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Seasonal Initial Concentrations and In-Field Decay Rates of *Escherichia coli* and Bovine *Bacteroidetes* in Beef Cattle Manure

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To the Graduate Council:

I am submitting herewith a thesis written by Jiangwei Liu entitled "Seasonal Initial Concentrations and In-Field Decay Rates of *Escherichia coli* and Bovine *Bacteroidetes* in Beef Cattle Manure." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biosystems Engineering.

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Seasonal Initial Concentrations and In-Field Decay Rates of *Escherichia coli* and Bovine *Bacteroidetes* in Beef Cattle Manure

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Degree

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DEDICATION

I would like to dedicate my work to my family, my friends and my advisor for their continuous support and steady encouragement.

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ABSTRACT

Eight naturally deposited beef cow manure patties were sampled during summer (July 19 to August 9, 2010), fall (October 26 to November 19, 2010), winter (January 14 to February 18, 2011), and spring (May 5-27, 2011) to determine whether hypothesized seasonal differences existed in the initial concentrations and decay rates of *Escherichia coli* (*E. coli*) and bovine *Bacteroidetes* (BoBac). *E. coli* concentrations were estimated as culturable colony forming units (CFU) and with a quantitative polymerase chain reaction (qPCR) assay targeting the 23S ribosomal gene. BoBac was quantified with a qPCR assay targeting a 16S ribosomal gene sequence associated with cattle manure.

Initial concentrations for culturable *E. coli* varied several orders of magnitude during each season, but were significantly lower when the animals grazed fresh forage (3.6 and 4.3 $\log_{10}$CFU/g-dry-manure in fall and spring, respectively) versus receiving hay and grain because of dormant pastures (6.4 $\log_{10}$CFU/g-dry-manure in winter). Average initial *E. coli* 23S gene abundance was also highly variable but lower in the spring and fall (7.1 and 8.5 $\log_{10}$copies/g-dry-manure) than in the winter (9.4 $\log_{10}$copies/g-dry-manure). Average initial BoBac 16S gene abundance was much less variable but again lower during grazing (9.9 $\log_{10}$copies/g-dry-manure in both spring and fall) versus during supplemental feeding (11.0 and 11.2 $\log_{10}$copies/g-dry-manure in summer and winter, respectively).

Linear regressions of aggregated log transformed concentration data were used to calculate seasonal decay rate coefficients. The decay rate for culturable *E. coli* was highest in the winter (-0.094 log_{10}CFU/g-dry-manure/day) and significantly lower in the fall and spring (-0.028 and +0.018 log_{10}CFU/g-dry-manure/day, respectively). The same was true for *E. coli* 23S gene abundance (-0.086, -0.026, and +0.023 log_{10}copies/g-dry-manure/day in winter, fall, and spring, respectively). The decay rates were far higher for BoBac 16S gene abundance which had an opposite seasonal trend, being much higher in the summer (-0.33 log_{10}copies/g-dry-manure/day) than in the winter (-0.10 log_{10}copies/g-dry-manure/day).

The fact that initial bacterial concentrations and decay rates vary seasonally should be considered when modeling the fate and transport of the regulatory fecal pollution indicator *E. coli* and the fecal pollution source tracking BoBac gene sequence.

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CHAPTER I

INTRODUCTION

1.1 Current Problems

The threat of water borne disease is a concern addressed by the U. S. Environmental Protection Agency (EPA). EPA uses *Escherichia coli* (*E. coli*) as a general "pathogen/fecal pollution indicator" to evaluate surface water quality because this bacteria correlates with the outbreak of gastrointestinal illness (U.S.EPA, 1986). Other non-regulatory bacteria are used as specific fecal pollution "source trackers." For example, bovine *Bacteroidetes* (BoBac) can be used to identify fecal waste pollution from cattle and are generally not associated with human or other animal fecal waste (Layton et al., 2006).

Manure from beef cattle is a potentially important source of *E. coli* and pathogenic bacteria that can deteriorate surface water quality for recreational use (Berry et al., 2010; Doran & Linn, 1979; Larney et al., 2003; Moore, 1982). In Tennessee, "pathogens" are a leading cause of wadeable stream recreation use impairment, accounting for 24% of all impaired stream miles (TDEC, 2010). Further, the leading source of surface water impairment in Tennessee is reported as agriculture and particularly "cattle grazing in the riparian zone" (TDEC, 2010).

When dairy and beef cattle are raised in confinement, fecal waste is usually collected, stored, and land applied during favorable weather conditions, when crop nutrient demands are high and under strict environmental regulations (Grewal et al., 2006; Islam et al., 2004; Shepherd et al., 2007; Sinton et al., 2007). This is not the case for pastured beef cattle, which far outnumber confined cattle in states like Tennessee. Manure from pastured beef cattle is deposited directly onto fields continuously in all weather conditions, and storm runoff from these pastures generally receives no treatment or storage to reduce potential pathogen concentrations. The potential health risk posed by movement of these contaminants (though subsurface rather than surface water movement) was recently demonstrated by a survey of 144 private water supplies in the Netherlands. This study revealed that 11% of water supplies on campsites in agricultural areas with large grazer densities were positive for fecal pollution indicators and 2.7% contained the pathogen *E. coli* O157:H7 (Schets et al., 2005).

Watershed models can be used to assess such environmental risks, to help understand the fate and transport of fecal bacteria from pastures, and to identify effective Best Management Practices (BMPs) to control such nonpoint sources of pollution. One such model, the Soil and Water Assessment Tool (SWAT), is designed for use in rural, largely agricultural watersheds (Benham et al., 2006; Jamieson et al., 2004; Jamieson et al., 2003). This model includes subroutines to model the fate and transport of two types of bacteria using set initial manure concentrations and decay or die-off rate coefficients. Such models can aid understanding of the survival of *E. coli* and beef cattle

indicator bacteria and ensuring surface water contamination. These are critical considerations for regulators who must reduce the health risk posed by livestock fecal waste pollution of surface waters.

In reality, beef cattle fecal bacteria likely have different decay patterns. For example, *E. coli* is commonly found in the lower intestine of warm-blooded organisms in low relative concentrations, but as a facultative anaerobe has the ability to survive in manure and soils under certain circumstances (Avery et al., 2004; Berry & Miller, 2005; Berry et al., 2007; Mubiru et al., 2000; Topp et al., 2003; Vinten et al., 2002; Wang et al., 1996a). Bacteria belonging to the phylum *Bacteroidetes* make up a far more significant part of fecal bacteria populations than *E. coli* (Fiksdal et al., 1985), representing approximately 30-40% of all fecal bacteria (Harmsen et al., 2000; Hayashi et al., 2002; Suau et al., 1999; Wang et al., 1996b). It's been reported that *Bacteroidetes* spp. found in the digestive tract of mammals have little potential for growth in the environment because they are strictly anaerobic (Bell et al., 2009; Fiksdal et al., 1985; Kreader, 1998).

One of the most important factors affecting the initial bacteria concentration in cattle manure is diet. This is an important consideration for water quality considerations because *E. coli* tends to be higher in fecal waste from cattle fed grain as opposed to pasture forages (Callaway et al., 2009; Lowe et al., 2010). Also, for pastured animals the diet varies in a predictable seasonal manner in states like Tennessee. In the summer, cattle feed on degraded pasture and are often given supplemental hay; in the

fall, cattle typically feed on pastures flush with cool season grasses; in the winter, pastures are dormant so beef cattle must be fed hay and/or grain silage; in the spring, cattle again feed on pastures flush with cool season grasses. Thus, it is important to measure initial bacteria concentrations and determine decay rates in different seasons that are associated with different feeding regimens, but this important work has not been conducted.

The vast majority of the studies on the fate of fecal bacteria from beef cattle involve inoculated organisms and/or studies confined to controlled labs. Few in-field survivability studies for *E. coli* in beef cattle manure directly deposited in pastures have been carried out and none have been conducted for BoBac. The decay rate coefficients derived from laboratory studies with constant environmental parameters (temperatures, humidity, light intensity, etc.) are generally different from field-based seasonal die-off rate coefficients (Himathongkham et al., 1999; Muirhead & Littlejohn, 2009; Oliver et al., 2006; Soupir et al., 2008; Van Kessel et al., 2007b; Wang et al., 2004).

1.2 Objectives and Hypotheses

The overall purpose of this work was to measure and compare the initial populations and in-field decay rates of *E. coli* and BoBac in beef cattle manure naturally deposited in a pasture during four seasons with different feeding regimens.

The specific objectives of this research were to:

1. Evaluate *E. coli* concentrations in beef cattle manure naturally deposited in a pasture using a most probable number (MPN) assay for culturable organisms and a quantitative polymerase chain reaction (qPCR) assay measuring the abundance of *E. coli* 23S gene sequences.
2. Evaluate BoBac concentrations in beef cattle manure naturally deposited in a pasture using a quantitative polymerase chain reaction (qPCR) assay measuring the abundance of *Bacteroidetes* 16S gene sequences.
3. Compare and contrast the initial *E. coli* and BoBac concentrations in naturally deposited beef cattle manure and the seasonal and feeding effects thereon.
4. Compare and contrast the decay rate coefficients for *E. coli* and BoBac and the seasonal and feed effects thereon.

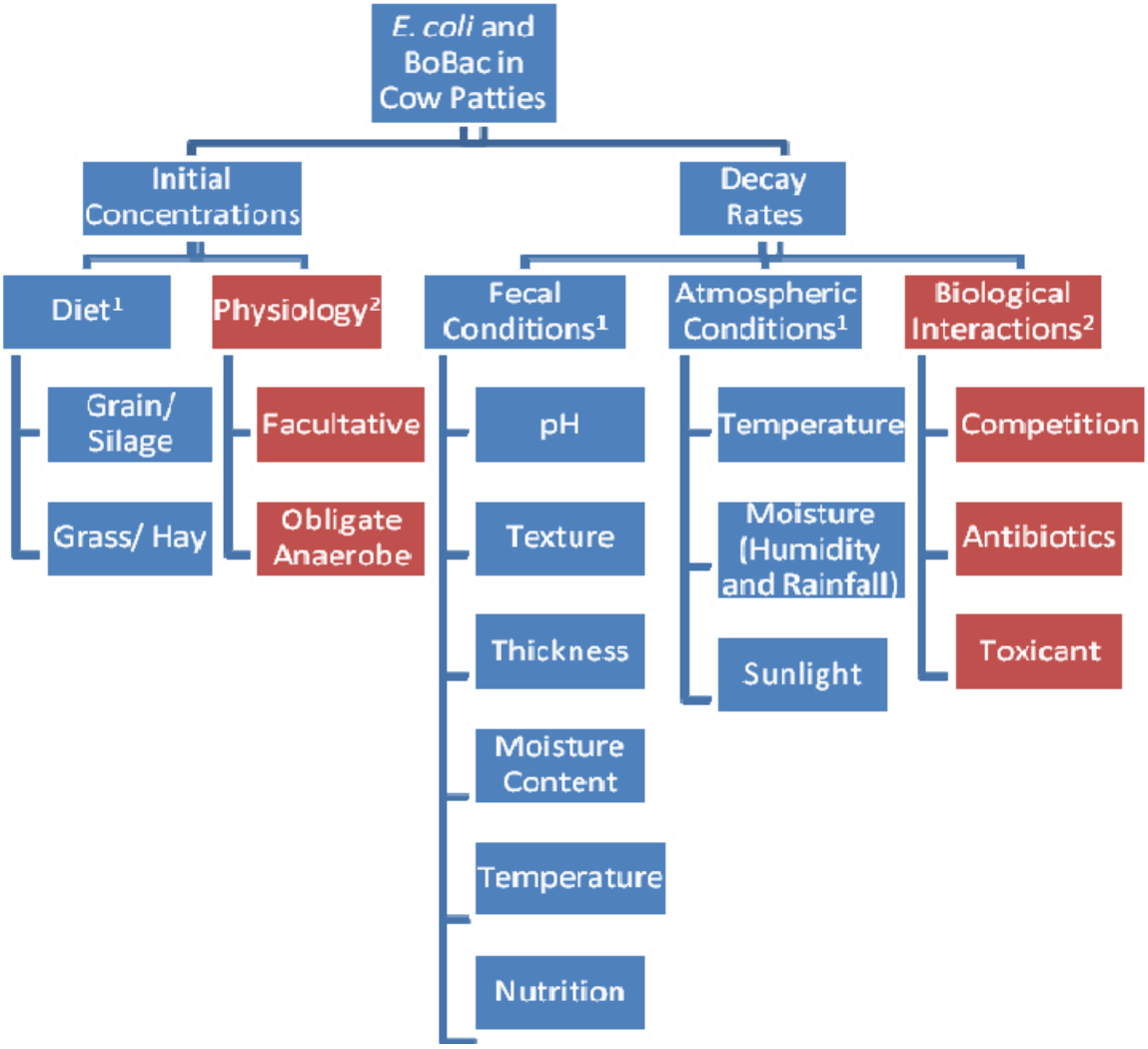
The null hypotheses of this study were that the seasonal initial concentrations and in-field decay rates did not vary for culturable *E. coli* and *E. coli* 23S gene abundance or BoBac 16S gene abundance. The alternate hypotheses were that the seasonal initial concentrations and in-field decay rates would vary seasonally.

CHAPTER II

LITERATURE REVIEW

Modeling livestock fecal bacteria transport and water resource contamination naturally begins with wash off from the source manure during rainfall runoff (Benham et al., 2006). In fact, significant correlations between the *E. coli* concentrations in source cow manure and associated runoff have been documented (Muirhead et al., 2005). Thus, the initial fecal bacteria concentration must be accurately estimated for the prediction of runoff concentrations. After manure is deposited on the land surface, die-off or decay rates continue to impact future rainfall runoff concentrations and pollution of water resources, but decay rates can be affected by many factors and are poorly documented, particularly using in-field manure sampling that accounts for integrated seasonal temperature and feeding regimen effects.

Figure 1 is as a conceptual model listing important variables affecting the initial concentrations and decay rates of *E. coli*, a regulatory water pollution indicator, and bovine *Bacteroidetes* (BoBac), a clade of bacteria that can be used to track the source of fecal waste pollution of surface waters. Some of the factors, such as initial concentrations, fecal moisture content and integrated atmospheric conditions and diet (referred to as seasonal changes in this study) have been examined in the literature.



[1] Varies by season.

[2] Varies by bacteria.

Figure 1. Conceptual model of the factors influencing the initial concentrations and decay rates of *E. coli* and BoBac in beef cattle manure.

2.1 Diet Influences on Cattle Manure *E. coli* Concentrations

Cattle, as ruminant animals, have evolved to digest cellulosic material (Huntington, 1997). However, grains are often supplied in order to increase animal production or due to the lack of fresh forage according to regular seasonal patterns. Although grain starch can be degraded by ruminal microbes, it generally is enclosed and protected by a protein coat and enters the small intestine; some directly reaches the cecum and colon due to the low pancreatic amylase activity of ruminants. *E. coli* strains have been shown to thrive in cattle fed large amounts of grain because of their ability to ferment sugar released from starch in the colon (Huntington, 1997).

Manure *E. coli* levels from cattle fed corn and soybean meal were more than 100-fold higher than cattle fed good quality hay (Diez-Gonzalez et al., 1998). When cattle were abruptly switched from a high grain diet to a forage diet, generic and acid-shock resistant *E. coli* populations declined 1,000-fold and 100,000-fold, respectively, within 5 days (Diez-Gonzalez et al., 1998). Other studies confirm that a switch from grain to hay caused a decrease in *E. coli* populations, although the affect was less pronounced. However, it is clear that switching cattle from grain to forage could potentially reduce *E. coli* populations, and thus their availability for rainfall runoff transport and subsequent water resource contamination (Callaway et al., 2003; Scott, 2000).

In a study of 200 cattle originally feeding on grain, 52% maintained on the grain ration were positive for *E. coli* O 157:H7, while only 18% of the cattle transitioned to hay were positive (Kudva et al., 1997). *E. coli* O157:H7 was significantly higher in manure from grain versus hay fed cattle, and survival was longest in manure from grain-fed cattle (Lowe et al., 2010). Lower average levels of verocytotoxin-producing *E. coli* shedding in beef finishing cattle were associated with animals being maintained on pasture. (Gunn et al., 2007). These outcomes suggest that diet affects *E. coli* initial concentrations in beef cattle manure.

However, there are also studies that do not report higher *E. coli* concentrations n manure from cattle fed grain as opposed to forage. One such study showed that the *E. coli* concentrations in cattle manure decreased with a sudden reduction of hay intake (Brownlie & Grau, 1967). Experimentally infected sheep feeding on poor quality grass shed much more *E. coli* than when switched to a corn/alfalfa diet (Kudva et al., 1997). In a separate study, grain versus hay feeding did not affect survival or acid resistance of *E. coli* O157:H7 in the rumen (Grauke et al., 2003). However, the different quantification and culture methodologies used in these studies make it difficult to generalize the findings (Jarvis & Russell, 2001). Conflicting results for the effect of diet on initial *E. coli* concentrations in cattle manure, and lack of such data for BoBac, indicate more study is necessary.

2.2 Factors Influencing *E. coli* and BoBac Survival in Cattle Manure

The integrated mechanisms and environmental factors that affect bacterial survival times (down to undetectable levels) in naturally deposited livestock waste are unclear. Lab-based experiments indicate a wide range of potential factors influence bacterial survivability, including soil properties, temperature, sunlight, humidity, rainfall, animal feed type, competition among organisms, bacterial density, waste application process, and management practices (Crane & Moore, 1986; Lowe et al., 2010).

Pathogenic *E. coli* strains are well studied in terms of survivability (measured as detectable culturable bacteria) in the laboratory, having been inoculated into livestock manure and subsequently shown to persist up to several months with very low decay coefficients under certain conditions (Bolton et al., 1999; Himathongkham et al., 1999; Kudva et al., 1998; Wang et al., 1996a). *E. coli* O157:H7 inoculated into bovine feces survived 42 to 49 days at 37°C and 49 to 56 days at 22°C at low manure moisture content (10%), and 63 to 70 days at 5°C at a high manure moisture content (74%) (Wang et al., 1996a). In another study, *E. coli* O157:H7 survival was longest (14 days) in feces from grain-fed cattle at 25°C (Lowe et al., 2010). Total *E. coli*, previously indicated to have slower decay rate than the pathogenic strain O157:H7, declined below the detection limit after 26 days in soil contaminated with swine manure at 25°C (Cools et al., 2001).

Few survivability studies have been conducted for *E. coli* in naturally deposited beef cattle manure. *E. coli* was detectable in a fecal waste contaminated soil up to 162 days after direct deposition by cattle (Avery et al., 2004). *E. coli* O157:H7 survived at a level of 1,000 CFU/g soil for up to 171 days after beef cattle runoff was discharged onto a bromegrass treatment area (Berry et al., 2007). Thus, the long-term survival of *E. coli* in bovine feces and contaminated pasture soils may be an important factor in contamination of surface water resources.

Some survivability study results have compared field and lab results, revealing contradictory findings. For example, *E. coli* O157:H7 in bovine feces decreased by 4.5-5.5 $\log_{10}$CFU/g-wet-manure within 99 days in closed plastic containers and by 4.0-5.5 $\log_{10}$CFU/g-wet-manure within 50 days in samples decanted onto grassland soil (Bolton et al., 1999). *E.coli* die-off rates were 0.205 and 0.230 natural log CFU/day in open and shaded field environments versus 0.08, 0.125, and 0.166 at 21, 27 and 32°C in a laboratory setting (Bolton et al., 1999; Van Kessel et al., 2007a). Even when exposed, survivability varies with the exposure conditions. For example, *E. coli* O157:H7 was detected for more than 1 year in non-aerated sheep manure, but only survived 4 months in similar aerated manure (Kudva et al., 1998).

Few survivability studies have been conducted for fecal source tracking organisms, and those that have been conducted for cattle manure indicate survivability may differ from *E. coli*. For example, ovine *Bacteroidetes* in unfiltered stream water microcosms

declined more slowly at lower versus higher temperatures. This trend for increasing decay rates with increasing temperature was opposite of the trend for fecal coliforms, which tended to decay faster at 4°C (Okabe & Shimazu, 2007). A feces-waste-derived human-specific *Bacteroidetes* 16S rRNA genetic marker has also been reported to persist longer (more than 24 days) at lower temperatures of 4 and 12 degree°C (Seurinck et al., 2005) and decay characteristics for fecal *Bacteroidetes* 16S ribosomal genes does appear to be similar between cattle, human and pig waste (Okabe & Shimazu, 2007). Other researchers report this same trend of increasing decay rates with increasing temperatures for an equine *Bacteroidetes* genetic marker spiked into surface water samples (Bell et al., 2009). However, *Bacteroidetes* appeared to survive in the anaerobic portion of an aerobic microcosm (aerobically incubated sewage sludge), which indicates the prolonged possibility of transport from a partially anaerobic environment like in-field cattle manure (Walters & Field, 2006).

In summary, there are a host of studies estimating *E. coli* degradation rates and survival periods in cattle manure, particularly for the pathogenic strain O157:H7. However, no studies have been undertaken to similarly examine BoBac decay rates. Such a study would best consider seasonal environmental effects like temperature as well as regular feeding pattern changes. Because the results of past *E. coli* studies have been divergent, such a study would best quantify both BoBac and culturalble *E. coli* and E. coli genetic markers for proper context and to aid interpretation of the results.

2.3 Bacterial Growth in Cattle Manure

E. coli can grow in manure, particularly during the first week after feces is deposited, as has been reported under both field and lab conditions (Conner & Kotrola, 1995; Soupir et al., 2008; Van Kessel et al., 2007a; Wang et al., 1996a; Wang et al., 2004). Van Kessel et al. (2007) found *E. coli* numbers in manually composited manure samples from three cow herds increased up to 1.5 orders of magnitude both in the field (at a site open to the sun and in shaded area under a tree) and in the lab at 21, 26 and 32 degree°C over a one week period. *E. coli* concentrations were observed to peak at day 4, 7, and 7 days in fall, spring, and summer, and two growth peaks occurred during winter at days 12 and 34 (Soupir et al., 2008). *E. coli* populations in dairy cow manure increased as much as 2.5 $\log_{10}$CFU/g-wet-manure in the three days following excretion and remained higher than the initial population until day 10 at 27°C (Wang et al., 2004). Wang et al. (1996) detected a 2 $\log_{10}$CFU/g-wet-manure increase of *E. coli* O157:H7 inoculated in manure after 2 days at 37°C.

However, there are also studies that do not report *E. coli* growth in manure or soil contaminated with manure. For example, *E. coli* levels of 7.7, 7.6 and 7.5 $\log_{10}$CFU/g-wet-manure were observed for soils contaminated with fresh cattle, sheep and pig manure 7 days after the animals had been kept in the field. By day 14 when all the animals were removed from the field, *E. coli* levels in the soil had decreased two to three orders of magnitude (Avery et al., 2004).

Studies were not found that examined whether *Bacteroidetes* growth occurred in cattle manure after deposit.

2.4 *E. coli* and BoBac Decay Rates in Cattle Manure

Log-linear regression is commonly used to derive bacterial decay coefficients (Crane & Moore, 1986; Oliver et al., 2006; Wang et al., 2004). Chick's Law (Crane & Moore, 1986) is often used to describe the first-order bacterial decay as $N(t)=N_0 e^{-kt}$ where N(t) is the bacterial quantity at time t, N_0 is the initial number of bacteria, k is first-order decay constant, and t is elapsed time.

Laboratory decay rates for *E. coli* in freshly excreted dairy cow manure increased as the temperature and moisture concentration increased, with rates of 0.11 and 0.32 $\log_{10}$CFU/g-dry-manure/day at 34°C/10% and 41°C/83%, respectively (Wang et al., 2004). The decay rates of *E. coli* O157:H7 in the top layer of fresh dairy manure were 0.11 $\log_{10}$CFU/g-dry-manure/day at 4°C/75% relative humidity (RH), 0.046 $\log_{10}$CFU/g-dry-manure/day at 20°C /50% RH, and 0.112 at 37°C/30% RH. In contrast, in the bottom presumably anaerobic manure layer, the decay rates were 0.054 $\log_{10}$CFU/g-dry-manure/day at 4°C/75% RH, 0.074 $\log_{10}$CFU/g-dry-manure/day at 20°C/50% RH, and 0.279 $\log_{10}$CFU/g-dry-manure/day at 37°C/30% RH (Himathongkham et al., 1999). For soils incorporated with fresh cattle manure and incubated in the laboratory, *E. coli*

decay rates were not affected by varying the soil moisture content (≈ 0.05 $\log_{10}$CFU/g-dry-manure/day at 25% and 50% soil moisture) (Oliver et al., 2006). The differences among these decay rates may be attributable to the different experimental conditions. For example, (Oliver et al., 2006) indicates that *E. coli* survived better in manure piles than within a slurry, died off more quickly within manure and slurries than in amended soil, and decayed faster within soil microcosms when introduced with sterile water rather than with cattle manure.

In-field studies revealed that the *E. coli* decay rate in naturally deposited cattle manure was 0.014 $\log_{10}$CFU/g-wet-manure/day in late April and higher at 0.020 $\log_{10}$ CFU/g-wet-manure/day in July (Mostaghimi, 1999). However, these results were not statistically different. Significant differences may have occurred in this study if the seasonal analysis was spread further apart, such as in mid-summer and mid-winter. For homogenized cow patties artificially applied to a mowed hay field, larger differences for die-off rates were detected in late winter (0.229 $\log_{10}$CFU/ g-dry-manure/day) versus spring (0.134 $\log_{10}$CFU/ g-dry-manure/day) (Soupir et al., 2008).

Very few studies have examined the decay rate for *Bacteroidetes* indicator species in naturally deposited manure. The decay rate for equine *Bacteroidetes* in unfiltered stream water increased from 0.17 to 0.81 $\log_{10}$copies/g-wet-manure/day as the temperature was increased from 5 to 25°C (Bell et al., 2009). Okabe and Shimazu (2007) also reported increased decay rates with increasing temperatures for bovine

Bacteroidetes in non-filtered river water (0.14 and 1.7 $\log_{10}$copies/L/day at 4 and 30°C, respectively).

2.5 Summary

The reviewed literature shows that it is difficult to apply controlled laboratory data to in-field conditions where beef cattle manure is deposited. Even so, very few studies have been conducted using naturally deposited manure, investigating for example integrated seasonal variability and feeding regime impacts on manure bacteria decay rates. A series of die-off rates based on direct fecal deposition (not within soil or artificially land applied) are needed to parameterize watershed models for bacterial transport and fate. No studies were found which simultaneously examined the in-field survivability of the regulatory fecal pollution indicator *E. coli* and source tracking bacteria for cattle manure.

CHAPTER III

MATERIALS AND METHODS

3.1 Sampling

Naturally deposited in-field beef cow manure samples were collected from the University of Tennessee East Tennessee Research and Education Center (Blount Beef Cow Unit, 4341 UT Farm Road in Louisville, Tennessee) in summer, fall, winter, and spring between 2010 and 2011. Samples were collected from 0-4 days following deposition, every two days for the following one to two weeks, and then weekly thereafter until the manure patty could no longer be clearly discerned or the detection limit for culturable *E. coli* (100 CFU/g-wet-manure), *E. coli* 23 S gene abundance (6.6 $\log_{10}$copies/g-wet-manure), or bovine *Bacteroidetes* 16S gene abundance (6.6 $\log_{10}$copies/g-wet-manure) was reached. More frequent samples were collected in the first several days following deposition to accurately establish the decay coefficients and observe whether growth of *E. coli* or BoBac occurred.

To initiate each seasonal study approximately 20 Angus beef cow-calf pairs were moved to a fresh pasture where they grazed freely for several hours. During this time eight newly deposited manure patties were randomly flagged and numbered. Samples on day 0 were obtained immediately after deposition. During the summer and fall sampling events the cattle were in small pastures and were moved to a larger adjacent

pasture to protect the patties from trampling. For the winter and spring sampling events the cattle were fed and grazed, respectively, in a large pasture where the designated patties were deposited and occasionally disturbed. Each sample (approximately 30 ml volume) included a vertical transect of manure from the top crust, if present, to the moist interior of the cow patty. Manure samples were collected in re-sealable plastic bags at the same time of day (9:00 am ± 1 hr), immediately placed on ice, and transported to the laboratory within one hour for analysis.

Weather data for the sampling periods were obtained from the adjacent McGhee Tyson Airport (Appendix A). Summaries of the data are provided in Table 1 below.

Table 1. A summary of weather data during seasonal sampling of in-field manure.

Season	Sampling dates	Temperature (°F)			Relative Humidity (%)	Rainfall (inch)
		average	max	min	average	total
Summer	7/19 to 8/9/2010	83	96	68	73.4	2.9
Fall	10/26 to 11/19/2010	53	85	27	67.8	5.4
Winter	1/24 to 2/18/2011	43	73	20	62.6	3.3
Spring	5/2 to 5/27/2011	67	93	37	70	4.2

3.2 Manure Solids Concentration

E. coli and BoBac concentrations were normalized to dry manure solids measured using Standard Method 2540D (APHA, 1998). Briefly, 2-9 grams of feces were placed on a pre-weighed pan and dried at 105°C for 24 hours. The sample was cooled and weighed again to calculate the percent solids using the weight loss.

3.3 *E. coli* Concentrations

Most Probable Number Assay. Culturable *E. coli* was analyzed in manure samples within six hours using Standard Method 9223B (APHA, 1998) providing a most probable number (MPN) quantification with the Colilert®/Quanti-tray® system by IDEXX Laboratories of Westbrook, Maine (Muirhead et al., 2004). During this assay, *E.coli* metabolizes 4-methyl-umbelliferyl glucoronide using the species specific enzyme b-glucuronidase to produce 4-methyl-umbelliferone, which fluoresces under long wave (365nm) ultra violet light.

To conduct this assay between 2 and 3 grams of feces were weighed and added to phosphate buffered saline (PBS: 1.16g sodium phosphate monobasic monohydrate, 5g sodium phosphate dibasic, 17g NaCl, 2L distilled H_2O, pH 7.4) to produce an initial concentration of 100 g/L. Serial dilutions were then made in PBS to obtain two dilutions used in the Colilert® assay (100 mg-wet-manure/L and 1,000 mg-wet-manure/L). The

diluted samples were poured into the Quanti-Tray® pouches, sealed using a Quanti-Tray® Sealer, and immediately placed in an incubator at 35°C for 24 hours. A total of 19 trays (8 trays for each cow patty sample dilution, two trays for a duplicate cow patty dilution series, and one negative control) were analyzed for each day the manure was sampled. Blue fluorescent wells were counted under 366 nm UV light the following day to obtain the MPN from the assay manual statistical table. The remaining 100 g/L solution and leftover manure were carefully labeled and frozen at -80°C for future analyses.

***E. coli* 23S gene quantitative PCR assay.** A quantitative real time polymerase chain reaction (qPCR) assay was employed to verify the MPN results and help establish *E. coli* growth potential in the manure as well as accurate decay coefficients (Knappett et al., 2011). This type of assay amplifies highly specific sequences of DNA and in the process creates a fluorescent signal. The initial gene sequence concentration is then quantified using a baseline fluorescence method with external standards (Orlando et al., 1998). In this study, E. coli 23S gene abundance was quantified directly in 100 mg-wet-manure/L dilutions without DNA extraction.

The *E. coli* qPCR reaction contained 12.5 µL PCR mix (Brilliant Agilent Technologies Absolute Blue Thermo), 0.75 µL each of the forward primer EC23Sf and reverse primer EC23Sr (20µM) (Table 2), 0.5 µL of EC23srv1bhq (10µM) linear fluorescent probe (Table 2) (Knappett, 2010; Knappett et al., 2011), 11 µL of sterile water and 2.5 µL

sample (100 mg/L manure sample dilutions) or quantification standard. External standards were a 10-fold dilution series of the EC3.3 plasmid isolated from *E. coli* (Layton et al., 2006). The PCR amplification and fluorescent probe detection was run on the Chromo4 Continuous Fluorescence Detector (Bio-Rad, Hercules, CA) with the following amplification protocol: 50°C for 2 minutes, 95°C for 15 minutes, and 45 cycles of 95°C for 30 s and 55°C for 45s. The threshold cycle (CT) value for fluorescence detection was manually set at 0.03. The standard curve and each sample were analyzed in duplicate. Each assay run included a negative control (sterile water).

Table 2. Probe and primer sequences for the qPCR assays used to detect *E. coli* and BoBac ribosomal genes in manure sample dilutions.

Assay	Primer/probe name: sequence (5'–3')
E. coli	EC23Sf; 5' GAGCCTGAATCAGTGTGTGTG 3' EC23Sr: 5' ATTTTTGTGTACGGGGCTGT 3' EC23Srv1bhq: 5' (FAM)CGCCTTTCCAGACGCTTCCAC(BHQ-1) 3'
BoBac	BoBac367f: 5' GAAG(G/A)CTGAACCAGCCAAGTA3' BoBac467r: 5' GCTTATTCATACGGTACATACAAG3' BoBac402Bhqf: 5'(FAM)TGAAGGATGAAGGTTCTATGGATTGTAAACTT(BHQ-1) 3'

3.4 Bovine *Bacteroidetes* Concentrations

Bovine *Bacteroidetes* 16S ribosomal gene abundance was quantified using qPCR directly in 100 mg-wet-manure/L dilutions (Layton et al., 2006). The reaction contained 12.5 µL PCR mix (Brilliant Agilent Technologies Absolute Blue Thermo), 0.75 µL each of the forward primer BoBac 367f (20µM) and reverse primer Bobac 467r (10µM) (Table 2), 0.5 µL of a linear fluorescent probe (Table 2) (Layton et al., 2006), 11 µL of sterile water and 2.5 µL sample or standard. A BoBac plasmid dilution series was used as an external standard (Layton et al., 2006). The qPCR assay was run on a Chromo4 Continuous Fluorescence Detector (Bio-Rad, Hercules, CA) with the following amplification protocol: 50°C for 2 min, 95°C for 10 min, and 50 cycles of 95°C for 30 s and 57°C for 45s. The threshold cycle (CT) value for fluorescence detection was manually set to 0.04. All assays were run in duplicate and each assay run included a negative control.

3.5 Data Analysis

Two methods were applied to analyze *E. coli* numbers. For the MPN method, the results for two sample dilutions were averaged if both concentrations were above the assay detection limit. Otherwise, the result for the larger dilution was used. The detection limits for the culturable cell assay was 100 CFU/g-wet manure. The theoretical detection limit for the *E. coli* 23S gene and the BoBac 16S gene was 6.6 $\log_{10}$copies/g-wet-manure .

For the qPCR assays, the *E. coli* and BoBac ribosomal gene concentrations were the average of duplicate reactions. When the concentrations of any of the three assays were below detection limit for several days only the first day was assigned a concentration value equal to the detection limit (BDL); the remaining below detection limit results were removed from the decay rate analysis. All concentration data were normalized to the manure solids content.

The initial bacteria concentration data were analyzed with an analysis of variance (ANOVA) to determine whether a difference existed in the populations between seasons ($\alpha = 0.05$). A subsequent Tukey Honestly Significant Difference (HSD) test was used to distinguished pairwise differences ($\alpha = 0.05$). These analysis were performed using SAS (version 9.3) statistical software.

Seasonal bacterial decay rates were established using the slope of a linear regression through the aggregated log transformed concentration data over time. This analysis was performed using SigmaPlot (version 11.0) software.

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CHAPTER IV

RESULTS AND DISCUSSION

E. coli is the regulatory fecal pollution indicator for evaluating surface water quality, and members of the *Bacteroidetes* family serve as promising source indicators for several types of fecal waste. But these different fecal bacteria may have different decay rate. The overall purpose of this work was to measure and compare the initial populations and in-field decay rates of *E. coli* and BoBac in beef cattle manure naturally deposited in a pasture during four seasons with different feeding regimens. The methodology was to collect one sample from each of eight randomly selected manure patties for several weeks following deposition. Manure solids content, culturable *E. coli* concentrations, and the abundance of *E. coli* 23S and BoBac 16S ribosomal genes are provided in Appendix B.

4.1 Initial Solids Content and *E. coli* and BoBac Concentrations

Initial manure solids content, culturable *E. coli* concentrations, *E. coli* 23S gene abundance, and BoBac 16S gene abundance from the first day of each seasonal sampling event are summarized in Table 3. The solids content and log transformed bacterial concentration data were analyzed with an ANOVA which lead to the rejection of the null hypothesis that these variables did not vary by season ($\alpha = 0.05$). A subsequent Tukey HSD analysis identified significantly different pairs of data ($\alpha = 0.05$) (Table 3).

Table 3. Seasonal means and standard deviations of the solids content and log transformed culturable *E. coli* and *E. coli* 23S and BoBac 16S gene abundance in eight freshly deposited beef cow patties.

Season Date	% Solids Content[1]	*E. coli*[1]		BoBac[1]
		culturable cells (log_{10}CFU per gram-dry-manure)	23S genes (log_{10}copies per gram-dry-manure)	16S genes (log_{10}copies per gram-dry-manure)
Summer 7/19/10	12.6 ± 1.4 a	5.0 ± 1.2 b	8.6 ± 0.3 b	11.0 ± 0.1 a
Fall 10/26/10	9.6 ± 0.8 c	3.6 ± 0.8 c	8.5 ± 0.5 b	9.9 ± 0.3 b
Winter 1/24/11	11.8 ± 1.0 ab	6.4 ± 0.8 a	9.4 ± 0.7 a	11.2 ± 0.2 a
Spring 5/2/11	10.3 ± 2.5 bc	4.3 ± 0.6 bc	7.1 ± 0.1 c	9.9 ± 0.5 b

1 The results of a Tukey HSD analysis on the log transformed results are summarized with underlined lower case letters; seasonal values followed by the same letter are not significantly different.

The initial culturable *E. coli* concentrations were highly variable but were significantly different by season/feeding pattern (Table 3). Results for the winter (6.4 ± 0.8 log_{10}CFU/g-dry-manure) when supplemental feed including hay and silage were provided were higher than for all other seasons. The average culturable *E. coli* concentration in summer when supplemental hay was provided (5.0 ± 1.2 log_{10}CFU/g-dry-manure) was approximately one order of magnitude higher than in fall (3.6 ± 0.8 log_{10}CFU/g-dry-manure) and spring (4.3 ± 0.6 log_{10}CFU/g-dry-manure) when the cattle consumed pasture forage. These results were corroborated with the *E. coli* 23S gene abundance, which was highest in the winter (9.4 ± 0.7 log_{10} copies/g-dry-manure) and lowest in the spring (7.1 ± 0.1 log_{10}CFU/g-dry-manure). Thus, the null hypothesis that the *E. coli* concentrations did not vary by season was rejected.

Culturable *E. coli* concentrations were much lower than the *E. coli* 23S gene abundance measured by qPCR. This was not unexpected because the qPCR assay measures intact gene sequences from both live and dead or un-culturable cells. *E. coli* has 7 copies of the 23S genes per genome (Stevenson & Schmidt, 2004), so at least 7 times higher 23S gene abundance was expected versus the culturable cell count. The ratios of the *E. coli* gene copy abundance to the culturable cell counts were 1,140;1, 57,829:1, 176:1 and 942:1 in summer, fall, winter, and spring, respectively. Thus, many gene copy numbers were being measured by the qPCR from non-culturable cells and/or dead cells.

Average initial BoBac 16S gene abundance was far less variable than *E. coli*, but varied in a similar manner to *E. coli* by season. BoBac was more abundant in summer (11.0 ± 0.1 $\log_{10}$copies/g-dry-manure) and winter (11.2 ± 0.1 $\log_{10}$copies/g-dry-manure) during supplemental feeding than in spring and fall (9.9 ± 0.1 $\log_{10}$copies/g-dry-manure) when the cattle grazed fresh pasture forages (Table 3). Thus, the null hypothesis that the BoBac 16S gene concentrations did not vary by season was rejected. Finally, the BoBac 16S gene concentrations were consistently 5 to 6 orders of magnitude higher than the culturable *E. coli* concentrations.

Seasonal manure solids content trends reflected variations observed in the bacteria concentrations. The manure solids content was highest during supplemental feeding in the summer and winter (12.6 and 11.8 ± 0.4%, respectively) when the *E. coli* and BoBac concentrations were highest; the manure solids content during grazing was lower (9.6 ± 0.4% 10.3 ± 0.6% in the fall and spring, respectively) when the *E. coli* and BoBac concentrations were lowest (Table 3).

The highest average initial culturable *E. coli* concentrations measured in this study (6.9 and 6.4 $\log_{10}$CFU/g-dry-manure in the winter and summer, respectively) were similar to the *E. coli* cell counts (6 to 7 $\log_{10}$CFU/g-dry-manure) in composite manure samples from three herds: 20-month-old beef heifers, mature beef cows and dry dairy cows collected on June 20, 2004 (Van Kessel et al., 2007a). However, the results herein are an order of magnitude lower than the 7.7 $\log_{10}$CFU/g-dry-manure from soil heavily

contaminated with beef cow manure (Avery et al., 2004). Another study of fresh dairy cow manure collected from the concrete base of a dairy shed holding pen reported a range of *E. coli* concentrations (5 to 7 $\log_{10}$CFU/g-dry-manure) similar to those observed in this study (Muirhead et al., 2005). The results herein were also similar to culturable *E. coli* concentrations (7.7 $\log_{10}$CFU/g-dry-manure) in fresh dairy cow waste collected before 9 am on July 27 and September 4, 2001 from cattle fed supplement grain because of degraded patures (Wang et al., 2004).

The initial *E. coli* concentrations were much lower (one to three orders of magnitude) in the fall and spring in this study compared with the previously cited literature (Chapter 2), most of which involved experiments in the summer season when supplemental feed was provided. The Literature Review revealed that one of the most important factors affecting bacterial concentrations in cattle manure is diet, which naturally and regularly varies between seasons for unconfined beef cattle in the southeastern United States. More *E. coli* are likely to be present in the cattle manure when the animals are fed grain versus grass forages (Callaway et al., 2006; Garauke et al., 2003; Lowe et al., 2010). No documented diet influence on BoBac concentrations were found in the Literature Review. This study clearly reveals lower concentrations of BoBac in cattle feeding on pasture forages versus those receiving supplemental hay and silage.

Water shed models like SWAT can be used to understand the fate and transport of fecal bacteria from livestock manure, but initial bacterial concentrations are required. This

study clearly confirms that the number of bacteria that would potentially be deposited into a watershed from beef cattle is not only related to the type of bacteria, but also to the season and feeding pattern. Models using fixed manure bacteria concentrations under all conditions are not accurate, and different concentrations should be accommodated for different seasons and/or feeding patterns (Parajuli et al., 2009a; Parajuli et al., 2009b; Zhu et al., 2011).

4.2 Bacteria Decay Rates

The literature did not reveal any simultaneous degradation rate studies of *E. coli* and bovine fecal source tracking bacteria. To better assess fecal pollution sources and recommend practices to improve surface water quality, which is commonly impaired with high *E. coli* concentrations potentially from grazing cattle, decay rate data are needed that reflect regular integrated seasonal factors.

During in-field sampling, the cow patties were not disturbed in summer and fall but were lightly tramped in winter and spring by the cattle. In the spring the patties were also disturbed by mowing. A total of nine samples were always collected from eight different fresh manure deposits (including one duplicate sample). For the summer, fall and winter, 8 samples were collected the same manure patties during each subsequent sampling day. In the Spring, as explained below, the initial patties were abandoned, but five other same age patties were subsequently sampled.

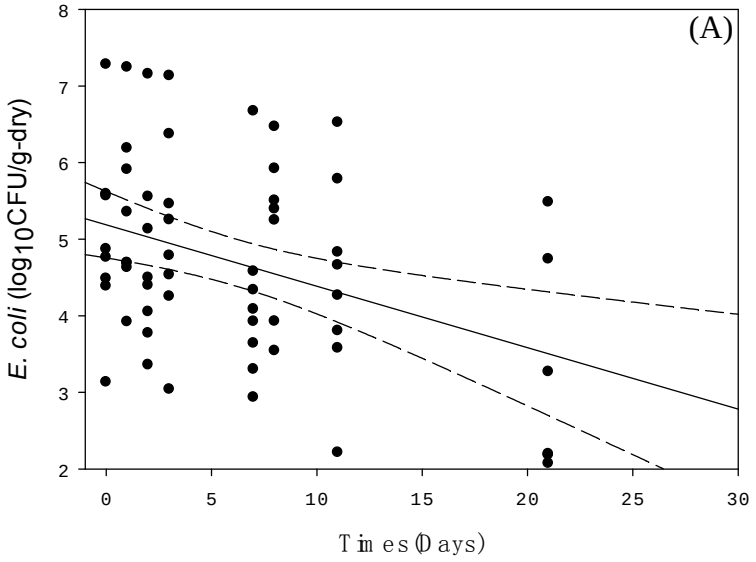
4.2.1 Summer

The summer season sampling began on July 19, 2010, lasted until August 9, 2010, and included 8 sampling events. The cattle were on pastured degraded cool season grass and being fed supplemental hay. Daily weather data for this time period are provided in Appendix A, Table A-1. In summary, the maximum and minimum average daily temperatures and relative humidity were 88 and 77°F and 84% and 64%, respectively. It rained 7 days during this 21-day period. Figure 3 provides all of the summer season results and includes log-linear regression lines with 95% confidence intervals for the slopes, which were used to establish decay rates.

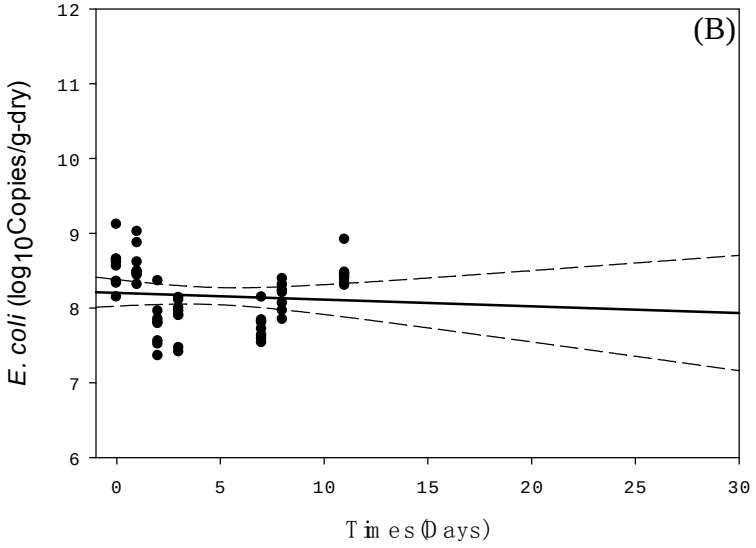
For culturable *E. coli*, fresh cattle manure collected on day 0 and day 21 displayed average *E. coli* concentrations of 6.4 $\log_{10}$CFU/g-dry-manure and 4.7 $\log_{10}$CFU/g-dry-manure, respectively. *E. coli* was still present in five of the eight patties after 21 days. High concentration variability (4 to 7 orders of magnitude) was present each sampling day. The decay rate was estimated with the slope of a log-linear regression of culturable E. coli concentration and time data (Figure 3). The rate of decline was 0.080 $\log_{10}$CFU/g-dry manure/day, with a standard error (S.E.) of 0.025, a p-value of 0.002. Thus, the slope of the best fit line, or decay rate, was significantly less than 0. However, a poor fit was confirmed by the low coefficient of determination (R^2) value of 0.15.

Figure 2. Summer season (A) culturable *E. coli* concentrations, (B) *E. coli* 23S gene abundance, and (C) BoBac 16S gene abundance.

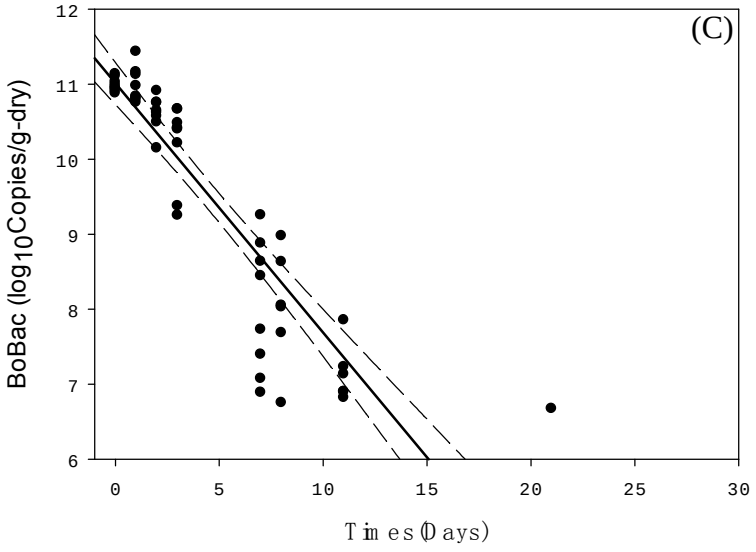
Regression lines are fit to the log transformed data and 95% confidence intervals for the line slopes are displayed. A statistical summary of the best fit lines include the standard slope ± 95% confidence interval, slope p-value (in comparison with 0), and the coefficient of multiple determination (R^2).



Slope = -0.080 ± 0.050
p-value = 0.002
R^2 = 0.148



Slope = -0.009 ± 0.048
p-value = 0.550
R^2 = 0.007



Slope = -0.333 ± 0.045
p-value < 0.001
R^2 = 0.812

Relatively lower daily concentration variability existed within the *E. coli* 23S gene abundance versus culturable *E. coli (*as expected, the *E. coli* 23S gene abundance consistently several orders of magnitude higher than the culturable *E. coli* concentrations). However, the decay rate of 0.009 $\log_{10}$copies/g-dry-manure/day also was not significantly different from zero (S.E. = 0.015, p-value = 0.55) (Figure 3-B).

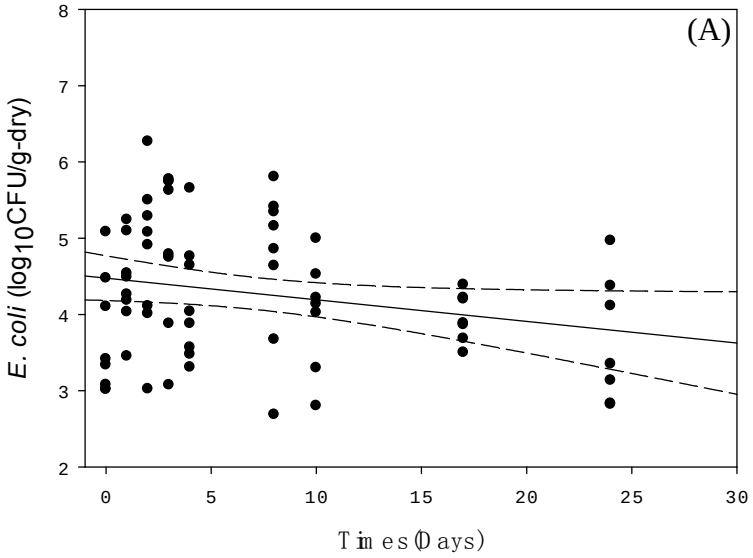
BoBac 16S gene concentrations continuously declined from approximately 10^{11} copies/g-dry-manure on the first day to below the detection limit in 7 out of 8 cow patties on day 11. The BoBac concentration data exhibited high variability only at low concentrations. The decay rate was high and significantly different from 0 (0.333 $\log_{10}$copies/g-dry-manure/day; SE = 0.022, p-value < 0.001) with a very good regression fit (R^2=0.81) (Figure 3-C). The decay rate was clearly much higher than for culturable *E. coli* and *E. coli* 23S gene abundance.

4.2.2 Fall

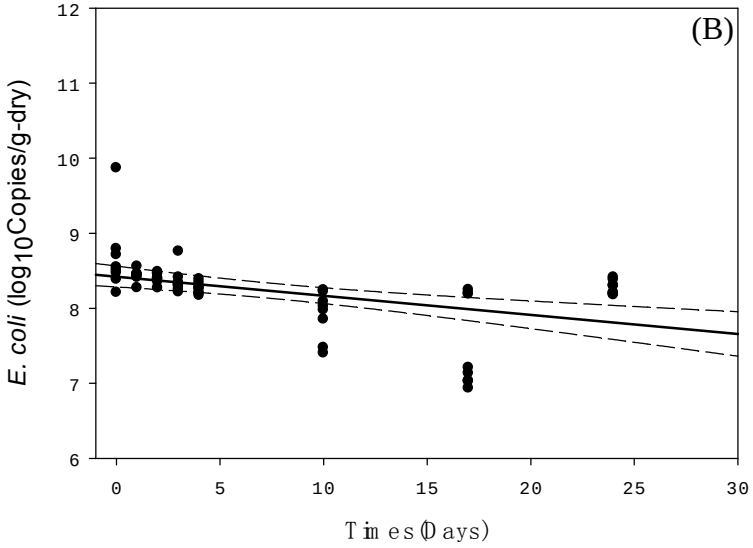
The fall season sampling began October 26, 2010 and lasted until November 19, 2010 and included 9 sampling events. The cattle were on very good quality pasture and were not receiving supplemental hay or grain. Daily weather data for this time period are provided in Appendix A, Table A-2. In summary, the maximum and minimum average daily temperatures and relative humidity were 72 and 39°F and 56 to 92%, respectively. It rained on 8 different days. The fall season data are presented in Figure 4.

Figure 3. Fall season (A) culturable *E. coli* concentrations, (B) *E. coli* 23S gene abundance, and (C) BoBac 16S gene abundance.

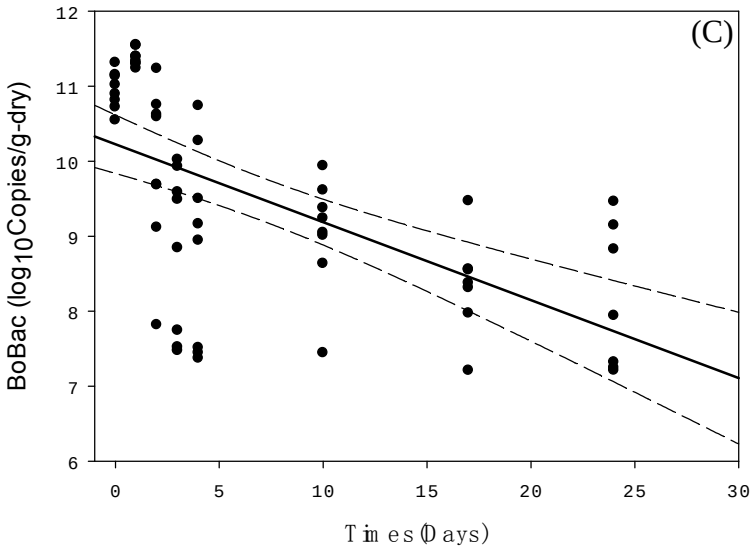
Regression lines are fit to the log transformed data and 95% confidence intervals for the line slopes are displayed. A statistical summary of the best fit lines include the standard slope ± 95% confidence interval, slope p-value (in comparison with 0), and the coefficient of multiple determination (R^2).



Slope = -0.028 ± 0.0028
p-value = 0.048
R^2 = 0.057



Slope = -0.026 ± 0.012
p-value < 0.001
R^2 = 0.213



Slope = -0.104 ± 0.036
p-value < 0.001
R^2 = 0.352

For cultivable *E. coli*, the average concentration decreased continually until day 24, but cells were still detectable in 6 of the 8 patties on day 24. High concentration variability, 2 to 4 orders of magnitude, existed within the data for each sampling day. The rate of decline was significantly different from 0, at 0.028 $\log_{10}$CFU/g-dry-manure/day (S.E. = 0.014, p-value = 0.048). The *E. coli* 23S decay rate was similar at 0.026 copies/g-dry-manure/day and was also statistically greater than 0 (SE = 0.006, p-value < 0.001). Both the cultural *E. coli* and *E. coli* 23S gene abundance displayed poor regression fits.

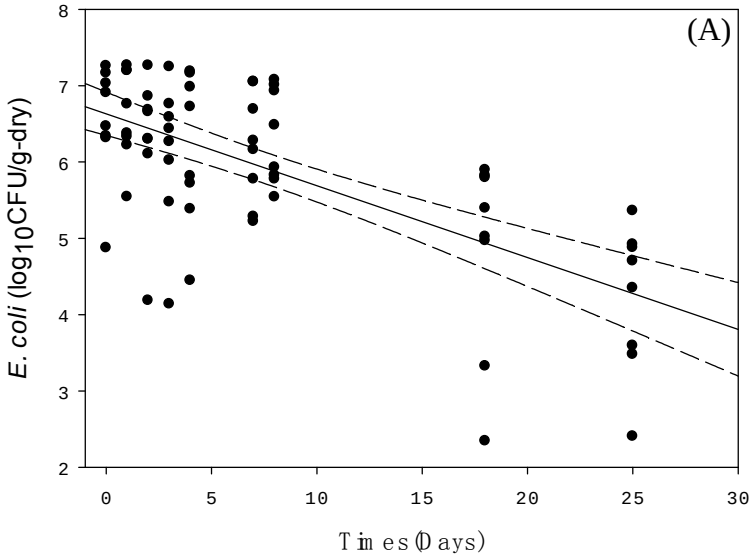
BoBac 16S gene abundance decayed rapidly with a 1 order of magnitude average decrease by day 2. BoBac 16S gene sequences were detectable in only 4 of the 8 patties by day 24 and showed high variability only at relatively low concentrations. The decay rate was significantly different from zero at 0.10 $\log_{10}$copies/g-dry-manure/day (SE = 0.018, p-value < 0.001).

4.2.3 Winter

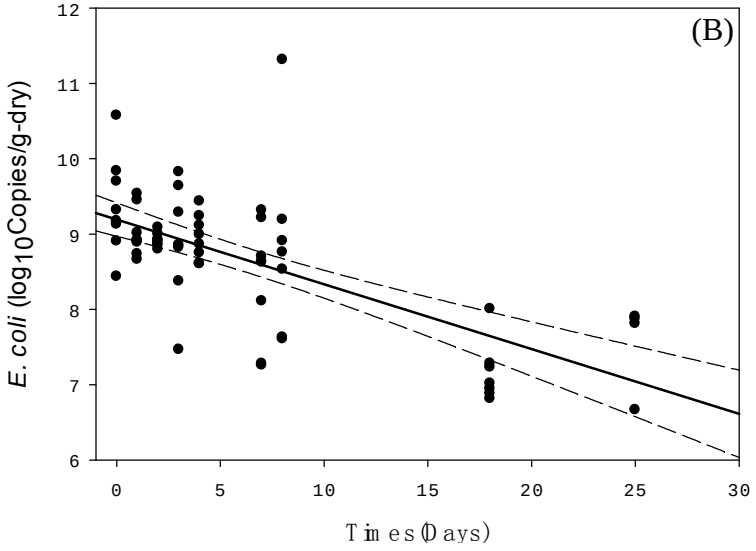
The winter season sampling began on January 4, 2011, lasted until February 18, 2011 and included 9 sampling events. The cattle were on dormant pasture and being fed supplemental hay and corn silage. Daily weather data for this time period are provided in Appendix A, Table A-3. In summary, the maximum and minimum average daily temperatures and relative humidity were 60 and 30°F and 34 to 86%, respectively. It rained and or snowed on 8 out of 25 days. Figure 4 provides the winter season results.

Figure 4. Winter season (A) culturable *E. coli* concentrations, (B) *E. coli* 23S gene abundance, and (C) BoBac 16S gene abundance.

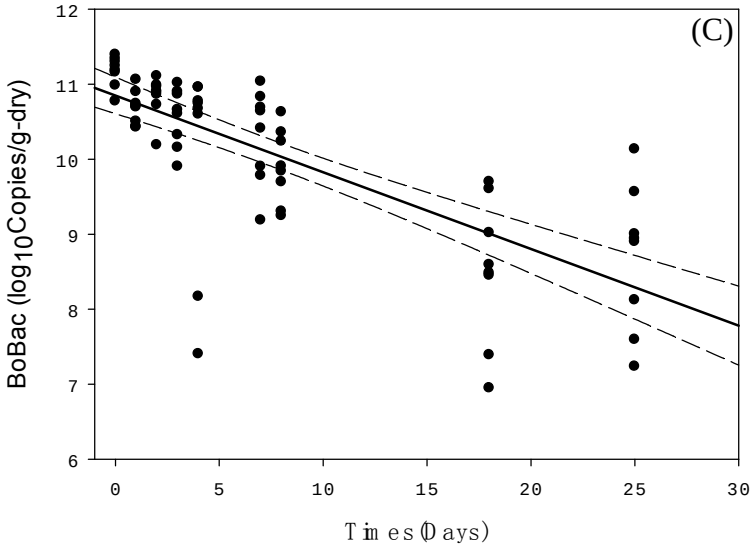
Regression lines are fit to the log transformed data and 95% confidence intervals for the line slopes are displayed. A statistical summary of the best fit lines include the standard slope ± 95% confidence interval, slope p-value (in comparison with 0), and the coefficient of multiple determination (R^2).



Slope = -0.094 ± 0.026
p-value < 0.001
R^2 = 0.432



Slope = -0.086 ± 0.024
p-value < 0.001
R^2 = 0.455



Slope = -0.102 ± 0.022
p-value < 0.001
R^2 = 0.549

The average culturable *E. coli* concentrations deceased throughout the 25-day winter sampling period but was measureable in 6 out of 8 patties the final sampling day. High variability, 2 to 4 orders of magnitude, existed within the data on each sampling day. The decay rate was 0.094 $\log_{10}$CFU/g-dry-manure/day (S.E. = 0.013, p-value < 0.001) and significantly different than zero and much higher than observed in fall and spring sampling seasons. This was corroborated with a significant decline in the *E. coli* 23S gene abundance at 0.086 $\log_{10}$copies/g-dry-manure/day (S.E. = 0.012, p-value < 0.001). BoBac 16S gene abundance declined at 0.102 $\log_{10}$copies/g-dry-manure/day (S.E. = 0.011, p-value < 0.001), a much lower rate than was observed in the summer.

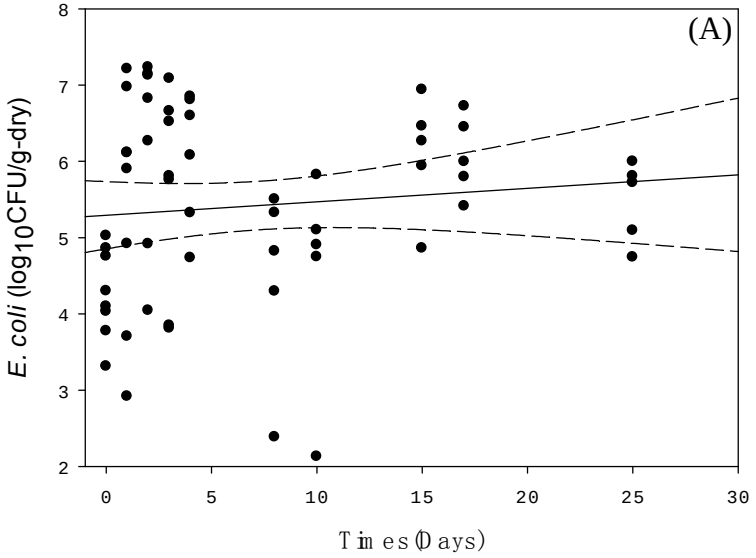
4.2.4 Spring

The spring season sampling began on May 2, 2011, lasted until May 27, 2011, and included 9 sampling events. The cattle were on fresh grass pasture with no supplemental feed. Daily weather data for this time period are provided in Appendix A, Table A-4. In summary, the maximum and minimum average daily temperatures and relative humidity were 77 and 56°F and 48 and 92%, respectively. It rained and or snowed on 13 out of 26 days. Figure 5 provides presents the spring season results.

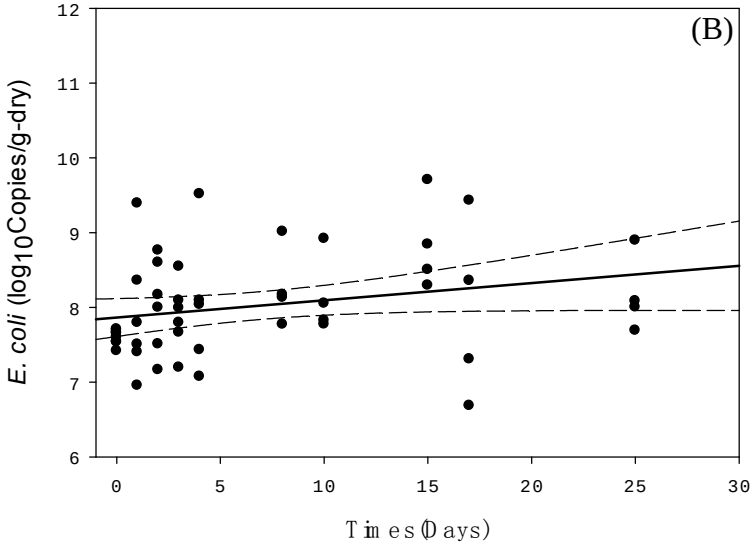
In spring, the samples were collected on the first day the cattle were moved to a pasture with fresh forage. Because the cows were adjusting to this change in diet, extremely small and liquid cow paddies were produced that spread out and degraded quickly.

Figure 5. Spring season (A) culturable *E. coli* concentrations, (B) *E. coli* 23S gene abundance, and (C) BoBac 16S gene abundance.

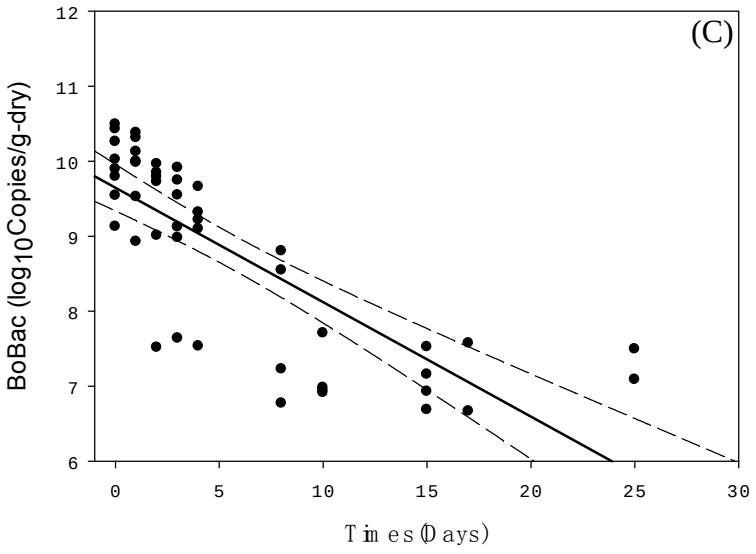
Regression lines are fit to the log transformed data and 95% confidence intervals for the line slopes are displayed. A statistical summary of the best fit lines include the standard slope ± 95% confidence interval, slope p-value (in comparison with 0), and the coefficient of multiple determination (R^2).



Slope = +0.018 ± 0.042
p-value = 0.403
R^2 = 0.012



Slope = +0.023 ± 0.025
p-value = 0.066
R^2 = 0.067



Slope = -0.153 ± 0.037
p-value < 0.001
R^2 = 0.611

In response to this anomalous result, a different set of 5 manure patties deposited on the afternoon of the same day the initial sampling occurred, May 2, 2011, were sampled from May 3, 2011 to May 27, 2011. The data from all the patties were combined to establish the decay rates in Figure 5.

The culturable *E. coli* abundance was very low on the first day of sampling, but high on the second sample day. After an intervening decline in the culturable *E. coli* abundance, a significant concentration increase was observed in most of patties on days 15 and 17, with an apparent decrease in the concentrations on day 25. This change in the decay behavior (decreasing until day 12 then increasing and decreasing again) may have been due to the patties being mashed by farm equipment on days 10 and 15. This flatten the manure patties, resulting in very thin layer of material, perhaps exposing *E. coli* to more oxygen and thus better survival/growth conditions (Figure 1). The *E. coli* 23S gene abundance also showed an increased on sampling days 12 and 15, and a decline thereafter. The cultivable *E. coli* and *E. coli* 23S gene abundance decay rates were not significantly different than 0.

BoBac decayed steadily during the spring sample season. The disturbance between days 10 and 15 had no apparent affect on BoBac, but could have been expected to increase the decay rate by making the patties more aerobic (Figure 1). The decay rate of 0.15 $\log_{10}$copies/ (g-dry-manure/day) (S.E. = 0.018, p-value < 0.001) was significantly different than 0 and the linear regression displayed a good fit (R^2 = 0.61).

4.2.5 Seasonal Comparison of Decay Rates

Figure 6 on the following page displays the estimated seasonal decay rates and corresponding 95% confidence intervals (slopes and slope confidence intervals from Figures 2-5). Based on these data the null hypothesis that the decay rates of bovine fecal bacteria do not vary by season were easily rejected. The *E. coli* die-off rates were higher (represented as a more negative decay rate) in winter than in all other seasons. Conversely, BoBac 16S gene concentrations decayed much faster during the summer. In fact, BoBac 16S gene concentrations decayed more quickly than culturable *E. coli* or *E. coli* 23S gene concentrations in all seasons except for winter when all of the decay rates were not statistically different (indicated by overlapping 95% slope confidence intervals). Persistence of the *Bacteroidetes* in this study fell within the wide variation of survival periods from previous research, including as little as 8 days (Kreader, 1998) and as much as 24 days (Seurinck et al., 2005) (x-intercepts for plot C in Figures 2-5). Referring back to the conceptual model in Figure 1, it can be surmised that the integrated seasonal atmospheric conditions and feeding patterns affect the decay rates.

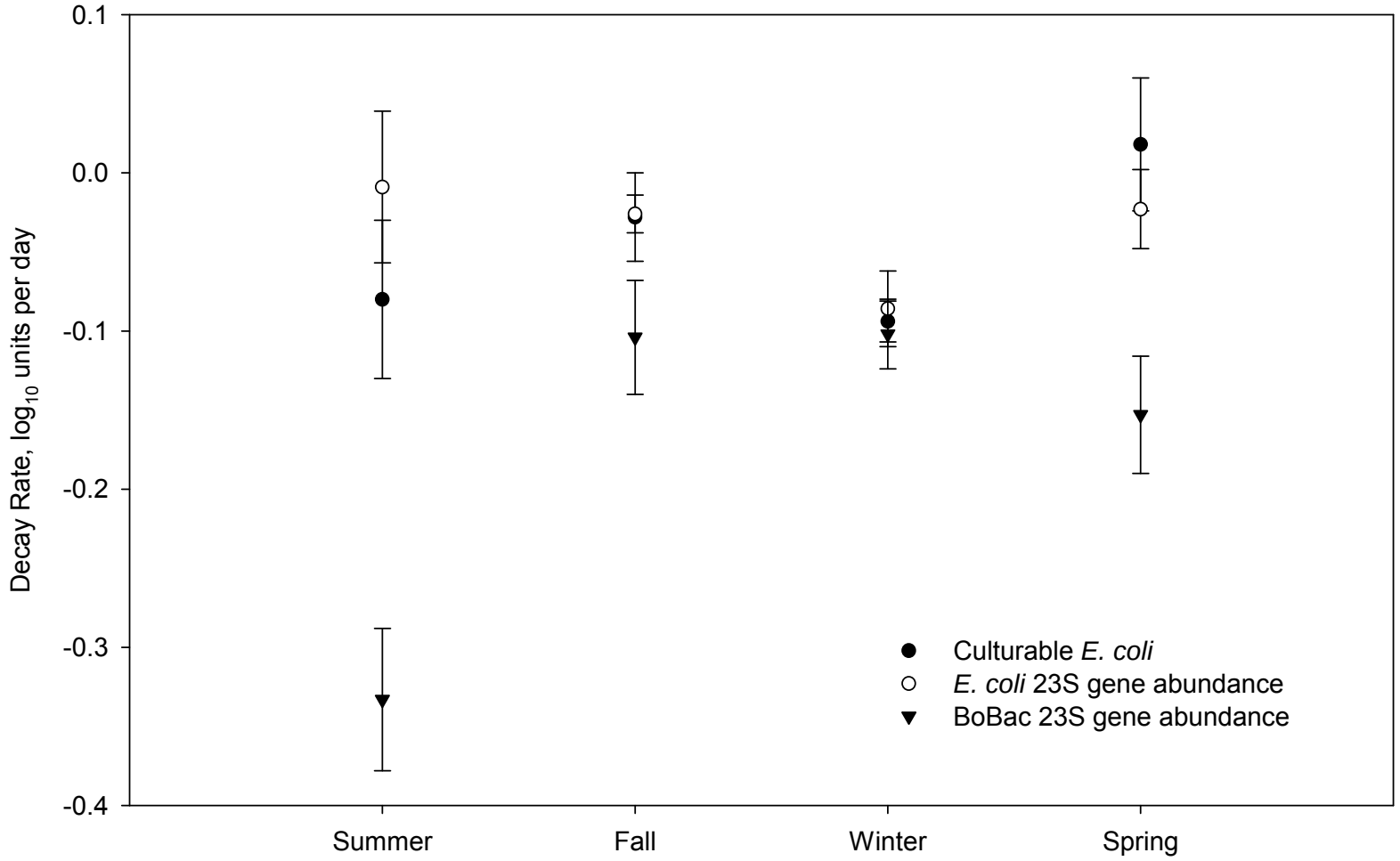


Figure 6. Seasonal decay rates for culturable *E. coli* (log_{10}CFU/g-dry-manure/day), *E. coli* 23S gene abundance (log_{10}copies/g-dry-manure/day), and BoBac 16S gene abundance ((log_{10}copies/g-dry-manure/day).

CHAPTER V

CONCLUSIONS

Initial concentrations for culturable *E. coli* varied several orders of magnitude during each season, but were clearly lower when the animals were grazing on fresh forage (3.6 ± 0.8 and 4.3 ± 0.6 $\log_{10}$ CFU/g-dry-manure in fall and spring, respectively) versus receiving supplemental feed because of degraded (5.0 ± 1.2 $\log_{10}$CFU/g-dry-manure, summer) or dormant (6.4 ± 0.8 $\log_{10}$CFU/g-dry-manure, winter) forage. Average initial *E. coli* 23S gene abundance was also highly variable and lower in the spring (7.1 ± 0.1 $\log_{10}$ copies/g-dry-manure) versus the winter (9.4 ± 0.7 $\log_{10}$copies/g-dry-manure). Average initial BoBac 16S gene abundance was less variable, but again significantly lower during grazing (9.9 ± 0.3 and 9.9 ± 0.5 $\log_{10}$ copies/g-dry-manure in the fall and spring, respectively) versus during supplemental feeding (11.0 ± 0.1 and 11.2 ± 0.1 $\log_{10}$ copies/g-dry-manure in summer and winter, respectively). A null hypothesis that the initial bacterial concentrations were not affected by season was easily rejected.

Linear regressions of log transformed concentration data were used to calculate seasonal decay rates. The decay rate for culturable *E. coli* was significantly higher in the winter (-0.094 ± 0.26 CFU/g-dry-manure/day) than in the fall (-0.028 ± 0.028 CFU/g-dry-manure/day). The same was true for *E. coli* 23S gene abundance (-0.086 ± 0.024 and -0.026 ± 0.012 copies/g-dry-manure/day in winter and fall, respectively). The decay rates were far higher for BoBac versus *E. coli*, but had an opposite trend, being much

higher in the summer (-0.333 ± 0.045 copies/g-dry-manure/day) than in the winter (0.102 ± 0.022 copies/g-dry-manure/day). A null hypothesis that the bacterial decay rates did not vary by season was easily rejected.

These seasonal initial concentration and decay rate data will provide a more accurate input for watershed models such as SWAT that are used to predict and guide remediation of pathogen contamination of surface water resources.

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APPENDIX A. WEATHER DATA

Table A1. Weather data during the summer in-field manure sampling period.

Date	Temperature (°F)			Relative Humidity (%)			Precipitation Events	
	High	Mean	Low	High	Mean	Low	Rain (in.)	Description
7/19/2010	90	80	70	100	78	56	0.7	Rain-Thunderstorm
7/20/2010	92	81	69	97	77	56	T	Thunderstorm
7/21/2010	91	83	74	94	73	52	0.08	Rain-Thunderstorm
7/22/2010	89	81	73	100	78	56	0.08	Rain-Thunderstorm
7/23/2010	94	84	73	100	73	46	0	
7/24/2010	96	86	75	94	68	42	0	
7/25/2010	96	88	80	85	64	43	0	
7/26/2010	96	86	76	94	72	49	T	Rain-Thunderstorm
7/27/2010	92	83	73.	93	72	50	0	
7/28/2010	91	83	75	94	77	59	0.39	Rain-Thunderstorm
7/29/2010	90	82	74	100	82	63	0.51	Rain-Thunderstorm
7/30/2010	91	82	73	100	72	44	0	Rain-Thunderstorm
7/31/2010	84	77	70	100	84	67	0.66	Rain-Thunderstorm
8/1/2010	92	83	75	100	76	52	T	Rain
8/2/2010	91	73	74	94	75	56	0	
8/3/2010	93	85	76	88	70	52	0	
8/4/2010	95	86	76	88	67	46	0	
8/5/2010	94	85	75	91	76	60	0.49	Rain-Thunderstorm
8/6/2010	88	81	73	94	78	61	0	Thunderstorm
8/7/2010	91	80	69	100	69	38	0	
8/8/2010	93	81	68	93	67	41	0	
8/9/2010	96	84	71	93	67	41	0	

Table A2. Weather data during the fall in-field manure sampling period.

Date	Temperature (°F)			Relative Humidity (%)			Precipitation Events	
	High	Mean	Low	High	Mean	Low	Rain (in.)	Description
10/26/2010	85	72	59	93	72	51	1.77	Rain-Thunderstorm
10/27/2010	70	64	57	100	87	73	0.69	Fog-Rain
10/28/2010	73.	63	52	100	61	22	0	Fog
10/29/2010	60	50	39	79	56	33	0	
10/30/2010	70	52	33	92	59	26	0	
10/31/2010	70	59	47	80	56	31	0	
11/1/2010	70	56	42	89	63	37	0	
11/2/2010	69	59	48	83	61	39	0	
11/3/2010	61	56	50	100	76	51	0.43	Rain
11/4/2010	58	51	44	100	71	41	0.61	Rain
11/5/2010	49	43	37	92	79	66	0.18	Rain
11/6/2010	47	39	31	92	65	37	T	Rain
11/7/2010	53	40	27	92	62	32	0	Fog
11/8/2010	65	48	30	92	58	24	0	
11/9/2010	71	53	34	82	57	28	0	
11/10/2010	72	55	37	92	62	31	0	
11/11/2010	74	56	38	92	61	29	0	
11/12/2010	71	55	38	92	66	40	0	
11/13/2010	69	54	39	92	64	35	0	
11/14/2010	55	49	43	93	82	71	0.02	Rain
11/15/2010	54	52	49	100	92	83	0.76	Rain
11/16/2010	68	58	48	100	78	55	0.9	Rain
11/17/2010	57	49	41	89	70	51	0	
11/18/2010	60	50	39	92	67	42	0	
11/19/2010	63	51	39	96	70	43	0	

Table A3. Weather data during the winter in-field manure sampling period.

Date	Temperature (°F)			Relative Humidity (%)			Precipitation Events	
	High	Mean	Low	High	Mean	Low	Rain (in.)	Description
1/24/2011	55	43	30	78	54	29	0	
1/25/2011	45	41	36	93	76	59	0.09	Rain
1/26/2011	42	37	31	96	86	75	0.48	Fog-Rain-Snow
1/27/2011	44	37	29	85	69	53	T	Snow
1/28/2011	51	42	32	70	51	32	0	
1/29/2011	63	45	27	85	61	36	0	
1/30/2011	68	51	33	96	69	42	0	
1/31/2011	61	54	47	80	70	60	T	
2/1/2011	62	55	47	93	78	62	0.42	Rain
2/2/2011	56	42	27	86	67	47	0	
2/3/2011	36	30	23	65	56	47	0	
2/4/2011	37	36	34	92	72	52	0.17	Rain-Snow
2/5/2011	49	40	31	100	79	57	0.03	Rain
2/6/2011	48	40	32	82	66	49	0	
2/7/2011	51	40	29	92	69	46	0.03	Rain
2/8/2011	39	31	23	82	65	47	0	
2/9/2011	40	31	21	92	66	40	0.03	Snow
2/10/2011	39	31	23	92	63	34	T	Snow
2/11/2011	44	32	20	81	52	23	0	
2/12/2011	51	37	23	55	44	33	0	
2/13/2011	61	45	29	72	49	25	0	
2/14/2011	67	56	45	53	34	15	0	
2/15/2011	58	45	31	85	55	24	0	
2/16/2011	67	51	34	75	54	32	0	
2/17/2011	73	55	36	85	60	35	0	
2/18/2011	67	60	53	89	63	36	0.05	Rain

Table A4. Weather data during the spring in-field manure sampling period.

Date	Temperature (°F)			Relative Humidity (%)			Precipitation Events	
	High	Mean	Low	High	Mean	Low	Rain (in.)	Description
5/2/2011	81	69	57	93	68	42	0	
5/3/2011	77	63	48	93	73	53	0.88	Rain
5/4/2011	58	50	41	100	72	43	0	Fog
5/5/2011	66	52	37	92	65	37	0	
5/6/2011	69	58	46	86	63	40	0	
5/7/2011	72	59	45	93	69	44	T	Rain
5/8/2011	82	71	59	90	68	45	0	
5/9/2011	85	72	59	100	72	43	0	
5/10/2011	86	74	62	93	72	51	0	
5/11/2011	86	74	62	84	66	48	0	
5/12/2011	87	76	65	87	65	42	0	
5/13/2011	84	74	63	87	71	55	0.02	Rain-Thunderstorm
5/14/2011	77	69	60	100	77	43	0.05	Rain
5/15/2011	63	59	55	80	72	64	T	Rain
5/16/2011	62	57	51	93	80	67	T	Rain
5/17/2011	54	51	47	100	83	66	001	Rain
5/18/2011	59	53	47	93	83	72	T	Rain
5/19/2011	72	63	53	93	70	47	0	
5/20/2011	76	67	58	90	70	50	0.01	Rain
5/21/2011	88	63	57	100	68	36	0	
5/22/2011	91	78	64	93	65	36	0.03	Rain-Thunderstorm
5/23/2011	88	75	62	100	71	42	0	
5/24/2011	89	79	68	79	57	34	T	Rain
5/25/2011	93	78	63	93	60	26	0	
5/26/2011	80	72	63	93	71	48	0.64	Rain-Thunderstorm
5/27/2011	80	72	63	87	69	51	T	Rain

APPENDIX B. SOLIDS CONTENT, *E. COLI* AND BOBAC RAW DATA

Table B1. Fractional manure solids concentrations during the summer in-field manure sampling period.

Cow Patty	Sample Day							
	0	1	2	3	7	8	11	21
1	0.118	0.118	0.178	0.426	0.518	0.806	0.612	0.707
2	0.117	0.151	0.271	0.181	0.670	0.708	0.444	0.638
3	0.127	0.139	0.208	0.189	0.324	0.698	0.507	0.662
4	0.140	0.157	0.293	0.246	0.720		0.604	
5	0.117	0.132	0.149	0.167	0.401	0.382	0.244	0.849
6	0.111	0.133	0.178	0.185	0.668	0.589	0.294	0.453
7	0.127	0.138	0.169	0.179	0.517	0.741	0.505	0.588
8	0.151	0.149	0.217	0.195	0.495	0.405	0.236	0.604

Table B2. Fractional manure solids concentrations during the fall in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	8	10	17	24
1	0.084	0.094	0.100	0.145	0.121	0.195	0.131	0.299	0.148
2	0.094	0.099	0.091	0.135	0.123	0.162	0.135	0.248	0.166
3	0.100	0.106	0.114	0.126	0.144	0.186	0.159	0.372	0.192
4	0.096	0.097	0.116	0.135	0.156	0.262	0.168	0.295	0.247
5	0.086	0.109	0.106	0.126	0.122	0.156	0.145	0.378	0.188
6	0.101	0.109	0.121	0.135	0.171	0.261	0.188	0.466	0.227
7	0.109	0.110	0.113	0.121	0.137	0.207	0.137	0.184	0.181
8	0.096	0.090	0.095	0.122	0.133	0.121	0.121	0.207	0.151

Table B3. Fractional manure solids concentrations during the winter in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	7	8	18	25
1	0.113	0.133	0.130	0.126	0.151	0.204	0.265	0.537	0.865
2	0.103	0.127	0.106	0.114	0.132	0.188	0.188	0.526	0.828
3	0.112	0.125	0.151	0.122	0.139	0.231	0.423	0.614	0.865
4	0.113	0.143	0.105	0.126	0.136	0.182	0.204	0.225	0.856
5	0.119	0.154	0.121	0.127	0.138	0.148	0.174	0.236	0.550
6	0.129	0.154	0.122	0.137	0.150	0.211	0.204	0.270	0.394
7	0.121	0.131	0.119	0.119	0.158	0.215	0.285	0.452	0.866
8	0.134	0.154	0.132	0.137	0.166	0.217	0.237	0.458	0.838

Table B4. Fractional manure solids concentrations during the spring in-field manure sampling period.

Cow Patty	Sample Day									
	0	1	2	3	4	8	10	15	17	25
1	0.087									
2	0.100	0.679								
3	0.078									
4	0.117	0.442	0.273	0.558						
5	0.152									
6	0.081									
7	0.089									
8	0.116	0.158	0.212	0.280	0.337					
9		0.149	0.142	0.216	0.164	0.155	0.337	0.279	0.459	0.346
10		0.125	0.124	0.165	0.148	0.679	0.791	0.833	0.827	0.327
11		0.138	0.180	0.197	0.221	0.238	0.788	0.826	0.808	0.353
12		0.133	0.174	0.198	0.342	0.762	0.805	0.838	0.864	0.495
13		0.121	0.113	0.143	0.206	0.412	0.745	0.728	0.697	0.480

Table B5. Manure culturable *E. coli* concentrations (CFU/gram-wet-manure) during the summer in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	7	8	11	21
1	8.72E+03	2.66E+04	5.59E+03	4.65E+02	1.00E+03	1.04E+03	2.81E+03	0.00E+00	0.00E+00
2	2.84E+03	7.43E+03	1.60E+03	3.25E+04	2.57E+03	8.12E+03	5.98E+03	1.68E+03	1.00E+02
3	3.85E+03	5.89E+03	2.35E+03	3.38E+03	4.58E+03	7.02E+03	5.79E+05	9.30E+03	0.00E+00
4	5.14E+04	2.40E+05	1.04E+05	5.79E+05	1.67E+05	2.72E+04		2.76E+04	
5	6.74E+03	6.39E+03	3.71E+03	5.67E+03	3.26E+03	3.36E+03	1.12E+06	1.55E+03	1.00E+02
6	1.50E+02	1.11E+03	4.05E+02	5.31E+04	8.82E+03	2.91E+03	1.04E+05	9.80E+05	8.40E+02
7	2.42E+06	2.42E+06	2.42E+06	2.42E+06	1.99E+06	2.42E+06	2.36E+05	3.08E+05	1.79E+05
8	5.87E+04	1.20E+05	2.94E+04	1.19E+04	5.80E+04	4.25E+02	1.00E+05	1.58E+04	3.30E+04

Table B6. Manure culturable *E. coli* concentrations (CFU/gram-wet-manure) during the fall in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	8	10	17	24
1	1.00E+02	1.43E+03	1.19E+04	8.08E+03	5.33E+03	5.02E+04	2.60E+02	4.75E+03	1.00E+02
2	2.05E+02	3.04E+03	1.17E+03	7.52E+04	9.30E+02	7.60E+02	1.42E+03	6.09E+03	3.70E+02
3	2.60E+02	1.94E+03	2.22E+04	1.50E+02	4.30E+02	8.03E+03	1.00E+02	2.85E+03	2.48E+03
4	1.00E+02	1.05E+03	3.64E+04	8.27E+03	3.15E+02	1.66E+05	2.31E+03	4.87E+03	2.29E+04
5	1.04E+04	1.90E+04	1.96E+05	7.40E+04	5.50E+04	2.24E+04	1.43E+04	2.76E+03	4.45E+03
6	3.02E+03	1.36E+04	9.82E+03	7.52E+04	9.80E+03	5.76E+04	6.30E+03	1.47E+03	3.10E+02
7	1.38E+03	3.10E+02	1.15E+03	5.11E+04	1.49E+03	1.00E+02	0.00E+00	0.00E+00	0.00E+00
8	1.00E+02	3.10E+03	0.00E+00	9.20E+02	4.90E+02	8.69E+03	2.00E+03	1.00E+03	0.00E+00

Table B7. Manure culturable *E. coli* concentrations (CFU/gram-wet-manure) during the winter in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	7	8	18	25
1	2.45E+05	2.86E+05	5.90E+05	4.86E+05	3.65E+04	2.95E+05	2.25E+05	4.98E+04	2.60E+03
2	3.01E+05	7.27E+05	2.11E+05	1.19E+05	8.59E+04	1.12E+05	1.26E+05	1.11E+03	6.24E+04
3	2.32E+05	2.96E+05	1.92E+05	3.64E+04	7.33E+04	3.85E+04	1.47E+05	1.52E+05	1.98E+05
4	1.20E+06	2.38E+05	2.08E+05	2.31E+05	1.30E+06	3.45E+05	2.42E+06	1.48E+05	4.31E+04
5	1.73E+06	2.42E+06	5.75E+05	7.27E+05	7.27E+05	7.27E+05	5.30E+05	1.85E+05	1.23E+04
6	9.64E+03	5.37E+04	1.86E+03	1.89E+03	4.17E+03	4.04E+04	1.21E+05	2.82E+04	1.00E+02
7	9.80E+05	2.42E+06	8.66E+05	3.26E+05	2.42E+06	2.42E+06	2.42E+06	1.00E+02	3.38E+03
8	2.42E+06	2.42E+06	2.42E+06	2.42E+06	2.42E+06	2.42E+06	2.42E+06	2.86E+05	6.94E+04

Table B8. Manure culturable *E. coli* concentrations (CFU/gram-wet-manure) during the spring in-field manure sampling period.

Cow Patty	Sample Day									
	0	1	2	3	4	8	10	15	17	25
1	5.20E+02									
2	2.05E+02	3.45E+03								
3	1.57E+03									
4	1.23E+04	3.66E+04	2.26E+04	3.93E+03						
5	8.69E+03									
6	8.75E+02									
7	1.11E+03									
8	8.42E+03	1.26E+05	1.41E+06	1.78E+05	7.11E+04					
9		2.42E+06	2.42E+06	9.80E+05	6.49E+05	1.03E+04	2.70E+04	2.42E+06	2.42E+06	2.22E+05
10		1.63E+05	2.28E+05	9.50E+04	1.77E+05	1.34E+04	4.42E+04	1.53E+06	8.16E+05	3.26E+05
11		1.30E+06	2.42E+06	6.49E+05	1.41E+06	5.02E+04	9.90E+04	7.21E+05	2.08E+05	4.37E+04
12		1.72E+05	2.42E+06	2.42E+06	2.42E+06	2.42E+05	5.34E+05	2.42E+06	2.42E+06	2.60E+05
13		1.00E+02	1.26E+03	9.25E+02	1.12E+04	1.00E+02	1.00E+02	5.28E+04	4.35E+05	2.65E+04

Table B9. Manure *E. coli* 23S gene abundance (copies/gram-wet-manure) during the summer in-field manure sampling period.

Cow Patty	Sample Day							
	0	1	2	3	4	7	8	11
1	2.67E+07	8.73E+07	1.15E+07	1.25E+07	2.42E+07	2.02E+07	7.40E+07	1.83E+08
2	4.21E+07	3.10E+07	6.18E+06	4.66E+06	2.01E+07	9.30E+07	1.13E+08	9.53E+07
3	1.77E+07	3.80E+07	1.47E+07	1.88E+07	1.85E+07	2.10E+07	1.71E+08	1.24E+08
4	6.25E+07	4.47E+07	2.65E+07	1.93E+07	2.27E+07	4.95E+07		1.21E+08
5	5.21E+07	3.94E+07	4.88E+06	1.33E+07	1.64E+07	2.55E+07	7.75E+07	6.90E+07
6	2.36E+07	5.43E+07	6.40E+06	2.54E+07	1.65E+07	2.29E+07	1.01E+08	7.57E+07
7	1.65E+08	1.45E+08	3.89E+07	2.28E+07	4.04E+07	2.22E+07	8.55E+07	1.39E+08
8	6.11E+07	4.59E+07	1.34E+07	1.77E+07	1.61E+07	2.58E+07	2.83E+07	1.94E+08

Table B10. Manure *E. coli* 23S gene abundance (copies/gram-wet-manure) during the fall in-field manure sampling period.

Cow Patty	Sample Day							
	0	1	2	3	4	10	17	24
1	5.17E+07	2.50E+07	2.49E+07	2.57E+07	2.20E+07	2.29E+07	5.26E+07	3.55E+07
2	2.79E+07	2.76E+07	2.77E+07	7.74E+07	3.01E+07	0.00E+00	0.00E+00	4.27E+07
3	1.62E+07	2.93E+07	2.83E+07	2.07E+07	2.25E+07	0.00E+00	0.00E+00	2.88E+07
4	3.11E+07	2.77E+07	2.77E+07	2.95E+07	2.30E+07	2.06E+07	0.00E+00	3.85E+07
5	4.42E+07	3.12E+07	3.08E+07	3.24E+07	1.97E+07	1.53E+07	0.00E+00	3.78E+07
6	7.48E+08	2.04E+07	2.72E+07	2.67E+07	3.76E+07	1.34E+07	0.00E+00	3.52E+07
7	2.64E+07	3.95E+07	3.24E+07	2.43E+07	2.69E+07	2.28E+07	2.91E+07	2.93E+07
8	3.37E+07	2.31E+07	1.77E+07	2.62E+07	2.45E+07	1.15E+07	3.18E+07	3.00E+07

Table B11. Manure E. coli 23S gene abundance (copies/gram-wet-manure) during the winter in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	7	8	18	25
1	5.63E+08	1.37E+08	1.02E+08	8.34E+07	6.17E+07	8.82E+07	2.14E+08	1.02E+07	0.00E+00
2	1.53E+08	1.05E+08	1.29E+08	2.21E+08	7.40E+07	3.09E+08	7.99E+06	0.00E+00	0.00E+00
3	2.33E+08	6.79E+07	1.07E+08	8.73E+07	1.02E+08	4.18E+06	8.71E+10	0.00E+00	5.59E+07
4	9.03E+07	6.57E+07	8.79E+07	2.97E+07	1.76E+08	9.16E+07	6.90E+07	2.27E+07	6.42E+07
5	1.59E+08	4.33E+08	1.01E+08	8.86E+07	1.35E+08	1.91E+07	7.02E+06	0.00E+00	0.00E+00
6	3.51E+07	1.25E+08	8.89E+07	9.16E+08	5.97E+07	0.00E+00	0.00E+00	0.00E+00	0.00E+00
7	8.31E+08	1.02E+08	1.19E+08	5.19E+08	2.74E+08	4.43E+08	1.63E+08	0.00E+00	0.00E+00
8	5.02E+09	5.27E+08	8.27E+07	0.00E+00	4.52E+08	9.11E+07	3.69E+08	4.75E+06	6.69E+07

Table B12. Manure *E. coli* 23S gene abundance (copies/gram-wet-manure) during the spring in-field manure sampling period.

Cow Patty	Sample Day									
	0	1	2	3	4	8	10	15	17	25
1	0.00E+00									
2	0.00E+00	0.00E+00								
3	0.00E+00									
4	4.05E+06	0.00E+00	0.00E+00	3.49E+07						
5	0.00E+00									
6	0.00E+00									
7	0.00E+00									
8	0.00E+00	0.00E+00	3.13E+07	4.39E+06	0.00E+00					
9		3.67E+08	5.66E+07	9.99E+06	1.79E+07	2.31E+07	3.80E+07	1.41E+09	1.24E+09	2.72E+08
10		0.00E+00	0.00E+00	2.05E+07	0.00E+00	9.21E+07	5.29E+07	5.80E+08	0.00E+00	3.97E+07
11		3.16E+07	1.79E+07	1.94E+07	2.73E+07	1.41E+07	4.68E+07	2.63E+08	1.64E+07	1.73E+07
12		8.33E+06	1.01E+08	6.97E+07	1.13E+09	7.86E+08	6.68E+08	1.65E+08	1.97E+08	4.98E+07
13		0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.47E+07	0.00E+00	1.00E+07	8.06E+06

Table B13. Manure BoBac 16S gene abundance (copies/gram-wet-manure) during the summer in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	7	8	11	21
1	1.16E+10	3.19E+10	6.66E+09	1.01E+09	1.43E+08	0.00E+00	0.00E+00	0.00E+00	0.00E+00
2	1.25E+10	2.03E+10	8.45E+09	4.55E+09	1.21E+09	7.95E+06	0.00E+00	0.00E+00	0.00E+00
3	1.10E+10	8.72E+09	8.72E+09	8.72E+09	1.73E+09	2.44E+08	3.38E+07	0.00E+00	0.00E+00
4	1.92E+10	8.93E+09	4.11E+09	4.37E+08	1.48E+08	1.79E+07		0.00E+00	0.00E+00
5	1.49E+10	1.26E+10	1.21E+10	2.72E+09	2.67E+09	1.73E+08	4.07E+07	1.75E+07	0.00E+00
6	8.41E+09	9.11E+09	1.00E+10	8.58E+09	1.57E+09	3.58E+07	6.59E+07	0.00E+00	0.00E+00
7	1.16E+10	9.02E+09	9.61E+09	4.63E+09	2.63E+09	9.27E+08	3.16E+08	0.00E+00	0.00E+00
8	1.23E+10	2.16E+10	9.62E+09	5.93E+09	1.44E+09	1.38E+08	3.84E+08	0.00E+00	0.00E+00

Table B14. Manure BoBac 16S gene abundance (copies/gram-wet-manure) during the fall in-field manure sampling period.

Cow Patty	Sample Day							
	0	1	2	3	4	10	17	24
1	1.72E+10	3.32E+10	1.70E+10	7.98E+06	3.82E+08	1.13E+09	1.06E+08	1.29E+07
2	7.41E+09	2.45E+10	4.37E+08	1.15E+09	0.00E+00	1.37E+08	0.00E+00	1.11E+08
3	1.36E+10	1.84E+10	5.54E+08	8.81E+07	0.00E+00	2.74E+08	8.83E+07	0.00E+00
4	1.00E+10	2.10E+10	6.52E+09	4.16E+08	1.36E+08	1.87E+08	1.07E+08	0.00E+00
5	4.51E+09	2.69E+10	1.38E+08	4.83E+08	2.27E+09	0.00E+00	0.00E+00	0.00E+00
6	1.43E+10	3.79E+10	4.70E+09	0.00E+00	0.00E+00	8.10E+07	4.38E+07	0.00E+00
7	3.83E+09	2.20E+10	7.38E+06	0.00E+00	7.50E+09	3.28E+08	3.78E+07	5.24E+08
8	6.23E+09	1.78E+10	3.98E+09	1.27E+09	1.94E+08	4.97E+08	6.11E+08	2.12E+08

Table B15. Manure BoBac 16S gene abundance (copies/gram-wet-manure) during the winter in-field manure sampling period.

Cow Patty	Sample Day								
	0	1	2	3	4	7	8	18	25
1	1.62E+10	7.28E+09	1.66E+10	1.32E+10	6.01E+09	1.38E+10	1.12E+10	5.59E+08	3.16E+09
2	2.05E+10	3.44E+09	8.20E+09	9.04E+09	7.83E+09	2.04E+10	4.29E+09	1.29E+07	8.30E+08
3	1.72E+10	6.41E+09	1.10E+10	5.54E+09	7.69E+09	5.95E+09	2.92E+09	1.73E+08	6.87E+08
4	2.78E+10	1.64E+10	1.02E+10	5.06E+09	1.23E+10	8.86E+09	3.53E+09	9.03E+08	7.43E+08
5	7.04E+09	7.60E+09	1.11E+10	1.01E+09	1.24E+10	1.18E+09	1.40E+09	1.17E+09	2.16E+07
6	2.82E+10	1.22E+10	6.47E+09	2.89E+09	7.04E+09	3.23E+08	1.01E+09	8.22E+07	6.77E+06
7	1.17E+10	3.50E+09	9.59E+09	8.73E+09	0.00E+00	9.38E+09	5.72E+08	0.00E+00	1.14E+08
8	2.35E+10	4.90E+09	2.04E+09	1.96E+09	2.44E+07	1.31E+09	4.16E+08	1.79E+08	1.14E+10

Table B16. Manure BoBac 16S gene abundance (copies/gram-wet-manure) during the spring in-field manure sampling period.

Cow Patty	Sample Day									
	0	1	2	3	4	8	10	15	17	25
1	2.70E+09									
2	1.83E+09	2.26E+09								
3	1.05E+08									
4	7.28E+08	3.74E+08	8.99E+06	2.42E+07						
5	1.60E+09									
6	2.18E+09									
7	3.09E+08									
8	9.10E+08	3.76E+09	1.31E+09	9.85E+08	7.03E+08					
9		1.42E+09	9.97E+08	2.05E+08	5.61E+06	5.47E+07	1.71E+07	0.00E+00	0.00E+00	0.00E+00
10		1.24E+09	1.13E+09	1.35E+09	2.44E+08	0.00E+00	7.55E+06	7.07E+06	3.09E+07	0.00E+00
11		1.85E+09	1.83E+08	1.09E+09	1.01E+09	0.00E+00	6.44E+06	0.00E+00	0.00E+00	0.00E+00
12		2.73E+09	9.21E+08	2.64E+08	4.27E+08	4.82E+08	7.01E+06	2.80E+07	0.00E+00	1.54E+07
13		1.24E+09	1.12E+09	1.51E+09	3.12E+09	0.00E+00	0.00E+00	2.11E+08	0.00E+00	4.34E+06

VITA

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