al. 2004; Sinton et al. 2007; Byappanahalli et al., 2004), interaction with native soil biota (Byappanahalli et al., 2000), nutrient requirements (Ishii and Sadowsky; Byappanahalli et al., 2012), soil texture preference (Cools et al. 2001), or preferred temperature ranges (Howell et al. 1996; Park et al., 2016) between the organisms.

Interestingly, *E. coli* in soil was found in significantly greater fractions in the 0 – 1 cm and 1 – 2 cm depth ranges of the LM treatments than the SM treatments 1-week after initial rainfall whereas the other soil layers did not significantly differ in their *E. coli* contents. We expected better survival in the SM treatments due to the protection from atmospheric interaction in the way of blocking UV radiation and retaining more water. The growth in the LM treatments was so substantial that it was apparently not noticeably deterred by the presence of the above two stressors. Certainly, the dense vegetation at the surface would've provided protection from direct UV contact in all treatments. It seems logical that the bulk of the organic matter and nutrients applied in the liquid manure would infiltrate into the upper soil layers and that the lower layers would not receive as great a portion due to straining by pores or adhesive processes that slowed the downward

movement. This partial explanation may help explain why the lower soil layers are not significantly different between manure consistencies one week after rainfall. The enterococci did not significantly differ by soil layer at this point of the study largely due to its overwhelming die-off which reduced the variability of fractions at each soil depth bringing them all closer to zero (Fig. 4.12).

In the 1- and 2-week rainfall events, the initial fractions of *E. coli* in the soil were significantly greater in the LM treatments than in the SM treatments and the *E. coli* fractions were also significantly greater in the 1-week pre-rainfall soils than the 2-week pre-rainfall soils (Table 4.13). The differences between consistencies are likely due to the major bacteria growth events that happened for both manure treatments, but occurred to a greater extent for the LM treatments. The differences between the 1- and 2-week fractions were due to die-off during this time period. The water content levels within the soil declined in time and were less for the 2-week rainfall than the 1-week rainfall (Figure 3.1). *E. coli* survival in soil has been shown to depend on soil water contents whereby concentrations decline with declining water content levels, but are able to rebound upon rewetting (Byappanahalli et al., 2004; Barry and Miller, 2005; Hodgson et al., 2016). The enterococci fractions did not significantly differ between the manure consistencies or the level of weathering in the subsequent rainfall events. As previously stated, this lack of perceived differences was likely attributable to the immediate exponential die-off of the enterococci indicator that was very similar across treatments.

The *E. coli* growth was seen greater for the 1-week LM treatment than for the 2-week LM treatment. This difference was likely attributable to the timing of the rainfall recurrence. After 1-week, the mean fractions of *E. coli* within the soil between the LM

treatments is comparable, but albeit 1/3 less for the 2-week treatment than the 1-week treatment (Fig. 4.12). At this point in the study, the 1-week weathering treatment receives rainfall and the 2-week weathering treatment does not. The simulated rainfall rehydrated the soil and delivered additional nutrients and bacteria into the soil from the remnants of the LM treatment applied the previous week. These additions likely spurred the continued growth of *E. coli* within the soil in this treatment as evidenced by the higher fraction measured during the following week. In the following week, the 2-week weathering rainfall occurred and provided water, nutrients, and additional bacteria to the soils of the 2-week weathering (Figure 4.12, panel b). As expected, the fraction of *E. coli* increased the week after the second rainfall in the LM 2 treatment, but then decreased substantially during the later weeks of the study. It is probable that the additional water and nutrients that entered the soil in the second rainfall came too late in LM 2 treatment relative to the LM 1 treatment and as such *E. coli* population dynamics in this 'starved' condition could not maintain constant levels due to the stresses encountered which began a phase of die-off. Additionally, when bacteria are stressed they enter a viable, but non-culturable state in which traditional detection methods do not work. This state may be a long-term survival strategy in which the cells become viable once again upon the return of favorable environmental conditions (Byappanahalli et al., 2006). In this case, the cells of both indicators may have been alive in large numbers within the soil and manure, but remain dormant due to water and nutrient restrictions. These explanations may apply to both indicators across treatments and at any point of the study as well. Finally, the LM 2 treatment experienced a significantly lower temperature than the other treatments although these lower temperatures only equated to a less than °2 C difference from other

treatments on average throughout the experiment (Figure 4.2). Still, the consistently lower temperatures for the LM 2 treatment may have stunted the growth of *E. coli* in the 2-week LM treatment relative to the 1-week LM treatment.

For the 1- and 2-week rainfalls, the larger increases of indicators were observed within the soil profile after rainfall in the SM treatments than in the LM treatments (Figure 4.11). This difference was attributable to the remaining solid manure remaining on the surface of the SM treatments that contained relatively high concentrations of both indicators which multiplied in the manure after each rainfall event (Figure 4.14). The majority of the liquid manure applied to LM 1 and LM 2 treated boxes was either infiltrated into the soil or lost in runoff so in the 1- and 2 week rainfalls, there was not a source at the surface that added bacteria to the soil with the infiltrating water.

4.6 Conclusions

With the application of manure to agricultural fields comes the potential for a massive release of manure-borne bacteria to surface and ground water sources. If microbial pathogens are present within an applied manure, this presence creates the risk of human illness resulting from the consumption and/or interaction with contaminated water. The associated risks of illness may be effectively forecasted by water managers via the use of watershed-scale water quality simulation software. While indeed effective, the simulations may be improved by the addition of results from scientific studies that aim to minimize the gaps in knowledge of the fate and transport of manure-borne bacteria. The present study aimed to evaluate and document the effects of manure consistency and

weathering on the release, survival, and removal of fecal bacteria from bovine slurry and solid manure.

As demonstrated in the present study, the massive removal of fecal indicator bacteria, *E. coli* and enterococci, with runoff and/or infiltrating water may occur at small-scale settings with a relatively small manure source. The removal of *E. coli* and enterococci with runoff and/or infiltration was not found to significantly differ by manure consistency when the manure was fresh. However, after 1- and 2-weeks of weathering this situation changed. The enterococci removal with runoff was found to be significantly affected by manure weathering with massive reductions in the number of enterococci bacteria removed as the time period since the initial rainfall event increased. The *E. coli* removal in runoff did not significantly differ by the level of weathering or consistency although large reductions were observed with an increased weathering period. For infiltrating water, the removal of both indicators in the subsequent rainfall events was significantly greater in liquid manure treatments than in solid manure treatments. These differences were attributed to the massive growth of *E. coli* within the soil which resulted in high fractions of the initially-applied bacteria being removed with infiltration. The enterococci did not experience growth within the soil, but soil populations following the first rainfall event were large relative to the total number applied. In subsequent rainfall events, great numbers of enterococci were still removed from the soil by infiltrating water. Additionally, the bacteria in the residual solid manure were felt likely to not be accessible in the later rainfall events due to weathering which effectively reduced the release of additional bacteria from the solid manure even though the indicator concentrations remained high within the manure.

The populations of *E. coli* and enterococci displayed opposite survival dynamics within the soil and manure. The *E. coli* experienced growth in all treatments although this growth was greater within soils that had received the liquid manure. The enterococci survived very poorly in the current study which calls into question the effectiveness of its use as a fecal indicator bacteria since these organisms are supposed to out-survive pathogenic bacteria. We obviously need to increase our understanding of enterococci population dynamics when the indicator bacteria is associated with land-applied bovine manure. The *E. coli* was detectable within the soil and manure in all treatments 28-days after the initial release event, but began to quickly decline in the absence of additional precipitation events which brought water and nutrients into the soil.

Future work should aim to model the results of this study so that factors affecting release and removal parameters can be better understood and implemented in microbial water quality forecasting. The effects of the delay of runoff and/or infiltration on the numbers of fecal bacteria eluted from manure-amended fields in either mode of transport should be investigated. It would also prove extremely useful to study the effects of scale on the removal of fecal bacteria from manured lands.

Chapter 5 – Modeling of bacteria indicator release and removal from liquid and solid manure-amended, vegetated soil exposed to simulated rainfall events

5.1 Introduction

The application of animal manures to agricultural fields is a wide-spread practice aiming to improve soil health, provide essential nutrients to plants, and to provide an efficient method of animal waste-management. With this common practice, comes the inherent risk of the spread of manure-borne fecal pathogens that are easily disseminated throughout the environment typically during precipitation events. In order to combat these risks, predictive watershed modeling is used to forecast the quality of surface waters used for recreational, drinking, or irrigation purposes.

The level of fecal indicator bacteria, *E. coli* and enterococci, are used as a metric of the microbial quality of a given water body as mandated by the U.S. EPA (USEPA, 2012). These two indicators are used because they act as non-pathogenic surrogates for the study of the release, survival, and removal of fecal pathogens from manure-amended lands (Bitton, 2005). Therefore, data derived from experiments focusing on the fate and transport of manure-borne *E. coli* and enterococci are useful to develop models so that the risks associated with the high levels of these bacteria can be quantified and mitigated.

Several models have been proposed to describe the release of fecal microorganisms from a manure source under precipitation action. These include the linear-exponential (EM) model, the Vadas-Kleinman-Sharpley (VKS) model, and the

Bradford-Schijven (B-S) release models (Bicknell et al., 1997; Vadas et al., 2004; Bradford and Schijven, 2002). All three release models have been found suitable for simulating the release of fecal bacteria from manure under simulated rainfall (Guber et al., 2006; Blaustein et al., 2015a). However, the relative accuracy of all three models seems to vary among studies. The manure consistency appears to be one of important factors affecting model performance. For instance, using manure slurry, Guber et al. (2006) found that the EM model performed the worst at simulating the release of fecal coliforms in runoff, whereas the VKS and B-S models performed so well that the authors did not explicitly assign a preference between the two models. Blaustein et al. (2016) compared the accuracy of the same three models when used to simulate the release of fecal bacteria from solid dairy manure. They found that the B-S model performed the best at simulating *E. coli* and enterococci release and that the EM model was more accurate than the VKS model for the same organisms. No studies have investigated the effectiveness of these models when manure consistency type was set as a factor.

Manure distributed on field and remaining on the soil surface undergoes weathering. It is known that manure weathering reduces the availability of fecal bacteria for release from the manure (Muirhead and Littlejohn, 2009; Drapcho, 2003; Sistani et al. 2010; Hodgson et al., 2009). However, model comparisons for the effectiveness at simulating the release and removal of fecal bacteria from weathered manure have not been performed. The effects of rainfall recurrence as an associated factor on the model parameters have only been investigated in one study using only the B-S release model (Kim et al., 2016).

Focusing only on the release of bacteria from manure during precipitation events may lead to underestimation of the total number of bacteria available for transport in runoff. After bacteria are released from the manure, they are transported with runoff and/or infiltration. Infiltrated bacteria are able to survive and grow in soil environments following manure application (Hodgson et al., 2016). In subsequent rainfall events, these bacteria may be released into runoff due to raindrop impacts on the soil surface which cause turbulent mixing of water and soil in a zone of soil dubbed the effective mixing layer or simply the soil mixing layer (Yang et al., 2016). One cannot exclude the active migration of bacteria from the mixing layer to runoff. Very little is known about the soil mixing layer in regard to the potential for release of manure-borne bacteria from the soil reservoir. In fact, current models such as the Soil and Watershed Assessment Tool (SWAT) use a 1-cm depth for the bacteria mixing layer. This choice is based on previous work that focused on nutrient movement in the environment. Researchers have reported positive correlations between the concentrations of bacteria within soil and those bacteria in runoff (Muirhead and Monaghan, 2012; Ling et al., 2009; Roodsari et al., 2005), but the choice of the depth of the soil sampling layer was not justified in these works.

The objectives of this study are: (i) simulate the removal of *E. coli* and enterococci with runoff and infiltration using the EM and VKS models; (ii) determine how the parameters are affected by manure consistency and weathering; (iii) evaluate the performance of each model; and (iv) determine the degree of bacterial mixing that occurred between the soil and runoff during three rainfall events each spaced 1-week apart.

5.2 Methods of data analysis

Runoff-removal modeling

For each rainfall simulation event, the concentrations of *E. coli* and enterococci in measured runoff and infiltration samples were multiplied by the water flux, based on the effluent flow rate, and the products were integrated to quantify the cumulative numbers of bacteria released in each effluent type. These cumulative numbers were converted into relative ratio values N_{Runoff}, or simply N, to N_0 by dividing the quantity in the runoff by the initial total quantity of bacteria for each bacteria group. The dependency of N/N_0 for each bacteria group on the rainfall depth were simulated using the following two microbial runoff-removal models:

4. The exponential release dependence equation that is used in the watershed-scale Hydrological Simulation Program – Fortran (HSPF) model for microbial fate and transport (Bicknell et al.1997)

$$\frac{N}{N_0} = 1 - e^{-k_e w} \quad (1)$$

where N is the total number of bacteria removed per unit area of manure with runoff, [N] = CFU m^{-2}; N_0 is the initial total number of bacteria in the applied manure, [N_0] = CFU; k_e is the rate constant parameter, [k_e] = cm^{-1} (for removal dependency on rainfall depth); w is rainfall depth after the initiation of rainfall, and [w] = cm rainfall.

5. The Vadas et al. (2004) equation was originally developed to describe organic phosphorous loss in runoff from surface-applied animal manures, but has since been adapted to predict bacterial release (Guber et al., 2006; Blaustein et al., 2016).

$$\frac{N}{N_0} = AW^n \quad (2)$$

where N is the total number of bacteria removed per unit area of manure with runoff, [N] = CFU m^{-2}; N_0 is the initial total number of bacteria in the applied manure, [N_0] = CFU; A is the rate constant parameter, [A] = cm^{-n} (for removal dependency on rainfall depth); W is the rainfall depth, [W] cm rainfall; and n is a dimensionless parameter.

For the remainder of this paper, Eq. 1 and Eq. 2 will be referred to as the EM model and the VKS model, respectively.

Model fitting was performed using Sigmaplot 13.0 (Systat Software, San Jose, California). Parameters were statistically compared using Paleontological Statistics Software Package (PAST) (Hammer et al., 2001). A student's t-test was used to compare mean parameter values in the first rainfall event. In the 1-and 2-week rainfall events, a two-way ANOVA was used to evaluate the effects of manure consistency and weathering on the parameter means. The level of statistical significance was set to 0.05.

Evaluation of model performance

The model performance was evaluated using three metrics: (i) the comparison of root-mean-square-error (RMSE) of the model fit to the data; (ii), the Akaike information criterion (AIC); and (iii) the comparison of the coefficient of determination (R^2) values.

RMSE were computed as:

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

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 and 2 have a different number of parameters (one and two, respectively). The corrected Akaike statistic is:

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

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

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

Effective depth of interaction and mixing model

The thin top layer of soil interacts with the runoff water flowing over it and has infiltrating water moving through it. Several mechanisms of interactions have been considered, and their joint action is conceptualized as mixing . Thereafter, the interacting soil layer is called the mixing zone. The original model of the solute exchange between flowing water and the soil mixing zone did not consider solute loss with infiltration, and assumed that ideal mixing occurred (Ahuja et al., 1981). The model of the ideal mixing assumes that the masses of the solutes in the mixing zone and in the runoff are added, and the sum is divided by the total volume of water in the soil and in the runoff layer to obtain the average "mixed" concentration. This concentration of solute is assumed to be characteristic of the concentration in the runoff.

The mathematical model of such ideal mixing is:

$$\frac{d[(EDI\theta+d)C]}{dt} = -CR \qquad (3)$$

Here R is the runoff rate (cm min^{-1}), t is the time in minutes, C is the solute concentration (M cm^{-3} or number of particles/cells N cm^{-3}), EDI is the effective interaction depth, cm, d is the thickness of the runoff layer, cm, and θ is the volumetric water content cm^3 cm^{-3}.

The value of d is usually much smaller than E, therefore, it can be neglected in the equation (3) and in its solution

$$\ln C = \ln C_0 - \frac{Rt}{EDI\theta} \qquad (4)$$

Here C_0 is the initial concentration of the solute in the mixing zone.

If the concentration in the runoff is measured several times, then the regression of time vs. ln C allows one to find the mixing zone thickness. Specifically, if the regression line obtained from experimental data has the equation

$$\ln C = A - Bt \tag{5}$$

then a comparison of equations (4) and (5) shows that

$$EDI = \frac{R}{B\theta} \tag{6}$$

In the case where the infiltration is considered, the ideal mixing model differs from the previous one only by accounting for the removal of the mixed concentration with both runoff and infiltration:

$$\frac{d[(EDI\theta+d)C]}{dt} = -C(R + I) \tag{7}$$

Where I is the infiltration rate, cm min^{-1}. To consider the infiltration along with the runoff, the equation for the mixing depth thickness (6) is modified accordingly:

$$EDI = \frac{R+I}{B\theta} \tag{8}$$

where B is the slope of the regression (5) obtained from experimental data. If the majority of the experimental points are collected during the later period when the sum of the runoff rate *R* and the infiltration rate *I* is equal to the total rainfall intensity, *P*, then Eq. (6) transforms into

$$EDI = \frac{P}{B\theta} \quad (9)$$

It was observed that in some cases the ideal mixing model tends to generate unrealistically high values of the mixing depth thickness (Ahuja and Lehman, 1983). Therefore, the incomplete or non-ideal mixing model was suggested that, in accordance with its name, assumed that only a fraction of the runoff and a fraction of the infiltration water actually mixes with the soil water containing the solute of interest. Under the incomplete mixing hypothesis, one considers not only the unknown effective depth of interaction (EDI), but also the fraction of the runoff (α) and the fraction of the infiltration water (γ) that are participating in the mixing. The equation of the partial mixing model is:

$$\frac{d[(EDI\theta+d)C]}{dt} = -C(\alpha R + \gamma I) \quad (10)$$

Its solution is (Ahuja and Lehman, 1983):

$$\ln C = \ln C_0 - \frac{(\alpha R+\gamma I)t}{EDI\theta} \quad (11)$$

If the regression (5) is developed than the relationship between the slope B and parameters of the incomplete mixing model are:

$$EDI = \frac{\alpha R+\gamma I}{B\theta} \quad (12)$$

One can see that the three parameters of the incomplete mixing model (α, γ, and EDI) cannot be found if only one value – the slope **(**B) - is known from the experimental data. Additional relationships and/or data are needed to determine all three parameters.

We will estimate the mixing fractions (α and γ) assuming the mixing zone thickness (EDI) is known and is equal to 1 cm. This assumption is based on the following information:

(1) a one centimeter-layer in our experiments is the layer where the concentrations are much higher than below the layer,

(2) the correlations were determined for the *E. coli* contents in the 1-cm layer and the *E. coli* concentrations in the runoff (Muirhead, 2009), and

(3) that small watershed water quality models (e.g. APEX and STWiR) are capable of using the 1-cm layer as the default mixing zone thickness.

Even if the value of E is known we still have two parameters α and γ to evaluate from the single equation (12). There were suggestions (Zhang et al., 1999) to use the convective- dispersive equation within the soil mixing zone and for soil depths below the zone. Using these suggestions is as complicated as using the incomplete mixing assumption. The dispersion coefficient has to be estimated and the boundary conditions are not well defined neither on the soil surface nor if the mixing with runoff occurs.

5.3 Results

Model parameters for indicator removal in runoff

The values for the model parameters *k*, *A*, and *n* from fits of the EM and VKS models to the runoff concentrations of both organisms are presented in Table 5.1. Both rate constant parameters *A* and *k* decreased with each subsequent rainfall event for both

organisms and in both consistency types while parameter *n* increased in each subsequent rainfall (Figure 5.1). Both *A* and *k* are similar across the consistencies and organisms in the initial removal event, but in both later rainfalls these parameters are larger for *E. coli* than for enterococci. Interestingly, the parameters *n* and *k* from the *E. coli* runoff concentration values are approximately equal for the LM and SM treatments during the 2-week rainfall event (Figure 5.1). During the initial rainfall event, the parameters did not statistically differ between consistencies in curves for *E. coli* (P = 0.445, P = 0.352, P = 0.407, for *k*, n, and *A*, respectively) or enterococci (P = 0.744, P = 0.485, and P = 0.473 for *k*, n, and *A*, respectively).

Table 5.1. Parameter values from fitting the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model to the runoff removal values.

Model; Parameter; Units	Week	*E. coli*		Enterococci	
		LM	SM	LM	SM
EM; *k;* cm^{-1} × 1000	0	43.28 ± 8.76	54.08 ± 9.88	79.63 ± 24.64	63.92 ± 16.37
	1	10.27 ± 5.96	15.37 ± 7.78	1.13 ± 0.29	1.33 ± 0.74
	2	2.3 ± 1.07	2.2 ± 0.59	0.09 ± 0.05	0.17 ± 0.11
VKS; *A;* cm^{-1} × 1000	0	88.7 ± 23.17	83.2 ± 13.80	112.88 ± 42.59	85.4 ± 19.63
	1	13.7 ± 7.67	20.8 ± 11.81	1.37 ± 0.35	1.3 ± 0.51
	2	1.83 ± 1.09	2.07 ± 1.07	0.09 ± 0.06	0.23 ± 0.19
VKS; *n;* none	0	0.49 ± 0.11	0.59 ± 0.04	0.7 ± 0.25	0.67 ± 0.11
	1	0.73 ± 0.03	0.81 ± 0.06	0.8 ± 0.10	0.88 ± 0.13
	2	1.25 ± 0.09	1.25 ± 0.28	1.33 ± 0.25	1.14 ± 0.31

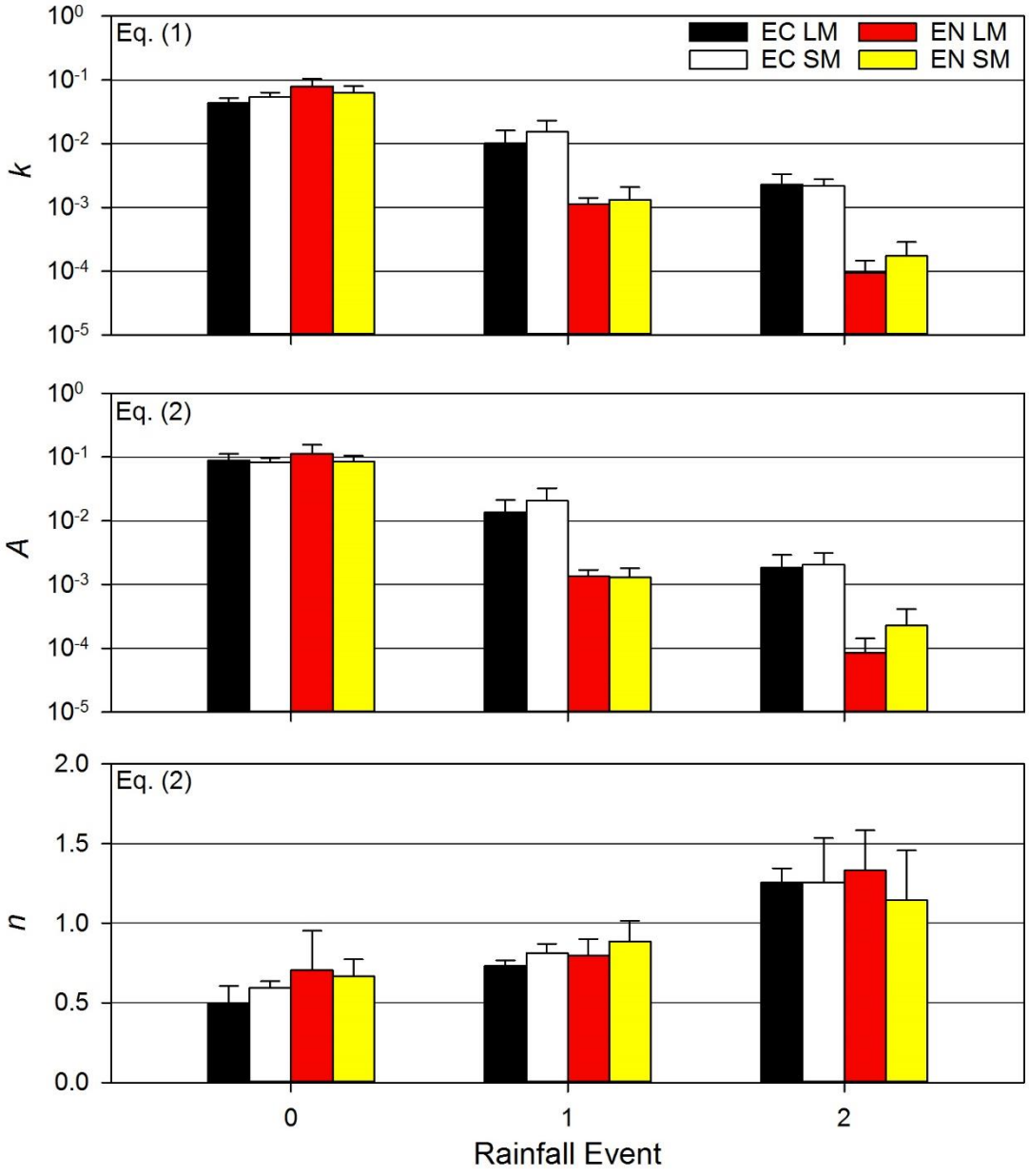

Figure 5.1. Runoff removal curve parameters from fitting the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model for *E. coli* (EC) and enterococci (EN) indicator bacteria by manure consistency and level of weathering for the liquid (LM) and solid (SM) manure consistencies.

During the 1- and 2 – week rainfall events, there were a number of significant differences between the parameter values reflecting the differences in weathering (Table 5.2). The parameter *k* significantly decreased from the one weathering week to two weathering weeks events for enterococci (P = 0.002) whereas this difference for *E. coli* was close to significant (P = 0.058). The parameter (*A*) from Eq. (2) significantly decreased in the 2-week rainfall event compared to the 1-week event for both organisms (P = 0.025 and P = 0.003 for *E. coli* and enterococci, respectively). The shape parameter (*n*) from Eq. (2) significantly increased between the 1- and 2-week rainfall events for *E. coli*, but not for enterococci. The manure consistency was not found to have a statistically significant effect on the parameter values between the 1- and 2-week rainfall events and no significant interactions between the factors were detected.

Table 5.2. Results from a two-factor ANOVA (p-values are displayed) on the effects of weathering and consistency on the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model parameters for the dependency of release of the *E. coli* and enterococci indicator bacteria based on the rainfall depth. No statistically significant interaction effects existed for the model parameters.

Model; Parameter; Units	Bacteria	Weathering	Consistency
EM; K_e; cm^{-1}	*E. coli*	0.058	0.480
	Enterococci	**0.002**	0.705
VKS; *A;* cm^{-1}	*E. coli*	**0.025**	0.606
	Enterococci	**0.003**	0.689
VKS; *n;* none	*E. coli*	**0.013**	0.798
	Enterococci	0.106	0.829

Values in bold are significant (P <0.05).

The model parameters of the EM and VKS models did not significantly differ between *E. coli* and enterococci except for *k* in the LM 2-week removal event where the removal values were smaller for enterococci (Table 5.3). The same parameter in the same time interval for solid manure was close to the level of significance. The coefficient *A* in the LM two-week weathering treatment also approached the level of significance. Both *k* and *A* seemed to follow a trend of increasing differences over time between *E. coli* and enterococci (Fig. 5.1).

Table 5.3. The probabilities of release parameters to be the same for *E. coli* and enterococci based on the runoff removal values.

	Liquid Manure			Solid manure		
Week	*A*	N	*K*	*A*	n	*k*
0	0.848	0.248	0.335	0.865	0.509	0.740
1	0.173	0.456	0.183	0.093	0.525	0.116
2	0.063	0.738	**0.046**	0.098	0.549	0.051

Values in bold are significant ($P < 0.05$).

Model parameters for indicator removal with infiltration

The values for parameters generated from fitting the EM and VKS models to the infiltration removal values can be seen in Table 5.4. The parameters *A* and *k* remained relatively similar throughout all treatments for *E. coli* infiltration removal, whereas, there was a gradual decrease for enterococci. Unlike with runoff removal, the parameter *n* generally decreased in time with the lowest values for both indicators seen in the 2-week rainfall event (Figure 5.2).

Table 5.4. Model parameter values for the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model from fitting the infiltration data

Model; Parameter; Units	Week	*E. coli*		Enterococci	
		LM	SM	LM	SM
EM; *k;* cm^{-1} × 1000	0	11.8 ± 5.56	3.35 ± 0.83	13.6 ± 5.75	7.18 ± 3.61
	1	9.67 ± 2.92	3.07 ± 0.73	1.8 ± 0.92	0.31 ± 0.12
	2	10.7 ± 1.25	2.43 ± 0.31	0.93 ± 0.29	0.11 ± 0.03
VKS; *A;* cm^{-1} × 1000	0	7.22 ± 3.27	0.95 ± 0.18	5.6 ± 1.55	1.08 ± 0.31
	1	7.83 ± 2.39	1.10 ± 0.29	0.44 ± 0.15	0.09 ± 0.04
	2	9.83 ± 1.33	1.53 ± 0.32	0.57 ± 0.19	0.09 ± 0.04
VKS; *n;* none	0	1.53 ± 0.20	1.74 ± 0.10	1.61 ± 0.20	1.92 ± 0.21
	1	1.14 ± 0.03	1.71 ± 0.12	1.93 ± 0.38	1.76 ± 0.12
	2	1.05 ± 0.04	1.34 ± 0.11	1.30 ± 0.08	1.4 ± 0.10

The model parameters generated from *E.* coli removal in the infiltration data did not significantly differ between consistency treatment in the initial rainfall event ($P = 0.101$, $P = 0.369$, $P = 0.180$ for *k*, *n*, and *A*, respectively). The parameters for the enterococci infiltration removal values for the initial event did not significantly differ between consistency for *k* or *n* ($P = 0.192$ and $P = 0.294$, respectively), but parameter *A* was significantly larger for the LM treatment than for the SM treatment ($P = 0.036$).

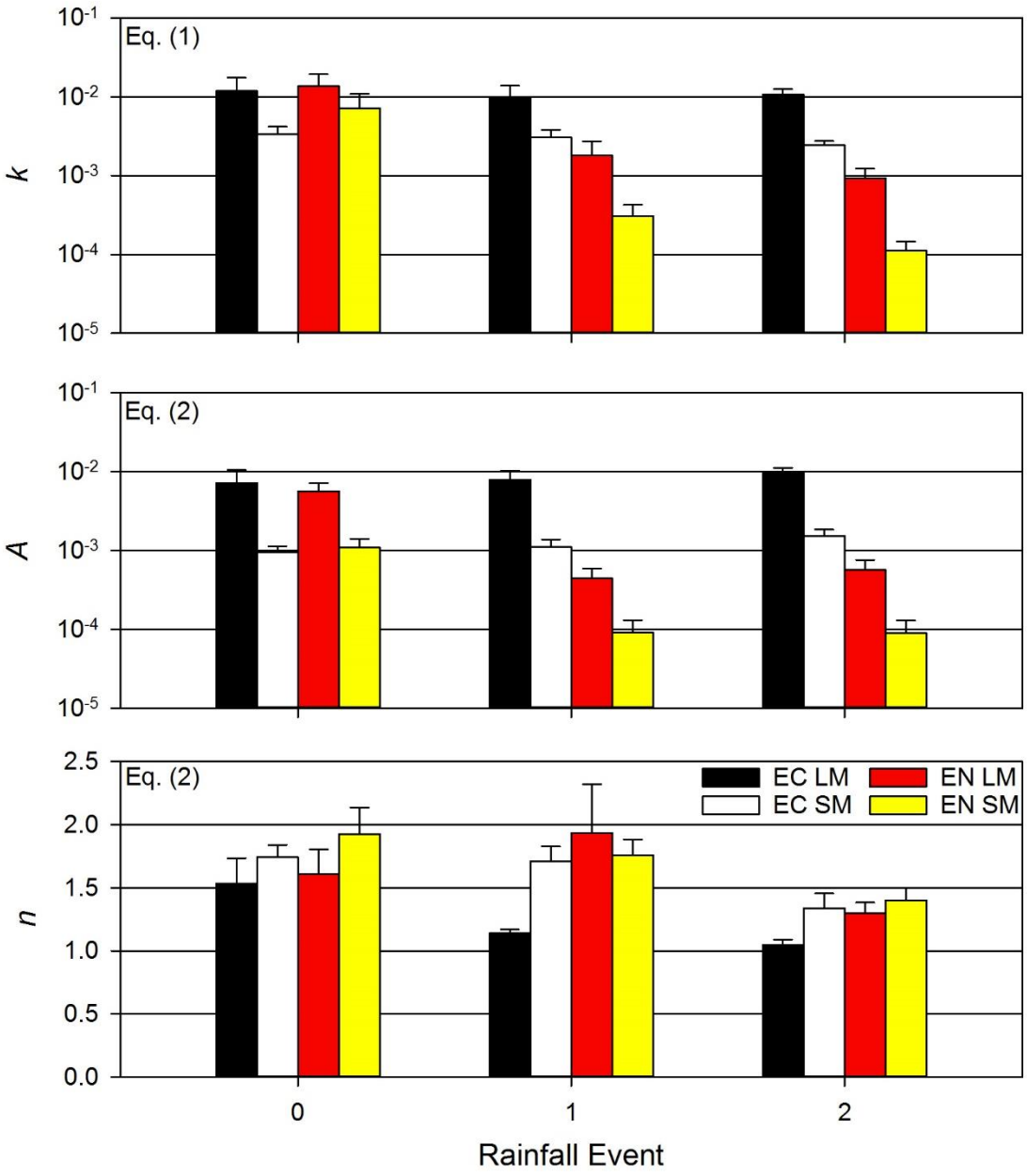

Figure 5.2. Infiltration removal curve parameters from fitting the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model for *E. coli* (EC) and enterococci (EN) indicator bacteria by manure consistency and level of weathering for the liquid (LM) and solid (SM) manure consistencies.

Between the 1- and 2-week rainfall events, the parameter *k* from both *E. coli* and enterococci infiltration removal values was significantly greater for the LM treatments than for the SM treatments (Table 5.5). The level of weathering did not significantly affect parameter *k* and there were no significant interactions between factors. The parameter *A* was significantly greater in the LM treatments than in the SM treatments for *E. coli*, but for enterococci this difference was just short of significance. The parameter *A* did not statistically differ by the level of weathering and the interactions with the other factors were not significant. The shape parameter *n* was significantly smaller during the 2-week rainfall event than during the 1-week rainfall event for both organisms. This parameter was also significantly greater in the SM treatments than in the LM treatments when fit to *E. coli* removal data, but *n* did not significantly differ by consistency for enterococci. No statistical interactions were found for consistency versus weathering for parameter *n*.

Table 5.5. Results from a two-factor ANOVA (p-values are displayed) on the effects of weathering and consistency on the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model parameters for the dependency of release of the E. coli and enterococci indicator bacteria based on the rainfall depth. No statistically significant interaction effects existed for the model parameters.

Model; Parameter; Units	Bacteria	Weathering	Consistency
EM; K_e; cm^{-1}	*E. coli*	0.866	**0.003**
	Enterococci	0.540	**0.026**
VKS; *A;* cm^{-1}	*E. coli*	0.288	**<0.001**
	Enterococci	0.648	0.056
VKS; *n;* none	*E. coli*	**0.028**	**0.001**
	Enterococci	**0.047**	0.859

Values in bold are significant ($P < 0.05$).

The infiltration parameters in most cases were not statistically different for the two indicator bacteria (Table 5.6). One exception was for parameter *k* during the 2-week rainfall event where the mean values of *E. coli* parameters in the SM treatment were significantly larger than those same values for enterococci. The other exception is for the mean values of parameter *A* in the LM treatments in the 1- and 2- week rainfall events. In the SM 1- and 2-week treatments, the differences between the values of *A* very closely neared the level of significance. The same observation may be made about *k* during the LM 2-week rainfall events.

Table 5.6. The probability of the model infiltration parameters to be the same for *E. coli* and enterococci in the liquid and solid manure treatments.

	Liquid Manure			Solid manure		
Week	*A*	*n*	*k*	*A*	*n*	*k*
0	0.685	0.500	0.575	0.851	0.197	0.292
1	**0.042**	0.166	0.124	0.051	0.333	0.054
2	**0.045**	0.179	0.057	0.070	0.665	**0.038**

Values in bold are significant ($P < 0.05$).

Evaluation of model performance: runoff

For both *E. coli* and enterococci in liquid and solid manure removal, the VKS model consistently had the lowest root-mean squared error (RMSE) values (Table 5.7 and Fig. 5.3). The mean RMSE values from both equations fit to the LM and SM runoff removal data were found to decrease with the increasing level of weathering and meant the models gave better fits through time. The results from the AICc analysis, which may penalize models for an excessive number of parameters, were also consistently more

favorable for the VKS model despite this equation having an additional parameter when compared to the EM model. The AICc values became smaller with each subsequent removal event indicating that the model fits were improving over time. The mean R^2 values for *E. coli* fitted to the EM and VKS models were 0.89 ± 0.03 and 0.99 ± 0.01, respectively. For enterococci, the mean R^2 values were 0.91 ± 0.04 and 0.99 ± 0.02, respectively. Based on the three model evaluation criteria described above, the VKS model's performance was superior to the EM model in simulating the removal of the indicator bacteria with runoff from manure applications of both consistencies and through all levels of weathering.

Table 5.7. The root mean square error (RMSE) and the Akaike information criterion (AICc) values for the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model fit to runoff removal data for both organisms.

		Liquid Manure			Solid Manure		
RMSE		Week 0	Week 1	Week 2	Week 0	Week 1	Week 2
EC	EM	36.93 ± 11.65	2.80 ± 1.46	0.55 ± 0.08	25.51 ± 4.07	5.38 ± 3.88	0.84 ± 0.05
	VKS	**10.18 ± 2.31**	**1.16 ± 0.57**	**0.30 ± 0.07**	**10.89 ± 1.06**	**3.11 ± 2.14**	**0.44 ± 0.16**
EN	EM	51.31 ± 14.38	0.26 ± 0.05	0.03 ± 0.02	29.63 ± 4.69	0.30 ± 0.09	0.08 ± 0.04
	VKS	**18.10 ± 5.04**	**0.15 ± 0.05**	**0.02 ± 0.01**	**16.18 ± 4.27**	**0.13 ± 0.03**	**0.03 ± 0.02**
AICC		Week 0	Week 1	Week 2	Week 0	Week 1	Week 2
EC	EM	-80.63 ± 11.53	-124.78 ± 6.58	-163.19 ± 3.50	-79.81 ± 3.78	-124.85 ± 16.09	-153.45 ± 1.19
	VKS	**-98.75 ± 8.4**	**-139.92 ± 5.40**	**-174.10 ± 5.59**	**-94.60 ± 2.59**	**-132.36 ± 14.96**	**-169.47 ± 11.58**
EN	EM	-67.47 ± 6.52	-162.93 ± 13.97	-231.95 ± 14.15	-76.65 ± 4.09	-178.15 ± 6.57	-215.23 ± 15.66
	VKS	**-87.87 ± 17.54**	**-171.16 ± 11.91**	**-241.62 ± 12.43**	**-89.48 ± 6.35**	**-192.16 ± 6.10**	**-229 ± .11.8**

Values in bold show the value from the equation with the lowest error as calculated by RMSE or AICC. All RMSE values are multiplied by 1000.

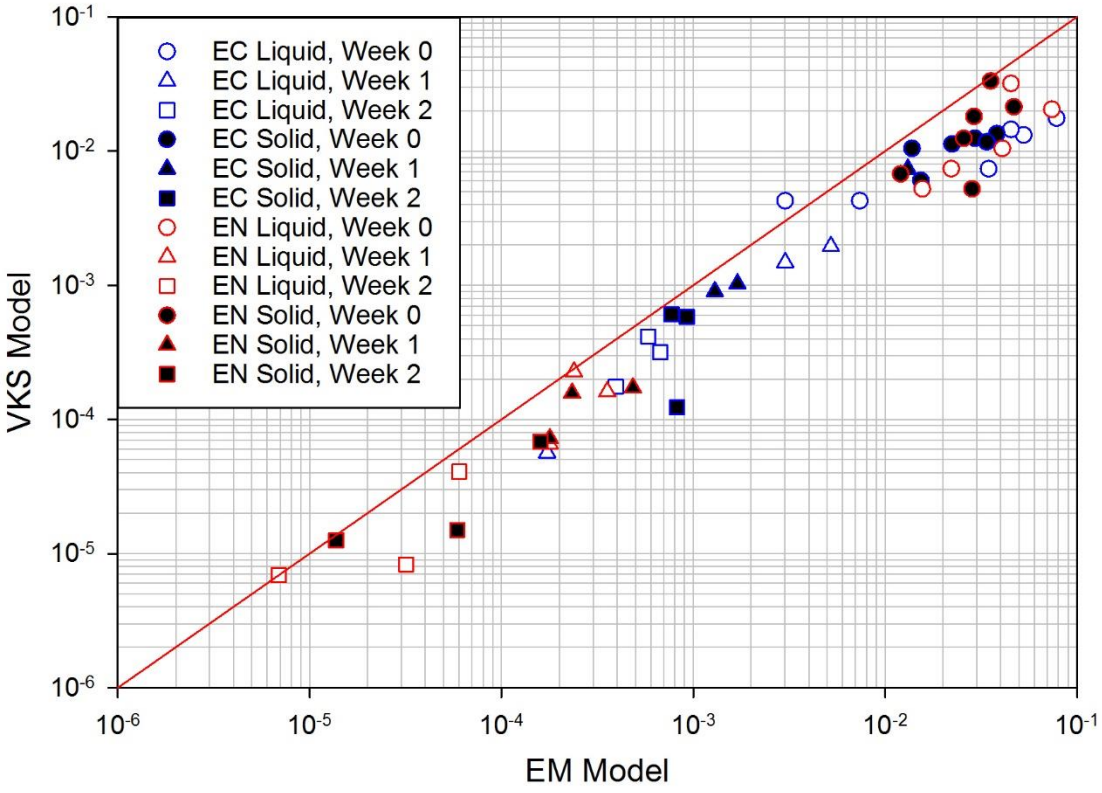

Figure 5.3. Plot of linear model root mean square error (RMSE) values vs the power model RMSE values for the removal of *E. coli* (EC) and enterococci (EN) in the runoff removal data after liquid and solid manure treatments. The red line shows a 1 to 1 ratio of error.

Evaluation of model performance: infiltration

For both organisms and across all treatment levels, the VKS model was found to produce the lower error in comparison to the EM model when fit to the infiltration removal data (Table 5.8). This observation was true for both evaluation metrics of the

RMSE and AICCc. The RMSE values followed a trend of decreasing values with each subsequent rainfall event (Figure 5.4). Likewise, the AICCc values became more negative (more preferable) by the final rainfall event than during the initial rainfall event. The mean R^2 values for *E. coli* for the EM and VKS models were 0.96 ± 0.005 and 0.99 ± 0.002, respectively. For enterococci, these mean R^2 values were 0.87 ± 0.011 and 0.99 ± 0.003 for the EM and VKS models, respectively. Based on the three model evaluation criteria described above, the VKS model's performance was superior to the EM model in simulating the fecal bacteria removal with infiltration from manure of both consistencies and through all levels of weathering.

Table 5.8 The root mean square error (RMSE) and the Akaike information criterion (AICc) values for the exponential-linear (EM) model and the Vadas-Kleinman-Sharpley (VKS) model fit to infiltration removal data for both organisms.

RMSE		Liquid Manure			Solid Manure		
		Week 0	Week 1	Week 2	Week 0	Week 1	Week 2
EC	EM	5.73 ± 2.81	2.68 ± 1.28	1.88 ± 0.47	2.73 ± 0.80	2.44 ± 0.97	1.01 ± 0.15
	VKS	**2.23 ± 1.28**	**2.05 ± 1.12**	**1.50 ± 0.52**	**0.55 ±0.17**	**0.63 ± 0.47**	**0.40 ± 0.11**
EN	EM	9.87 ± 5.86	1.84 ± 1.58	0.33 ± 0.14	8.54 ± 4.76	0.27 ± 0.14	0.05 ± 0.01
	VKS	**2.96 ± 1.33**	**0.23 ± 0.14**	**0.07 ± 0.04**	**1.59 ± 0.63**	**0.11 ± 0.08**	**0.03 ± 0.01**
AICC		Week 0	Week 1	Week 2	Week 0	Week 1	Week 2
EC	EM	-120.47 ± 8.35	-119.7± 13.60	-137.45 ± 6.68	-137.22 ± 11.25	-133.75 ± 9.53	-149.70 ± 3.13
	VKS	**-145.20 ± 8.78**	**-123.52 ± 16.18**	**-140.12 ±7.40**	**-168.56 ± 10.41**	**-170.51 ± 17.01**	**-168.24 ± 6.19**
EN	EM	-113.56 ± 10.37	-147.50 ± 32.80	-181.23 ± 14.41	-127.13 ± 17.73	-193.06 ± 22.91	-216.64 ± 7.31
	VKS	**-132.89 ± 16.63**	**-173.88 ± 30.52**	**-209.99 ± 11.17**	**-152.65 ± 14.64**	**-217.33 ± 27.03**	**-228.40 ± 7.31**

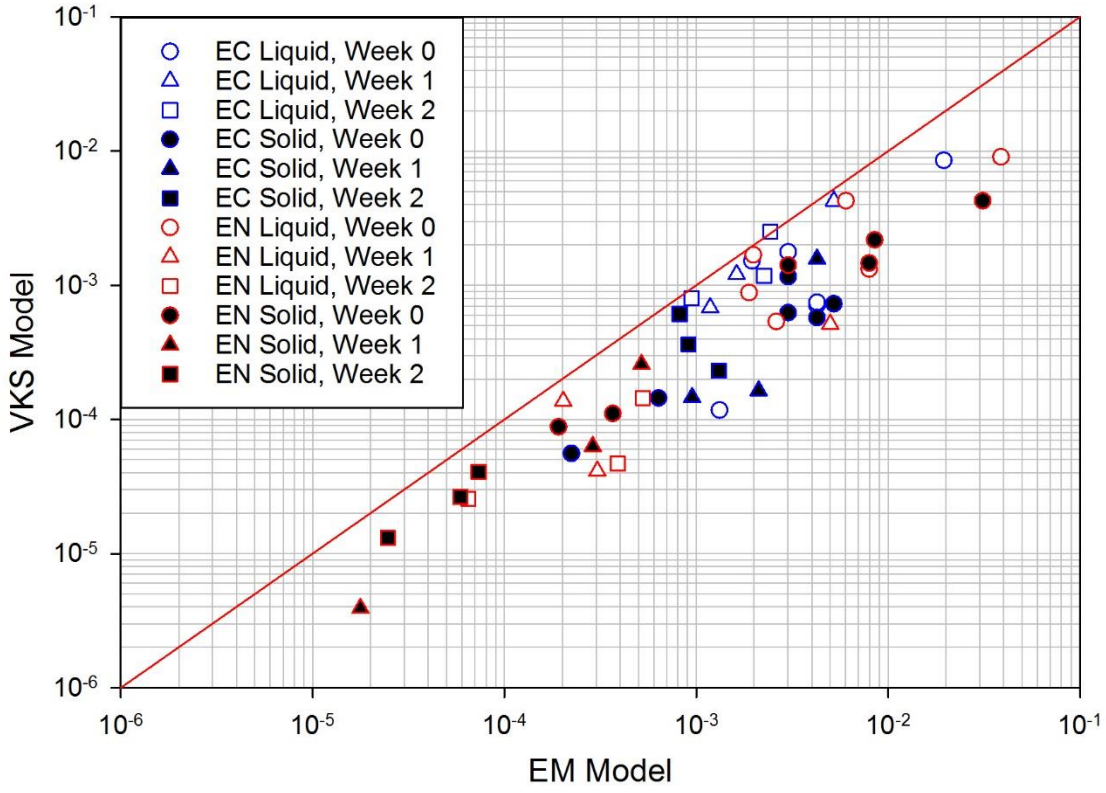

Figure 5.4. Plot of linear model root mean square error (RMSE) values vs the power model RMSE values for the removal of *E. coli* (EC) and enterococci (EN) in the infiltration removal data after liquid and solid manure treatments. The red line shows a 1 to 1 ratio of error.

Effective depth of interaction and the mixing model

To make an assumption regarding the relationship between parameters α and γ, we developed Table 5.9 and 5.10. It may be observed that the N/N_0 ratio per ml is on

average numerically similar for runoff and infiltration. This similarity indicates that an assumption $\alpha = \gamma$ may be a good first approximation. Under this assumption:

$$B = \frac{\alpha(R+I)}{EDI\theta} \quad (13)$$

or

$$\alpha = \frac{B\theta}{(R+I)} \quad (14)$$

where B is in min^{-1}, R and I are in cm min^{-1}, and θ=0.49 is the saturated volumetric water content.

Figures 5.7 and 5.8 show the dependencies of indicator concentrations over time after the liquid manure application during the first rainfall event. Only the data for the 20 min period following the initiation of runoff are shown, since this period has runoff and infiltration fluxes with minimal variations, and using the constant values of *R* and *I* in Eq. (13) and (14) would seem justified. The determination coefficients for regression shown in Figures 5.5 and 5.6 are collected in Tables 5.11 and 5.12 for the initial and the 1- and 2-week rainfall events, respectively. The coefficients of determination are reasonably high during the initial rainfall event and, therefore, the use of a linear regression equation (5) appears to be justified. The only exception to this observation is for enterococci in replication 1 of the LM 1 treatment had an R^2 = 0.001 (Table 5.11).

During the first and second rainfall events, the coefficients of determination were substantially lower for both organisms (Table 5.12). The fitting of *E. coli* data during the 1-week rainfall event still produced moderate to strong linear fits of the runoff concentrations versus the times after the initiation of runoff and/or infiltration, but during

the 2-week rainfall event, the linear fitting produced generally low to moderate R^2 values. The linear regressions with the enterococci data produced very poor to strong fits during the 1-week rainfall event, but the fits were consistently very poor when attempts were made to predict the 2-week runoff concentrations (Table 5.12).

The values of α, or the "degree of mixing", were found to scale linearly with the effective depth of interaction (EDI) (i.e. if one wanted to calculate α and B for an EDI of 5-cm simply multiply α by 5 as per Eq. 13 and Eq. 14). The values of α from the runoff data in the first rainfall event are substantially less than one when setting the EDI to 1 cm (Table 5.11). The average values of α were 0.139 and 0.093 for *E. coli* and enterococci, respectively. The subsequent rainfall events produced lower values of α compared to the initial event and the mean values of 0.044 and 0.056 for *E. coli* in the 1- and 2-week rainfalls and 0.053 and 0.063 for enterococci in the same respective rainfalls were closer to one another than the values of α observed during the initial event Similar to α, the *B* parameter decreased by roughly half between the initial and 1-week rainfall events and then retained a similar value between the 1- and 2-week rainfall events (Table 5.11 and 5.12).

Table 5.9. The volumes of water and the indicator fractions removed from the Liquid 1-week (LM 1) and the Liquid 2-week (LM 2) treatments with runoff or infiltration in the initial rainfall event.

Week 0	LM 1			LM 2			Average	Mean M/M0 ml^{-1}
Total runoff (ml)	13773	12512	7719	11693	11033	18719	12575	
EC N/N0	0.14	0.08	0.17	0.24	0.24	0.15	0.17	$1.35 \cdot 10^{-5}$
EN N/N0	0.50	0.44	0.16	0.48	0.11	0.14	0.30	$2.42 \cdot 10^{-5}$
Total infiltration (ml)	3746	3792	12144	9333	11151	7008	7862	
EC N/N0	0.28	0.02	0.06	0.06	0.08	0.04	0.09	$1.15 \cdot 10^{-5}$
EN N/N0	0.04	0.01	0.06	0.03	0.23	0.06	0.07	$9.22 \cdot 10^{-6}$

Table 5.10. The volumes of water and indicator fractions removed from the Liquid 1-week (LM 1) and the Liquid 2-week (LM 2) treatments with runoff or infiltration in the 1- and 2-week rainfall events.

Week 1 and Week 2	LM 1 (1 – week recurrence)			LM 2 (2 – week recurrence)			Average Week 1	Average Week 2	Mean N/N_0 ml^{-1} Week 1	Mean N/N_0 ml^{-1} Week 2
Total runoff (ml)	1386	14115	9536	6316	2906	4141	8346	4454		
EC (N/N0) ×1000	2.01	46.24	112.16	243.45	242.58	146.00	53.47	210.68	$6.41 \cdot 10^{-6}$	$4.73 \cdot 10^{-5}$
EN (N/N0) ×1000	1.66	8.53	7.43	1.55	0.25	0.28	5.87	0.70	$7.04 \cdot 10^{-7}$	$1.56 \cdot 10^{-7}$
Total infiltration (ml)	14240	6838	13310	12310	13695	11322	11463	12442		
EC (N/N0) ×1000	43.37	24.12	101.29	41.86	71.26	70.94	56.26	61.35	$4.91 \cdot 10^{-6}$	$4.93 \cdot 10^{-6}$
EN (N/N0) ×1000	35.70	2.20	4.47	11.06	1.81	5.49	14.12	6.12	$1.23 \cdot 10^{-6}$	$4.92 \cdot 10^{-7}$

All values of N/N0 have been multiplied by 1000. Mean N/N_0 ml^{-1} were calculated with uncorrected values (i.e. not multiplied by 1000).

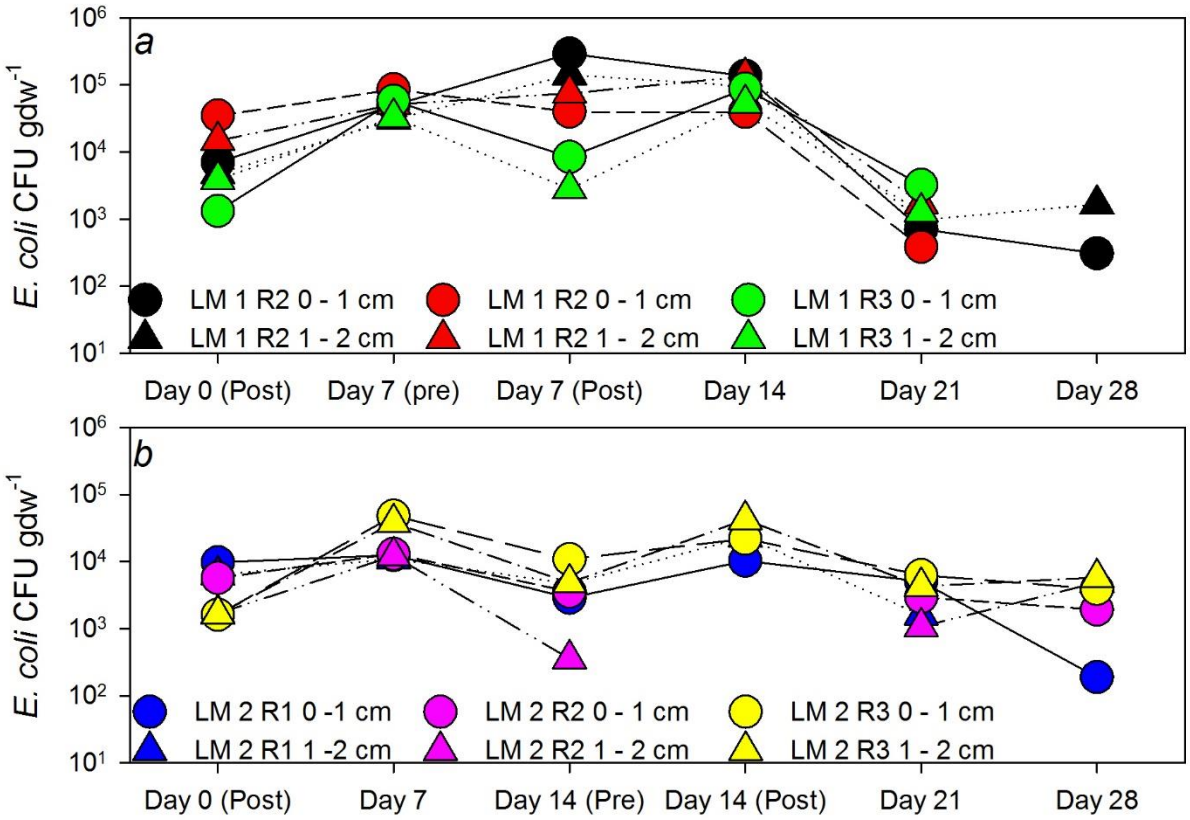

Figure 5.5. The concentrations of *E. coli* in the top 0 – 1 cm (circles) and 1 – 2 cm (triangles) soil depth ranges over the course of the study. Panel *a* shows the LM 1 treatment and Panel *b* shows the LM 2 treatment. R1, R2, and R3 are the replications of treatments.

The concentrations of *E. coli* in the 0 – 1 cm and 1 – 2 cm soil depth layers remained at relatively high levels in both the liquid manure treatments throughout the study (Figure 5.5). The concentrations between these two soil depth layers were very

similar throughout the study although the 0 – 1 cm range tended to have higher concentrations than the 1 – 2 cm range. The *E. coli* concentrations increased in the soil for all treatments between the initial rainfall event and the following week. After the 1-week rainfall event, there was some divergence in the *E. coli* population dynamics in the LM 1 treatment (Figure 5.5, Panel a) where replication 1 had increased *E. coli* concentrations in comparison to the concentrations prior to rainfall, replication 2 remained largely the same, and replication 3 decreased nearly 1 log in the 0 – 1 cm soil depth range and decreased over 1-log in the 1 – 2 cm soil depth range. After this initial period, the population dynamics between the replications were similar although the *E. coli* displayed greater persistence for approximately a week longer in replication 1 than the other two replications. In the LM 2 treatment, the *E. coli* concentrations declined after Day 7, but then increased again after the 2-week rainfall event for replications 1 and 3. In replication 2, *E. coli* was not detected in the top 2 centimeters of soil after the second rainfall event (Day 14), but was detected in both subsequent weeks.

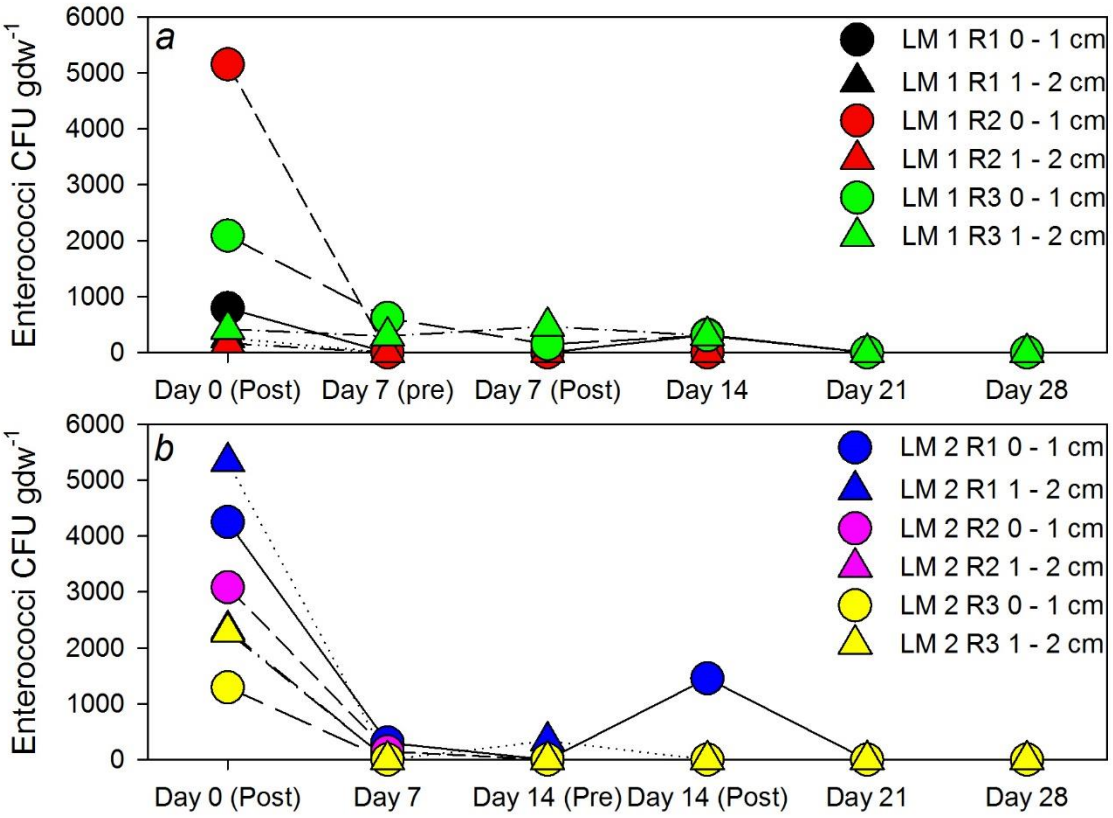

Figure 5.6. The concentrations of enterococci in the top 0 – 1 cm (circles) and 1 – 2 cm (triangles) soil depth ranges over the course of the study. Panel *a* shows the LM 1 treatment and Panel *b* shows the LM 2 treatment. R1, R2, and R3 are the replications of the treatments.

The enterococci concentrations declined in the 0 -1 cm and 1 – 2 cm soil layers following the first rainfall event in both liquid manure treatments which was indicative of an immediate die off of the indicator (Figure 5.6). The concentrations remained similar across the replications of both the LM treatments except during the 2-week rainfall event in replication 1 where the 0 – 1 cm soil depth range displayed an increased enterococci

concentration from a non-detectable range prior to the rainfall event to about $1.5 \cdot 10^3$ CFU gdw^{-1} following the 2-week rainfall event.

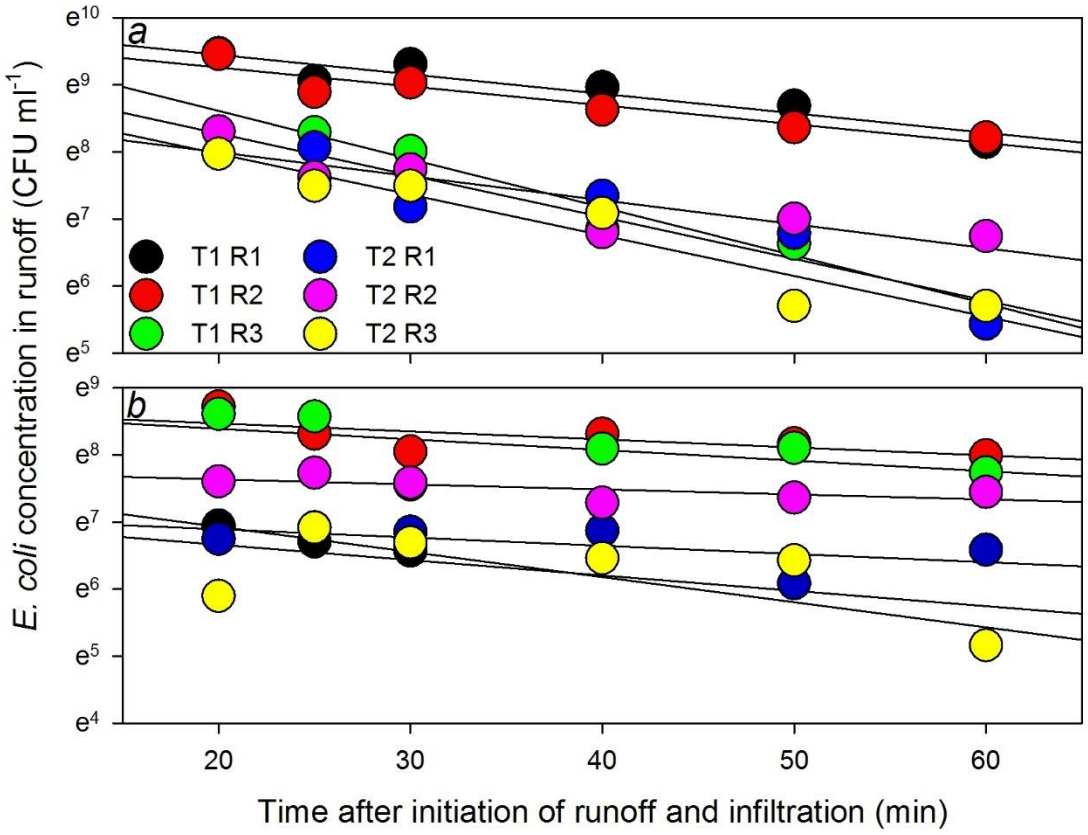

Figure 5.7. The relationship of the *E. coli* concentrations in runoff versus the time after runoff and infiltration initiation for the liquid manure treatments for the 1-week (LM 1) and the 2-week (LM 2) rainfall events. Panel *a* displays the initial rainfall event and Panel *b* shows the 1- and 2-week rainfall events. R1, R2, and R3 are the replications of the treatments.

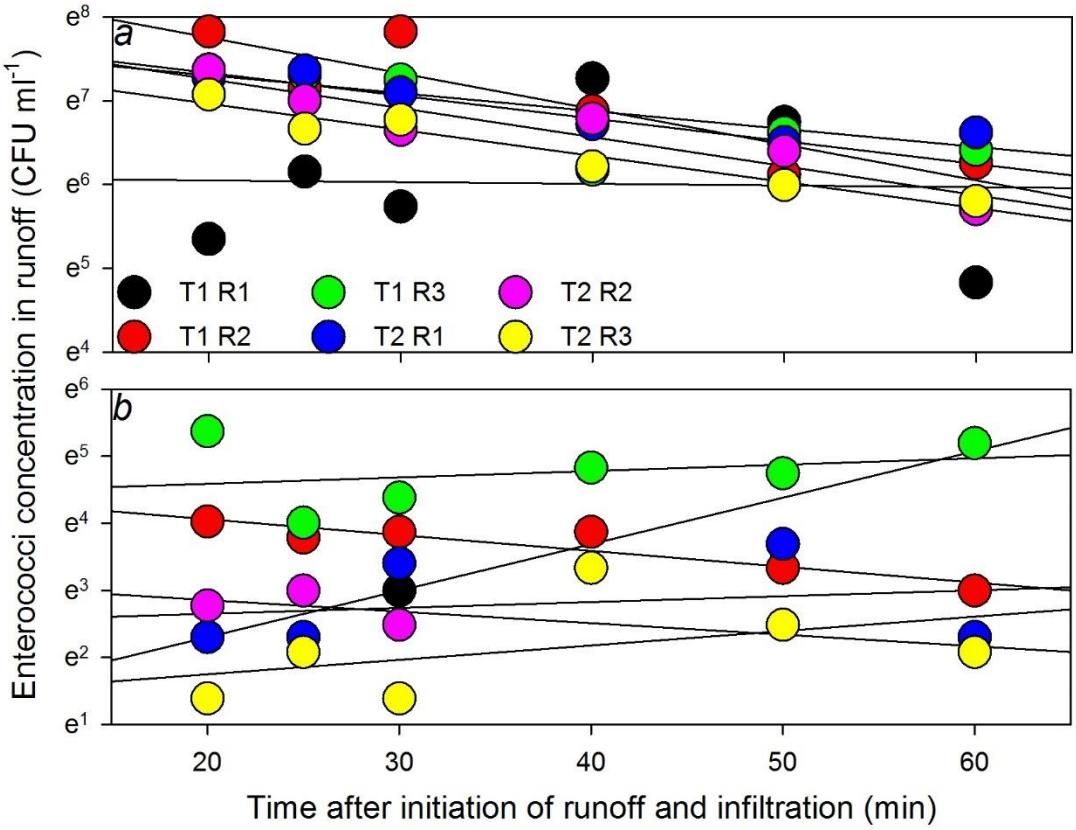

Figure 5.8. The relationship of the enterococci concentrations in runoff versus the time after runoff and infiltration initiation for the liquid manure treatments during the 1-week (LM 1) and 2-week (LM 2) rainfall events. Panel *a* displays the initial rainfall event and Panel *b* shows the 1- and 2-week rainfall events. R1, R2, and R3 are the replications of the treatments.

Table 5.11. The parameters of the regression equations and the parameters for incomplete mixing for runoff data from the initial rainfall events for the liquid manure treatments during the 1-week (LM 1) and 2-week (LM 2) rainfall events. R1, R2, and R3 are the replications of the treatments.

Treatment and replication	*E. coli*			Enterococci		
	R^2	B (min^{-1})	α	R^2	B (min^{-1})	A
LM 1 R1	0.888	0.029	0.122	0.001	0.002	0.001
LM 1 R2	0.898	0.028	0.124	0.79	0.043	0.022
LM 1 R3	0.925	0.072	0.221	0.652	0.027	0.014
LM 2 R1	0.709	0.031	0.11	0.838	0.021	0.011
LM 2 R2	0.755	0.026	0.086	0.88	0.035	0.018
LM 2 R3	0.92	0.061	0.173	0.942	0.031	0.016

Table 5.12. The parameters of the regression equations and the parameters for incomplete mixing for runoff data from the 1- and 2-week rainfall events for the liquid manure treatments during the 1-week (LM 1) and 2-week (LM 2) rainfall events. R1, R2, and R3 are the replications of the treatments.

Treatment and replication	Rainfall recurrence	*E. coli*			Enterococci		
		R^2	B (min^{-1})	α	R^2	B (min^{-1})	α
LM 1 R1	1-week	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
LM 1 R2	1-week	0.495	0.012	0.043	0.843	0.024	0.086
LM 1 R3	1-week	0.329	0.016	0.044	0.084	0.009	0.026
LM 2 R1	2-week	0.367	0.012	0.042	0.051	0.009	0.031
LM 2 R2	2-week	0.471	0.007	0.033	0.735	0.017	0.078
LM 2 R3	2-week	0.308	0.023	0.085	0.205	0.021	0.079

N.D. Not determined.

5.4 Discussion

The parameters *k* and *A* from the EM and VKS models, respectively, are the rate constants that account for the microbe and manure properties. The rate constants are considered the rates of manure dissolution and account for such properties the age, type, and consistency of the manure. The parameter *n* from the VKS model describes the shape of the removal curve with smaller values describing a more precipitous initial removal of bacteria in response to the treatments as opposed to the larger values which describe a more gradual removal in response to the treatments.

The parameters *k*, *A*, and *n* after fitting to the runoff removal data were not found to significantly differ between the manure consistency for either organism in the first rainfall event which indicates that these indicators were removed in runoff at similar rates for all the manure consistency types. The rate constants tended to be higher for the LM treatments than the SM treatments in the first week which agrees with another recent study that reported higher median values of their rate constant parameters when fitting bacterial release from swine slurry than when fitting cattle solid pats to the Bradford-Schijven (B-S) release model (Kim et al., 2016). Similarly, Guber et al. (2006) reported increased values of *A* with decreased manure solids content. The values for parameters *k* and *A* in the current study are similar, but a few times were smaller to what was reported in Blaustein et al. (2016) for removal of *E. coli* and enterococci from solid diary manure using the same models to fit their data. The values for parameter *n* are slightly greater for

their study. It follows that their higher values of *k* and *A* coincided with a greater release of both indicators than the present study.

The similarity between the values of *n* between the consistencies indicates a similar shape of the release curves which was found to be largely a two-stage process. The values of *n* in the present study are somewhat similar to those reported in the high manure solids replication (15.8%) in Guber et al. (2006). However in their study, the other three replications which have manure solids contents of 7-8% exhibited values of *n* that were orders of magnitude smaller than the present study indicating that they observed a much greater initial release of bacteria. Blaustein et al. (2016) reported values of *n* as 0.736 and 0.788 for *E. coli* and enterococci removal from solid diary manure which is very similar to the values reported in the present study of 0.70 and 0.67 for *E. coli* and enterococci, respectively, for the SM treatments during the initial rainfall event.

During the 1- and 2-week rainfall events, weathering significantly impacted the runoff parameters for each organism in almost all cases (Table 5.2). These impacts were manifested in a sizeable decrease in the values of *k* and *A* with an increased level of weathering. The values for *n* increased with the level of weathering which indicated that the two-stage runoff removal curves were becoming less concave or the rate of indicator removal decreased with increased rainfall depths. In this situation, the advantages of the VKS model are lessened due to less complexity in the shape of the release curve which diminish the differences in the effectiveness of both models.

Some insights into the change in shapes of the release curves can be obtained by noting that

$$n = \frac{d\left(ln\frac{N}{N_0}\right)}{d(lnw)}$$

Since $d\left(ln\frac{N}{N_0}\right) \cong \Delta N/N$ and $d(\ln(w)) \cong \Delta w/w$, one has

$$n \cong \frac{\Delta N/N}{\Delta w/w}$$

The value of n shows the relative change in the removed bacteria per unit of the relative change in rainfall depth. With the same relative change in rainfall depth, $\Delta w/w$, the larger n indicates a larger relative increase in removed bacteria.

The causes for the decreased values of *k* and *A* and the increased values of *n* may be explained by the die-off processes occurring within the soil and manure which would cause decreased concentrations and, therefore, less bacteria available for removal (Sistani et al., 2010). In addition to the reduction in the bacteria concentrations, weathering processes have sealed away bacteria within the internal structure of the manure which would need a prolonged rainfall at a high intensity to fully elute the manure solids (Hodgson et al., 2016). All indications are that the general availability of indicator bacteria available for removal in the rainfall water is changing.

During the initial rainfall event, the parameters *k* and *A* were greater for enterococci than for *E. coli* albeit by a small amount. During the 1-week and 2-week rainfall events, the *k* and *A* parameters were greater, sometimes significantly, for the *E. coli* than for the enterococci across all treatments (Table 5.1 and Fig. 5.1). This result was expected based on the poor survival of enterococci within the soil and manure which resulted in fewer cells available for release and removal. The values for parameter *n*

increased with the level of weathering for both organisms in both consistencies, but remained similar for the two organisms. Guber et al. (2007) found different release rates for *E. coli* and enterococci in both runoff and infiltration from bovine-slurry applied soil boxes under simulated rainfall. These authors found higher release rates for *E. coli* than for enterococci. This finding contradicts the results of the present study with both models having greater values of the release parameters for the enterococci in the LM treatments during the first week than the *E. coli* in the LM treatments. Similarly, Blaustein et al. (2016) found the release rates calculated from the EM, VKS, and B-S release models to be greater for *E. coli* than enterococci when fitting their runoff removal data. Both Guber et al. (2007) and Blaustein et al. (2016) attributed differences in the release of these two organisms to the different affinities of each indicator to either the liquid phase (*E. coli*) or solid phase (enterococci) of manure.

The average values of the exponents in the VKS model for the first week were 0.49 and 0.59 for *E. coli* and 0.7 and 0.67 for enterococci in the LM and SM treatments, respectively. These average exponent values are very close to the exponent in the modified universal soil loss equation as developed for sediment loss (Williams et al., 1975; U. S. BR, 2006) and later assumed as the default value of the exponent in the manure erosion model (Williams et al., 2006). This similarity makes sense for both manure types since the LM treatments are quickly infiltrated into the soil and the rainfall action must erode the soil surface layer in order to suspend bacteria into runoff. Additionally, the bacteria have been shown to associate with soil particles in runoff (Muirhead et al., 2006) so it is not surprising that sediment and bacteria release and removal are similar in some ways. For the bacteria within the solid manure, the rainfall

erodes the manure structure in a similar manner to the soil-rainfall interactions and results in the release of bacteria either as single cells or attached to manure colloids (Pachepsky et al., 2009; Muirhead et al., 2005).

During the first rainfall, the parameters *k* and *A* were much smaller for the infiltration removal data than the parameter values for runoff (Table 5.4). Conversely, the *n* parameter was approximately 3 times greater for the infiltration data across all the treatments than it was for the runoff. The parameter differences between the runoff and the infiltration are attributable to the slower movement of bacteria and water through the soil as opposed to water moving over the soil surface (Bradford et al., 2013). The higher value of *n* for removal with infiltration compared to runoff is also because of the absence of a major initial removal process similar to the observed behavior with runoff where the initial removed concentrations were much greater than the initial infiltration concentrations.

The infiltration parameters in this study are purely based on the water removed from the soil boxes. In reality, the rate constants are probably much larger and the *n* parameter much smaller when one considers the bacteria that remained within the soil since these bacteria infiltrated the soil, but were not completely removed from the soil. These types of measurements would have proved impossible in the current study. However, Blaustein et al., (2015) induced the release of *E. coli* and enterococci from solid dairy manure atop a mesh screen under simulated rainfall and collected runoff and infiltration samples. Although both runoff and infiltration were individually analyzed for bacterial content, these authors pooled the release of both modes of water transport into one cumulative release value in which they modeled using the EM model as well as the

Bradford-Schijven release model. At the 5% slope treatment, they recovered 97-99% of water as infiltration so the comparisons of their model parameter values and our model parameter values for infiltration are justified. They reported values of k as being 0.099 and 0.115 cm^{-1} for *E. coli* and enterococci, respectively. These values are 29.5 and 16 times greater than the respective averages of k from the infiltration removal data of the SM treatments in the present study which were $3.5 \cdot 10^{-3}$ and $7.18 \cdot 10^{-3}$ for *E. coli* and enterococci, respectively. The greater values of k reported in Blaustein et al. (2015) were most likely due to the absence of a soil body which microbes would have had to infiltrate and percolate through before being removed and measured with the infiltration.

The data on the microbial masses of bacteria released from manure is usually only available for runoff, but not for infiltration which limits the scope of the comparisons that may be made with other researchers. It would be interesting to compare the results of this study to other studies that model the release/removal of fecal bacteria with infiltration to see how the parameters change under other conditions such as with different soil textures and/or the differences in the thickness of the soil layer.

The differences in release kinetics between the *E. coli* and enterococci from the manure suggest microbe-specific release processes for different bacteria from manure at least at fine spatiotemporal scales (Blaustein et al., 2015). The *E. coli* and enterococci have been described to have preferences in either the liquid or solid phases of manure (Blaustein et al. 2015; Guber et al., 2007; Hodgson et al., 2009) which affects the rate at which they are released as well as when they are released in a given precipitation event. For instance, if *E. coli* reside in the liquid phase of manure, their release will be quick and occur largely in the earlier part of a rainfall event as incoming rainfall replaces liquids

within the manure (Guber et al. 2007). On the other hand, if enterococci preferentially exist in/on the solid phase of manure, their release will be slow and gradual and will be dependent more heavily on the duration and intensity of the rainfall. Additionally, the differences in cell physiology (i.e. gram-positive versus gram-negative, the presence of extra-cellular structures, cell size, surface chemistry etc…) have been suggested to play a role in the release, removal, and transport of bacteria from manure (Blaustein et al., 2015a; Lombard et al., 2011). While differences in model parameters were observed between both indicators in the present study, the final fractions removed with infiltration and runoff remained similar by the end of the initial rainfall event. It was not until subsequent rainfall events that substantial differences in removal were observed which was primarily due to rapid die-off of enterococci in soil and manure whereas *E. coli* grew in both mediums. As scale increases, the variability of the numbers of microorganisms in manure seem to increase as well (Guber et al., 2011). The increased variability of numbers of fecal bacteria in manure applied to agricultural fields may mask the differences in the release and removal of different bacterial groups/species from the manure under rainfall or irrigation (Blaustein et al., 2015). More experiments are needed to assess the large-scale release and removal of fecal bacteria from manure amendments on agricultural fields.

The VKS model was found to be superior to the linear exponential model in simulating the removal of *E. coli* and enterococci from the liquid and solid manure treatments in all rainfall events as determined by consistently lower RMSE and AICc values as well as higher R^2 values (Table 5.3. and Figure.5.3.). Similarly, Guber (2006) reported smaller RMSE values for the VKS model than for the linear exponential model

when fitting runoff removal curves for fecal coliforms eluted from vegetated lysimeter plots under simulated rainfall. The authors attributed the poor performance of the linear model to having only one parameter that evidently could not account for the two different release mechanisms. These results conflict with the findings of Blaustein et al. (2016) who reported smaller RMSE and AICCc values for the LM model than for the VKS model when fitting these equations to their runoff data for *E. coli* and enterococci. However, in their work, the VKS model was found to better simulate the release of total coliforms than the LM model.

The VKS model provided superior simulation results in comparison with the EM model for the removal of both organisms with infiltration (Table 5.8). Similar to the runoff removal data, the gaps between the RMSE of both models became smaller over time. Once again these RMSE reductions may be due to the decreased importance of parameter n over time as a parameter needed to describe more complex release behaviors.

The differences in the RMSE values between the two models became less with increased weathering. These RMSE reductions were due to the decreased complexity of the removal data during subsequent rainfall events. The parameter *n* grew closer to 1.0 across all treatments and when the *n* value reaches a values of 1.0, the linear exponential model and VKS model provides almost identical results in their simulations.

An evaluation of the degree of soil-runoff mixing was performed by analyzing the concentrations of *E. coli* and enterococci in the runoff and relating these concentrations to the flow rates of runoff and infiltration. This analysis was only performed for the LM treatments since the SM treatments would not have been a good representation of soil

mixing processes as unknown amounts of manure solids remained at the soil surface after the initial rainfall event. The values of α were substantially lower than 1 for both indicators during the first rainfall event. Values less than 1 indicates a largely incomplete mixing process that may be interpreted in one of two ways: α × 100 % of the area has 100% mixing occurring or there is not enough time for complete mixing to occur and only α × 100 % occurs within the duration of the 60 minutes of runoff collection. The latter scenario seems to make more sense since the values of α for *E. coli* and enterococci differ at 14.3% and 12.5%, respectively. In the former scenario, the different values of α are not logical since the same area was used for both indicators. Ahuja and Lehman (1983) proposed two physical reasons for the incomplete mixing of rain water and soil solution within the EDI: (i) the degree of mixing decreases within depth below the soil surface because of the decrease with depth in hydraulic turbulence produced by raindrops; and (ii) the soil solution present within the soil aggregates does not mix instantaneously with rain water. When infiltration is restricted, the chemicals within soil may be transferred to runoff from an appreciable depth below the surface as the solute concentration in overland flow has been found to be negatively correlated with infiltration rates (Snyder and Woolhiser, 1985; Ahuja and Lehman, 1983). In the present study, there was no restriction of infiltration and infiltration volumes remained high although they were almost always lower than the runoff volumes during the initial rainfall event (Table 5.9).

The mean values of α were larger for *E. coli* than for enterococci during the first rainfall. These differences may be because the *E. coli* concentration in the applied manure was higher than the enterococci concentration so more *E. coli* cells were simply

available for transport. Alternatively, Nola et al. (2008) stated the microbial attachment to surfaces seemed to be a competitive process between *E. coli* and enterococci in which enterococci is favored to win. This premise would have result in more *E. coli* being incorporated into runoff from the soil than enterococci.

The values of α decreased in the subsequent rainfall events probably due to some combination of increased attachment of cells to soil at the surface, increased infiltration rates relative to the first week, and surface crusting processes that, while letting water infiltrate through cracks, reduced the effectiveness of the rainfall action at breaking apart the soil. Biofilm formation within the soil may likely have occurred to some extent following the first rainfall event. The adhesive properties of biofilms may increase the retention of bacteria within the soil by providing attachment sites for the extra cellular polymeric substances, the cell walls, the lipid membranes, as well as the cytoplasm (Engstrom et al., 2015; Strathmann et al., 2007). Moreover, the longer delays prior to the initiation of runoff probably resulted in a sizeable number of bacteria infiltrating into the soil that were later released at the surface. Vadas et al. (2011) used nine different studies on concentrations of phosphorus in the runoff to conclude that when both the delay to the initiation of runoff as well as the rainfall:runoff ratio are large, that smaller concentrations of phosphorous are likely removed with the runoff. This finding is based on the loss of released phosphorous, either from soil or manure, to infiltration in the time it takes for the soil to saturate. Thus, in this time, relatively high concentrations of released soil and manure constituents are not measured in runoff as they have been removed in infiltration prior to the initiation of runoff (Vadas et al., 2011). During this time dilution also occurs due to the large volumes of rainfall applied relative to the runoff

volumes collected. Dao et al. (2008) demonstrated the similarity of the release of *E. coli* and enterococci with that of phosphorous from a manure source so it is reasonable to think that the same phenomena as described in Vadas et al. (2011) for phosphorous may occur for manure-borne bacteria.

Similar to the value of α, the value of *B* also declined during subsequent rainfall events. Many of the same explanations for the smaller α values help explain the lower *B* values. In addition, the microbial die-off for both indicators likely lowered the number of cells available for release from the soil into the runoff. This result is very evident with the enterococci concentrations, but the *E. coli* concentrations within soil remained relatively high at the start of each subsequent rainfall event (Figs. 5.5 and 5.6). Still, these concentrations probably pale in comparison to the concentrations within the top soil layer immediately following liquid manure application and are thought to likely be orders of magnitude higher. The values of both α and *B* were likely diminished during all the rainfall events by the presence of the dense surface vegetation in the soil boxes which would have greatly attenuated the force associated with rain drop impacts on the soil and manure surfaces. When the delivery of this kinetic energy to the surface soil is impeded, the transfer of solutes to overland flow is less efficient (Shi et al., 2011).

Improved estimations of the soil mixing layer depth is important to improve the accuracy of models that incorporate the soil as a reservoir for fecal bacteria in the environment. Underestimating the depth of the soil mixing layer can result in underestimation of the concentration of solutes from the soil that can exchange with runoff. Ahuja and Lehman (1983) showed that the release of soil constituents to runoff may be from soil depths as great as 2 cm which in the present study comprised the two

soil depth ranges with the highest concentrations of fecal bacteria. Other authors have noted high concentrations of fecal bacteria in the uppermost soil layers following the release of indicator bacteria from animal manures. Blaustein et al. (2016) found that the top 2 cm had the greatest concentrations of fecal bacteria following application of solid dairy manure to vegetated soil and then exposed to simulated rainfall. Fenlon et al. (2000) applied manure slurry to a lysimeter site and measured the *E. coli* concentrations in the soil depth ranges of 0 – 2.5 cm, 2.5 – 5 cm, and 5 to 20 cm and found consistently higher concentrations in the top 2.5 cm of soil over the 29-day study. The explanations for the large abundance of fecal bacteria within the top soil layers relative to the lower layers includes bioclogging or cell aggregation and straining of cells by small soil pores (Engstrom et al., 2015). The fecal bacteria released from manure are also known to associate to some degree with manure colloids and particulates and have been shown to be transported along with them (Dao et al., 2008; Pachepsky et al. 2009). Attached bacteria may infiltrate and percolate to different extents within the soil as governed by the depth-dependent movement of manure colloids that have entered the soil (Bradford et al., 2006). It is understandable to conclude that the straining of planktonic and particle-associated cells seems to occurs most heavily in the top layers of the soil.

The top 1 cm is often assumed to be an adequate estimation of the effective depth of interaction between the runoff and the soil (Muirhead, 2009; Muirhead and Monaghan, 2012; Cho et al., 2016; Neitsch et al., 2011; Benham et al., 2006). This layer has been reported to be as thin as 0.2 – 0.3 cm (Ahuja et al., 1981) however, sampling soil in depth increments less than 1 – cm is not practical and so the top 1 cm is what is commonly sampled (Muirhead, 2009). High concentrations of *E. coli* remained within the top 2-cm

of soil in the present study and in other similar studies (Blaustein et al. 2016; Fenlon et al., 2000; Ling et al., 2009). Perhaps the EDI used in studies or employed in field applications should be a site- and situation specific value. In scenarios with dense vegetative cover and/or when infiltration is restricted, such as in fine-textured soils, the EDI values should be decreased or increased, respectively. The soil slope, rainfall intensity, and soil water content have also been shown to substantially increase the EDI (Yang et al., 2016). With an increased EDI, there is more soil loss to runoff and, therefore, greater release of soil-bound bacteria as well as any planktonic cells existing within the EDI (Sharpley et al., 1988; Muirhead et al., 2005). This release highlights the importance of incorporating depth-dependent analysis of soils of interest as a modification of EDI since it is not very useful without the knowledge of solute concentrations at the depth ranges that it spans. The depth-dependent observations of fecal bacteria within soil following manure application and with or without simulated rainfall is currently severely lacking in both laboratory and field settings.

5.5 Conclusions

The removal of *E. coli* and enterococci with the runoff and infiltration from manure-amended vegetated soil under simulated rainfall was modeled using two bacterial release models. The parameters gained from fitting the one-parameter EM model and the two-parameter VKS model to the experimental data were then compared and model evaluation was performed. The rate constants k and A, from the EM and VKS models, respectively, were determined from fitting runoff data. The parameter values were similar throughout all rainfall events, however, the values of A were consistently higher than the corresponding values of k. The values of n from the VKS model increased with each

subsequent rainfall event and corresponded with the simpler patterns displayed by the runoff removal data over time. The increasing *n* values were primarily attributed to the microbial die-off and the manure weathering processes that inhibited the release of bacteria from the manure and soil.

The above parameter trends for runoff were in complete contrast to those trends observed for infiltration. The parameter values of *k* and *A* from fitting the infiltration data were similar, but the *k* values were consistently greater than the *A* values. The *n* values for infiltration almost always decreased in time such that the lowest values were observed during the 2-week rainfall event. This decreased value meant that a more precipitous removal of bacteria with infiltration was occurring during the 2-week rainfall event than during the initial rainfall event. These differences were attributed to the massive population growth displayed by *E. coli* within the soil following the first rainfall event.

Nearly all the runoff parameters for both organisms were significantly impacted by the level of weathering whereas the consistency did not significantly affect any runoff parameter during any rainfall event. Conversely, the infiltration parameters were largely unaffected by the weathering processes, but the consistency was found to have more significant impacts on indicator removal with infiltration. The exception to this finding was when the *n* parameter was significantly smaller during the 2-week rainfall then during the 1-week rainfall.

The VKS model was found to perform better in simulating the removal of *E. coli* and enterococci in both the runoff and infiltration throughout all the rainfall events in the present study. The addition of a second parameter, *n*, which governed the shape of the

removal curves evidently resulted in consistently lower RMSE and AICc values and higher R^2 values than the one-parametric EM model. The greater error associated with the one-parametric model likely occurred due to the inability of the *k* as a single parameter alone to adequately describe multiple release/removal mechanisms.

In the present study, it was shown that the soil-borne populations of both *E. coli* and enterococci could be mobilized during rainfall through a consortia of processes that occurred within the soil mixing zone. Once mobilized, these bacteria either infiltrate into the soil or are transported with runoff. We showed that the mixing of the fecal bacteria between the soil and runoff, as mediated by rainfall, is largely governed by an incomplete mixing process whereby only a small fraction of the soil bacteria are mixing with the runoff. The small degree of mixing was primarily attributed to the dense vegetative cover that reduced the ability of the incoming rainfall to free the soil-bound bacteria. Despite the relatively low degree of mixing, there were massive numbers of *E. coli* removed from the soil boxes within the runoff even at the small scale of this study. At the field-scale, it can be expected that even if the α values are low that massive numbers of fecal bacteria from the soil reservoir may still be transported within runoff to surface waters. More research is needed to study the mixing layer concept as it is applied specifically to the transport of microorganisms in the environment. A more complete understanding of processes within the mixing layer will help to improve predictive watershed-scale models aimed at water quality-forecasting and, therefore, may minimize the risk of human illness derived from manure-borne bacteria.

Chapter 6 – General Conclusions

The release, survival, and removal of fecal indicator bacteria from manure is an important component of microbial fate and transport because it helps determine the degree of potential microbial contamination in surface waters that may pose risks to human health. The release of bacteria from manure is governed by factors such as manure consistency, level of weathering, rainfall intensity and duration, rainwater solution chemistry, and the preference of microbe habitats within the manure structure. The survival of fecal microbes in soil after release will be determined largely by the environmental conditions within the soil habitat such as temperature, moisture and nutrient availability, texture class, the level of competition from native soil biota, and in the case of microbes at the soil surface, inactivation due to sunlight. It is important to understand the distinction between release and removal. The removal of fecal microbes from pastures or fields depends on their release and hydrologic conditions. If runoff or infiltration events do not occur, the released microorganisms are not removed from the land and their numbers will remain dependent on the rate of die-off. The removal of fecal microbes is dictated by the volume of water applied, the antecedent moisture conditions of the soil and manure, the rates of runoff and infiltration, the interaction between runoff and the soil surface, types and abundance of surface vegetation, and the time delay between the start of precipitation and the initiation of runoff.

Studies were conducted to evaluate the effects of rainfall intensity on the survival of fecal bacteria in the soil after microbial release, determine how manure consistency and the level of weathering affected the release, survival, and removal of fecal bacteria, and investigate how the levels of fecal microorganism contamination vary by soil depth. The secondary objective was to fit the experimental results to existing models and, thereby, obtain parameters that might be used in the future to develop improved microbial release and transport simulation models for field application. Once the study results were fitted, an evaluation of model performance was performed to determine which model should be used to simulate fecal bacteria release and removal processes. Results and observations obtained from this work are detailed below.

Chapter 3. The laboratory rainfall simulation study was conducted to assess the effect of rainfall intensity on the profile distributions of manure-borne indicator organisms, *E. coli* and enterococci in soil. Conclusions are as follows:

- Rainfall intensity had a significant impact on initial concentrations of indicator bacteria released from manure onto soil
    - Increasing rainfall intensity resulted in reduced concentrations of the released bacteria
- Concentrations of indicator bacteria decreased with increasing depth
    - *E. coli* and enterococci persisted in soil layers up to a 10 cm depth.
    - Most of the released bacteria remained within the 0 – 1 cm surface layer
        - This layer is important because it is regarded as the soil mixing layer for resuspension of manure particles and bacteria during subsequent rainfall-runoff events

- Bacterial survival was the greatest in the surface layer
    - *E. coli* experienced initial growth phases in all treatments and was detected in high concentrations in the soil 28 days after the release event
    - Enterococci experienced rapid initial population declines and was determined to not be as hearty as *E. coli* in our study
    - The total numbers of *E. coli* cells increased at all rainfall intensities between simulated rainfall and 1-week afterwards, whereas enterococci declined in each treatment and at each depth.
    - Rainfall intensity was identified as a factor in the persistence of both indicators within soil after being released.

Chapter 4 - The laboratory rainfall simulation study was conducted to assess the effects of manure consistence and weathering duration between irrigations on the release and removal of manure-borne indicator organisms, *E. coli* and enterococci in soil. Conclusions are as follows:

- The release and removal of fecal indicator bacteria did not significantly differ between liquid and solid manure consistencies in either runoff or infiltration when the manure was fresh.
- The duration of the manure weathering between the rainfall events was found to significantly impact the numbers of bacteria removed within runoff and infiltration.
    - The enterococci removal within runoff was significantly reduced with the increased duration of manure weathering.

- The *E. coli* removal within runoff was not significantly affected by the duration of weathering or the manure consistency.
- Both indicator organisms were removed in significantly higher numbers with infiltration in the liquid manure treatments than in the solid manure treatments
    - The enterococci did not experience population growth after manure application, but the numbers within soil remained sufficiently high so that the numbers removed in time were not significantly different over the time of observations.

- The *E. coli* and enterococci survival dynamics were different, and there were differences observed between survival within the liquid and solid manures.
    - The *E. coli* experienced growth in all treatments, but the growth was larger in the liquid manure treatments and persisted for all treatments throughout the 28 days of the study.
    - The enterococci survival within soil was very poor for all treatments, and it was not possible to detect enterococci by the end of the study.
    - The weathering significantly reduced the number of both indicators within the soil.
    - There were significant differences in indicator populations detected within depth ranges throughout the study.
    - The highest concentrations of indicators were found within the top 1 cm of soil although this result is somewhat masked when the relative fractions ($N_{soil}/N_{applied}$) are reported.

- Both indicators were detected in the manure 28-days after the start of the experiment and after a total of two rainfall events separated by either 1- or 2-weeks.

Chapter 5 - The data analysis was performed to parameterize and compare removal of indicator bacteria from manured soils under simulated rainfall in laboratory conditions. Liquid and solid manure were used and were evaluated after one and two weeks of weathering. The onclusions are as follows:

- The Vadas- Kleinman -Sharpley model was determined to be superior to the EM model for simulating the release/removal of both *E. coli* and enterococci from both manure types and at every level of manure weathering.
- The parameters of both models did not statistically differ by consistency during the initial rainfall event.
- The level of weathering significantly impacted certain runoff parameters for both organisms, but the fitting parameters did not significantly differ between consistency types. Conversely, the infiltration parameters were mostly similar for the two levels of weathering and were significantly different in most cases between consistency types.
- The rate constants from both models after being fit to the runoff data decreased with the weathering duration, but the shape parameter *n* from the Vadas-Kleinman -Sharpley model increased with time as the concavity of the shape of the release curves was reduced.
- The degree of soil and runoff mixing were determined to be very low when the mixing depth was set to 1 cm.

- The values of the degree of mixing ranged from 0.086 to 0.221 for *E. coli* and 0.001 to 0.022 for enterococci in the first rainfall event and indicated a largely incomplete mixing had occurred.
- The degree of mixing decreased with subsequent rainfall events, ranging from 0.033 to 0.085 for *E. coli* and 0.026 to 0.086 for enterococci.

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