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Impacts of poultry manure application on bacteria and phosphorus in soils and drainage tile-waters

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Impacts of poultry manure application on bacteria and phosphorus

in soils and drainage tile-waters

by

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A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSPHY

Major: Environmental Science

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Iowa State University

Ames, Iowa

2015

DEDICATION

I dedicate this dissertation to my daughter, Isabelle Rose Guzman Hruby. May all the streams she plays in be clean, and may she never remember how many hours I had to spend writing when she was a baby.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

CFU	Colony forming unit
CP	Chisel-plowed
EC	*Escherichia coli*
ENT	Enterococci
FIB	Fecal indicator bacteria
g	gram
h	hour
IDNR	Iowa Department of Natural Resources
ISU	Iowa State University
L	liter
NT	No-till
qPCR	quantitative polymerase chain reaction
P	phosphorus
PM	Poultry manure
SALM	*Salmonella*
TMDL	Total maximum daily load
USDA	United States Department of Agriculture
US EPA	United States Environmental Protection Agency

xii

ACKNOWLEDGEMENTS

I would like to thank my advisor and committee chair, Dr. Michelle Soupir, for her guidance and support, and for providing the understanding and flexibility that made it possible for me to pursue my academic interests, while working and raising a family. I am also grateful to my committee members, Dr. Robert Ewing, Dr. Rameshwar Kanwar, Dr. Thomas Moorman, and Dr. Mack Shelley, for their guidance and valuable feedback throughout the course of this research. Thanks especially to Dr. Ewing for his attention to language, Dr. Kanwar for enabling my adventures in the Middle East, to Dr. Moorman for teaching me new methods, and to Dr. Shelley for his statistical expertise and his willingness to dive into a messy subject!

Much of the water quality research that has been completed at Iowa State has benefitted from the field expertise of Carl Pederson, and the laboratory expertise of Loren Shiers, Beth Douglass, and Leigh Ann Long. I am grateful to all four of them for their amazing devotion to understanding the environment, for teaching me so much, and for putting up with the demands of so many students.

I would like to thank all the graduate students who tried, sometimes unsuccessfully, to keep me young! I wouldn't have survived without coffee with Pramod, rubberband fights with Josh and Ov, awkward jokes with Jason, geeking with Dan, yoga with Amy, lab races with Ross, Xiao's singing and dancing, and Rohith's quiet focus! I also owe endless gratitude to all of the undergrads who sampled in the rain, stayed late in the lab, and put up with the smells! Thanks to all the students, who helped me along the way including, Josh Claypool, Mindy Homan, Samantha Riess, Jason Garder, Promod Pandey, Rohith Gali,

ABSTRACT

Poultry manure (PM) contains nutrients that are highly beneficial for crop production, but loss of nutrients, pathogenic bacteria, hormones, and other PM constituents to groundwater and surface waters can disrupt aquatic ecosystems, raise concerns for recreational water users, threaten drinking-water systems and human health, and have economic consequences for producers. While transport of manure-derived contaminants via runoff has been widely studied, transport of contaminants through artificial drainage systems in less well understood. The principle objective of this study was to compare the effects of poultry manure application rate and tillage practices (chisel-plow and no-till) on drainage tile-water quality. The secondary objective was to further understand the processes and pathways which impact the fate and transport these contaminants. While the focus of this dissertation is on fecal indicator bacteria (*Escherichia coli* and enterococci), the pathogen, *Salmonella*, and phosphorus, concentrations of nitrate and hormones in tile-water samples were also assessed. Results of this research can be used to inform farm-management decisions and watershed planning efforts.

Test plots used for this study are located at Field 5 at the Iowa State University's Agricultural and Biological Research Station, approximately 8 mile (13 km) west of Ames, Iowa. These plots contain calcareous loamy Canisteo-Clarion-Nicollet series soils derived from glacial till. Each plot is drained along its midline by a tile 1.2 meters deep, which outlets into a sump from which flow volume is determined. From 1998-2009, 9 plots were split in half, with each side receiving corn or soybeans in alternate years, and each plot was chisel-plowed annually, prior to planting. In 2010, these plots were transitioned to a continuous-corn rotation, and three established no-till plots were added to the study. From

2010 to 2012, PM was delivered to the site each spring, and broadcast to achieve target rates of 112 and 224 kg/ha nitrogen (PM1 and PM2). Actual PM application ranged from 5 – 40 kg/ha depending on the nitrogen content of the manure. Control plots received no manure (PM0). Each manure application treatment was replicated on three chisel-plowed (CP) plots and one no-till (NT) plot.

To determine the effect of manure application rates and tillage on bacteria concentrations in tile-waters, water samples were collected weekly from drainage tile outlets and following precipitation events from 30 days before manure application to 100 days post application, when tiles were flowing. In 2011 and 2012, additional tile monitoring was employed to capture the hydrographic response to precipitation, and in 2012, smoke-testing was used to determine macropore densities. Manure and tile-water samples were analyzed for the pathogen, *Salmonella spp.* (SALM), fecal indicator bacteria (FIB), *E. coli* (EC), and enterococci (ENT). All three bacterial species were detected in tile-waters, with the highest plot-normalized concentrations observed under the NT PM2 plot in 2010 (3.7×10^4 cfu/100 mL EC, 3.6×10^6 cfu/100 mL ENT, and 1.6×10^4 cfu/100 mL SALM). Macropore density above the tile was also highest (13.9 pores/m) in the NT PM2 plot, in 2012. Individual and 30-day geometric mean ENT concentrations correlated more strongly to SALM than EC.

To determine whether over-wintering of bacteria had occurred, soil samples were obtained each spring at 0-15 cm and 15-30 cm depths. In 2012, soil samples were collected 21, 42, and 158 days after manure application (DAM), to assess the effects of time, manure application rates, and tillage on frequency of detection and concentrations of bacteria. Detection of all three bacteria species in spring soil samples indicated over-wintering of

bacteria was possible. Despite dry conditions in 2012, all three bacteria species were detected 158 DAM. The highest SALM concentration (790 cfu/g dry weight) and detection rate (25%) was found in PM2 plots. SALM detection was higher in CP plots (20%) compared to NT plots (5%). In contrast, tillage practices had no apparent effect on EC or ENT survival, as indicated by both soil sample analyses, and decay rates estimated from tile-water bacteria concentrations. Decay rate constants (μ) ranged from 0.044 to 0.065 day^{-1} for EC, and 0.010 to 0.054 day^{-1} for ENT.

Soil and tile-water samples were also used to determine the effects of manure application rate and tillage on orthophosphate (PO_4-P) losses to tile drainage waters. Spring soil samples (0 – 15 cm and 15 – 30 cm) were analyzed for soil test phosphorus (STP) using the Bray's 1-P method. Water samples were obtained from drainage tiles following precipitation events in 2010 and 2011, and more frequent sampling throughout selected hydrograph events was conducted in 2011 and 2012. STP levels increased significantly under the PM2 treatment over the three year period, while significant differences were not observed under the PM1 treatment or the control plots (PM0). Despite the similarity between 0 – 15 cm STP levels in chisel-plowed (CP) and no-till (NT) plots, orthophosphate (PO_4) concentrations in drainage tile-waters were significantly higher under NT plots (>0.1 mg/L PO_4-P) than under CP plots ($\leq$0.04 mg/L PO_4-P) at $P < 0.05$. Event hydrographs from 2011 and 2012 showed short-lived peaks in PO_4-P concentrations, which may be explained by macropore transport; however, elevated levels of PO_4-P remain throughout the hydrograph in NT plots, suggesting that matrix flow accounts for much of the PO_4-P transport to tiles. Further analyses of soils data indicates that NT soils had

elevated pH levels relative to CP plots, indicating the potential for soil to retain phosphorus may be impacted by chemical conditions in the soil.

In general, this study shows that applying poultry manure at a low (~112 kg/ha N) application rate to chisel-plowed ground is likely to result in smaller bacteria and orthophosphate losses to tile-waters than application at a higher rate (~224 kg/ha N) on no-till plots. Even when heavy manure application and no-till practices were combined, tile-waters only exceeded recreational water quality standards (which do not apply directly to tile outlets) for *E. coli* under exceptionally wet conditions. However, results of this study also show that *Salmonella* can be present in waters containing little or no fecal indicator bacteria.

Producers and watershed managers can take away several important lessons from this research. This study confirms what is already widely accepted, that using lower application rates for poultry manure, and using fertilizers with lower levels of P to balance crop needs, would reduce contaminant losses, benefitting both producers and the environment. An increase in tillage, on the other hand, increases the risk of runoff and soil compaction, and may have other drawbacks; therefore, more modest changes to tillage, such as occasional disking, may be recommended to reduce bacterial water quality impacts by disconnecting macropores and helping to distribute phosphorus within the soil column. Control of pH, and other techniques for increasing the ability of soils to retain phosphorus, may prevent losses to tiles as well as surface-runoff. Although additional research is necessary, the results also suggest that adaptation of the Iowa P-Index to account for the potential for greater losses of P via the subsurface should be considered. Furthermore,

transport of bacteria and phosphorus via tile drainage should be considered when designing

watershed models and water quality improvement plans.

CHAPTER 1: INTRODUCTION

1.1 Introduction

"Water is the most critical resource issue of our lifetime and our children's lifetime. The health

of our waters is the principal measure of how we live on the land."

–Luna B. Leopold, Former USGS Chief Hydrologist

Less than three percent of the water on Earth is freshwater, and even less is currently accessible for use as drinking water (Shiklomenov 1993). While desalination of sea-water is possible, current technologies are energy intensive. As global populations rise, protection of valuable water resources from contamination will be increasingly important. In the United States (US), passage of the Clean Water Act of 1972 has led to significant improvements in the quality of point-source discharges; however, non-point source pollution from urban and rural sources continues to strain both surface and groundwater resources. As well as being a major consumer of freshwater, agriculture is a major source of non-point source pollution in the US (USEPA 2004) and around the world (Wang 2006). Potential contaminants from crop and livestock production include plant nutrients, such as nitrogen and phosphorus, microbial contaminants, such as bacteria and viruses, and organic compounds, including hormones, pesticides, and pharmaceuticals (USEPA 2013b). Degradation of water quality can have significant negative impacts on aquatic life and ecosystems, recreational use, and the health of humans and livestock, and these impacts correlate to significant economic losses. To ensure sustainability of water resources for future generations, it is imperative that we learn how to better manage agricultural areas, minimize contamination, and protect all water users from the risks associated with these contaminants.

1.2 Agricultural Impacts on Water Quality

The Midwest contains some of the most productive land in the world, supporting 65% of the annual corn and soybean production in the United States (USDA 2010). To replenish soils with nutrients necessary for crop production, nitrogen and phosphorus are widely applied in the form of commercial fertilizers or animal manure. Some of these nutrients are lost to surface and groundwaters, where they can be harmful to aquatic life and human health, and have economic consequences for both farmers and downstream water users. Significant efforts to reduce soil erosion and runoff have benefited water quality; however transport of nutrients through tile drainage in this region remains a significant challenge.

1.2.1 Nutrients

Nitrogen (N) in fertilizers comes in many forms. In animal manures, nitrogen is present in both inorganic and organic forms. Once applied to soil, organic N is slowly converted to inorganic ammonium (NH_4^+) and nitrate (NO_3^-) over time. Inorganic ammonium can be readily taken up by plants, converted to ammonia gas and lost to the atmosphere, or converted to nitrate under aerobic conditions. While ammonium adsorbs strongly to soil particles, nitrate is highly mobile in water, and can be quickly transported to surface waters when drainage tiles are present.

Nitrate ingestion can impact human and animal health, and poses significant challenges for drinking-water systems. Nitrate has been associated with methemoglobinemia (blue-baby syndrome), which is the basis of the US EPA's drinking-water standard of 10 mg/L nitrate as N. Nitrate ingestion can also contribute to the formation of *N*-nitroso compounds that have been associated with increased risks of certain cancers and negative reproductive outcomes; however, these risks are not yet well understood (Ward et al. 2005). High nitrate concentrations in feed and drinking water have also been shown to impact livestock and poultry health (Bruning-Fann

and Kaneene 1993). Drinking-water systems that draw water from surface waters or shallow, unprotected, aquifers are most vulnerable to nitrate contamination. These communities may have the option of using treatment systems or switching to deep, protected aquifers; however, there are significant costs associated with treatment or drilling wells, and deep aquifers are more likely to contain natural contaminants, such as radionuclides, sulfate, and metals.

Nitrogen, in the form of ammonium, is more likely to be transported to surface waters via direct discharge or runoff containing sediments, manure, or fertilizer, than via the subsurface; however, it can also have significant impacts. Ionized ammonium (NH_4^+) is converted to unionized ammonia (NH_3) in water, under specific temperature and pH conditions. Ammonia is toxic to aquatic life, and acute and chronic surface water quality standards for ammonia are based on the life-cycle stage of invertebrates and fish (IAC 2012). Both ammonia and ammonium interfere with drinking-water treatment systems that depend on chlorine for disinfection. As ammonia levels in source waters increase, water treatment facilities are forced to use additional chlorine, increasing taste-and-odor problems, the potential for formation of disinfection byproducts including trihalomethanes, and the cost of treatment (Sawyer 2008; DMWW 2014).

Phosphorus (P) applied to crops is generally supplied by manure, or commercial fertilizers derived from phosphate rocks. Plants generally absorb phosphorus in the form of orthophosphate, but they can also use some forms of organic phosphorus. Phosphates often bind to particles with ion exchange capacity such as iron and aluminum oxides and clay minerals in acidic soils, or become incorporated into calcium carbonate minerals, such as apatite, in calcareous soils (Iyamuremye and Dick 1996). Given the tendency for phosphorus to attach to soils, efforts to reduce phosphorus in surface waters have focused on reducing soil erosion;

4

however, recent research highlights the potential for drainage tiles to transport phosphorus to surface waters, especially in areas of intense manure application (Sharpley et al. 2004).

Together, nitrate and phosphates, stimulate growth of algae and cyanobacteria in surface water bodies, threatening both aquatic and human health. When these organisms die, oxygen consumption by decomposing bacteria results in hypoxic conditions the can negatively impact populations of fish and other aquatic organisms (Carpenter et al. 1998). Areas of hypoxia at the mouths of large river systems, such as the Mississippi, have been spreading since the 1960s, significantly disrupting aquatic ecosystems (Diaz and Rosenberg 2008). In addition, cyanobacteria (blue-green algae) blooms, can release toxins into the water that cause skin rashes and other illness in humans. The presence of these toxins can lead to closure of recreational areas and disrupt tourism. In the summer of 2014, high concentrations of microcystin were measured in drinking-water derived from Lake Erie as a result of a cyanobacteria bloom. This caused the city of Toledo, Ohio, to ban use of tap water for drinking, cooking, or tooth-brushing for two days. Recent research has shown that while total phosphorus loading to Lake Erie has been reduced over the past decade as a result of improved wastewater treatment, there has been in increase in phosphorus in the dissolved form, which is thought to have contributed to the 2014 bloom (Kane et al. 2014). Increased phosphorus transport to drainage tiles has been documented in fields with high surface soil phosphorus concentrations, such as heavily manure soils (Beauchemin et al. 2003; Madison et al. 2014), and macropores may facilitate phosphorus transport (Geohring et al. 2001).

1.2.2 Pathogens and fecal indicator bacteria

Livestock and poultry manures have been shown to contain enteric pathogens that can infect humans, such as *Salmonella*, *Campylobacter*, and enterohemorrhagic *Escherichia coli* (*E.*

coli) (USEPA 2013b). Human health can be impacted by ingestion of contaminated recreational or drinking-waters or by consumption of foods contaminated by irrigation or land application (Rogers and Haines 2005; Craun et al. 2010; Dale et al. 2010; USEPA 2013b). In addition, transport of these pathogens can compromise the bio-security of poultry facilities and may lead to contamination of meat or egg products (CDC 2010; Castiglioni Tessari et al. 2012). Concern over microbial contaminants is further exacerbated by detection of antibiotic resistant bacteria associated with livestock and poultry manure (West et al. 2011; Hoang et al. 2013; Cook et al. 2014; Garder et al. 2014), and documented increased risk of resistant infections in those living in proximity to confined animal feeding operations (Carrel et al. 2014).

Historically, bacterial pathogens have been difficult to monitor given the large number of potential pathogens, low pathogen concentrations in environmental media, and the expense of analytical techniques. As an alternative, the US Environmental Protection Agency (EPA) has recommended the use of fecal indicator bacteria, including fecal coliforms, *E. coli* and enterococci, based on epidemiological studies that showed increased risk of illness associated with concentrations of these indicators in recreational waters (Pruss 1998). In Iowa, and nationwide, the number of streams that exceed water quality standards based on concentrations of fecal indicator bacteria (FIB), has steadily increased (USEPA 2004; Iowa Department of Natural Resources 2012), leading states to invest considerable resources into modeling and watershed improvement efforts. While the original epidemiological studies linking FIB concentrations and illness from recreational exposure were conducted in areas with microbial impacts from human waste discharge (Stevenson 1953), only a handful of studies have documented increased risks associated with FIB concentrations in agricultural watersheds and fewer still are able to determine the exact source of the microbial contamination (Harwood

2005). Some genetic source-tracking methods appear promising (Seurinck et al. 2005; Casarez et al. 2007); however, these often require large, locally-sourced, and continuously updated genetic libraries from known fecal sources (Rogers and Haines 2005). To better understand and improve predictions of risks associated with microbes, a need for studies focused on specific sources has been identified (Benham et al. 2006).

1.2.3 Hormones

Along with nutrients and bacteria, all animal wastes contain naturally produced steroidal hormones including androgens, such as testosterone and androstenedione, gestagens (mainly progesterone), and estrogens, including estrone, estriol, and estradiol (which comes in two configurations, 17*alpha* and 17*beta*) (Finlay-Moore et al. 2000; Raman et al. 2001; Kjaer et al. 2007; Bartelt-Hunt et al. 2011; Bevacqua et al. 2011). For example, reported concentrations of estradiol in broiler litter range from 14 - 904 µg/kg dry weight (Hanselman et al. 2003). Hormones can also be introduced into manure via feed or pharmaceuticals. Plants, such as soybeans produce phyto-estrogens, while fungi can form estrogenic mycotoxins, such as zearalanone produced by *Fusinarium* which can colonize corn (*Zea maize* L.). Zearalanol and trenbolone acetate are steroid hormones administered to livestock (mostly cattle).

Both synthetic and artificial hormones have been shown to be endocrine-disrupting compounds and introduction of hormones into the environment in sufficient quantities could pose risks for aquatic life (Jensen et al. 2006) and humans (Frizzel et al. 2011). For example, after 21 days of exposure in waters with a concentration of 40 ng/L 17*beta*-estradiol derived from poultry litter, vitellogenin was detected in mature male fathead minnows, indicating feminization, and at 74 ng/L 17*beta*-estradiol, 90% of adolescent males had become female or feminized (Yonkos et al., 2010). Even when concentrations of individual hormones in the

environment are lower than established no-observed-effect-levels, it is possible that the potency of mixtures of hormones may be greater than the sum of their individual components (Rajapakse et al. 2002).

1.3 Manure Management

Iowa is a leader in US production of animals, animal products, and grains: 20.5 million swine, 52 million layer chickens, 4.4 million turkeys, and 3.9 million cattle (USDA/NASS 2013) are housed in confinement operations (Figure 1.1) and open feedlots, annually. In 2013, 13.6 million acres were used for corn production and 9.3 million acres for soybeans (USDA/NASS 2013). Much of the corn produced in Iowa is used for ethanol production (43%), 15% goes to distiller's dried grains with soluble (DDGS) from ethanol production, approximately 14% is used for animal feed, 8% is exported, 7% goes to residential use, and 13% is processed for other uses (ICGA 2014). Based on growing international demand for meat products from countries, like China, demand is projected to continue growing (Hansen and Gale 2014).

The production of animals and row crops is highly integrated, for both economic and agronomic reasons. As feed is a major expense for animal producers, it makes sense for these producers to grow their own. In turn, fertilizer is the greatest expense for corn producers, and commercial fertilizer prices are tied to the price of fuel. Manure from animal feeding operations contains high levels of organic matter and plant nutrients, making it a valuable soil amendment, especially for lands used for corn production that have high nitrogen needs. In addition, use of manure as fertilizer reduces the need for treatment or disposal of large quantities of animal waste. While application of manure as fertilizer makes good agronomic sense, nutrients, bacteria, and other contaminants can be lost to surface and groundwater resources. Thus, careful manure management must be used to reduce these impacts. Careful attention to the nutrient

needs of crops is also beneficial to producers, by helping them to get the most economic value

from manure.
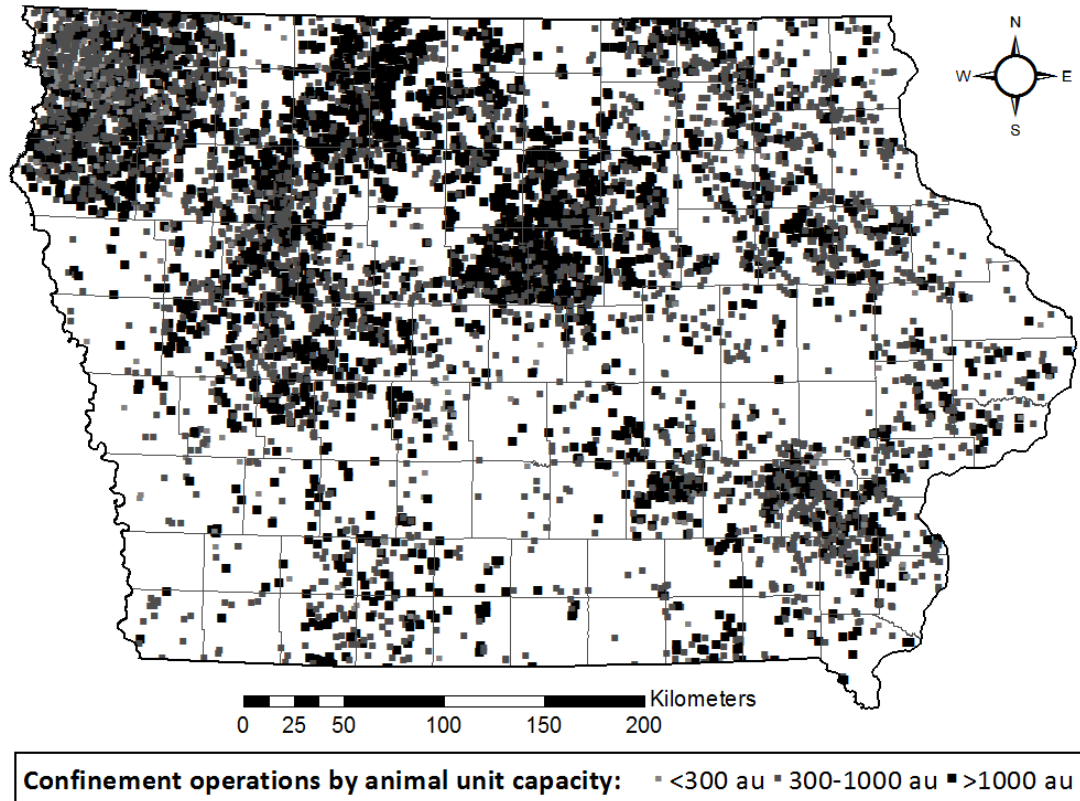
Figure 1.1 Confined animal feeding operations in Iowa by animal unit capacity (IDNR, 2014).

Extensive research has been conducted over the past three decades on crop nutrient

management at Iowa State University, throughout the Midwest, and across the globe. Testing of

manure, soil, and corn stalks has been employed to help producers manage plant nutrients

(Mallarino and Wittry 2000; Kang et al. 2008; Mallarino 2008; Kyveryga and Blackmer 2012).

Numerous studies have been conducted to identify the best practices for storing and applying

manure that minimize nutrient losses, while ensuring that producers meet yield goals (Kanwar et

al. 1988; Miller et al. 2003; Sharpley et al. 2004; Bakhsh et al. 2005; Chi Kim et al. 2010;

Delgado et al. 2011; Shipitalo et al. 2013). Phosphorus indices have been developed to help

prevent over-application of phosphorus-rich manures in high-risk areas (Mallarino et al. 2002; Bechmann et al. 2007). In addition, researchers have evaluated the potential benefits of in-field, edge-of-field, and watershed-scale practices that can help mitigate potential nutrient losses, many of which were summarized in the 2013 Iowa Nutrient Management Strategy (IDALS/IDNR/ISU 2013).

Studies focused on bacterial contamination from manure application have predominantly focused on transport via runoff (Soupir et al. 2006; Jenkins et al. 2008; Brooks et al. 2009; Harmel 2009; Guzman et al. 2010; Sistani et al. 2010; Delgado et al. 2011). Recently, however, multiple field studies have documented the potential for bacterial transport through the subsurface (Coelho et al. 2007; Thiagarajan et al. 2007; Pappas et al. 2008; Chi Kim et al. 2010; Samarajeewa et al. 2012; Garder et al. 2014). Additionally, increased transport of bacteria to drainage tiles has been observed under reduced tillage practices facilitated by flow through undisturbed macropores (Coelho et al. 2007; Thiagarajan et al. 2007; Pappas et al. 2008; Chi Kim et al. 2010; Samarajeewa et al. 2012; Garder et al. 2014).

Vaccination, management of conditions to reduce animal stress, fly and vermin control, diet control, composting and other manure treatments methods have been recommended to reduce bacteria loading from manure application (Spiehs and Goyal 2007). While careful timing of application to avoid imminent precipitation, saturated conditions, or snowmelt have been shown to reduce bacterial loading to surface and subsurface waters (Joy et al. 1998; Pappas et al. 2008), simple recommendations for manure application method have not been established. Although surface application has been shown to enhance bacterial decay resulting from exposure to ultra-violet light, injection or mixing of manure into soils reduces the potential for runoff (Hutchison et al. 2004b; Samarajeewa et al. 2012).

Numerous in-field practices have also been proposed for minimizing the risks of pathogen transport to water, including application of biochar (Abit et al. 2014) and introduction of cover-crops (Rothrock et al. 2012). Edge-of-field practices, including grass and prairie filter strips have been shown to decrease risks from runoff (Coyne et al. 1998; Guber et al. 2009), and may have benefits for subsurface drainage as well. Finally, watershed scale efforts, such as strategically placed wetlands, could help reduce impacts of upstream manure application (Hill and Sobsey 2001; Ibekwe et al. 2002).

Despite significant progress in our understanding of the fate and transport of plant nutrients and bacteria, and improvements in manure management and land-use practices, many questions remain, and water quality issues continue to arise in agricultural areas; therefore, additional research is needed to understand the causes and solutions to these problems.

1.5 Research Objectives

To fully evaluate the potential environmental impacts of livestock and poultry waste application, each type of manure must be tested in all common combinations of storage, application, and land-management methods. Additionally, these combinations must be tested on a variety of soil types under a wide range of climatic conditions. Although laboratory tests are necessary to isolate the effects of individual factors on contaminant fate and transport, field tests conducted using realistic agronomic practices integrate multiple environmental variables and allow for the quantification of impacts under natural conditions. The research presented in this dissertation was motivated by a desire to provide such source-specific data, specifically, the effects of spring poultry manure application to cornfields on a tile-drained landscape in the Upper Midwest. The principle objective of this study was to compare the effects of poultry manure application rate and tillage practices (chisel-plow and no-till) on concentrations of

nutrients, bacteria, and hormones in soils and drainage tile-waters. As the study progressed, the following additional objectives were added:

- To determine how long bacteria survive in soils after poultry manure application.

- To determine whether concentrations of fecal indicator bacteria, correlate to pathogenic bacteria concentrations in tile-waters, and to evaluate whether recreational water quality standards are likely to protect public health in downstream waters.

- To evaluate various method of quantifying bacteria in manure, soil, and water.

- To quantify the density of macropores above drainage tiles under a range of manure application rates and tillage practices.

This 3-year study was conducted under a wide range of moisture conditions, and tile flow regimes. Therefore, our findings can be used to improve predictive models and risk assessments, and to inform producers and watershed managers interested in minimizing the impacts of poultry manure application on water quality. This dissertation addresses all of the objectives outlined, above; however, practical constraints lead us to focus primarily on FIB, *Salmonella*, and phosphorus as described, below.

1.6 Study Design

This study is a continuation of a long-term evaluation of the effects of poultry manure on soil and water quality conducted at Field 5, a set of experimental plots 8 miles (13 km) west of Ames, Iowa. These plots are part of Iowa State University's Agricultural and Biosystems Engineering Research Station. This site is located on loamy Clarion-Webster-Nicollet series soils derived from glacial tills, and each plot is drained by a subsurface tile, accessible via a sump at the lower end of the field. The set-up of these plots is conducive to side-by-side comparison of the effects of various poultry manure application and land-management practices.

In previous years, 9 chisel-plowed plots at this site were used to evaluate the effects of two poultry manure application rates on nitrate concentrations in drainage tile-waters under a corn-soy rotation (Nguyen et al. 2013). The study design was amended in 2010 in response to increased corn production statewide, combined with greater adoption of reduced tillage practices. Starting in 2010, three adjacent plots historically maintained as no-till plots were added to the study, and the crop rotation was converted to continuous corn.

The original plan for the 3-year (2010-2012) study proposed to evaluate two pathogens (*Salmonella* and *Campylobacter*), two species of fecal indicator bacteria (*E. coli* and *Enterococcus spp.)*, hormones (including alpha- and beta-estradiol), and common plant nutrients (nitrogen and phosphorus) in soil and tile-waters. Initial tests of the proposed method for culturing *Campylobacter* were unsuccessful, and limitations on time and sample quantity available for analysis led to the decision to focus on *Salmonella* and the fecal indicators. Two methods for quantification of *Salmonella spp.* were compared: growth on selective agar (using XLD agar) and quantitative polymerase chain reaction (qPCR) using *invA* primers.

The yeast estrogenicity screen (YES) assay (Routledge and Sumpter 1996) proved to be an effective screening tool for estrogenic hormones, as confirmed by LC/MS/MS analyses conducted by the Water Resources Laboratory at the University of Nebraska-Lincoln following the procedure described by Snow et al. (2013). Preliminary results from 2010, show that the synthetic hormones, 17*alpha*-trenbolone and *beta*-zearalanol, occurred at higher concentrations under no-till plots, less than 30 days after manure application, at high tile flow rates (Figure 1.2). Additionally, the natural steroidal hormone, 17*alph*a-estradiol, was observed in tiles draining chisel-plowed plots following manure application (Figure 1.2). None of the following hormones were detected in the 15 tile-water samples tested using LC/MS/MS: testosterone, epitestosterone,

progesterone, 17*alpha*-hydroxyprogesterone, androsterone, 4-androstenedione, androstanedione, melengesterol acetate, trendione, 17*beta*-trenbolone, estriol, estrone, ethynyl estradiol, 17*beta*-estradiol, *alpha*-zearalanol, *alpha*-zearalenol, and *beta*-zearalenol.
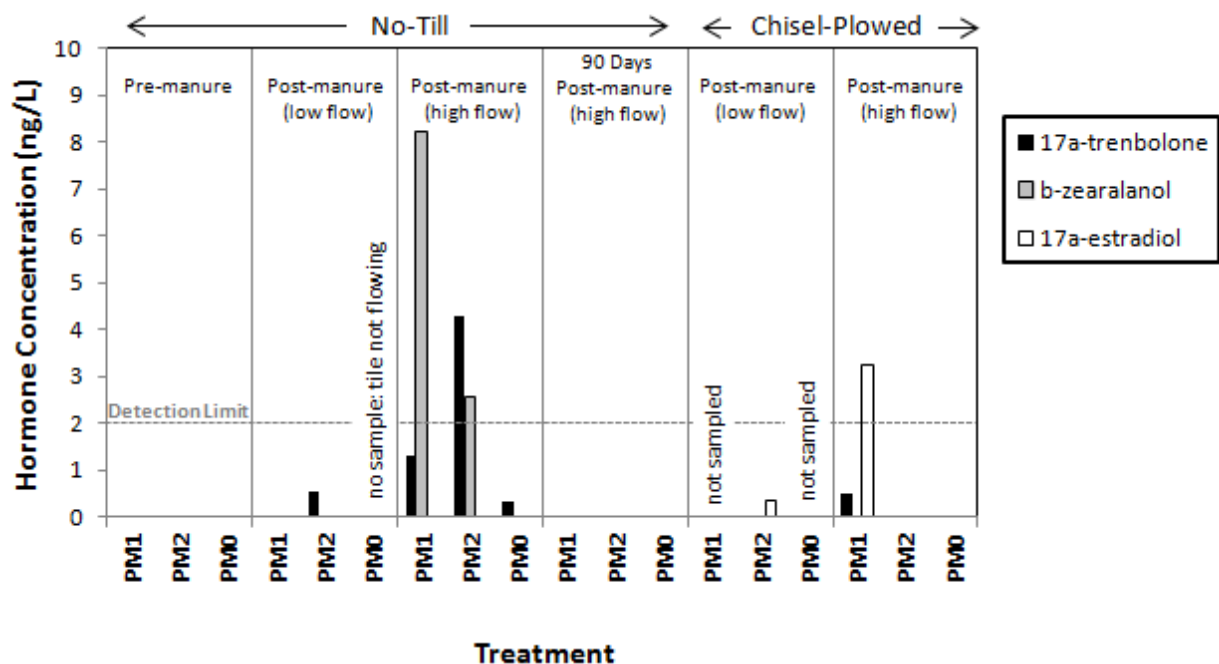
Figure 1.2 Hormone concentrations measured using LC/MS/MS for 2010 tile-water samples collected before and after poultry manure application at low and high tile flow rates. PM1 is the low application rate, PM2 is twice the PM1 rate, and PM0 represent controls with no manure.

These results suggest that the potential for hormone transport is restricted to periods of high flow shortly after manure application, and does not extend to 90 days after application. The presence of the synthetic hormone, 17*alpha*-trenbolone was surprising given that it is mainly administered to cattle. All measured concentrations of 17*alpha*-trenbolone were below the range of concentrations (7–16 ng/L) shown to decrease fecundity of fathead minnows (*Pimephales promelas*) (Jensen et al. 2006). *Beta*-zearalanol, has been detected in layer hens and eggs fed *Fusarium* toxin-contaminated maize (Danicke et al. 2002). Although not sufficient for statistical analyses, these results suggest differences in transport of hormones under no-till and chisel-

plowed plots. In contrast, results of a similar study of poultry manure impact on drainage tile-waters by Jenkins et al. (2009) did not find significant effects of tillage on hormone transport. Further quantification of the impacts of poultry manure on the hormone content of tile-waters would be very interesting; however, a larger number of manure and water samples would be required, and a thorough hormone analysis using LC/MS/MS can be costly.

Concentrations of both nitrate and orthophosphate were measured throughout the study. While the long-term focus of research at Field 5 has been on nitrate losses from poultry manure application, phosphorus contributions have not been previously reported; therefore, we chose to focus our efforts on phosphorus. We anticipate future publication of nitrate results.

During the course of our investigation, significant differences in seasonal precipitation allowed for characterization of effects of manure application under both extremely wet and extremely dry conditions. Precipitation and tile flow data were collected at the site. Manure and soil samples were sampled each spring prior to manure application. Three additional sets of soil samples were also obtained in 2012. Tile-water samples were collected directly from tile outlets throughout the 2010-2012 growing seasons (April-September), when flow was available. 2010 was a wet year, and tiles flowed throughout the majority of the season. Standing water was observed in the fields twice in 2010. In contrast, 2011 and 2012 were dry years, and tiles did not continue flowing throughout the season. In 2011 and 2012, several attempts were made to collect tile-water quality data hourly throughout precipitation-induced hydrographs using auto-sampling devices; however, the lack of precipitation in these years limited the success of these efforts. Detailed descriptions of sampling and analytical procedures can be found in the following chapters.

1.5 Thesis Organization

The studies presented here characterize the potential impacts of poultry manure application to cornfields on soil and drainage-tile-water quality. In Chapter 2, the tile-water bacteria concentrations are evaluated to determine effects of time, tillage practices (chisel-plowed or no-till), and manure application rates (at low and high agronomic rates based on nitrogen needs of corn). In Chapter 3, the potential for long-term (~1 year) survival of manure-derived bacteria in soils is assessed, using soil samples collected in spring of three consecutive years. In addition, effects of time, tillage, and application rate on bacterial survival in soils are assessed from four sets of soil samples obtained in 2012, and tile-water bacterial concentrations from 2010 are used to estimate decay rates. In Chapter 4, the effects of repeated poultry manure application on soil test phosphorus levels and tile-water orthophosphate concentrations are evaluated.

Chapter 5 summarizes the conclusions of these studies, highlighting the implications for manure and watershed management. In general, while past research has shown that reducing tillage has numerous benefits, the increased potential for transport of both microbial contaminants and phosphorus from no-till fields to tile-waters should be considered as part of a holistic approach to resource management.

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CHAPTER 2: SALMONELLA AND FECAL INDICATOR BACTERIA CONCENTRATIONS IN DRAINAGE TILE-WATERS FROM PLOTS RECEIVING POULTRY MANURE

2.1 Abstract

Application of poultry manure (PM) to cropland as fertilizer is a common practice in artificially drained regions of the Upper Midwest. To assess the potential for PM to contribute pathogenic bacteria to downstream waters, information is needed on the impacts of manure management and tillage practices on bacteria transport to drainage tiles under a wide range of field conditions is needed. In this 3-year study (2010-2012), PM was applied annually in spring, prior to planting corn. PM application rates ranged from 5 – 40 kg/ha to achieve target rates of 112 and 224 kg/ha nitrogen (PM1 and PM2). Control plots received no manure (PM0). Each treatment was replicated on three chisel-plowed (CP) plots and one no-till (NT) plot. Water samples were collected weekly from drainage tile outlets and following precipitation events from 30 days before manure application to 100 days post application, when tiles were flowing. In 2011 and 2012, additional tile monitoring was employed to capture the hydrographic response to precipitation, and in 2012, smoke-testing was used to determine macropore densities. Manure and tile-water samples were analyzed for the pathogen, *Salmonella spp.* (SALM), fecal indicator bacteria (FIB), *E. coli* (EC), and enterococci (ENT). All three bacterial species were detected in tile-waters, with the highest plot-normalized concentrations observed under the NT PM2 plot in 2010 (3.7×10^4 cfu/100 mL EC, 3.6×10^6 cfu/100 mL ENT, and 1.6×10^4 cfu/100 mL SALM). Macropore density above the tile was also highest (13.9 pores/m) in the NT PM2 plot, in 2012. Individual and 30-day geometric mean ENT concentrations correlated more strongly to SALM than EC.

2.2 Introduction

Over 15 billion eggs are produced annually in Iowa (UDSA-NASS 2014), resulting in the generation of over 5.6 million Mg of fresh layer manure (Naber 1990). Poultry manure (PM) is an excellent source of phosphorous, nitrogen, potassium, and other nutrients essential for plant growth, and is therefore regularly applied to cropland in lieu of, or in addition to, commercial fertilizer (Sistani et al. 2010). Along with beneficial nutrients, PM commonly contains pathogenic bacteria, including *Salmonella* (SALM) (Kraft et al. 1969; Rodriguez et al. 2006; Berghaus et al. 2013). Once released to the environment, pathogens can be transported to recreational, irrigation, or drinking waters and pose a risk to human health (Rogers and Haines 2005; Craun et al. 2010; Dale et al. 2010; USEPA 2013a), or compromise the bio-security of poultry facilities (CDC 2010; Castiglioni Tessari et al. 2012). For example, PM is suspected as the source of *Salmonella* Hartford and *Plesiomonas shigelloides* that sickened approximately 30 people in New York after food was prepared with contaminated water (CDC 1998). In this instance, the water was obtained from a shallow well surrounded by tilled, manure-amended farmland, following a rainfall event (CDC 1998). Zoonotic pathogens originating from manure-amended lands have also been shown to cause increased risk of gastrointestinal illnesses associated with exposures to recreational waters (Mead et al. 1999; Rogers and Haines 2005).

In addition to pathogens, PM commonly contains non-pathogenic bacteria, including *E. coli* (EC) and enterococci (ENT) (Terzich et al. 2000; Rogers et al. 2011). Studies of human exposures have confirmed the increased risk of negative health outcomes from swimming in waters containing these and other fecal indicator bacteria (FIB) (Pruss 1998). Thus, the US Environmental Protection Agency (US EPA) first established microbial recreational water quality standards using FIB under the Clean Water Act in 1976, and the agency has continued to

update these criteria based upon available research. But while FIB are sometimes good predictors of potential risk, they are rarely accurate predictors of pathogen concentrations in the environment (Field and Samadpour 2007; Payment and Locas 2011). Even when FIB correlate to pathogen concentrations in bacterial sources, differential survival rates and transport behaviors between species complicate interpretation of indicator data in downstream or down-gradient samples. For example, Winfield and Groisman (2003) found that SALM are much better adapted than EC to survive outside their host species. Alternative indicators, such as wastewater chemicals and genes unique to pathogenic bacteria, have been tested (Haack et al. 2009); however, despite their shortcomings, EC and ENT have remained the preferred indicators for assessing the risk to human health (US EPA, 2012). Following U.S. EPA requirements, Iowa's single-sample maximum water quality standards for EC are 235 cfu/100 mL for primary contact and children's recreation and 2880 cfu/100 mL for secondary contact recreation. Geometric mean standards are 126 cfu/100 mL EC for primary contact and children's recreation and 630 cfu/100 mL EC for secondary contact recreation (IAC 2012).

Nationwide, FIB contributed to 10,654 impairments (15% of surveyed waters) based on exceedances of recreational water quality standards (US EPA, 2012). To address these impairments, states are required to develop watershed improvement plans based on total maximum daily load (TMDL) calculations. Watershed-scale models commonly used to evaluate microbial fate and transport often underestimate microbial contributions via drainage tiles, or assume that all leached bacteria die-off (Gassman et al. 2007). To improve these models, source-specific data sets that document the variability of bacterial concentrations in tiles under common agricultural practices are needed, along with research relating pathogens to the more readily-modeled FIB (Benham et al. 2006). Quantitative microbial risk assessments (QMRAs) also

require source-specific bacterial distributions to more accurately determine potential exposures of water users to pathogens (Soller et al., 2010).

Most PM generated in Iowa confinements is stockpiled, and solid manure is broadcast on cropland as fertilizer. Transport of bacteria from applied PM can occur via runoff or through the subsurface. In regions with poor natural drainage, including formerly glaciated landscapes, tile-drainage systems are commonly installed to remove excess water and facilitate plant growth. These systems move water quickly to surface waters, decreasing the soil's natural capacity for filtration. Bacterial transport in runoff from application of various types of manures, including poultry, has been widely studied (Soupir et al. 2006; Jenkins et al. 2008; Brooks et al. 2009; Harmel 2009; Guzman et al. 2010; Sistani et al. 2010; Delgado et al. 2011). Laboratory-scale studies have shown that SALM and other pathogens can be transported through over 1 meter of soil, and transport is controlled by soil types, hydrodynamic forces, physical filtration, and interactions between bacterial surface-charges and air, water, and soil interfaces (Haznedaroglu et al. 2009; Bech et al. 2010; Chen 2012). Studies show increased bacterial transport under saturated conditions; however, transport has also been shown to occur under unsaturated conditions when preferential flow paths, such as vermicular macropores, are present (Beven and Germann 1982; Abu-Ashour et al. 1998; McMurry et al. 1998; Bottinelli et al. 2013). Laboratory studies are often designed to isolate factors that influence bacteria transport. By design, these studies do not account for the complex interactions between manure-borne bacteria and native microbial populations or predators, nor do they account for variability of temperature and moisture conditions within and between field seasons.

The results of multiple field-studies, of both cattle and swine manures, highlight the potential for bacterial transport to groundwater and tile-waters (Coelho et al. 2007; Thiagarajan

et al. 2007; Pappas et al. 2008; Chi Kim et al. 2010; Samarajeewa et al. 2012; Garder et al. 2014). Increased transport has been observed under reduced tillage practices when macropores provide direct conduits to drainage tiles (Joergensen et al. 1998; Shipitalo and Gibbs 2000; Samarajeewa et al. 2012). While effects of manure application rates have been mixed (Coelho et al. 2007; Pappas et al. 2008), timing of manure application relative to precipitation has been shown to play an important role in determining transport to drainage tiles (Samarajeewa et al. 2012). Recent studies of PM-amended soils have reported survival of FIB and pathogens for weeks and months after application (Rogers et al. 2011; Cook et al. 2014), highlighting the potential for continued release of bacteria to subsurface waters. Additionally, application of PM has been shown to increase drainage and earthworm activity (Endale et al. 2010).

Despite the documented potential for bacteria transport to tile-waters, no previous studies have addressed the impacts of PM on microbial tile-water quality. Here, we present analyses of both SALM and FIB concentrations in drainage tiles waters under realistic field conditions, following PM application. The objectives of this study were to evaluate the effects of the following factors on tile-water bacterial concentrations: time relative to manure application, PM application rate, and tillage practices. In addition, macropore densities were quantified for CP and NT plots using smoke tests to explain observed differences between bacterial concentrations within this study, and with results of comparable studies. Concentrations of EC were evaluated in the context of current recreational water standards, and correlations between individual and geometric mean EC and ENT to SALM concentrations were assessed. This 3-year study was conducted under a wide range of moisture conditions, and flow regimes. Therefore, our findings can be used to improve predictive models and risk assessments, and to inform producers and watershed managers interested in minimizing the microbial impacts of PM application.

2.3 Materials and Methods

2.3.1 Study site

Field experiments were conducted from 2010 through 2012 at Iowa State University's Agronomy and Agricultural Engineering Research Farm, west of Ames, Iowa. The site is located in the Des Moines Lobe landform region, a landscape formed by the last glacial maximum that occurred in the state during the late Pleistocene Epoch, between 18,000 – 15,000 years ago. The research plots are located on soils with a Canisteo-Clarion-Nicollet association, which are loamy soils formed in glacial till under prairie vegetation, characterized as moderately permeable, with drainage classifications ranging from well-drained to poorly drained. Soil texture typically ranges from 30-45% sand, 35-42% silt, and 20-30% clay content (NRCS, 2014). Topsoil (0 – 30 cm) measurements for all plots (2010 – 2012) range from 2.0 – 4.4% organic matter content. Plot slopes range from 0 – 5 percent.

Drainage tiles are installed along the midline of each plot at a depth of approximately 1.2 meters. At the edge of each plot, tiles outlet into sumps, which are protected from the elements and accessible for sampling. Although recreational water quality standards do not apply to tile outlets, a segment of the South Skunk River, downstream from this study site, is listed as impaired due to ongoing exceedances of both the geometric mean and the single-sample maximum standards for Class A1 primary contact recreational waters (IDNR 2012).

A long-term study of the effects of PM on water quality began at this site in 1998, with nine chisel-plowed (CP) plots under a corn-soybean rotation. For 12 years, PM was applied in the spring to the portion of each plot that was to be planted in corn as (Nguyen et al. 2013). Starting in 2010, all CP plots were converted to a continuous corn rotation, and 3 established NT plots were included as part of the PM study. PM and urea ammonium nitrate (UAN) were

applied each spring, prior to planting, at application rates based on nitrogen (N) goals as

described in Table 2.1. The PM2 goal achieves the maximum recommended rate of N

application for continuous corn production in Iowa (Sawyer et al. 2006).

Table 2.1 Plot treatments with nitrogen fertilization goals on a per hectare (ha) basis.

Treatment	Treatment Description	Nitrogen Fertilization Goal (kg/ha/year)
PM0 - NONE	control, no manure or fertilizer	0
PM0 - UAN	control, urea ammonium nitrate fertilizer	224
PM1	PM applied at a low rate	112
PM2	PM applied at double the low rate	224

Figure 2.1 shows the layout of the study plots. Plot areas range from 0.08 to 0.51

hectares (ha). Tiles are spaced 36.3 meters (m) apart. The experimental treatments on CP plots

were arranged in a randomized design with 3 plots receiving PM1 treatment, 3 plots receiving

PM2 treatment, and 3 plots receiving no manure as controls (PM0). Two of the control plots

were fertilized with urea ammonium nitrate (PM0 - UAN), and one that received no fertilizer

(PM0 – NONE). Individual NT plots were fertilized with PM1, PM2, and PM0 – UAN

treatments. The NT PM1 and PM2 plots are split, so that separate tiles capture the upper and

lower portions of the plots, allowing for replication of tile-water samples. No manure was

applied to either type of PM0 plot; thus, we will not distinguish between PM0 treatments for the

remainder of this report. The six possible combinations of tillage and treatment are as follows:

CP PM0, CP PM1, CP PM2, NT PM0, NT PM1, and NT PM2.
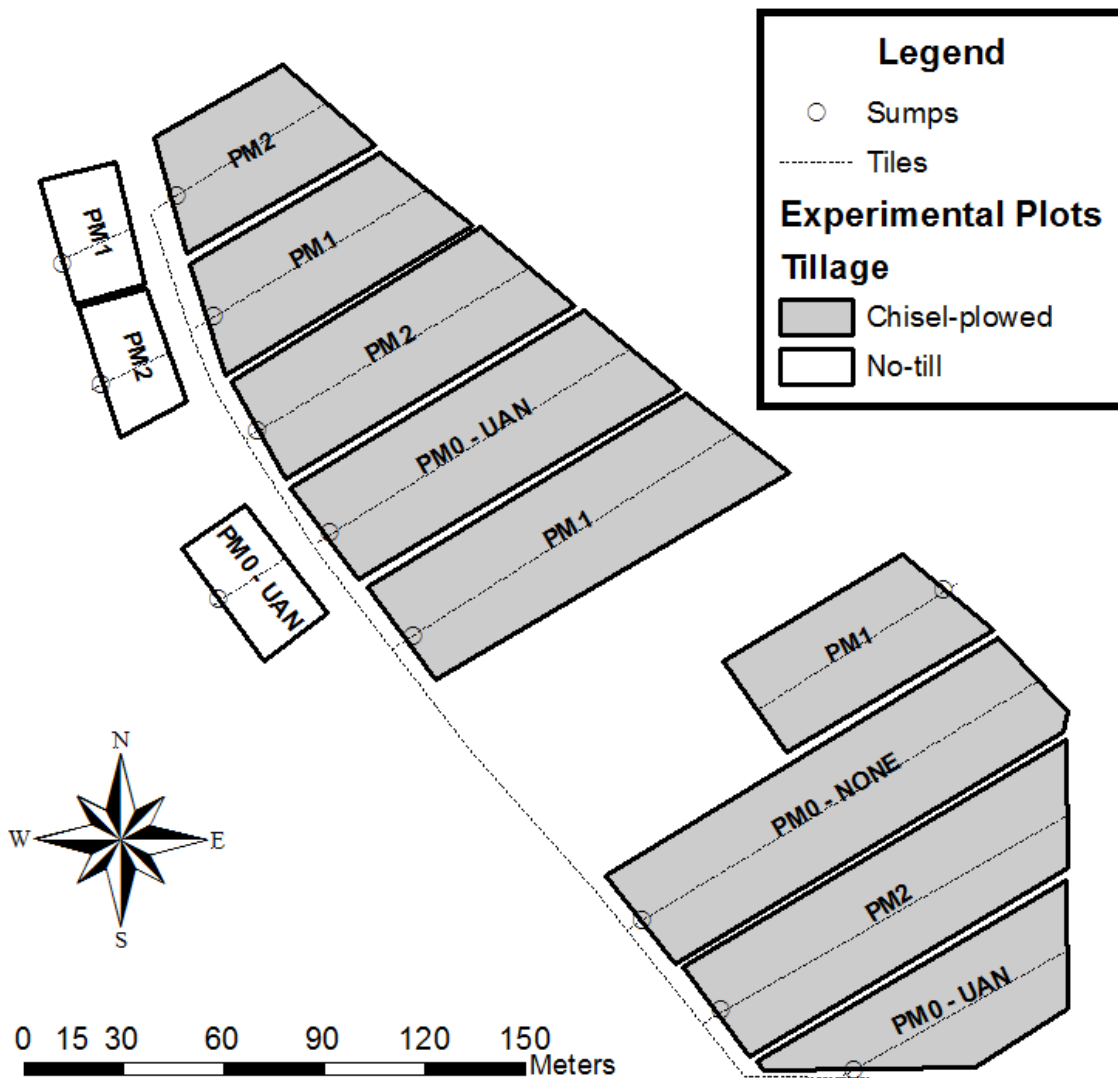
Figure 2.1 Map of experimental plot design with tillage practices (chisel-plowed and no-till), fertilization treatments (PM1, PM2, PM0-UAN, and PM0-NONE), and locations of tiles and sumps.

2.3.2 Precipitation and tile flow measurements

Rainfall data were collected using two tipping-bucket rain gauges with HOBO data-loggers (Onset Computer Corp., Pocasset, Mass.) located at the site. Volume-triggered float pumps were located in each sump, and connected to in-line flow meters (Neptune Technology Group, Tallassee, AL), with Hobo Pendant Event Data Loggers (Onset Computer Corp., Pocasset, MA), to provide continuous volume-based tile flow data. To supplement continuous readings, instantaneous tile flow rates were obtained directly from each tile, at the time of sampling, by measuring the time to fill a 1-liter (L) bottle.

2.3.3 Manure sampling and application

PM was transported directly from confinements housing layer hens, and stockpiled at the study site each spring. Three representative manure samples were obtained from the stockpile prior to application. These samples were placed in plastic bags and stored on ice, then transported to Minnesota Valley Testing Laboratory in Nevada, Iowa, for moisture content and nutrient analyses. Manure was re-sampled one day prior to scheduled application, and transported on ice to ISU's Water Quality Research Laboratory for moisture content and microbial analyses. New moisture content measurements and previously-measured nitrogen content data were used to calculate appropriate tonnage of manure to be applied per plot. An additional 8 – 12 manure samples were obtained from the spreader during application in 2011 and 2012 for better characterization of the microbial content of the manure. Manure was applied using a dry-spreader as described in Hanna and Richard (2008). Application occurred on May 24th and 25th, 2010, June 1st, 2011, and May 15th, 2012, at the rates listed in Table 2.2, followed immediately by tillage in CP plots. Corn was planted within a few days after manure application.

Table 2.2 PM application rates by wet mass and nitrogen equivalents. Nitrogen application rates are calculated assuming 60% availability of N in the first year. Numbers in parenthesis are standard deviations.

| | | Tillage Practice | | | |
| | | Chisel-Plowed | | No-Till | |
Year	Treatment	Manure (Mg/ha)	Nitrogen (kg N/ha)	Manure (Mg/ha)	Nitrogen (kg N/ha)
2010	PM1	9.3 (2.4)	72 (20)	12	90.2
	PM2	24 (0.1)	187 (1)	24	184
2011	PM1	13 (0.3)	120 (4)	12	109
	PM2	27 (1.6)	249 (25.8)	40	380
2012	PM1	6.0 (0.14)	132 (5.54)	5.0	103
	PM2	10 (0.15)	231 (5.96)	13	298

2.3.4 Water sampling

Grab samples were collected weekly from drainage tile outlets and following precipitation events during the 30 days prior to manure application and up to 100 days after manure application (DAM) when flow was available. Grab sampling was conducted from May 14th to September 1st in 2010, April 19th to July 27th in 2011, and April 15th to July 21st in 2012. Grab samples were collected in 1 L sterile polypropylene bottles, placed on ice, and transported to ISU's Water Quality Research Laboratory. Samples were stored at 4 °C until they were processed for bacteria concentrations within 24 hours.

In 2011, auto-sampling devices (Teledyne ISCO, Lincoln, Nebraska) were installed in 6 of the tile sumps (one representing each combination of tillage and treatment). These devices sampled directly from tiles via clear rubber tubing placed directly into the tile. Tubes were flushed with tile-water, prior to collection of tile-water samples in 1 liter sterile propylene bottles. Samples were extracted from auto-sampling devices within 12 hours of collection, transported to the laboratory at 4 °C, and analyzed immediately for bacteria. Samples were obtained hourly during and immediately following precipitation events, then at longer intervals

as flow receded. Data presented here were obtained following precipitation events, which began

on 9 June, 2011, and 20 June, 2012. Unlike grab sampling, where sampling personnel were

available to wait until sufficient volumes had been collected, auto-samplers were not able to

collect large volumes during low flows; therefore, hourly samples were analyzed first for ENT,

followed by EC and SALM when sufficient volumes were available.

2.3.5. Bacterial enumeration

Manure and water samples were analyzed using membrane-filtration and growth on

selective agars following previously described methods (Dufour et al. 1981; Eaton et al. 1995;

Messer and Dufour 1998). Manure sub-samples (10 g wet weight) were first mixed with 150 mL

phosphate buffered solution (PBS) and placed on an orbital shaker for 30 minutes prior to being

serially diluted. Multiple subsamples of water were filtered, with volumes varying up to three

orders of magnitude, including dilutions when necessary to achieve bacteria plate counts ideally

between 20 and 80 colonies. EC, ENT, and SALM were cultured on Difco^{TM} modified mTEC,

mEnterococcus, and XLD agars, respectively, per manufacturer's instructions. Samples were

analyzed in triplicate whenever possible and measured values of laboratory replicates were

averaged for analytical and reporting purposes. For quality control, blanks with a minimum of

25 mL PBS were evaluated with each batch of samples. All bacteria concentrations are reported

in colony forming units (cfu) per volume.

2.3.6. Salmonella concentrations in manure using qPCR

Concentrations of SALM in manure samples from 2011 were quantified using the qPCR

method for detection of the *inv*A gene, as adapted from Ahmed et al. (2009). *Salmonella*

enteritidis ATCC 13076 was used as a positive control strain, and *Enterococcus faecalis* (ATCC

29212) was used as a negative control. Controls were inoculated into 10 mL Tryptic Soy Broth,

incubated in a water bath shaker overnight at 37 °C, and enumerated using culture methods described above. Deoxyribonucleic acid (DNA) was extracted from controls using MoBio PowerWater kit, and DNA concentrations were measured using an Eppendorf BioPhotometer. DNA was extracted from manure using the MoBio PowerSoil kit following manufacturer's instructions. DNA eluates (100 μL) were then frozen at -20 °C until use. DNA extraction and qPCR were performed in separate laboratories. Primer concentrations were optimized by running all 6 combinations of forward and reverse primers at 125, 300, and 500 nM, and annealing temperature was optimized by performing a gradient analysis from 50 °C to 74 °C. The PCR mixture contained 12.5 μL QuantTect SYBR PCR Master Mix, 300 nM of each primer, 5 μL of DNase and RNAase free deionized water, and 2.5 μL of template DNA. After optimization, qPCR reactions were performed with a 57 °C annealing temperature on an Opticon2 thermal cycler (MJ Research, St. Bruno, Qeubec, Canada). The equation of the standard curve relating cycle threshold, C(T), to the log of the DNA concentration (log quantity) was:

$$\text{Log Quantity} = -0.30 * C(T) + 5.62$$

The r^2 value of the standard curve was 0.994. Confirmation of the PCR products was completed using gel electrophoresis, which did not produce any bands, other than the one expected, with 244 base pairs. Environmental samples often contain proteins and other organic molecules that bind to DNA, inhibiting detection. Inhibition was measured by spiking DNA samples with known concentrations of SALM. Tests indicate that inhibition resulted in a 20-37% reduction of the expected concentrations for DNA samples extracted from manure. Given the variability of potential inhibition effects, results were not corrected for inhibition. All samples were run in

triplicate and intra-assay variability was assessed. Gel electrophoresis was used to confirm PCR product.

2.3.7. Macropore assessment

Smoke testing was conducted 23 days after manure application (DAM) on 7 June 2012, to determine the number and distribution of macropores directly connected to the tiles. Smoke tests were conducted on 6 plots representing each combination of tillage and treatment. Smoke candles (Superior Signal Company LLC, Spotswood, NJ) were ignited, providing approximately 1133 m^3 of smoke each, and smoke was pumped into the tile outlets using an unused liquid manure application tank. Up to 4 candles were used per tile. Locations of visible smoke, indicating direct conduits to the tile line, were marked with flags along the full length of each plot.

2.3.8. Data analyses

For the purposes of statistical analyses, all samples without detectable levels of bacteria were assigned the value of the detection limit. For tile-water samples, volumes of 100 mL were most often filtered; thus, the most common detection limit was 1 cfu/100 mL. Lower concentrations were reported when the average of laboratory replicate samples was below 1 cfu/100 mL, or if sample volumes greater than 100 mL were filtered. The detection limit varied for manure samples as a result of variable moisture content prior to initial dilution.

Normalization was required to compare the effects of time, tillage, and treatment on grab sample bacterial concentrations in tile-waters from unequal plot sizes. Each plot is 36.3 m in width; however, due to the geometry and topography of the experimental field, plot lengths vary from 11.8 to 139 m. Tile flow rates were consistently higher under longer plots. Regression analyses by sample date confirmed linear relationships between plot length (m) and tile flow

rates (L/s), as shown in Figure 2.2. Slopes ranged from 0.0005 at low flows (June 10, 2010), to

0.0050 at relatively high flow (June 26, 2010). Based on these relationships, bacterial

concentrations were normalized by dividing measured concentrations by the tile length, and

multiplying these values by the average tile length for all plots (65.5 m).
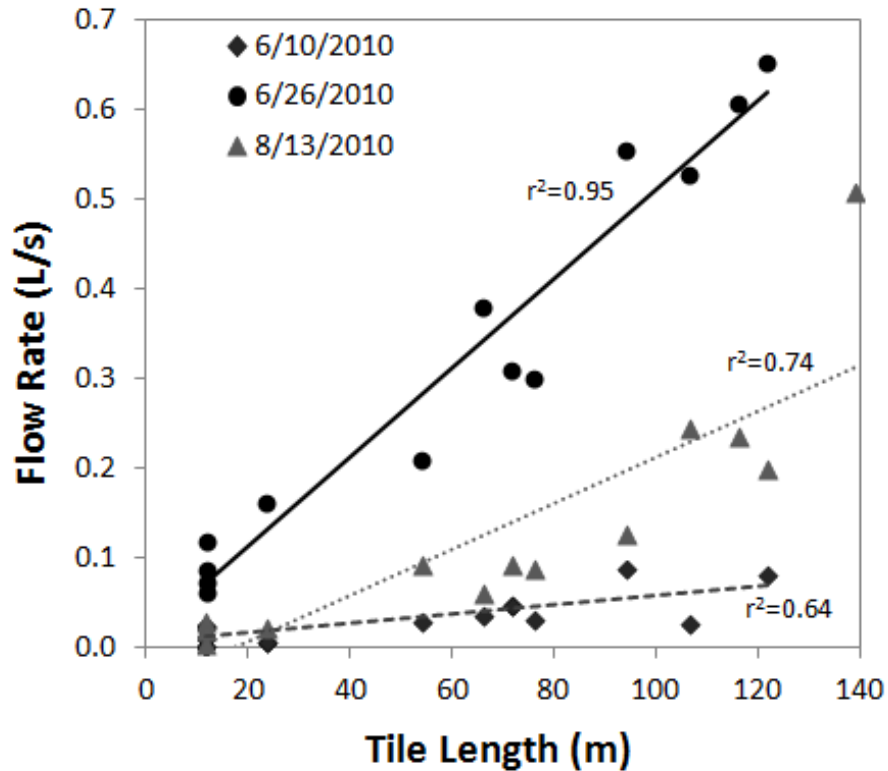
Figure 2.2 Tile length (m) vs. tile flow rates (L/s) representing low, intermediate, and high flow rates during 2010.

Bacterial concentrations in water are generally log-normally distributed; therefore, the

geometric mean is the preferred statistic for summarizing these data (APHA et al. 1989). Many

microbial water quality standards, including Iowa's recreational standards, include 30-day

geometric means (IAC 2012). To compare the effect of time using data from all 3 field seasons,

30-day periods were defined relative to the date of manure application, for each year. Samples

were grouped into one 30-day period prior to manure application (-30 - 0), and three 30-day

periods after application: 0 - 30 DAM, 30 - 60 DAM, and 60 - 90 DAM. Both individual, and

30-day geometric mean EC concentrations, were compared to Iowa's recreational water quality

standards.

Nonparametric Wilcoxon rank sum tests were performed using JMP software (SAS

Institute, Cary, NC) to determine differences between distributions of normalized tile-water

bacteria concentration by year, 30-day period, tillage practice (CP or NT), and plot treatment

(PM1, PM2, or PM0). In addition, geometric means values of normalized bacteria

concentrations were determined for groups defined by these four variables. Comparison of

geometric means addresses the problem of uneven replication between CP and NT plots. To

evaluate the importance of these variables for predicting geometric mean concentrations, a

factorial (completely randomized) analysis of variance model was estimated using SPSS

software. Student's t-tests were performed (JMP) to compare macropore densities between CP

and NT plots. Correlations between individual and geometric means of normalized FIB and

SALM concentrations in tile-waters were estimated using Spearman's rank-order analyses, and

linear regression analyses were performed using JMP software. Significance of all statistical

evaluations was determined using the $p < 0.05$ level.

2.4 Results and Discussion

2.4.1 PM constituents and bacterial application rates

Average moisture contents of PM sampled prior to application were 48%, 61%, and 30%

in 2010, 2011, and 2012, respectively, and average total nitrogen contents of manure ranged

from 1.3 to 3.7 percent by weight. Bacteria concentrations in PM were highly variable, as

reported in Table 2.3. Average concentrations of bacteria in PM sampled at the time of

application were highest for ENT each year (ranging from 5.0×10^4 to 1.5×10^5 cfu/g dry

weight), followed by SALM (ranging from 2.0×10^2 to 1.4×10^5 cfu/g dry weight), and EC (ranging from 2.4×10^1 to 2.6×10^3 cfu/g dry weight). These values represent PM that was transported directly from the confinement and stockpiled outdoors for up to four weeks; therefore, we expected concentrations below those previously reported for fresh PM. Literature values are indeed higher for EC and ENT, but not SALM. Terzich et al. (2000) reported EC concentrations between 10^5 and 10^{10} for fresh PM. ENT concentrations ranging from 10^5 to 10^8 cfu/g dry weight were reported by (Graham et al. 2009). Hutchison et al. (2004b) reported SALM concentrations up to 2.2×10^4 cfu/g in fresh PM, and 8.0×10^3 cfu/g for stored manure samples.

Table 2.3 Average bacterial concentrations in PM at the time of application (2010-2012) on a dry weight basis. Values in parentheses are standard deviations. Salmonella spp. were analyzed by qPCR only in 2011.

Year	*E. coli* (cfu/g)	Enterococci (cfu/g)	*Salmonella spp.*	
			by culture (cfu/g)	by qPCR (copies/g)
2010	2.4×10^1 (3.1×10^1)	5.0×10^4 (6.3×10^3)	1.5×10^4 (1.0×10^3)	NA
2011	5.2×10^2 (1.7×10^2)	1.5×10^5 (1.8×10^5)	9.7×10^3 (6.4×10^3)	3.8×10^4 (9.2×10^4)
2012	2.6×10^3 (5.6×10^3)	5.9×10^4 (7.6×10^4)	2.0×10^2 (4.7×10^2)	NA

Concentrations of SALM were also determined for 2011 manure samples using qPCR. Concentrations of the *inv*A gene, unique to SALM, were detected above both the lowest standard and negative controls for all manure samples analyzed by qPCR. The mean and standard deviation for concentrations of SALM as determined by qPCR analyses for 2011 manure samples are presented in Table 3. Despite potential losses from inhibition, the mean concentration of SALM in manure determined using qPCR was 0.6-log higher than the mean

concentration determined by cultural analysis. Although studies have confirmed a linear

relationship between genetic markers for SALM and culturable SALM (Bech et al. 2010; Rogers

et al. 2011), qPCR does not differentiate between viable and non-viable organisms. Therefore,

the difference between the results of these methods of SALM quantification may be due to any

of various factors, such as differences in the viability of the organisms counted, or interference

with SALM growth by other organisms on the XLD agar.

Masses of applied manure ranged from 5 to 13 Mg ha/yr for PM1 treatments, and 10 to

40 Mg ha/yr for PM2 treatments. Calculated nitrogen application rates averaged 108 kg N ha/yr

for the CP PM1 plots, just below the 112 kg N ha/yr fertilization goal, and 223 kg N ha/yr for the

CP PM2 plots, almost matching the 224 kg N ha/yr goal. Application rates for the NT PM1 plots

fell short of the goal, with an average of 101 kg N ha/yr, while rates on the NT PM2 plots

exceeded the goal, with an average of 287 kg N ha/yr. Table 2.4 summarizes the bacterial

loading (cfu/ha) by year, tillage, and treatment.

Table 2.4 Annual application rates for manure, nitrogen, and E. coli, enterococci, and Salmonella spp. by year, tillage and treatment.

Year	Tillage	Treatment	Bacterial Application Rate (cfu/ha)		
			E. coli	Enterococci	*Salmonella spp.*
2010	CP	PM1	4.2×10^7	8.1×10^{10}	2.5×10^{10}
		PM2	1.1×10^8	2.1×10^{11}	6.5×10^{11}
	NT	PM1	5.2×10^7	1.0×10^{11}	3.1×10^{10}
		PM2	1.1×10^8	2.1×10^{11}	6.4×10^{10}
2011	CP	PM1	3.3×10^9	3.1×10^{12}	6.2×10^{10}
		PM2	6.9×10^9	6.5×10^{12}	1.3×10^{11}
	NT	PM1	3.0×10^9	2.8×10^{12}	5.6×10^{10}
		PM2	1.1×10^{10}	9.8×10^{12}	2.0×10^{11}
2012	CP	PM1	5.3×10^9	1.2×10^{11}	4.1×10^8
		PM2	9.3×10^9	2.1×10^{11}	7.2×10^8
	NT	PM1	5.7×10^9	1.1×10^{12}	4.4×10^8
		PM2	1.7×10^{10}	3.2×10^{12}	1.3×10^9

2.4.2 Rainfall and tile flow

The average total rainfall for the field season (March to October) from 1998 to 2012 is 78 cm. Rainfall amounts for 2011 and 2012 represented below-average years, with 50 and 42 cm of precipitation, respectively, while 2010 was the wettest year at the field site since 1993, with 123 cm rainfall. Standing water was observed at the site twice during the 2010 field season, first on 4 July (41 DAM) and again on 9 August (77 DAM). Runoff was not observed at any time; however, it is possible that runoff occurred on 9 August, 2010.

Figure 2.3 shows grab-sampling dates, tile flow rates from a representative CP plot, and corresponding rainfall intensities for 2010, 2011, and 2012. The last sample collected in 2010 was on 1 September (100 DAM). In 2011, tile flow was not observed after 5 August (35 DAM), and in 2012, tiles did not flow after 25 June (41 DAM). The maximum measured flow rate for the CP plots was 3,500 L/hr, but fewer than 20 percent of flow rates were above 500 L/hr. Flow rates for NT plots rarely exceeded 1,000 L/hr, with fewer than 20 percent of flow rates exceeding 50 L/hr. Grab samples were obtained throughout the entire range of tile flow rates.
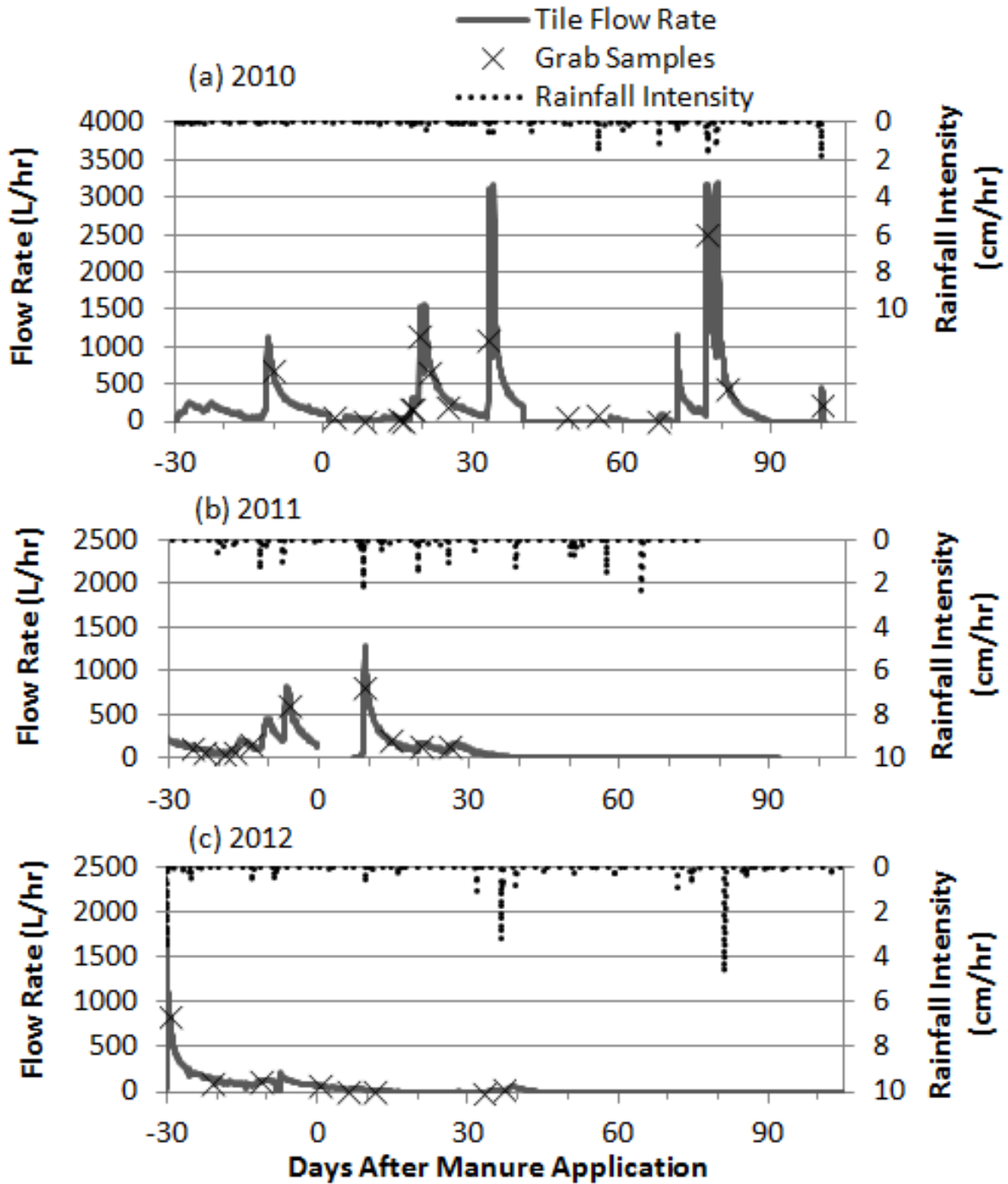
Figure 2.3 Digital tile flow records (L/hr) for a representative chisel-plowed plot along with timing of grab samples and rainfall intensities for (a) 2010, (b) 2011, and (c) 2012.

2.4.3 Non-normalized tile-water bacterial concentrations

2.4.3.1 Sample variability

Uncertainty of concentrations, as determined from laboratory replicates, increased with bacterial concentrations. For samples where the average value of 3 replicates was greater than 100 cfu/100 mL, the mean relative standard error was 10% for EC and ENT, and 24% for SALM. While the selective agars and incubation procedures used to enumerate EC and ENT adequately suppress other bacteria species, observations of the XLD agar plates after incubation reveal growth of numerous non-SALM bacteria. The growth of EC on XLD agar was indicated by pale colonies and yellow color of the medium, and confirmed by isolation and growth on modified mTEC agar. When EC concentrations were high, this growth interfered with growth and enumeration of SALM. Thus, SALM concentrations in tile-waters reported in this study are likely lower than actual concentrations.

2.4.3.2 Variability between sample years

Forty-one grab-sample collection trips were conducted over 3 years, with a maximum of 14 samples per trip. Greater intensity and frequency of precipitation events in 2010 facilitated the collection of 165 grab samples. In comparison, lack of precipitation and inconsistent tile flow resulted in collection of 82 grab samples in 2011 and 65 in 2012. Figure 2.4 shows the distributions for all measured tile-water bacteria concentrations by year. EC concentrations varied almost 5 orders of magnitude in 2010 and just 3 orders of magnitude in 2011 and 2012. Both primary and secondary recreational water quality standards were exceeded in 2010, with 24% of samples exceeding the single-sample maximum concentration of 235 cfu/100 mL EC for primary recreation, and 4% of samples exceeding the single-sample maximum concentration of 2880 cfu/100 mL EC for secondary recreation. In 2011, no water quality standards for bacteria

were exceeded in the study tiles, and in 2012, the concentration of EC was greater than 235

cfu/100 mL in only one sample (2% of samples). Variability of tile-water ENT concentrations

was greater than EC, while SALM concentrations varied less between years. Nonparametric

Wilcoxon rank sum analyses reveal differences between 2010 and both 2011 and 2012

($p<0.001$), but not between 2011 and 2012 for all bacterial species (Figure 2.4).
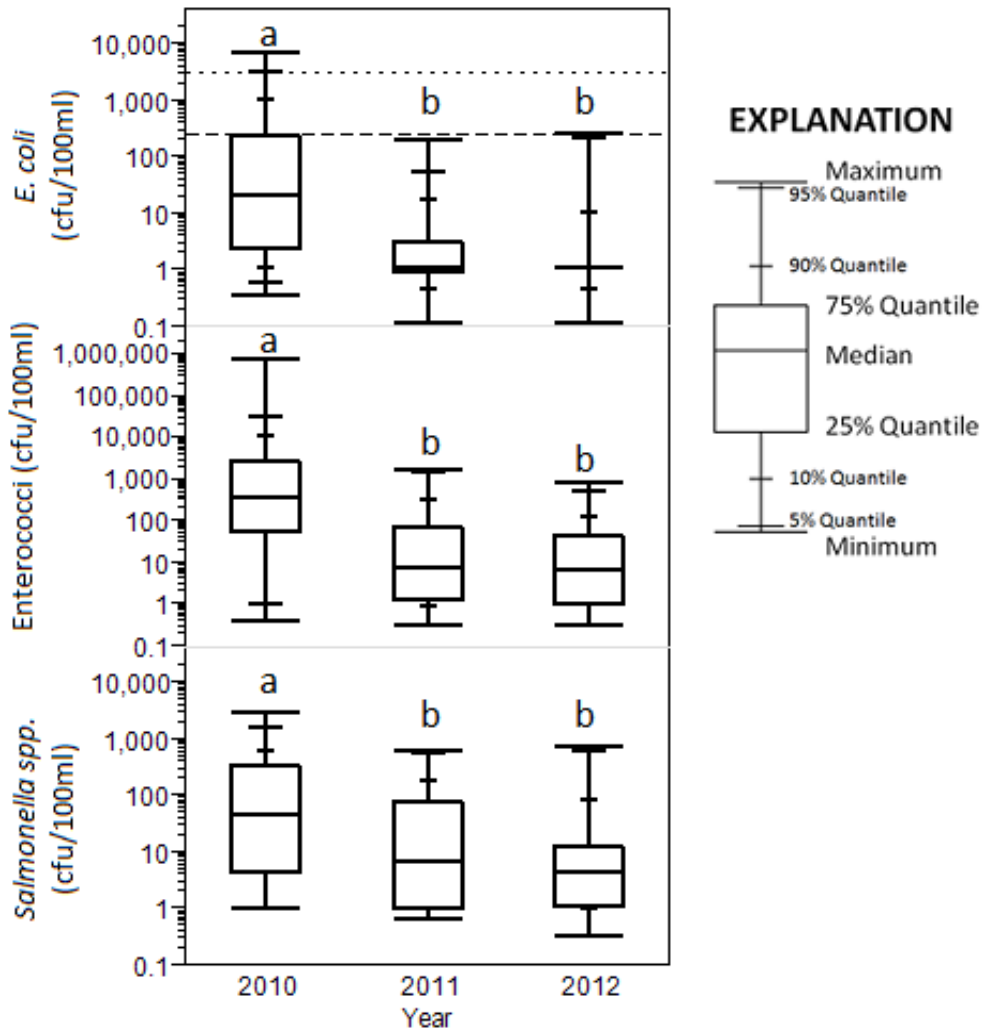
Figure 2.4 Distributions of non-normalized bacteria concentrations for E. coli, enterococci, and Salmonella spp. by year. Data are represented by quantile boxplots, and lettering indicates differences determined by pairwise Wilcoxon rank sum analyses. Single-sample maximum water quality standards for primary recreation (dashed line) and secondary recreation (dotted line) are displayed for E. coli.

2.4.3.3 Hydrological response

Monitoring of tile-waters throughout the hydrograph response to rainfall events in 2011 and 2012 revealed that bacterial concentrations generally mirror changes in tile flow rates, as illustrated in Figure 2.5a. During precipitation events that were intense enough to cause pooling at the surface, bacteria were detected at high concentrations in tile-waters ahead of the wetting front, as illustrated in Figure 2.5b. Presumably, these bacteria were released at or near the soil surface during rainfall and transported directly to the tiles via macropores. From these data, we can infer that direct transport of bacteria through macropores also occurred in 2010, and we suspect that this process was responsible for the highest concentrations of bacteria observed in that year. However, much dryer conditions in 2011 and 2012 prevented additional data collection under similarly saturated conditions.

In addition to characterization of the variability in bacterial concentrations in response to precipitation, monitoring throughout the hydrograph can be used for estimating loading potential. ENT losses during the first 45 hours after rainfall began on 20 June, 2012 (36 DAM), were 6.4×10^8 cfu/ha from the NT PM2 plots, compared to 1.1×10^5 cfu/ha for the NT PM0 plot. Total precipitation for this event was 6.26 cm. Flow-weighted ENT concentrations (Novotny and Olem 1994) were 800 cfu/100 mL for the NT PM0 plot, and 3.3×10^4 cfu/100 mL for the NT PM2 plot. It should also be noted that the total tile-water volume discharged from the NT PM0 plot (53 liters) was lower than for the NT PM2 plot (77 liters) over the same time-period. In 2011 and 2012, low tile flow rates and power-failures, prevented further comparison of bacterial loading potential between plots.
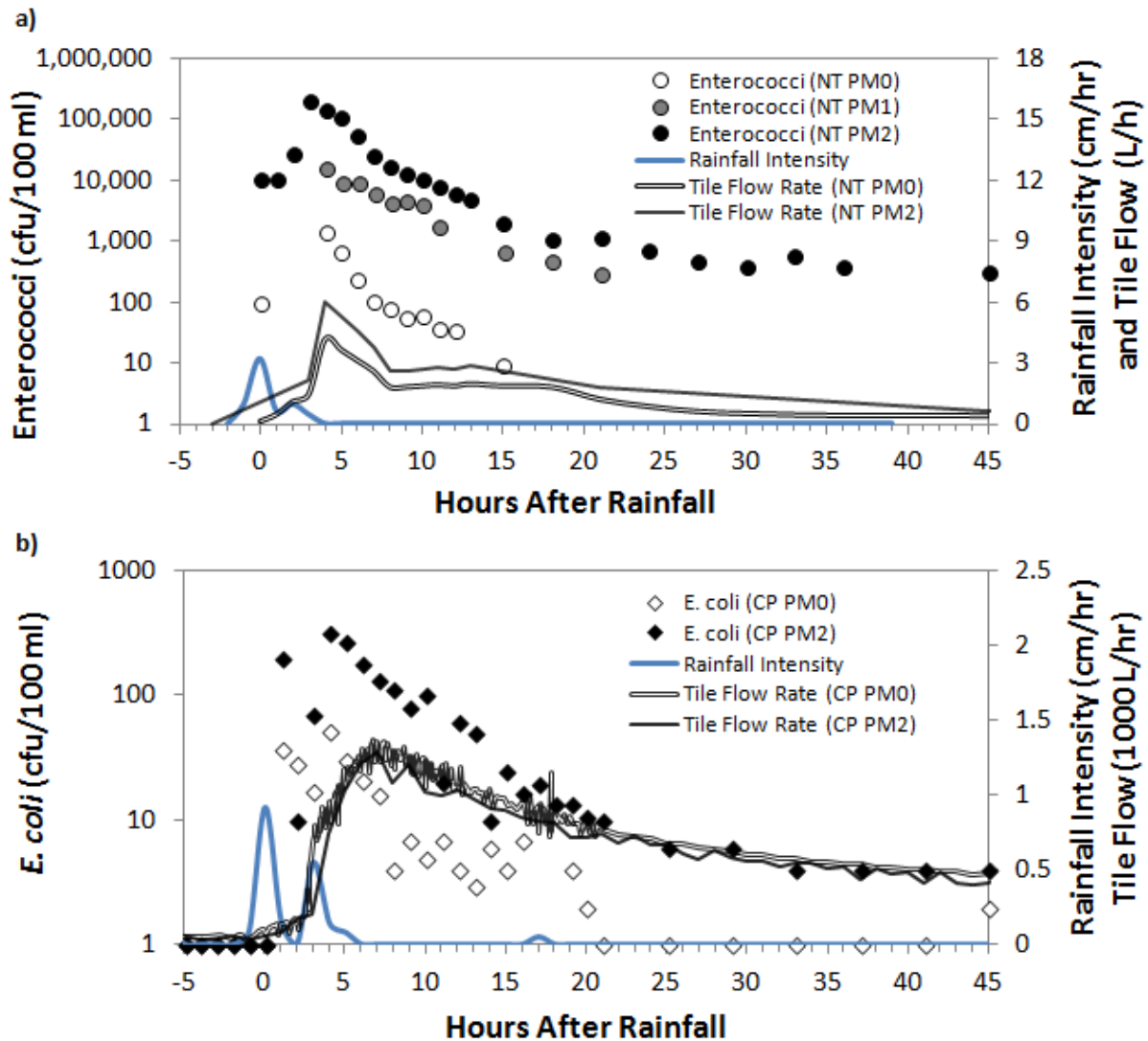
Figure 2.5 Results of bacterial analyses of auto-sampled tile-water during events occurring, a) 36 days after manure application on June 20, 2012, and b) 8 days after manure application on June 9, 2011. Corresponding tile flow rates and rainfall intensities are shown on the secondary axes.

2.4.4 Effects of time relative to manure application

It is well-understood that maximizing the time between manure application and precipitation reduces the potential for impacts on water quality. Our data support this general rule, but reveal that high concentrations of bacteria are observed in tile-waters even during periods of little rainfall. Additionally, tile-water bacteria concentrations can remain elevated above pre-manure values for over 90 days given sufficient precipitation, as observed in 2010.

Figure 6 shows plot-normalized concentrations of EC, ENT, and SALM for tile-waters samples in 2010 grouped by tillage and treatment. Three small rainfall events, totaling 1.6 cm, occurred in the first 10 days after manure application (DAM), and only a few CP plots flowed throughout those 10 days. After a 1.8 cm rainfall at 11 DAM, tile flows increased sufficiently to obtain samples, but flow rates remained very low. EC was measured at concentrations above pre-manure samples in both CP and NT plots. Despite the very low flow rates, EC concentrations were highest at 11 DAM for NT plots. In contrast, the peak EC concentrations were not seen under CP plots until 19 DAM, when a 2.1 cm rainfall event saturated soils, resulting in a tile hydrograph response.

The ENT data followed trends similar to the EC data (Figure 6). ENT concentrations in tile- waters under NT plots were often an order of magnitude higher than their CP equivalents, especially during the first 30 DAM. High plot-normalized ENT concentrations also occurred in NT plots 8 days before peak concentrations were measured in CP plots. The NT PM2 samples from 11 DAM were not sufficiently diluted; therefore, the actual concentration was higher than the reported ENT concentration for those samples ($>3.2 \times 10^6$ cfu/100 mL). ENT concentrations in drainage from NT PM2 plots remained 5 orders of magnitude above pre-manure levels, even at 41 DAM.

SALM concentrations did not peak until 49 DAM (Figure 2.6). This lag in SALM concentrations was likely due to interference from EC on XLD agar plates, as discussed above. Due to this interference, SALM concentrations during the first 45 DAM should be regarded as minimum values. As EC concentrations in tile-waters dropped, SALM concentrations increased. Like EC and ENT, SALM concentrations from NT plots consistently exceeded concentrations from CP plots.
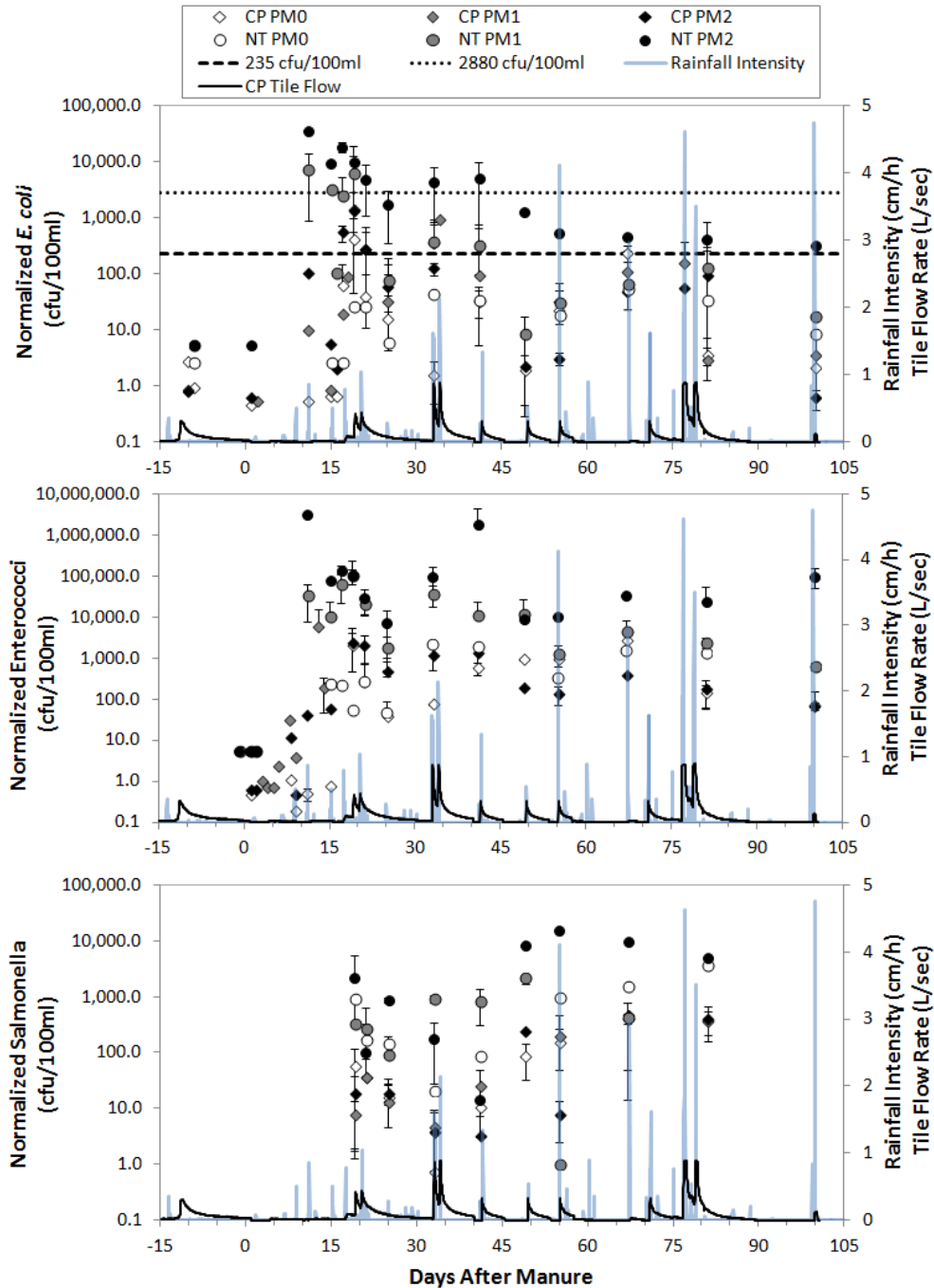
Figure 2.6 Concentrations of plot-normalized E. coli, enterococci, and Salmonella spp. for tile-water samples from 2010 under chisel-plowed (CP) and no-till (NT) plots with PM0, PM1, and PM2 treatments. Where field replicates were available, mean values are presented with bars indicating standard deviations. Corresponding rainfall intensities (cm/hr), and tile flow rates (L/s) for a representative CP plot, are displayed. The one-time sample maximum standards for primary recreational waters (235 cfu/100 mL E. coli), and secondary contact recreational use waters (2880 cfu/100 mL E. coli) are shown for reference.

In 2010, pre-manure concentrations of bacteria never exceeded 10 cfu/100 mL even during the relatively high flows 10 days prior to manure application. This indicates that any bacteria that survived the winter in soils, or were introduced by wildlife, did not contribute significantly to tile-water concentrations. Bacterial concentrations after manure application exceeded pre-manure concentrations in the control plots (PM0), as shown in Figure 2.6. This may be explained by cross-contamination from neighboring fields during tillage and cultivation of the plots, as well as by cross-contamination at the surface during periods of standing water caused by heavy rainfall (observed 33 - 34 and 76 - 78 DAM in 2010), and by subsurface transport of bacteria between plots when tile-flow was overwhelmed.

To further evaluate the effects of time relative to manure application on tile-water bacteria concentrations, data from all three years were combined. Table 2.5 displays the summary statistics, including geometric means, for the 30 days prior to application and the four 30-day periods after application. Inclusion of non-detects tended to increase the geometric mean concentrations relative to the medians, except late in the summer when non-detections predominated. Wilcoxon rank sum analyses indicate that bacteria concentrations from the 30 days prior to manure application were significantly lower than in the 30 days post-manure application ($p < 0.001$). Differences were not observed between samples collected in the first and second month after application. In the third month (60-90 DAM), concentrations of all 3 bacterial species remained significantly higher than pre-manure values.

2.4.5 Effects of tillage and treatment

Plot-normalized tile-water bacteria concentrations from all three years were also analyzed using the Wilcoxon rank-sum test to evaluate differences between tillage and treatment practices. Summary statistics are displayed for plot-normalized bacteria concentrations in Table 2.6 by

tillage type. NT plots had significantly higher concentrations of all three types of bacteria ($p <$

0.001) for all three bacteria species.

Table 2.5 Summary statistics for plot-normalized E. coli, enterococci, and Salmonella spp.
concentrations from 2010-2012 for 30-day periods before and after manure application.
Lettering indicates differences between 30-day periods for each bacterial species as determined
by Wilcoxon rank sum analyses (p < 0.05).

Days After Manure	Statistic	*E. coli* (cfu/100 mL)		Enterococci (cfu/100 mL)		*Salmonella spp.* (cfu/100 mL)	
	N	78		70		60	
	Min	0.060		0.18		0.16	
-30 - 0	Median	0.099	a	5.5	a	1.8	a
	GeoMean	1.8		7.6		3.2	
	Max	930		8000		1100	
	N	136		129		92	
	Min	0.052		0.019		0.47	
0 - 30	Median	6.8	bc	58	bc	27	bc
	GeoMean	15		98		25	
	Max	3.7×10^4		$>3.2 \times 10^6$		4400	
	N	54		53		51	
	Min	0.31		0.70		0.16	
30 - 60	Median	20	c	510	bd	21	c
	GeoMean	22		440		29	
	Max	8300		3.6×10^6		1.6×10^4	
	N	26		23		19	
	Min	0.38		59		130	
60 - 90	Median	47	c	1600	d	460	b
	GeoMean	26		1000		600	
	Max	740		4.6×10^4		1.0×10^4	

Table 2.6 Summary statistics, including geometric mean (GeoMean) concentrations of E. coli, enterococci, and Salmonella spp., concentrations in cfu/100 mL from no-till (NT) and chisel-plowed (CP) plots. Lettering indicates differences between practices as determined by Wilcoxon rank sum comparisons (all p values were <0.001).

Tillage	Statistic	*E. coli*		Enterococci		*Salmonella spp.*	
	N	208		185		154	
	Min	0.052		0.18		0.16	
CP	Median	1.0	a	15	a	55	a
	GeoMean	3.1		1.3		1.6	
	Max	2,500		1.6×10^4		690	
	N	102		107		72	
	Min	2.5		1.8		37	
NT	Median	36	b	1,800	b	160	b
	GeoMean	74		1,300		150	
	Max	3.7×10^4		3.6×10^6		1.6×10^4	

Table 2.7 displays summary statistics for tile-water bacteria concentrations by treatment. Median and geometric mean concentrations were higher for manured plots (PM1 and PM2) than for control plots (PM0). Differences were significant (*p* at 0.05) between EC and ENT concentrations from the control plots (PM0) and the manure-amended plots (PM1 and PM2), and between PM0 and PM1 treatments for SALM, but no significant differences were seen between PM1 and PM2 treatments for any of the three bacteria species. Differences between treatments did not appear when data were analyzed separately by year, or when all pre-manure application data were excluded.

Table 2.7 Summary statistics for tile-water E. coli, enterococci (ENT), and Salmonella spp. concentrations (cfu/100 mL) from plots with no manure applied (PM0), the low manure application rate (PM2), and the high manure application rate (PM2). Lettering indicates significant differences between manure treatments as determined by Wilcoxon rank- sum analyses (p<0.05).

Treatment	Statistic	*E. coli*		Enterococci		*Salmonella spp.*	
	N	93		85		71	
	Min	0.052		0.19		0.16	
PM0	Median	2.0	a	21	a	9.2	a
	GeoMean	2.5		20		9.6	
	Max	760		5.8×10^5		3,600	
	N	108		108		82	
	Min	0.063		0.19		0.56	
PM1	Median	8.0	b	220	b	19	b
	GeoMean	11		140		21	
	Max	1.3×10^4		1.3×10^5		5,200	
	N	109		99		73	
	Min	0.060		0.18		0.36	
PM2	Median	8.6	b	110	b	15	ab
	GeoMean	21		180		2.3	
	Max	3.7×10^4		$>3.6 \times 10^6$		1.6×10^4	

The combined effects of tillage and treatment on the distributions of individual tile-water bacteria concentrations are displayed in Figure 2.7. The single sample maximum water quality standard for secondary recreation (2880 cfu/100 mL) was exceeded only in tile-waters below NT plots, while exceedance of the single sample maximum water quality standard for primary recreation (235 cfu/100 mL) was rare under the control treatment (PM0) and more common under NT manure-amended (PM1 and PM2) plots. The significance of tillage on ENT and SALM concentrations in tile-waters is also visible in Figure 2.7, while the magnitude of change caused by treatment effects is relatively moderate for ENT and negligible for SALM.
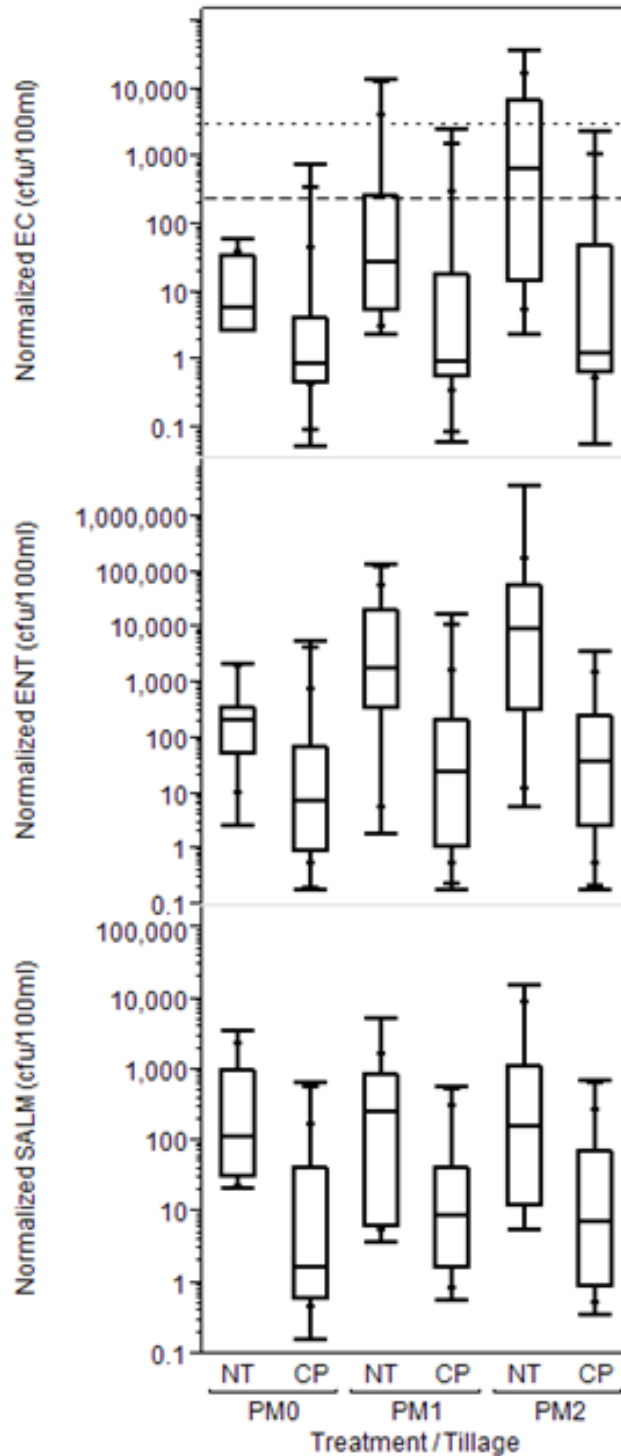
Figure 2.7 Quantile distribution of tile-water E. coli (EC), enterococci (ENT), and Salmonella spp. (SALM) concentrations from 2010 – 2012 under chisel-plowed (CP) and no-till (NT) plots treated with no poultry manure (PM0), a low manure application rate (PM1), and a high manure application rate (PM2). Primary (dashed line) and secondary (dotted line) recreational water quality standards for E. coli are included.

2.4.6 Thirty-day geometric mean concentrations

Thirty-day geometric mean normalized bacteria concentrations were calculated for each combination of year, tillage practice, and treatment, for the 30 days prior to manure application and the four 30-day periods after application, when groupings had sufficient data for calculation of the geometric means. Figure 2.8 shows the geometric mean bacteria concentrations for the first 30 days after manure application in 2010 and 2011. Insufficient data were available to calculate geometric means for the first 30 days of 2012 due to low flows. These data confirm that the combination of PM application, no-till, and significant precipitation results, result in the highest geometric mean EC concentrations in tile-waters. Iowa's primary and secondary recreational water quality standards for geometric mean EC concentrations were exceeded only during the first two months after application in 2010, under NT plots with manure applied. While the geometric mean standards were not exceeded in 2011, the geometric mean normalized SALM concentrations greater than 100 cfu/100 mL were observed under NT plots, indicating that the risk of downstream exposure remained.

A factorial analysis of variance model, combining the effects of year, 30-day period relative to the date of manure application, tillage, and treatment variables, predicted EC, ENT, and SALM 30-day geometric mean concentrations with r^2 values of 0.913, 0.847, and 0.971, respectively. Tillage, treatment, and 30-day period were significant factors ($p \leq 0.001$) for all three bacteria species. While we found differences between the wet year (2010) and the dry years (2011 and 2012) for individual bacteria measurements, year was significant ($p < 0.001$) only for estimating 30-day geometric mean concentrations of EC, but not for estimating ENT ($p = 0.905$) or SALM ($p = 0.132$). Since geometric means give less weight to extreme values,

which were associated with precipitation events in 2010, it is not surprising that year was less of

a factor for predicting geometric mean concentrations.
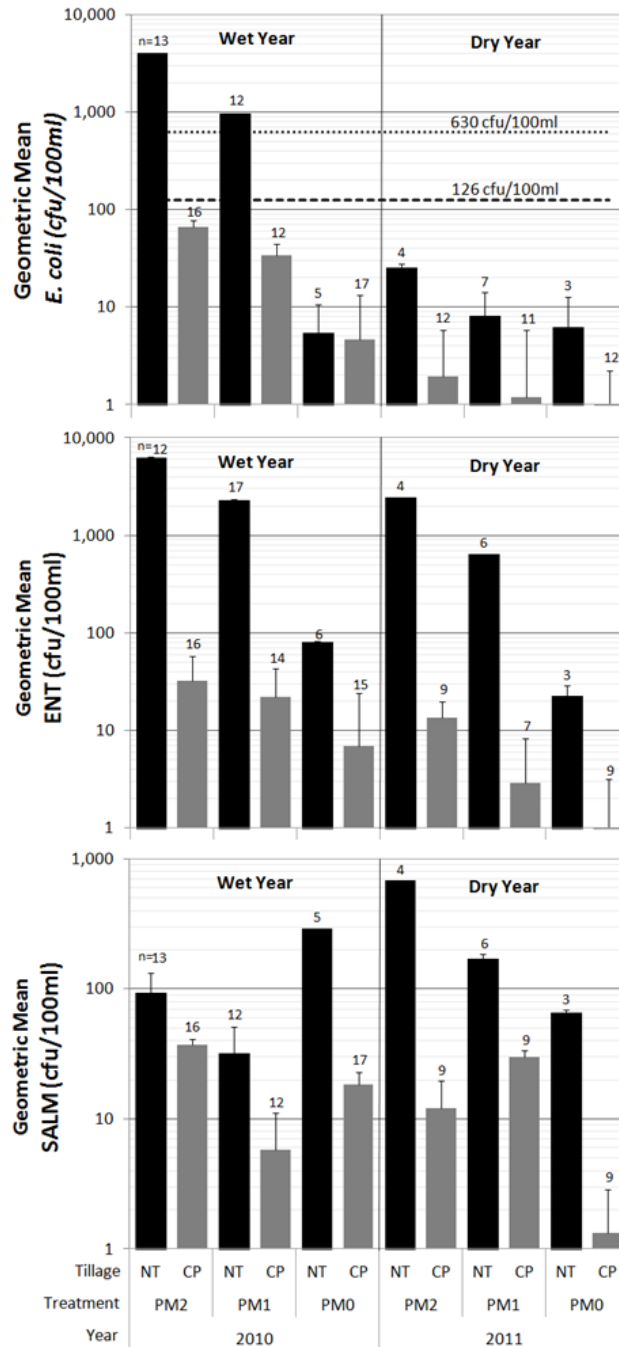
Figure 2.8 Geometric means of plot-normalized tile-water bacteria concentrations from the first 30 days after manure application in 2010 and 2011. Whiskers represent positive standard deviations with number of samples, above. Primary (dashed line) and secondary (dotted line) recreational water quality standards for E. coli are displayed for reference.

2.4.7 Comparison to other studies

Reported effects of manure application rate on tile-water bacteria concentrations are variable and appear to depend on the magnitude of the differences between rates, the species of bacteria, and the type of manure. A three-year study of the effects of swine-manure application by Pappas et al. (2008) compared flow-weighted FIB concentrations in tile-waters under fields adjacent to our study site. For EC, the Pappas et al. (2008) study noted differences between manure-amended plots and control plots, but did not observe differences between EC concentrations in tile-waters under varying swine manure application rates. In contrast, EC concentrations did correlate to application rate of liquid swine manure in Coelho et al. (2007). Pappas et al. (2008) report that ENT concentrations in tile-waters following liquid swine manure application were significantly higher when the application was double the agronomic rate, compared to the recommended agronomic rate or to the control plots, while no differences were observed between the control and the recommended rate.

Previous studies report increased concentrations of bacteria in tiles lines under NT plots when compared to tilled plots (Pappas et al. 2008; Samarajeewa et al. 2012; Hoang et al. 2013). However, bacteria concentrations observed in tile-waters below NT plots with PM2 treatment in this study were substantially higher than previously reported. These results were unexpected, given that storm events were smaller, and estimated bacterial loading from manure in this study were lower, than in comparable studies. In this study, the maximum plot-normalized concentration of EC from a NT plot with PM2 treatment was an order of magnitude higher than the maximum concentration measured after spring-applied liquid swine manure (1.6×10^2 plot-normalized EC cfu/100 mL), as reported in Pappas et al. (2008) despite higher levels of EC (8.2×10^{11} cfu/ha) applied in that study. Also, the maximum measured ENT value (6.6×10^5 cfu/100

mL) in our study was observed 41 days after manure application in 2010, following a rainfall event that peaked at 0.28 cm/h, under a NT plot with the PM2 treatment. This maximum concentration is two orders of magnitude greater than that reported by Hoang et al. (2013), of 5.0 $\times 10^3$ cfu/100 mL ENT. This concentration was observed after a rainfall simulation, with an intensity of 5.1 cm/h, on a NT field, in spring, following injection of liquid swine manure at a rate of 7.3×10^{12} cfu ENT/ha, which is an order of magnitude higher than the application rate estimated for our study in 2010. Similarly, the maximum plot-normalized concentration of ENT was three orders of magnitude higher than those reported (plot-normalized for comparison) by Pappas et al. (2008) from swine manure-amended fields. The estimated bacteria application rate $(1.2 \times 10^{12}$ cfu/ha ENT) for the Pappas et al. (2008) study was within one standard deviation of the application rate $(3.2 \times 10^{12}$ cfu/ha ENT) for this study. These results may indicate that injection of liquid manure may result in less leaching of bacteria, or that other factors facilitating bacteria fate and transport were present at the time of our study.

2.4.8 Macropore densities and distribution

Six smoke tests were conducted in the spring of 2012 to test the hypothesis that macropores were responsible for exceptionally high bacterial concentrations in tile-waters under NT plots. Testing occurred two weeks after manure application, tillage, and planting. Smoke was detected along the full length of each plot, indicating that no major tile-blockages existed at that time. Macropores large enough and sufficiently connected to the tile to allow visible smoke plumes were observed above the tile lines in bands less than one meter wide. Earthworms (*Lumbricus terrestris*) were seen exiting several of the macropores during the smoke test. Higher densities of surface-connected macropores were detected above tile lines in the NT plots than the CP plots (Student's *t*-test, $p = 0.045$), as seen in Figure 2.9. Macropore densities for NT

plots ranged from 3.6 – 13.9 pores/m^2, while under the CP plots, less than one pore/m^2 was observed. These observed macropore densities under NT plots are high compared to observations with densities approximately one surface-connected pore per square meter for both spring and late fall investigations of fields with Floyd sandy loam soils in north-central Iowa with a history of swine manure application (Fox et al. 2012; Hoang et al. 2013).
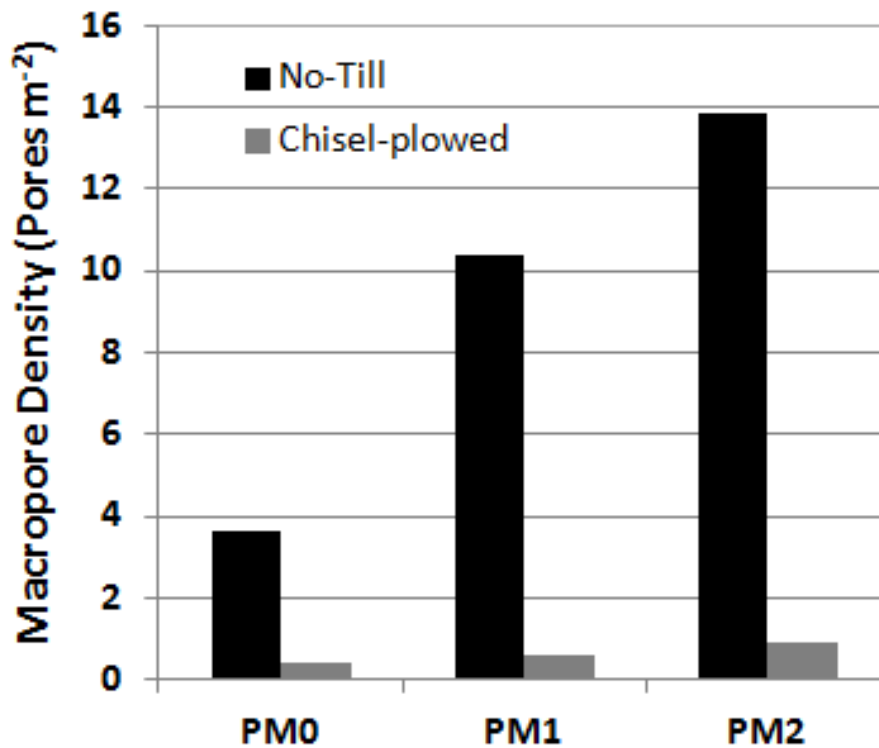
Figure 2.9 Macropore densities (pores m-2) for no-till and chisel-plowed plots treated with no manure (PM0), the low PM application rate (PM1), and the high PM application rate (PM2).

Macropore densities were shown to increase with manure application rate, although the magnitude of the change is smaller for CP plots (Figure 2.9). These results are consistent with the findings of Bottinelli et al. (2013), who found the area occupied by macropores to be more

sensitive to tillage effects than was the rate of PM application after seven years of treatment. In another long-term study of the effects of cattle manure in Alberta, CA, Miller et al. (2002) found significant increases in saturated hydraulic conductivity and the number of pores >1120 μm in response to manure application, but little impact from manure application rate on unsaturated hydraulic conductivity. The potential for macropores to act as an important pathway for bacteria transport is illustrated by the results of Miller et al. (2002), who measured an increase in infiltration and percolation through macropores ≥61% of the total flow under saturated conditions.

For water bodies that routinely violate microbial water quality standards and that drain watersheds with dense drainage systems and widespread manure application, some tillage prior to application may be recommended to disconnect macropores from the surface. However, conventional tillage practices are known to cause compaction and disrupt soil aggregation, resulting in decreased infiltration, increased runoff, increased soil erosion, and decreased yields. Tillage also affects nutrient transport; therefore, all land-management objectives should be considered before such recommendations are made, and other factors, such as timing of manure application, should also be considered.

2.4.9 Risk of exposure to Salmonella and the use of fecal indicator bacteria

The dose of *Salmonella* necessary to cause infection has been shown to be serovar-dependent and highly variable. Reported infective doses for *Salmonella* range from 200 to 1.0×10^6 cells (Huang et al. 1999), and Haas and others (1999) report the ID50 (dose necessary to infect 50% of experimental subjects) at 2.3×10^4 cells. A recent study determined that recreational water users swallow between 18 – 51 mL per swimming event, with children ingesting higher volumes than adults (Schets et al. 2011). In a worst-case scenario, where

recreational water users ingest 51 mL of water, ingestion of surface-waters with SALM concentrations above 392 cfu/100 mL, could result in infection. Figure 2.10 shows results of individual non-normalized FIB and SALM concentrations for all tile-water grab samples. Concentrations of SALM above 392 cfu/100 mL were observed in 12% of all tile-water samples analyzed for SALM. SALM above 392 cfu/100 mL occurred in 9% of samples for which EC was below the one-time sample maximum primary recreational water quality standard for EC (126 cfu/100 mL). Furthermore, 42 samples with no detection of EC contained SALM, 3 of which were above 392 cfu/100 mL.
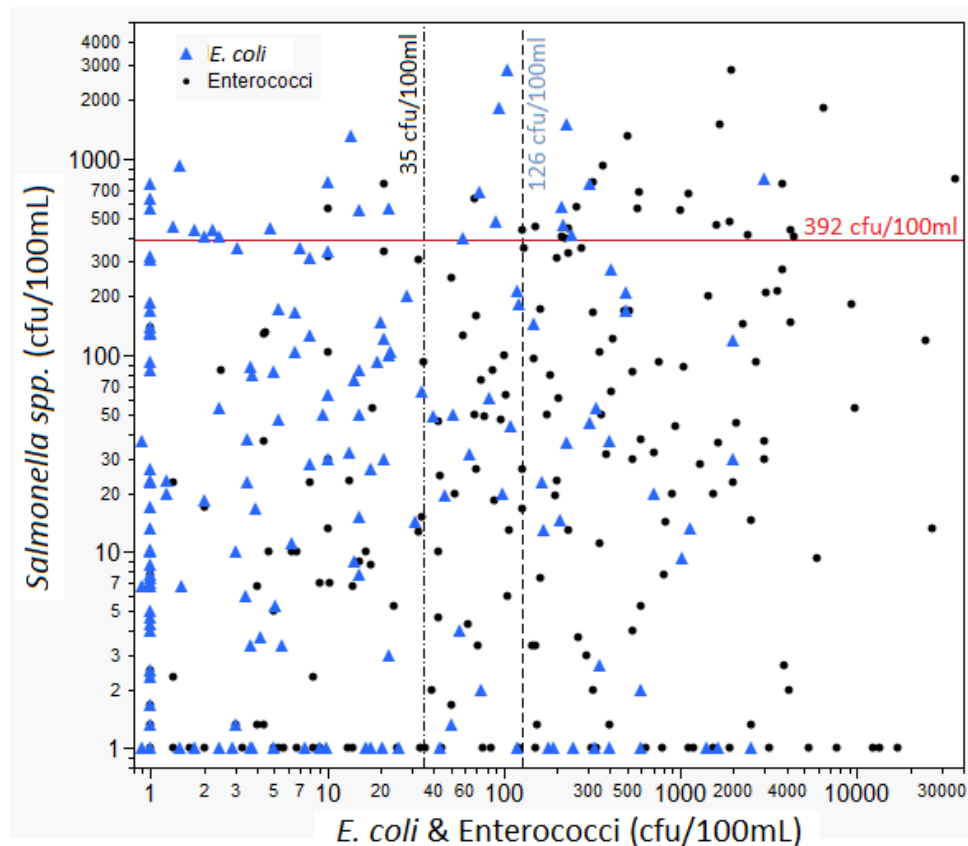
Figure 2.10 Plot of non-normalized tile-water fecal indicator bacteria (E. coli and enterococci) vs. Salmonella spp.. The red horizontal line indicates a dose of Salmonella sufficient to cause infection (392 cfu/100mL) assuming ingestion of a minimum of 51 mL undiluted tile-water. EPA's proposed geometric mean standard for enterococci (35 cfu/100mL), is represented by a dash-dot line, and Iowa's current single-sample maximum recreational water quality standard for E. coli (126 cfu/100mL) is represented with the dashed line.

In a Canadian study of pathogen and EC concentrations in agricultural watersheds, Edge et al. (2012) also concluded that existing recreational water quality standards based on EC did not guarantee pathogen-free waters. In their study, 79.8 % of samples with geometric mean EC concentration ≤100 cfu/100 mL were positive for 1 or more pathogens, including *Salmonella*, *Campylobacter*, *E. coli* O157:H7, *Cryptosporidium*, or *Giardia* (Edge et al., 2012). EPA has recommended ENT be used as an alternative indicator to EC, with a geometric mean standard of 35 cfu/100 mL, and a statistical threshold value (90th percentile) of 130 cfu/100 mL ENT (USEPA 2012). In this study, only 2% of the samples containing less than 35 cfu/100 mL ENT, contained SALM above 392 cfu/100 mL (as shown in Figure 2.11).

While correlation analyses of individual and geometric mean values were equally significant ($p < 0.001$) between both FIB species (EC and ENT) and SALM, the values of Spearman's rho (ρ) indicate stronger relationships between ENT and SALM than between EC and SALM (Table 2.8). R^2 values for linear regression of log bacteria concentrations are 0.06 for EC vs. SALM and 0.18 for ENT vs. SALM. R^2 values improve when log values of geometric mean concentrations are compared (0.24 for EC vs. SALM, and 0.45 for ENT vs. SALM). 95% of both the individual and geometric mean SALM concentrations are within 5 orders of magnitude of the best fit lines for FIB.

Table 2.8 Results of Spearman's rank correlation analyses for individual non-normalized bacterial concentrations and geometric mean concentrations in tile-waters. P <0.001 for all correlations.

Correlation	Spearman's ρ for individual measurements	Spearman's ρ for geometric mean values
E. coli vs. *Salmonella spp.*	0.2907	0.5281
Enterococci vs. *Salmonella spp.*	0.4266	0.6857
E. coli vs. Enterococci	0.7513	0.7883

Our results suggest that ENT is the better indicator of the pathogen in this environment. Cook and others (2014) came to a similar conclusion based on analyses of EC, ENT, and SALM concentrations in PM-amended soils. However, these relationships may not, be representative of environments dominated by other manure sources, soil types, or land-uses. Our study was conducted on loamy soils, which have been shown to favor ENT survival, while sandy soils favor EC survival (Cools et al. 2001). In an urban watershed primarily influenced by stormwater, Krometis et al. (2010) found Spearman's coefficients of 0.51 for incidence of EC and *Salmonella* and 0.48 for ENT and *Salmonella* ($p < 0.0001$). Regardless of the FIB used, a lack of indicator bacteria is not a guarantee of *Salmonella*-free tile-water.

The maximum tile-water SALM concentrations observed in this study indicate that the potential for infection exists, even if tile-waters are diluted 100-fold. However, further studies are necessary to quantify the risks posed by PM application to downstream recreational areas. Additional study is also necessary to determine how bacterial survival and transport from manure application fields impacts the spread of pathogens back to the farm environment.

2.5 Conclusions

This study was conducted under highly variable weather conditions and a range of common management practices, allowing for extensive characterization of microbial concentrations in drainage tiles under PM-amended cornfields. Our results have implications for manure management and tillage practices, watershed-modeling efforts, risk assessments for pathogens in recreational waters, biosecurity, and public health. This study clearly shows that bacteria from spring application of PM are transported to drainage tile lines, even under unsaturated conditions, and that elevated bacteria concentrations can persist for more than 3 months in years with above-average precipitation. Concentrations of bacteria in tile-drainage

from PM-amended fields were greatest when PM was applied to NT plots. The difference between bacterial concentrations in tile-waters below NT and CP plots may be explained by the presence of macropores, which were shown to occur at higher densities in NT plots than previously reported. In tile-drained watersheds, PM application may contribute to exceedances of recreational water standards, and such contributions should be incorporated into watershed models and risk assessments. Additionally, our results indicate that ENT is a better predictor of SALM than EC in this setting. However, caution should be taken when using either FIB, because low concentrations of either indicator, does not ensure that waters are free of *Salmonella*.

2.6 Acknowledgements

2.7 References

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CHAPTER 3: SALMONELLA AND FECAL INDICATOR BACTERIA SURVIVAL IN SOILS AMENDED WITH POULTRY MANURE

3.1 Abstract

Minimizing the risks associated with manure-borne pathogenic microorganisms requires an understanding of the survival of bacteria under realistic field conditions. The objective of this 3-year study was to assess the fate of *Salmonella* (SALM) and fecal indicator bacteria, *E. coli* (EC) and enterococci (ENT), in glacial till-derived soils, after application of poultry manure (PM) to cornfields under conventional chisel-plowed (CP) or no-till (NT) management. From 2010 - 2012, soil samples were obtained each spring at 0-15 cm and 15-30 cm depths, to determine whether over-wintering of bacteria had occurred. Sampling was followed by application of PM at low (PM1) and high (PM2) rates, based on nitrogen application goals. CP plots were tilled, prior to planting corn (*Zea mays L.*). In 2012, soil samples were collected 21, 42, and 158 days after manure application (DAM), to assess the effects of time, manure application rates, and tillage on frequency of detection and concentrations of bacteria. Detection of all three bacteria species in spring soil samples indicated over-wintering of bacteria was possible. Despite dry conditions, all three bacteria species were detected 158 DAM in 2012. The highest SALM concentration (790 cfu/g dry weight) and detection rate (25%) was found in PM2 plots. SALM detection was higher in CP plots (20%) compared to NT plots (5%). In contrast, tillage practices had no apparent effect on EC or ENT survival, as indicated by both soil sample analyses, and decay rates estimated from tile-water bacteria concentrations. Decay rate constants (μ) ranged from 0.044 to 0.065 for EC, and 0.010 to 0.054 for ENT.

3.2 Introduction

Salmonellosis affects 1.2 million Americans annually (Scallan et al. 2011), with tens of millions of cases worldwide each year (WHO 2013). Infections can be caused by consumption of contaminated seafood, meat, eggs, dairy, juice, fresh produce, or water (Dale et al. 2010; FDA 2012). Quality control plans, mandatory testing of eggs, and hazard analysis at critical control points (HACCP), are used to reduce the spread of *Salmonella* in the Unites States (US). Despite these efforts, persistence of *Salmonella* (SALM) in the farm environment continues to be a concern for dairy, livestock and poultry operations, and growers of vegetables and leafy greens (CDC 2010; Jacobsen and Bech 2012; Berghaus et al. 2013). Additionally, the risk of transport of manure-derived bacteria to surface and groundwater resources is a concern worldwide (Unc and Goss 2004; Dale et al. 2010; WHO 2012; USEPA 2013a).

Iowa is the leading producer of eggs in the US, and is also a major producer of broiler chickens (UDSA-NASS 2014). Poultry manure (PM) from confinement operations is typically stockpiled, and applied in the spring or fall, to provide nutrients necessary for production of corn (*Zea mays L.*) (Moore 1998; Sharpley et al. 1998; Jn-Baptiste et al. 2013). Along with beneficial nutrients, PM commonly contains *Salmonella* (SALM) and other enteric pathogens, (Kraft et al. 1969; Rodriguez et al. 2006), which can be transported between farms and to water resources. In this region of the US, a majority of the soils are derived from sediments deposited during the Pleistocene glacial period (Brady 1984), and as much as 50% of the agricultural fields have had subsurface drainage tiles installed to artificially lower the water-table in these poorly-drained soils. While these tiles generally reduce the potential for transport of soil and other contaminants from agricultural fields via runoff (Skaggs et al. 1994), they have been shown to transport

significant loads of leached contaminants, including bacteria, to surface waters (Bakhsh et al. 2005; Kjaer et al. 2007; Pappas et al. 2008; Hoang et al. 2013).

Common species of enteric bacteria, including fecal coliforms, *Escherichia coli* (EC) and enterococci (ENT), are often used as indicators of the presence of fecal contamination in the environment. These fecal indicator bacteria (FIB) are preferred over direct detection of pathogenic organisms, because, 1) they are present at high concentrations in human and animal waste, 2) they are generally commensal (not pathogenic), and 3) they are more easily, and less expensively, detected using traditional culture methods. Epidemiological studies have shown associations between FIB and human illness after exposure to environmental waters (Pruss 1998). However, a growing body of literature suggests that FIB are not always well correlated to pathogen occurrence, and the fate and transport of these species can vary widely depending on their source and local environmental conditions (Payment and Locas 2011). In aquatic environments, SALM has been detected when FIB are absent or present at low concentrations (Morinigo et al. 1990; Polo et al. 1998; 1999). Given the risks associated with the spread of SALM in the environment, it is crucial to evaluate the effectiveness of FIB as indicators of potential risks from this pathogen, in a wide variety of settings.

Numerous laboratory studies have been conducted to assess the potential for survival of SALM, and other bacteria, in manure-amended soils. Key factors shown to impact bacterial survival include temperature, soil type, soil moisture, nutrient availability, and protection from UV exposure (Chandler and Craven 1980; Crane and Moore 1986; Guan and Holley 2003; Holley et al. 2006; You et al. 2006; Lang and Smith 2007; Garcia et al. 2010). Bacterial survival in soils is also dependent on interactions with plants and plant roots, protozoan predators, and native microbial communities (Jiang et al. 2002; Brandl et al. 2005; You et al. 2006; van Elsas et

al. 2007; Garcia et al. 2010; Ibekwe et al. 2010; Liang et al. 2011; Rothrock et al. 2012; Farhangi et al. 2013; Erickson et al. 2014a).

With such a wide range of complex interactions occurring in natural settings, it is especially important to collect field data from a variety of locations under a range of climate conditions. In addition, various common agricultural practices, such as storage practices, method of application, timing of application, and tillage practices, have been shown to impact bacterial survival, and must be considered (Hutchison et al. 2004b; Arrus et al. 2006; Coelho et al. 2007; Semenov et al. 2009; Samarajeewa et al. 2012; Amin et al. 2013; Hoang et al. 2013). While numerous studies have documented persistence of SALM, and other pathogens, in soils amended with swine and bovine manures, few have evaluated bacterial survival on artificially drained glacial till-derived soils (Gessel et al. 2004; Rogers et al. 2011; Samarajeewa et al. 2012; Hoang et al. 2013; Garder et al. 2014). Islam et al. (2004) observed longer survival times of SALM in soils amended with composted poultry manure in comparison to composts from other manure sources; however, studies of untreated poultry manure are rare. All previous field studies of bacterial survival in soils after PM application, have been conducted in the Piedmont region of the southeastern US, and have evaluated the effects of broiler litter application on cotton fields or grassed plots (Jangid et al. 2008; McLaughlin et al. 2011; Jenkins et al. 2012; Cook et al. 2014; Erickson et al. 2014a). Of these studies, only one detected *Salmonella* in poultry litter samples (Cook et al. 2014).

The objective of our study was to evaluate the long- and short-term survival of SALM and FIB (EC and ENT) in Canisteo-Clarion-Nicollet series soils under cornfields amended with poultry manure. Soil samples were collected each spring for 3 years (2010-2012) to evaluate if bacterial survived (over-wintering) a full year after PM application. Additionally, we compared

bacterial occurrence and concentrations in soils 35 days before, and 21, 42, and 158 days after

manure application (DAM) in 2012, to determine the effects of time, tillage and treatment.

Finally, bacterial decay rates were estimated using records of bacterial concentrations in drainage

tile-waters collected during peak flows in a year with above-average precipitation (2010).

3.3 Materials and Methods

3.3.1 Study site

Field experiments were conducted from 2010 through 2012 at Iowa State University's

(ISU's) Agronomy and Agricultural Engineering Research Farm west of Ames, Iowa. The site is

located in the Des Moines Lobe landform region, a landscape formed by the last glacial

maximum that occurred in the Upper Midwest during the late Pleistocene Epoch, between

18,000 – 15,000 years ago. The research plots are located on soils with a Canisteo-Clarion-

Nicollet association, which are loamy soils formed in glacial till under prairie vegetation,

characterized as moderately permeable, with drainage classifications ranging from well-drained

to poorly drained. Soil texture typically ranges from 30-45% sand, 35-42% silt, and 20-30% clay

content (NRCS, 2014). Topsoil (0 – 30 cm) measurements for all plots (2010 – 2012) range

from 2.0 – 4.4% organic matter content. Plot slopes range from 0 – 5 percent. Tile drains are

installed along the midline of each plot at a depth of approximately 1.2 meters to enhance

drainage.

A long-term study of the effects of PM on water quality began in 1998, with nine chisel-

plowed (CP) plots under a corn-soy rotation. For 12 years, PM was applied in the spring, to the

portion of each plot that was to be planted in corn, as described by Nguyen et al. (2013). In

2010, all CP plots were converted to a continuous corn rotation, and 3 established NT plots were

included as part of the PM study. PM and urea ammonium nitrate (UAN) were applied each

spring, prior to planting, at application rates based on nitrogen (N) goals (Table 3.1). The PM2

goal achieves the maximum recommended rate of N application for continuous corn production

in Iowa (Sawyer et al. 2006).

Table 3.1. Plot treatments with nitrogen fertilization goals.

Treatment	Treatment Description	Nitrogen Fertilization Goal (kg/hectare/year)
PM0 - NONE	control, no manure or fertilizer	0
PM0 - UAN	control, urea ammonium nitrate fertilizer	224
PM1	PM applied at a low rate	112
PM2	PM applied at double the low rate	224

Figure 3.1 shows the layout of the study plots. Plot areas range from 0.08 to 0.51

hectares (ha). Tiles are spaced 36.3 meters apart. The experimental treatments on CP plots were

arranged in a randomized design with three plots receiving PM1 treatment, three plots receiving

PM2 treatment, and three plots receiving no manure as controls (PM0). Two of the control plots

were fertilized with urea ammonium nitrate (PM0 - UAN), and one that received no fertilizer

(PM0 – NONE). Individual NT plots were fertilized with PM1, PM2, and PM0 – UAN

treatments. No phosphorus was applied to either type of PM0 plot; thus, we will not distinguish

between PM0 treatments for the remainder of this article. Six possible combinations of tillage

and treatment are as follows: CP PM0, CP PM1, CP PM2, NT PM0, NT PM1, and NT PM2.
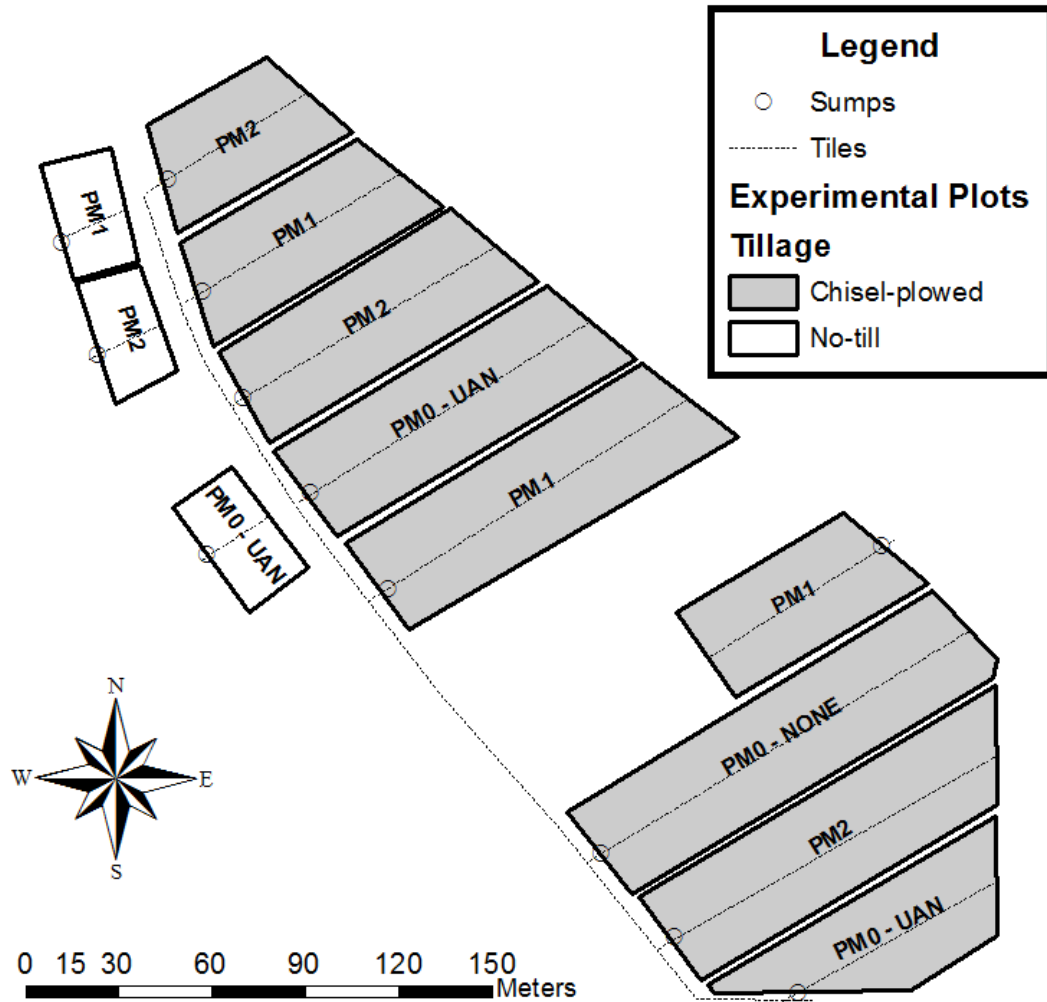
Figure 3.1 Map of experimental plot design with tillage practices (chisel-plowed and no-till), fertilization treatments (PM1, PM2, PM0-UAN, and PM0-NONE), and locations of tiles and sumps.

3.3.2 Precipitation and soil temperature and moisture measurements

Rainfall data were collected using two tipping-bucket rain gauges with HOBO data-loggers (Onset Computer Corp., Pocasset, Mass.) located at the site. Daily average soil temperature data from 10.2 cm depth, and soil moisture percentage from a loam soil at 5 cm depth was obtained from the Soil Climate Analysis Network Site 2031 (NRCS 2014). Where continuous soil moisture data were missing, soil moisture data from the Iowa Soil Erosion Project were used (IDEP 2014).

3.3.3 Manure sampling, application, and analysis

Poultry manure was collected from a confinement facility housing layer-hens (*Gallus gallus domesticus*), and transported to the study site, where it was stockpiled for up to four weeks, prior to application. Three representative manure samples were collected from the stockpile, placed in plastic bags and stored on ice, and then transported to Minnesota Valley Testing Laboratory in Nevada, Iowa, for nutrient analyses. Nitrogen content was used to calculate appropriate tonnage of manure to be applied per plot (Hanna and Richard 2008). Manure was resampled the day prior to scheduled application to the field plots, and transported on ice to ISU's Water Quality Research Laboratory for moisture content and microbial analyses. An additional 8 – 12 manure samples were collected directly from the spreader during manure application in 2011 and 2012 for improved characterization of the microbial content of the manure. Application occurred on the 24th and 25th of May, 2010, June 1st, 2011, and May 15th, 2012, followed immediately by tillage in chisel-plowed plots. Corn was planted within 3 days after manure application.

3.3.4 Soil sampling and analyses

Soil samples (0-15 cm and 15-30 cm deep) were collected using hollow-core samplers (Oakfield Model L Tube Sampler with a 30 cm tube). Soil samples were collected on May 17, 2010 (368 DAM), April 2, 2011 (374 DAM), April 10, 2012 (314 DAM in 2011 and 35 days prior to the 2012 application), and July 5, 2012 (21 DAM), July 26, 2012 (42 DAM), and October 20, 2012 (158 DAM). For each plot, 10 samples were obtained along diagonal transects, composited, and stored in plastic bags. Decontamination of all sampling equipment between plots was completed using a 90% ethanol solution. Samples were placed on ice, and transported to ISU's Water Quality Research Laboratory for moisture content and microbial analyses as described below.

Differences between distributions of bacteria concentrations in 2012 soil samples, were determined by non-parametric Wilcoxon rank sum tests using JMP software (SAS Institute) by date relative to manure application (-35, 24, 42, and 158 DAM), treatment (PM0, PM1, and PM2), tillage (CP and NT), and sample depth ($0 - 15$ cm and $15 - 30$ cm). For these analyses, all non-detections were assigned the value of the detection limit. Because differences between bacteria concentrations are not apparent when detection limits are low ($<33\%$), differences between frequency of positive detections were also assessed for the categories listed above, by Fisher Exact Tests using JMP software (SAS Institute).

3.3.5 Tile-water sampling and analyses

Water samples were collected directly from drainage tile outlets for 100 DAM in 2010 as described in Chapter 2. Samples were collected in 1 L sterile polypropylene bottles, placed on ice, and transported to ISU's Water Quality Research Laboratory, where they were stored at 4°C until they were processed for EC and ENT within 24 hours as described below. Average

concentrations of laboratory replicates were normalized to represent the average plot area, which is 65.5 m in length and 36.3 m in width. Mean bacterial concentrations from field replicates, were used for the following decay analysis, with the exception of the NT PM0 treatment, which had no replication. Average tile-water bacterial concentrations obtained during peak tile flows in response to precipitation events, were assumed to represent maximum levels of bacteria on and in the soil after PM application. Reductions in these concentrations over time were then used to estimate bacterial decay rates as described by Chick's Law:

$$N=N_0\exp(-\mu t)$$

where N is the bacterial concentration at time t (days), N_0 is the initial bacteria concentration, and μ is the die-off rate constant. Bacteria were not detected in tile-water until 11 DAM, and the first peak flow occurred 19 DAM; therefore, t_0 was defined at 19 DAM. Decline of peak tile-water bacterial concentrations were fit with an exponential decay model in semi-log space, and R^2 values, root mean square error (RMSE), and p values were determined by linear regression using JMP (SAS Institute). T_{90} values, the number of days for a 1-log reduction in tile-water concentrations of EC and ENT, are reported as determined from estimated decay curves.

3.3.6 Enumeration of Salmonella and fecal indicator bacteria

Manure, soil, and water samples were analyzed using membrane-filtration and growth on selective agars using previously described methods (Eaton et al. 1995; Messer and Dufour 1998; EPA 2002). Manure and soil sub-samples (10 g wet weight) were mixed with 150 mL phosphate buffered solution (PBS) and placed on an orbital shaker for 30 minutes to disperse aggregates and bioflocculated cells. Manure and soil solutions, and tile-water samples, were filtered in volumes ranging from 1 – 10mL, including dilutions when necessary, to achieve bacteria plate counts ideally between 20 and 80 colonies. EC, ENT, and SALM were cultured on modified

mTEC, mEnterococcus, and XLD agars, respectively (Difco[TM]). Samples were analyzed in triplicate and average values of replicates were used as the basis for further statistical analyses. For quality control, blanks with a minimum of 25 mL PBS were evaluated with each batch of samples. All bacteria concentrations are reported in colony forming units (cfu) per gram on a dry weight basis for soil and manure, and cfu/100 mL for water samples. Cultures of *E. coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), and *Salmonella enteritidis* (ATCC 13076) were used as positive controls. Soil and manure samples were analyzed for moisture content following the ATSM D2216 standard procedure (ASTM 1998). Variations in soil sample volumes resulted in detection limits of 12, 19, and 14 cfu/g dry weight for 2010, 2011, and 2012 soil samples, respectively.

3.4 Results and Discussion

3.4.1 Field conditions

The average total rainfall for the field season (March to October) from 1998 to 2012 was 78 cm. In 2011 and 2012 rainfall was below-average, with 50 and 42 cm of precipitation, respectively. In contrast, 2010 was the wettest year at the field site, since 1993, with 123 cm of rainfall. Soil moisture data from the nearby weather station reflect changes in precipitation up until February, 2012, after which, soil moisture data are not available. An alternative source of soil moisture data, the Iowa Daily Erosion project, reported an average volumetric soil moisture content at this location of 27-30% until early July (42 DAM), then average monthly soil moisture dropped to 15-20% for the remainder of the 2012 growing season (IDEP 2014). Soil moisture content of samples consistently averaged 15% for all sample dates. Average daily soil temperatures ranged from -5°C to 33°C from 2009 to 2012 (Iowa Mesonet, 2014). Figure 3.2

shows the daily average 10.2 cm soil temperatures along with the dates of manure application, 5 cm soil moisture measurements, and timing and temperatures of soil samples.
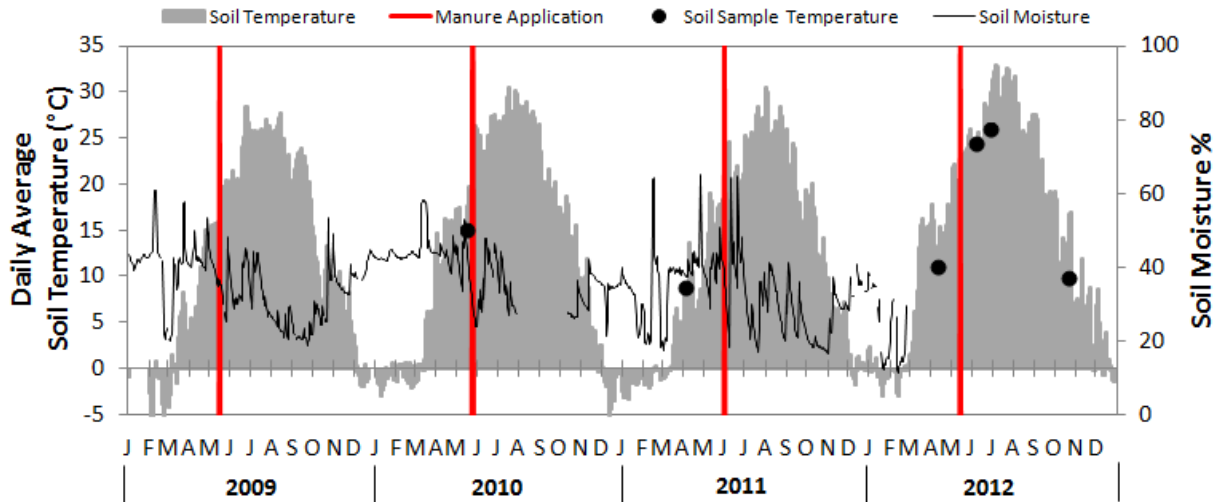
Figure 3.2 Timing of soil samples and manure application with daily average 10.2 cm soil temperatures (°C), and daily average 5 cm soil moisture content (NRCS, 2014).

3.4.2 Poultry manure

Average moisture content of poultry manure samples collected prior to application were 48%, 61%, and 30% in 2010, 2011, and 2012, respectively, and average total nitrogen content of manure ranged from 1.3 to 3.7 percent. Manure was applied to field plots at rates ranging from 5 to 13 Mg ha/yr for PM1 treatments, and from 10 to 40 Mg/ha/yr for PM2 treatments (Table 3.2). Nitrogen application rates averaged 108 kg N/ha⁄yr for the CP PM1 plots, just below the 112 kg N/ha⁄yr fertilization goal, and 223 kg N/ha⁄yr for the CP PM2 plots, nearly matching the 224 kg N/ha⁄yr goal. Application rates for the NT PM1 plots fell short of the goal, averaging 101 kg N/ha⁄yr, while rates on the NT PM2 plots exceeded the goal, averaging 287 kg N/ha⁄yr.

Table 3.2 Poultry manure application rates, by total wet mass and plant available nitrogen. Numbers in parentheses are standard deviations for rates on chisel-plowed plots.

Year	Treatment	Chisel-Plowed		No-Till	
		Manure Rate (Mg/ha)	Nitrogen Rate (kg N/ha)	Manure Rate (Mg/ha)	Nitrogen Rate (kg N/ha)
2010	PM1	9.3 (2.4)	72 (20)	12	90.2
	PM2	24 (0.13)	187 (1)	24	184
2011	PM1	13 (0.25)	120 (4)	12	109
	PM2	27 (1.6)	249 (26)	40	380
2012	PM1	132 (5.54)	132 (6)	5	103
	PM2	231 (5.96)	231 (6)	13	298

Bacteria concentrations in poultry manure were highly variable, as reported in Table 3.3. Average concentrations of bacteria in poultry manure sampled at the time of application were highest for ENT each year (ranging from $5.0 \times 10^4 – 1.5 \times 10^5$ cfu/g), followed by SALM (ranging from 2.0×10^2 - 1.4×10^5 cfu/g), and EC (ranging from 2.4×10^1 to 2.6×10^3 cfu/g). These EC and ENT concentrations are lower than previously reported for fresh PM, while SALM concentrations exceed previously reported values. Terzich et al. (2000) reported EC concentrations between 10^5 and 10^{10} cfu on a dry weight bases for fresh PM. ENT concentrations ranging from 10^5 to 10^8 cfu/g dry weight were reported by (Graham et al. 2009). Hutchison et al. (2004a) reported SALM concentrations in fresh PM samples up to 2.2×10^4 cfu/g, and 8.0×10^3 cfu/g for stored manure samples. Numerous variables could result in the differences in SALM concentrations observed between our study and the Hutchison study, including, the timing of PM collection, variations in on-farm management, and variations in bacterial shedding rates between flocks. Hutchison et al. (2004b) report collection of most samples over the winter months, when animals were housed, implying that animals were allowed

outside during summer months. Our samples were collected in spring from birds that were

raised continuously in roofed confinements.

Table 3.3 Average bacterial concentrations in poultry manure at the time of application.

Year	N	Stat	Concentrations (cfu/g dry weight)		
			E. coli	Enterococci	*Salmonella spp.*
2010	3	Max	5.9×10^1	5.6×10^4	2.1×10^4
		Mean	2.4×10^1	5.0×10^4	1.5×10^4
		SD	3.1×10^1	6.3×10^3	1.0×10^3
2011	3	Max	7.1×10^2	3.4×10^5	1.4×10^4
		Mean	5.2×10^2	1.5×10^5	9.7×10^3
		SD	1.7×10^2	1.8×10^5	6.4×10^3
2012	10	Max	1.8×10^4	6.8×10^4	1.5×10^3
		Mean	2.6×10^3	5.9×10^4	2.0×10^2
		SD	5.6×10^3	7.6×10^4	4.7×10^2

Two prior studies focused on microbial impacts of poultry manure application on soils

did not detect SALM in poultry litter samples. Jenkins et al. (2008) enriched litter slurries prior

to culturation. McLaughlin et al. (2011) used both culture and qPCR (using *spaQ* primers)

methods for detection and enumeration of SALM. Cook et al. (2014) also did not detect

Salmonella spp. above a limit of 100 cells/g, in DNA extracted from poultry litter samples;

however, they did confirm detection of SALM in over 50% of their poultry litter samples using

enrichment, followed by qPCR analyses for the *ttr* gene.

Average bacterial loads range from 1.1×10^8 to 1.7×10^{10} cfu/ha for EC, 1.1×10^{11} to 1.1

$\times 10^{13}$ cfu/ha for ENT, and 3.8×10^8 to 2.0×10^{11} cfu/ha for SALM (Figure 3.3). These values

for EC and ENT are comparable to those reported for a study of the effects of PM application by

Jenkins and others (2008) (on the order of 10^{11} and 10^{12} cfu/ha, respectively). However, SALM

was not detected in the poultry manure in that study.
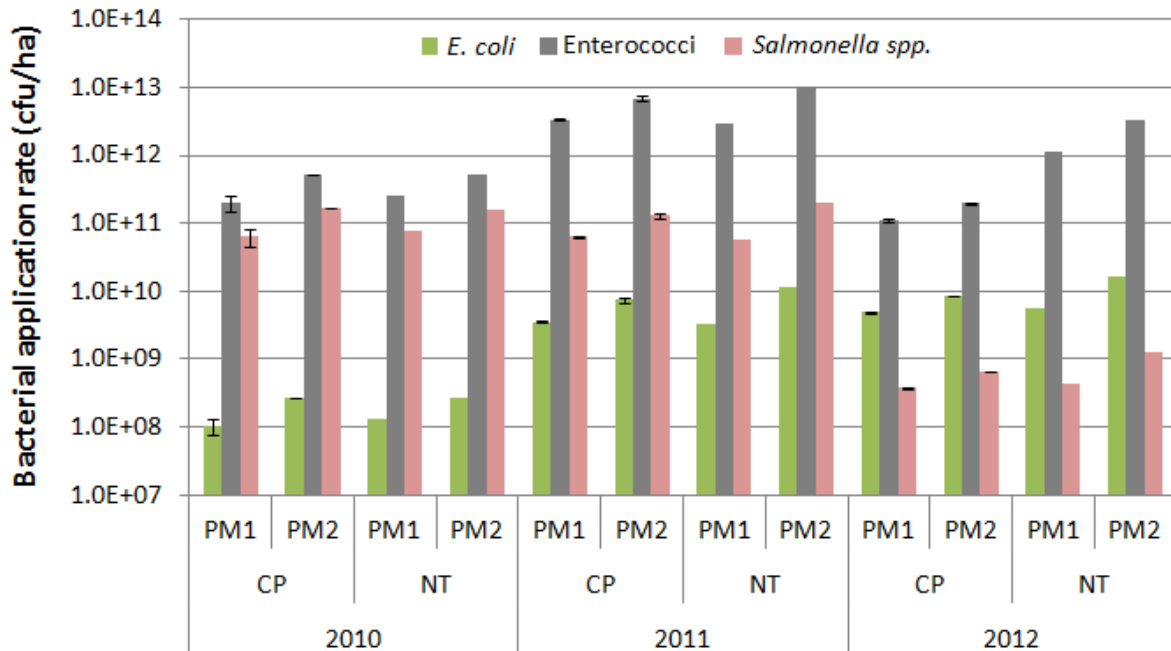
Figure 3.3 Estimated bacterial application rates on a per-hectare (ha) basis by treatment, tillage, and year for poultry manure (PM) amended plots based on average values reported in Table 3.3. Average rates are shown for replicated chisel-plowed (CP) plots, with error bars indicating one standard deviation above and below the mean. No-till (NT) plots were not replicated.

3.4.3 Bacterial survival in soils

3.4.3.1 Long-term survival

Bacteria concentrations in soil samples indicate that, under some conditions, manure-derived bacteria can survive almost a year after PM application (Table 3.4). EC and SALM were not detected in any spring soil samples from 2010. Only one ENT colony was detected in 2010, in a shallow (0-15 cm) sample from a CP PM1 plot, resulting in a reported concentration of 12 cfu/g. The other two laboratory replicates of that sample had no detections; therefore, the average of the replicates was considered below the detection limit. Furthermore, ENT were not detected in either of the other two CP PM1 plots.

In contrast to spring of 2010, all three species of bacteria were detected in 2011 and 2012 spring soil samples (Table 3.4). No bacteria were detected in the control plots (PM0), suggesting

that a history of PM application was responsible for the presence of bacteria in soils from manure-amended plots. No deep sample (15-30 cm) data are available for 2011 due to a laboratory processing error. While EC were not detected in shallow 2011 spring soil samples, EC were detected in both shallow and deep spring samples from 2012, in all PM2 plots, with a maximum concentration of 320 cfu/g. ENT were detected more frequently than either EC or SALM; however, average concentrations did not exceed 74 cfu/g. The maximum average SALM concentration was 37 cfu/g. No discernible patterns were observed with regard to tillage or sample depth in spring soil samples.

Lower inputs of bacteria in 2009 may be responsible for the lack of bacteria detections in 2010 spring samples. In 2009, CP plots were split under a corn-soy rotation; thus, only half of each plot received PM application (Nguyen et al. 2013). NT plots received commercial fertilizer rather than manure in 2009. Bacteria concentrations in PM were not measured in 2009; thus, we cannot make a direct comparison to bacterial loading rates.

Soil conditions at the time of sampling, and over the preceding year, may also have contributed to the variability of spring bacteria concentrations. Wet conditions in 2010 delayed soil sampling; thus, the average 10.2 cm soil temperature at the time of sampling was 15.1°C, which was higher than the soil temperatures in subsequent years of 8.8 and 11.2°C, respectively (Figure 3.2). Soil moisture conditions at the time of sampling consistently averaged 15% for all three years. Antecedent soil moisture in 2010 was higher than in 2011 and 2012. If antecedent soil moisture was the primary factor controlling bacterial concentrations in spring soil samples, one would expect higher concentrations in 2010. This was not the case; therefore, it does not appear that soil moisture variations can explain the differences between spring soil bacteria results. Given that soil moisture contents generally remained between 15-45%, it is possible that

conditions limiting survival were not reached, or that regrowth occurred. Although their study was conducted on soils with different parent materials, Chandler and Craven (1980) observed declines in *S.* Typhimurium and EC only after soil moisture dropped below 15%, with rapid declines below 10%. These authors also noted the ability of both organisms to regrow when soils were remoistened after 14 days. It is also interesting to note that Chandler and Craven (1980) observed a decline in EC when soil moisture contents exceeded 40%, but did not observe the same effects for SALM.

Table 3.4 Bacteria concentrations in shallow (0-15 cm) and deep (15-30 cm) soil samples from 2011 and 2012, approximately one year after application. Results from manure-amended plots are shaded gray. Results from 2010 are not shown because average of laboratory replicated samples never exceeded the detection limit.

Year	Tillage	Treatment	Depth (cm)	Avg. bacteria concentrations (cfu/g dry weight)		
				E. coli	Enterococci	*Salmonella spp.*
2011	CP	PM0	0 - 15	<19	<19	<19
		PM1	0 - 15	<19	26	<19
		PM2	0 - 15	<19	29	<19
	NT	PM0	0 - 15	<19	<19	<19
		PM1	0 - 15	<19	<19	34
		PM2	0 - 15	<19	74	37
2012	CP	PM0	0 - 15	<14	<14	<14
		PM1	0 - 15	<14	30	17
		PM2	0 - 15	222	<14	<14
	NT	PM0	0 - 15	<14	<14	<14
		PM1	0 - 15	<14	19	19
		PM2	0 - 15	19	19	<14
	CP	PM0	15-30	<14	<14	<14
		PM1	15-30	<14	17	15
		PM2	15-30	321	23	<14
	NT	PM0	15-30	<14	<14	<14
		PM1	15-30	<14	<14	<14
		PM2	15-30	63	<14	<14

While soil moisture variations did not appear to play a major role in overwintering of PM-derived bacteria, there is some evidence that temperatures affected the ability of these bacteria to survive. In general, survival of fecal bacteria in soils is generally favored by cool temperatures (5°C) in comparison to warm temperatures (22°C), as demonstrated for *Salmonella enterica* Typhimurium by Zibilske and Weaver (1978). Additionally, although bacteria are not always deactivated by freezing, the stress of repeated freeze-thaw cycles has been shown to decrease SALM survival (Olson et al. 1981). Between 2009 and the spring of 2010, 11 freeze-thaw cycles were recorded in the 10.6 cm soil temperatures. From 2010 to 2011, only three freeze-thaw cycles were recorded, and five freeze-thaw cycles were recorded from 2011 to 2012.

Regardless of the exact cause of differences between spring soil bacteria concentrations, these data confirm that PM application can result in elevated soil bacteria concentrations 1 year after application. In a similar study, McLaughlin et al. (2011) compared bacteria concentrations in soils with and without a history of poultry (broiler) manure fertilization. These authors did not detect the pathogens, *Salmonella spp.* or *Campylobacter spp.*, or find differences between EC or ENT concentrations in manure-amended and non-manured fields, one year after fertilization. Application rates in the McLaughlin et al. (2011) study ranged from 2.2 to 13.4 Mg/ha per year, which are comparable to our PM1 treatment, and lower than our PM2 treatment. McLaughlin et al. (2011) report 30-year average ambient temperatures (17 and 21°C) and rainfall amounts (14.2 and 12.5 cm) for the months of April and May, respectively, when soil samples were collected. Differences in initial PM composition, soil types, and warmer ambient temperatures, may explain the lack of manure-derived bacteria in the McLaughlin et al. (2011) results in comparison to our results.

3.4.3.2 Short-term survival in a dry year

In 2012 pre- and post-manure application samples, frequency of detection was generally low, with detection of ENT>SALM>EC. ENT were detected in 69% of all soil samples from 2012, while only 18% of samples had detectable SALM, and 15% had detectable *E. coli*. Table 3.5 displays detection frequencies and soil bacteria concentrations for the four sets of samples collected in 2012. Concentrations of EC in post-manure-application soil samples were lower than pre-manure samples as determined by Wilcoxon rank sum analysis, and detection frequencies between sample sets were different as determined by Fisher Exact Test analysis. Four samples (17%) had EC detections 158 days after application, however, average concentrations of replicates did not exceed the detection limit (14 cfu/g). While maximum SALM concentrations exceeded pre-manure application concentrations in all three post-manure application samples, no statistical differences between samples sets were observed due to the low detection frequencies, and no differences between detection frequencies were observed, either. Only ENT concentrations 158 DAM were statistically higher than pre-manure soil samples. Significant differences between detection frequencies were observed for ENT ($p = 0.009$), with detection frequencies increasing over time.

Low detection rates of EC and SALM in 2012 may be explained by a sustained lack of soil moisture over the growing season, or other factors. While continuous soil moisture measurements were not available, the Iowa Daily Erosion project reports an average volumetric soil moisture content at the site of 27-30% for the first 42 DAM, followed by 15-20% moisture for the remainder of the growing season. Only 3.4 cm of rain fell in the first 21 DAM in 2012. The largest precipitation event post manure application (6.3 cm) occurred 36 DAM. After that event, negligible amounts of precipitation fell until 71-75 DAM, when three storms, totaling 3.4

cm occurred. Almost no precipitation was recorded until 102 DAM, when a small storm totaling

0.9 cm occurred. Although maximum ENT and SALM concentrations were higher 21 DAM

than in pre-manure soil samples, higher maximum concentrations were measured in later

samples, suggesting that regrowth may have occurred, as documented in Chandler and Craven

(1980). It is also possible that these bacteria could have transitioned in and out of a viable-but-

not-culturable (VBNC) state, further complicating interpretation of these data. Future studies

with more frequent sampling, paired with continuous soil moisture measurements, may help

answer these questions.

Table 3.1 Summary statistics for E. coli, enterococci, and Salmonella spp. in soil samples from 35 days before, and 21, 42, and 158 days after manure application in 2012. Median and maximum bacterial concentrations are expressed in colony forming units per gram dry weight. The number of samples for each category is 24. Letters indicate statistically significant differences (p<0.05) evaluated by pair-wise comparison of pre- and post-manure sample sets using Wilcoxon rank sum analyses.

Days After Manure	Statistic	*E. coli*	Enterococci	*Salmonella spp.*
-35	Det. Freq.	29%	50%	21%
	Median	<14 b	<14 a	<14 a
	Max.	840	40	28
+21	Det. Freq.	13%	54%	17%
	Median	<14 a	14 a	<14 a
	Max.	19	4500	52
+42	Det. Freq.	0%	75%	25%
	Median	<14 a	14 a	<14 a
	Max.	<14	2600	790
+158	Det. Freq.	17%	96%	8%
	Median	14 a	130 b	<14 a
	Max.	14	56,000	190

Greater differences between pre- and post-manure samples may have been detectable if

samples had been obtained less than 21 days after PM application. Gessel and others (2004)

found concentrations above background levels of *Salmonella anatum* (p=0.0530) in soils at 2 cm depths, 4 days after surface application of liquid swine manure and disking, but not in samples collected 7 or more DAM. No differences between shallow and deep soil samples were observed in our soil samples; however, it is possible that focusing on shallower samples would have elicited higher concentrations, especially under NT plots. As described in Chapter 2, competition from the growth of EC on selective agar (XLD) plates used to enumerate SALM, likely reduced counts of SALM, especially for samples from manured plots where EC were detected at high concentrations.

Short-term EC and ENT survival in PM-amended soils more closely resembles results previously reported for beef manure amended soils than for swine-manure amended soils. Rogers et al. (2011) reported no detections of EC 41 and 56 days after injection with liquid swine manure, while EC were detected 120 DAM in beef manure-amended soils in a controlled laboratory at 25°C. ENT also survived longer in beef manure-amended soils (120 DAM), than swine manure-amended soils (no ENT 41 and 56 DAM). SALM survival in soils (158 DAM) is longer than previously reported for other manure types under similar conditions. Rogers et al. (2011) reported finding cultivable *S. enterica* Typhimurium in swine manure-amended soil for 120 days, and 55 days in beef manure-amended soils. Baloda et al. (2001) reported *S. enterica* Typhimurium survival up to 14 days in swine manure-amended soils, Johnson et al. (1996) reported survival of SALM 27 days after application of swine manure at a depth of 15 cm, and Zibilske et al. (1978) reported SALM survival 42 DAM at 22°C. While manure type and application method both have been shown to effect bacterial survival (Semenov et al. 2009; Rogers et al. 2011), it is clear that the magnitude of these effects differs between bacteria species.

3.4.3.3 Effects of application rate

Analysis of post-manure application samples from 2012, reveal some effects of manure treatment. Wilcoxon rank sum tests revealed significantly higher concentrations of ENT under plots that received the high (PM2) application rate, compared to PM0 and PM1 treatments (Table 3.6). No other significant differences were observed for post-manure soil bacteria concentrations (Table 3.6), which is not surprising because EC and SALM detection did not exceed 25%. Results of Fisher Exact test analyses of detection frequencies, by treatment category, indicate that the increase in detection was significant for EC and ENT ($p = 0.01$), but not SALM ($p = 0.13$).

Table 3.2 Occurrence data for bacterial species in post manure application soil samples from 2012 by treatment. The number of samples for each category is 24. Median and maximum bacterial concentrations are expressed in colony forming units per gram dry weight. Letters indicate significant differences in bacterial concentrations by treatment class.

Treatment	Statistic	*E. coli*		Enterococci		*Salmonella spp.*	
	Det. Freq.	0%		54%		20%	
PM0	Median	<14	a	<14	a	<14	a
	Maximum	<14		154		59	
	Det. Freq.	4%		79%		4%	
PM1	Median	<14	a	27	a	<14	a
	Maximum	14		56,000		41	
	Det. Freq.	25%		92%		25%	
PM2	Median	<14	a	87	b	<14	a
	Maximum	19		6300		790	

In contrast to the results of spring soil samples (~1 year post application), ENT and SALM were detected in soils from control plots (PM0) in post-manure samples from 2012 (Table 6). For ENT, the frequency of detection in control plots increased over time, from 25%

21 DAM, to 38% 42 DAM, to 100% 158 DAM. Conversely, SALM detection in control plots was highest 21 DAM (38%), and decreased to 13% 42 and 158 DAM. If cross-contamination during manure application and tillage caused contamination of control plots, one would expect the effect to be greatest in the samples immediate following these activities, which matches the pattern observed for SALM. The fact that none of the sample from the NT control plots had SALM supports this theory. Unfortunately, as discussed above, potential interference from EC, complicates interpretation of SALM data, although there was no inverse relationship between EC and SALM occurrence in these control samples. Transport between plots could also have been facilitated by wildlife. Numerous birds and rodents were observed in these fields over the course of the experiment. Nearly equivalent percentages of samples from CP (56%) and NT (50%) control plots had ENT detections, suggesting that tillage was not the cause of contamination. Determination of the principle strains of bacteria present in the manure, soils, and visiting wildlife might help answer these questions.

3.4.3.4 Tillage effects

The effects of tillage practices on soil bacteria detections and concentrations were also evaluated for post-manure application samples in 2012. Because manure was incorporated shortly after PM application to CP plots, minimizing exposure of bacteria to ultra-violet light, we expected bacteria to survive better in these plots than in NT plots where manure remained at the soil surface, as has been previously demonstrated (Tyrrel and Quinton 2003; Hutchison et al. 2004b; Rogers and Haines 2005). In fact, only SALM survival appeared to benefit from tillage, with 20% detection in CP post-manure soil samples, compared to 6% for NT samples (Table 7). The difference in detection frequency between CP and NT soils for SALM was significant at $p = 0.019$. While occurrence of EC and ENT were higher under NT plots, the difference in detection

frequencies was less than 4%, and was not significant (Table 7). The chi-square approximation based on Wilcoxon analyses for evaluation of differences between SALM concentrations in soils from CP and NT plots (0.052), was lower than for EC (0.564) and ENT (0.716), though it was not significant at $p = 0.05$.

Table 3.3 Frequency of detection of bacterial species in post-manure application soil samples from 2012 by tillage class. Lettering indicate significant differences at p<0.05 using Fisher Exact test analyses.

Tillage	Statistic	*E. coli*	Enterococci	*Salmonella spp.*
CP	N	54	54	54
	Det. Freq.	9% a	74% a	20% a
	Median	14	37	14
	Max	19	6,300	790
NT	N	18	18	18
	Det. Freq.	11% a	78% a	5% b
	Median	14	16	14
	Max	14	56,000	14

Previous studies also reported little impact from tillage on soil bacteria concentrations after PM application. Jenkins et al. (2008) found no differences between EC concentrations in soil samples from CP and NT plots one day after poultry litter application and rainfall simulation. Cook et al. (2014) also found no effect of tillage on ENT concentrations in soils after poultry litter application. These authors also report finding more soil samples positive for SALM under CP plots than in NT soils. These findings imply that UV radiation and desiccation increases die-off of SALM in untilled soils; however, these factors appear to be less important for survival of EC and ENT.

3.4.4 Decay rate estimation

In comparison to 2012, 2010 was a very wet year. Soil moisture contents exceeded 25% for the entire 2010 growing season (IDEP 2014). Post-manure EC and ENT concentrations in drainage-tile-waters remained above pre-manure values 100 DAM (Chapter 2). Because tile-water bacterial concentrations generally increase with increasing tile flow rates in response to precipitation, only peak tile-water bacterial concentrations measured during the top 10% of tile flow rates were used to estimate bacterial decay rates. Maximum tile-water bacteria concentrations were seen earlier (11 DAM) under NT plots than under CP plots (19 DAM). Figures 3.4 and 3.5 show the average peak concentrations of EC and ENT, associated tile flow rates, rainfall intensity, estimated decay curves, and decay equations. Decay constants and T_{90} values are displayed in Table 3.8 for ease of comparison.

Decay rates were higher for EC than ENT under all combinations of tillage and manure treatment. The lowest decay constants for ENT under CP PM1 and NT PM2 treatments are suspect given their poor correlations. However, it is also possible that these values reflect regrowth of ENT under repeated wetting, as described by Yamahara et al. (2009) in marine beach sands. Without additional data, it is difficult to explain why regrowth would occur in some plots and not in others. Further study is necessary to determine which strains of ENT are present, and whether biofilm formation or other processes that enhance bacterial growth, occur in these soils.
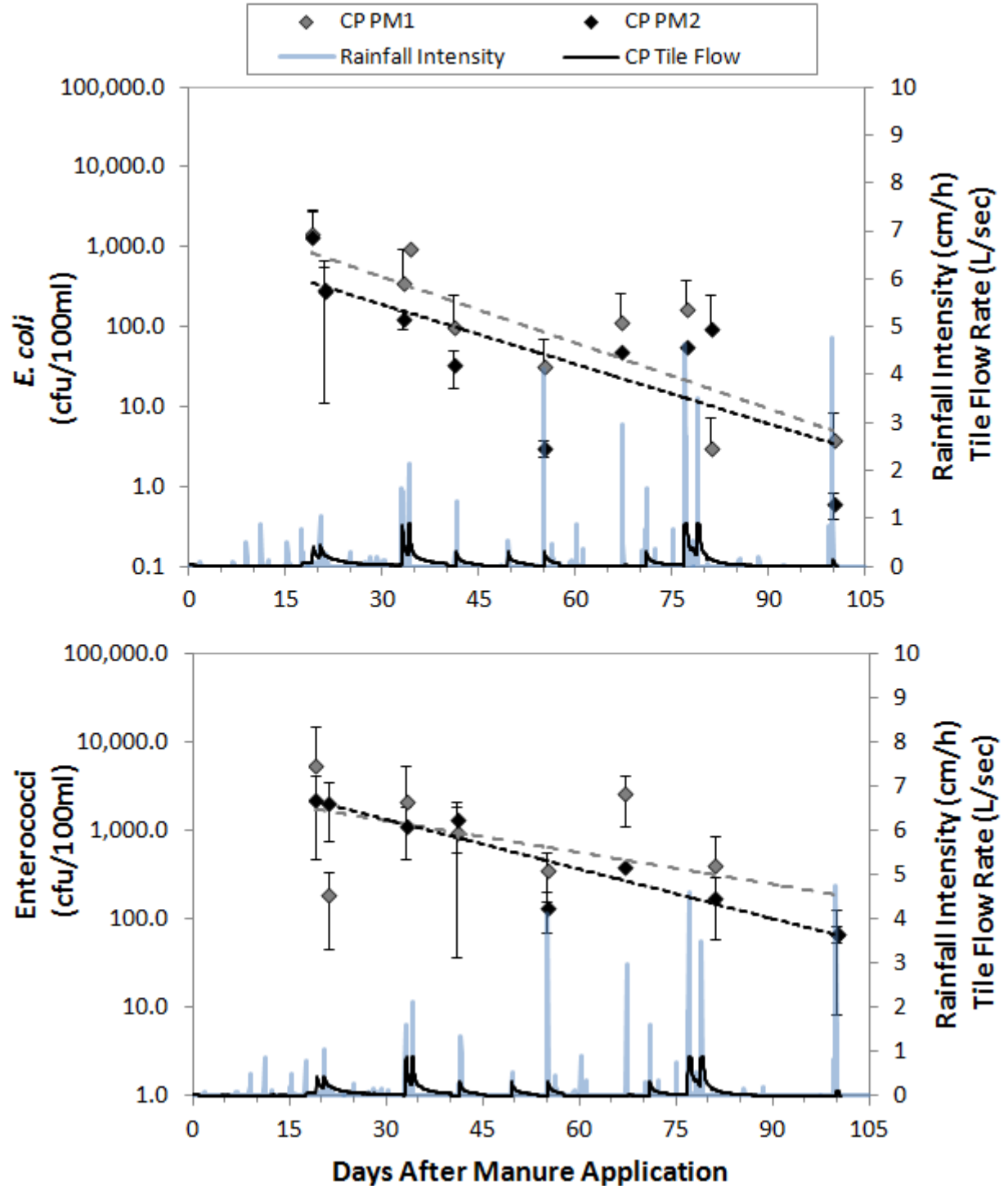
Figure 3.4 Bacterial decay estimated from 2010 tile-water concentrations of E. coli and enterococci after poultry manure application to chisel-plowed (CP) plots. PM1 and PM2 represent low and high rates of poultry manure application. Error bars represent standard deviations of field replicates.
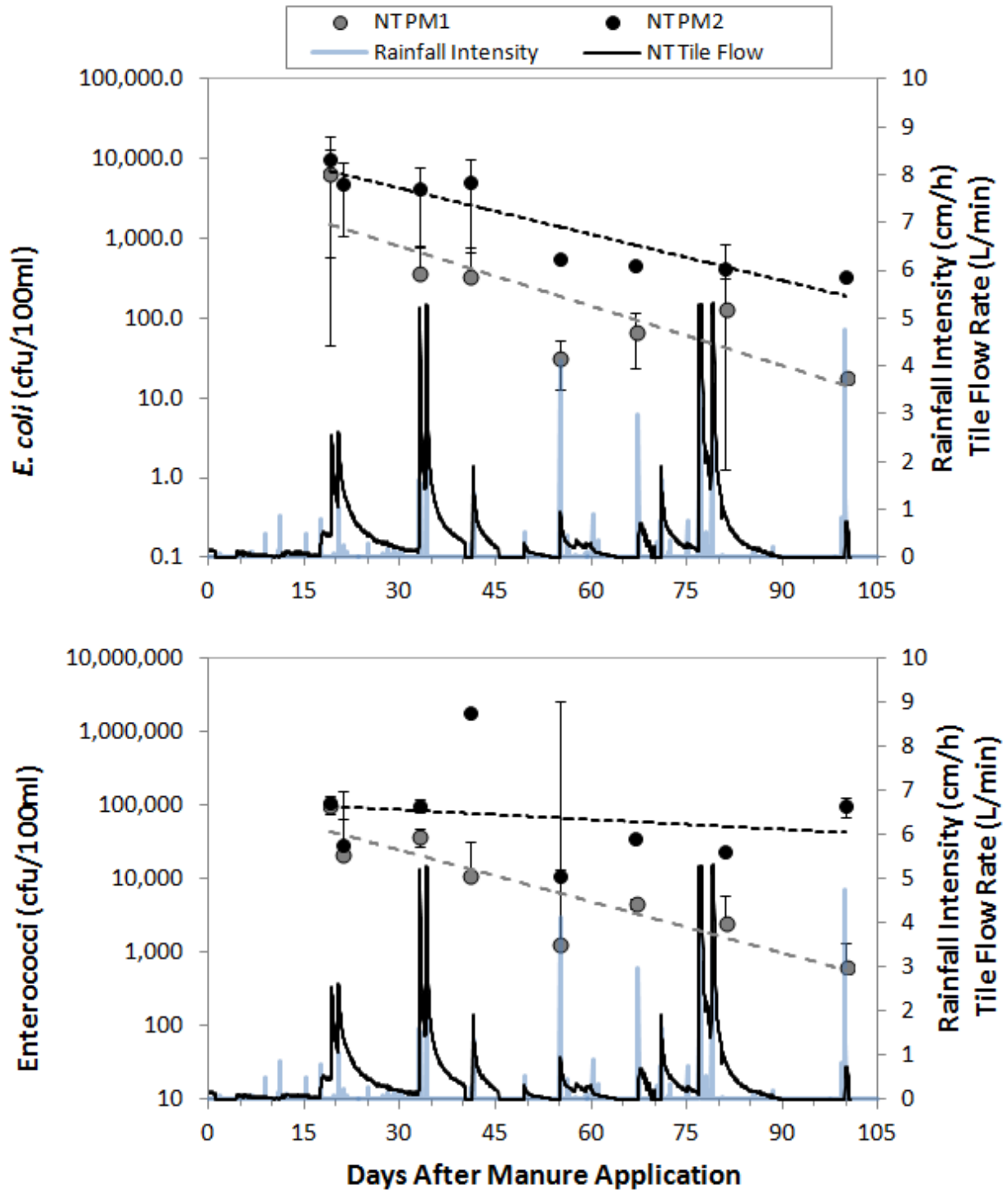
Figure 3.5 Bacterial decay estimated from 2010 tile-water concentrations of E. coli and enterococci after poultry manure application to no-till (NT) plots. PM1 and PM2 represent low and high rates of poultry manure application. Error bars represent standard deviations of field replicates.

Table 3.8 Descriptive values for estimations of decay based on tile-water concentrations of E. coli and enterococci under chisel-plowed (CP) and no-till (NT) plots with low (PM1) and high (PM2) rates of manure application in 2010. Decay constants (μ), number of days for 1-log (or 90%) reduction in bacterial concentrations (T90), R^2 values, root mean square error (RMSE), and p values are presented.

Till-age	Treat-ment	*E. coli*					Enterococci				
		μ (d^{-1})	T_{90}	R^2	RMSE	*p*	μ (d^{-1})	T_{90}	R^2	RMSE	*p*
CP	PM1	0.065	35	0.55	217	0.0135	0.028	83	0.27	556	0.1894
	PM2	0.063	37	0.90	73	0.0001	0.043	54	0.93	218	0.0001
NT	PM1	0.057	41	0.84	231	0.0036	0.054	44	0.66	10940	0.0144
	PM2	0.044	53	0.79	1217	0.0020	0.010	230	0.02	21274	0.7619

Decay estimates were derived from tile-water bacteria concentrations obtained 19 – 100 days after PM application; therefore, estimated decay rates most likely represent the second phase of decay as defined by (Benham et al. 2006). In controlled laboratory studies, Rogers et al. (2011) identified a second phase of decay beginning 10.7 to 16.9 days after inoculation in swine manure-amended soils for EC, and 7.09 to 16.9 days for ENT in beef manure-amended soils (at 10°C and 25°C).

Although estimating decay from tile-water concentrations is an indirect method, it circumvents problems caused by accumulation of sediment on membrane filters, which limits sample size. Also, this method effectively integrates large volumes of soil, rather than relying on the low ratio of soil samples to potential contributing soil volumes in the field. The results of our estimation of decay appear reasonable with respect to previously published values under similar, but not identical, conditions. Estimated decay rates from 2010 are slightly higher than decay rates reported for the second phase of die off by Rogers et al. (2011) for soils amended with swine and beef manure in a laboratory setting. Rogers et al. (2011) reported decay constants ranging from 0.029 – 0.048 day^{-1} for EC, and 0.011 – 0.030 day^{-1} for ENT, in soils incubated at

25° C for 120 days. Soils in our study experienced a range of temperatures from 15°C to 30°C over the course of the 2010 growing season.

Decay constants were lower than those reported by Cook and others (2014), who sampled 0 – 15 cm Crider silt-loam soils after poultry-litter application to grassed plots in 2011 and 2012. Cook and others (2014) reported decay constants ranging from 0.17 to 0.31, corresponding to T_{90} values ranging from 7.41 to 13.31 days. Cook et al. (2014) used soil measurements from 1 to 148 days after application to estimate decay; therefore, their results are likely to represent the combined effects of the first- and second-phase of decay as described by Benham et al. (2006), while our results are only from the second-phase.

While we were unable to determine a decay rate for SALM, the survival of SALM for over 80 days is consistent with the results of Rogers et al. (2011), who calculated a second-phase decay rate of 0.065 – 0.13 days^{-1} for *Salmonella enterica* serovar Typhimurium in swine manure-amended soils and 0.14 – 0.19 days^{-1} for beef manure-amended soils.

Tillage practices do not appear to impact EC or ENT decay rates estimated using 2010 tile-water bacteria concentrations. This is consistent with our analyses of 2012 soil data, which revealed no significant effects of tillage on EC and ENT. Cook and others (2014) also reported no consistent differences between ENT decay rates in NT and CP soils amended with poultry litter (broilers) in their 2-year study.

3.6 Conclusions

Bacteria concentrations measured in Canisteo-Clarion-Nicollet silt-loam soil samples, following PM application, confirm that SALM and FIB can survive for as long as one year, in the Midwestern USA. Although detection frequencies in 2012 soil samples were at or below 25% for EC and SALM, all three bacterial species were detected 158 days after manure

application. Both detection frequency and concentration of EC and ENT in soils increased with higher rates of PM application; however, the effect of application rate on SALM was not conclusive. Incorporation of SALM into soils via tillage favored persistence of SALM, while surface-application on NT soils decreased survival. In contrast, tillage did not impact EC or ENT concentrations in soils, nor were differences observed in EC and ENT decay rates estimated from tile-water bacterial analyses. ENT detection frequencies and concentrations were consistently greater than both SALM and EC in manure, soil, and water samples. Thus, ENT could potentially be used as an indicator of risk from PM-derived SALM. However, detection of ENT in control plots (PM0) suggests that background levels of ENT should be established before inferring the presence of pathogens. Additionally, both soil samples and estimation of decay rates indicate that regrowth of bacteria is possible, and further research is needed to better define the conditions under which regrowth is possible. Overall, our results indicate that soils act as a long-term reservoir for manure-derived *Salmonella*, and that the potential for release of these pathogens to the environment should be considered when making manure management decisions.

3.7 Acknowledgements

3.8 References

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CHAPTER 4: EFFECTS OF 3 YEARS OF POULTRY MANURE APPLICATION ON PHOSPHORUS IN SOILS AND DRAINAGE TILE-WATERS UNDER CHISEL-PLOWED AND NO-TILL CORNFIELDS

4.1 Abstract

Phosphorus (P) is an essential nutrient for plant growth, but P losses from agricultural systems contribute to degradation of surface-waters. The objectives of this study were to assess the effects of low and high rates of poultry manure (PM) application to chisel-plowed (CP) and no-till (NT) plots on soil test phosphorus (STP) levels over three years (2010-2012), and to evaluate orthophosphate losses in tile drainage waters. Soil sampling at $0-15$ cm and $15-30$ cm depths was completed each spring, followed by poultry manure (PM) application at low (PM1) and high (PM2) rates based on agronomic nitrogen goals for continuous-corn production. Control plots received no manure (PM0). Water samples were obtained from drainage tiles following precipitation events in 2010 and 2011, and more frequent sampling throughout selected hydrograph events was conducted in 2011 and 2012. STP levels (measured as Bray's 1-P) increased significantly under the PM2 treatment over the three year period; while significant differences were not observed under the PM1 treatment or the control plots (PM0). Despite the similarity between $0-15$ cm STP levels in chisel-plowed (CP) and no-till (NT) plots, orthophosphate (PO_4) concentrations in drainage tile-waters were significantly higher under NT plots (>0.1 mg/L PO_4-P) than under CP plots (≤0.04 mg/L PO_4-P) at $P < 0.05$. Event hydrographs from 2011 and 2012 short-lived peaks in PO_4 concentrations, which may be explained by macropore transport; however, elevated levels of PO_4 remain throughout the hydrograph in NT plots, suggesting that matrix flow accounts for much of the PO_4 transport to tiles. Orthophosphate losses to tiles from NT PM2 plots were estimated at 0.26 kg/ha PO_4-P the growing season in a wet year.

4.2 Introduction

It has long been understood that phosphorus (P) is a major cause of eutrophication in surface waters (Ryden et al. 1973; Sylvan et al. 2006). Significant research efforts have been devoted to understanding fate and transport processes of P, impacts on surface water bodies from various forms of P, relative contributions from different sources of P, and effects of various management practices in agricultural watersheds. Given the relative immobility of P in soils, most research and management has targeted reduction of P losses via erosion and runoff (Sharpley et al. 1994; Sharpley et al. 2004). In 1993, the first P Index was proposed as a tool for assessing risk of P losses (Lemunyon and Gilbert 1993). Since then, the P-Index concept has been adopted widely in the US (Mallarino et al. 2002), as well as Canada and Europe. Recently, the importance of P losses from agricultural drainage has been well documented (Gaechter et al. 1998; Sims et al. 1998; Tan and Zhang 2011; Ruark et al. 2014), and modifications of current P indices have been proposed to better reflect these contributions (Beauchemin and Simard 1999; Ulen et al. 2011; Reid et al. 2012).

The Upper Mississippi River Basin, including most of Iowa, has been estimated to contribute 40,400 metric tons (26%) of the total phosphorus discharged to the Gulf of Mexico annually (USEPA 2007), which is a major cause of eutrophication (Sylvan et al. 2006). In addition, P contributes to excess algae growth, especially in lakes and reservoirs, leading many of these freshwater bodies to be listed as impaired (Carpenter et al. 1998). Eutrophication restricts water use for fisheries, recreation, industry, and drinking water supplies. For example, in 2014, a large bloom of toxic cyanobacteria occurred in Lake Erie, threatening the public water supply of Toledo, Ohio. Water quality records from 1996-2006, indicate that algae blooms in

Lake Erie are correlated to increases in dissolved reactive phosphorus in the agriculturally-dominated Maumee River (Kane et al. 2014).

Significant efforts to reduce phosphorus and nitrogen (N) losses are ongoing, including development of water quality criteria (USEPA 2014b) and the US Environmental Protection Agency's (EPA) Total Maximum Daily Load (TMDL) program (USEPA 2014a). Nutrient reduction strategies often promote the use of best available technologies to reach point-source reduction goals, and in Iowa, these plans currently rely on voluntary methods for reduction of non-point source N and P (IDALS/IDNR/ISU 2013). P-based manure management plans can be used for both regulatory and non-regulatory purposes, and continue to be an important tool for helping livestock and poultry producers minimize nutrient impacts to surface waters while meeting crop needs (IDNR 2004; Sharpley and Wang 2014). As such, it is imperative that P indices accurately assess potential P losses to both surface and subsurface pathways.

Poultry manure contains high levels of nutrients and organic matter, essential for crop growth, and can contain up to 21,850 mg/kg P (43.7 lb/ton P) (Mallarino 2008). As with other manure sources, there is an imbalance between N and P concentrations in manure, such that application of manure at N-based rates leads to the build-up of soil test P (STP) over time (Sims 1998). In some areas of the Upper Mississippi River Basin, STP levels have increased dramatically in recent decades (Potash and Phosphate Institute 2001). Several studies have documented increased concentrations of P in runoff from pastures and fields with high STP levels (Pierson et al. 2001; Klatt et al. 2003; Allen et al. 2006), and studies of drainage tile P losses, indicate that P losses to tiles increase linearly with STP above a saturation point (Heckrath et al. 1995; Klatt et al. 2003). Soil composition and texture are important factors for determining P sorption capacity, especially the presence of fine clay particles, aluminum and

iron-oxides, and calcium, and can be further influenced by redox and pH conditions (Iyamuremye and Dick 1996; Nolin et al. 1999).

Use of conservation tillage practices reduce sediment erosion, increase soil biological activity, and often improve crop yields (Olson and Ebelhar 2009; Endale et al. 2010; Lafond et al. 2011). However, reduced tillage combined with regular manure application leads to stratification of P in the soil profile (Duiker and Beegle 2006; Abdi et al. 2014), and increased numbers of macropores (Endale et al. 2010), which can increase the risks of subsurface transport of P (Simard et al. 2000; Kleinman et al. 2009; Cade-Menun et al. 2013). Several studies of tile-waters below crop-fields, pastures, and grasslands, amended with liquid manures have documented significant loading of P in tile-waters, especially where macropores are present (Thomas et al. 1997; Gaechter et al. 1998; Stamm et al. 1998; Geohring et al. 2001; Chi Kim et al. 2010; Hernandez-Ramirez et al. 2011; Madison et al. 2014). However, fewer studies have assessed P losses to drainage waters from solid poultry manure application (Endale et al. 2010; Ulen et al. 2014).

Here, we compare the effects of two rates of annual poultry manure application on chisel-plowed and no-till cornfields, and relate STP values to orthophosphate concentrations in drainage-tile-waters. Analyses of water quality changes throughout the tile hydrograph in response to precipitation events were used to assess the role of preferential flow in P transport. Results of this study can be used to improve P-based manure management strategies in drained agricultural areas of the Upper Midwest.

4.3 Methods

4.3.1 Site description

Field experiments were conducted from 2010 through 2012 at Iowa State University's Agronomy and Agricultural Engineering Research Farm near Ames, Iowa. The site is located in the Des Moines Lobe landform region, a landscape formed by the last glacial maximum that occurred in the Upper Midwest during the late Pleistocene Epoch, between 18,000 – 15,000 years ago. The research plots are located on soils with a Canisteo-Clarion-Nicollet association, which are loamy soils formed in calcareous glacial till under prairie vegetation, characterized as moderately permeable, with drainage classifications ranging from well-drained to poorly drained. Soil texture typically ranges from 30-45% sand, 35-42% silt, and 20-30% clay content (Natural Resources and Conservation Service 2013). Plot slopes range from 0 – 5 percent. Tile drains are installed along the midline of each plot at a depth of approximately 1.2 meters to enhance drainage.

A long-term study of the effects of PM on water quality began in 1998, with nine chisel-plowed (CP) plots under a corn-soy rotation. For 12 years, PM was applied in the spring, to the portion of each plot that was to be planted in corn, as described by Nguyen et al. (2013). Starting in 2010, all CP plots were converted to a continuous corn rotation, and 3 established NT plots were included as part of the PM study. PM and urea ammonium nitrate (UAN) were applied each spring, prior to planting, at application rates based on nitrogen (N) goals as described in Table 4.1. The PM2 goal achieves the maximum recommended rate of N application for continuous corn production in Iowa (Sawyer et al. 2006).

Table 4.1 Plot treatments with nitrogen fertilization goals.

Treatment	Treatment Description	Nitrogen Fertilization Goal (kg/hectare/year)
PM0 – NONE	control, no manure or fertilizer	0
PM0 – UAN	control, urea ammonium nitrate fertilizer	224
PM1	PM applied at a low rate	112
PM2	PM applied at double the low rate	224

Figure 4.1 shows the layout of the study plots superimposed over a map of soil types. Plot areas range from 0.08 to 0.51 hectares (ha). Tiles are spaced 36.3 meters apart. The experimental treatments on CP plots were arranged in a randomized design with 3 plots receiving PM1 treatment, 3 plots receiving PM2 treatment, 3 three plots receiving no manure as controls (PM0). Two of the control plots were fertilized with urea ammonium nitrate (PM0 - UAN), and one received no fertilizer (PM0 – NONE). Individual NT plots were fertilized with PM1, PM2, and PM0 – UAN treatments. Manured NT plots are split in half, with separate tiles draining the upper and lower portions of these plots. The control NT plot has a single drainage tile. No phosphorus was applied to either type of PM0 plot; thus, we will not distinguish between PM0 treatments for the remainder of this report.

4.3.2 Precipitation and tile flow measurements

Rainfall data were collected using two tipping-bucket rain gauges with HOBO data-loggers (Onset Computer Corp., Pocasset, Mass.) located at the site. Volume-triggered float pumps were submerged in each sump, and connected to in-line flow meters (Neptune Technology Group, Tallassee, AL), with Hobo Pendant Event Data Loggers (Onset Computer Corp., Pocasset, Mass.), to provide continuous volume-based tile flow data. To supplement continuous readings, instantaneous tile flow rates were obtained directly from each tile, at the time of sampling, by measuring the time to fill a 1-liter (L) bottle. During occasional pump

failures, missing flow data were estimated using data from adjacent plots, which were calibrated using instantaneous flow measurements.

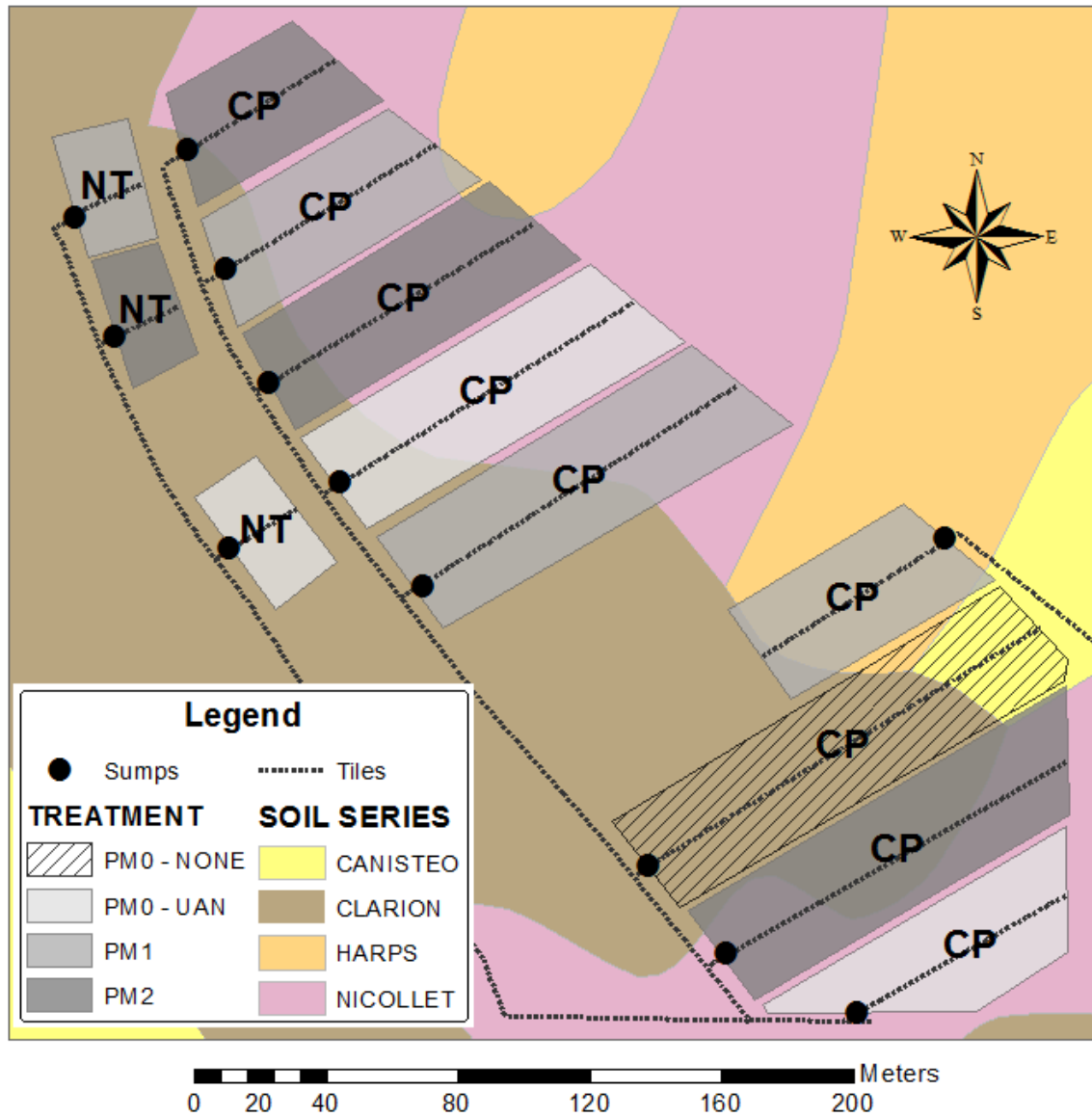*Figure 4.1 Map of study site with locations of sumps, tiles, and chisel-plowed (CP) and no-till (NT) plots, superimposed over Natural Resources Conservation Service soil types. Treatments include control plots with no fertilizer (PM0), control plots with commercial fertilizer (PM0-UAN), plots with the low rate of poultry manure (PM1), and plots with the double rate of poultry manure applied (PM2).*

4.3.3 Manure sample collection and analysis

PM was transported directly from confinements housing layer-hens, and stockpiled at the study site each spring. Three representative manure samples were obtained from the stockpile prior to application in 2010, and 8 – 12 manure samples were obtained from the spreader during application in 2011 and 2012 for better characterization of the manure. PM samples were placed in plastic bags and stored on ice, then transported to Minnesota Valley Testing Laboratory in Nevada, Iowa, for moisture content and N, P, and potassium (K) analyses.

Manure was applied using a dry-spreader as described in Hanna and Richard (2008). PM was applied based on N application goals outlined in Table 1, assuming 60% N availability in the first year. Application occurred on May 24 and 25, 2010, June 1, 2011, and May 15, 2012, followed immediately by tillage in CP plots. Corn was planted within a few days after manure application.

4.3.4 Soil sample collection and laboratory analysis

Soil samples, 0-15 cm and 15-30 cm deep, were collected using hollow-core samplers (Oakfield Model L Tube Sampler with a 30 cm tube). Soil samples were collected on May 17, 2010, April 2, 2011, and April 10, 2012. For each plot, 10 samples were obtained along diagonal transects, composited, and stored in plastic bags. All sampling equipment was thoroughly cleaned between plots using a 90% ethanol solution. Samples were placed on ice, and transported to Iowa State University (ISU). Subsamples were sent to the Soil and Plant Analysis Laboratory for P, pH, OM, potassium (K), and nitrate (NO_3-N) analyses using standard methods recommended for the North Central Region of the United States (NCERA 2012). Specifically, soil test phosphorus (STP) was analyzed using Bray's 1-P method, and concentrations were reported in mg P per kg soil (ppm).

4.3.5 Drainage tile-water sampling and laboratory analysis

In 2010 and 2011, water samples were collected weekly from drainage tile outlets, and following precipitation events, during the 30 days prior to manure application, and up to 100 days after manure application (DAM), when flow was available. This grab sampling was conducted from May 14th to September 1st in 2010 and from April 19th to July 27th in 2011. Water samples were collected in 1-liter (L) sterile polypropylene bottles, placed on ice, and transported to ISU's Water Quality Research Laboratory. Samples were stored at 4°C until they were processed for PO_4-P on a Lachat auto-analyzer at ISU's Agriculture and Biosystems Engineering Water Quality Research Laboratory using a molybdate method with a detection limit of 0.005 mg P/L.

In 2011, auto-sampling devices (ISCO samplers) were installed in 6 of the tile sumps (one representing each combination of tillage and treatment). These devices sampled directly from tiles via clear rubber tubing placed directly into the tile. Tubes were flushed with tile-water, prior to collection of tile-water samples in 1 liter sterile propylene bottles. Samples were obtained at hourly intervals during and immediately following storm event on July 10, 2011, then at 3-hour intervals, as tile flow receded. Samples were transported to ISU's Water Quality Research Laboratory, and stored at 4°C prior to analyses. Unfiltered samples were analyzed for PO_4-P, chloride (Cl), and nitrate (NO_3-N), on an AQ2 Discrete Analyzer (Seal Analytical, Mequon, Wisconsin), following EPA method 365.1 (Rev. 2) (USEPA 1993).

4.3.6 Statistics

Student's *t*-tests were evaluated using JMP software (SAS Institute, Cary, NC) to compare means of STP levels and tile-water orthophosphate concentrations by sample depth, tillage, and treatment. For analysis of the combined effects of tillage and treatment on spring

STP levels, data from all three years were used. STP data from manure-amended plots (PM1 and PM2) were combined for analysis of the differences between STP levels in shallow (0-15 cm) and deep (15-30 cm) spring soil samples from CP and NT plots. Grab sample tile-water PO_4-P concentrations from 2010 and 2011 were evaluated to determine the effects of tillage and treatment. Statistical significance for all t-tests was assessed at the $\alpha = 0.05$ level.

Shallow and deep STP data from CP plots were fit with a linear model (JMP) to describe the change in STP concentrations over time from annual PM1 and PM2 application rates. R^2 values, root mean square errors (RMSE), and p values were reported to document the accuracy of each linear regression.

4.4 Results and Discussion

4.4.1 Poultry manure characteristics and application rates

Moisture content of PM at the time of delivery varied widely between years, with average total moisture in 2010 at 48%, 61% in 2011, and 30% in 2012. Nutrient contents of PM also varied, ranging from 1.3 to 3.7 % average total nitrogen, 2.6 – 3.7 % average phosphorus reported as P_2O_5, and 1.6 -2.7 % average potassium reported as K_2O. Results of PM analyses were used to calculate nitrogen and phosphorus application rates on a per-hectare basis, as displayed in Table 4.2. For CP plots, application rates fell short of nitrogen-based goals in 2010, and exceeded these goals in 2011 and 2012. For NT plots, PM1 plots fell just short of nitrogen-based goals in all three years, while PM2 plots exceeded nitrogen-based goals in 2011 and 2012.

Table 4.2 Annual nitrogen and phosphorus application rates for chisel-plowed and no-till plots by treatment type. Average rates are displayed for replicate chisel-plowed plots with standard deviations in parentheses, and individual rates are shown for individual no-till plots.

Year	Treatment	Chisel-Plowed Avg. Application Rate (kg/ha)		No-Till Application Rate (kg/ha)	
		N*	P	N*	P
2010	PM1	72 (20)	65.5 (17.9)	90.2	81.7
	PM2	187 (1)	170 (0.87)	184	167
2011	PM1	120 (4)	62.4 (2.18)	109	56.3
	PM2	249 (26)	130 (13.5)	380	197
2012	PM1	132 (6)	35.4 (1.31)	103	27.5
	PM2	231 (6)	61.6 (1.75)	298	70.7

*Application rate assuming 60% availability of N in the first year.

4.4.2 Spring soil test phosphorus concentrations

Results of spring (pre-manure) STP concentrations for CP and NT plots are displayed in Figure 4.2 by sampling depth and year. STP concentrations are higher in shallow (0 - 15 cm) manure-amended samples than in deep samples (15 – 30 cm). This is consistent with results of long-term soil sampling of the CP plots at this site under a corn-soy rotation (Hoover et al. in press), and with other studies of soil phosphorus in fields receiving regular poultry manure application (Endale et al. 2010). The NT PM2 plot was used for an experiment on the effects of stockpiling of poultry manure on soil and tile-water concentrations in 2002, so high STP levels measured in 2010 may have been an artifact of historical experimental practices.

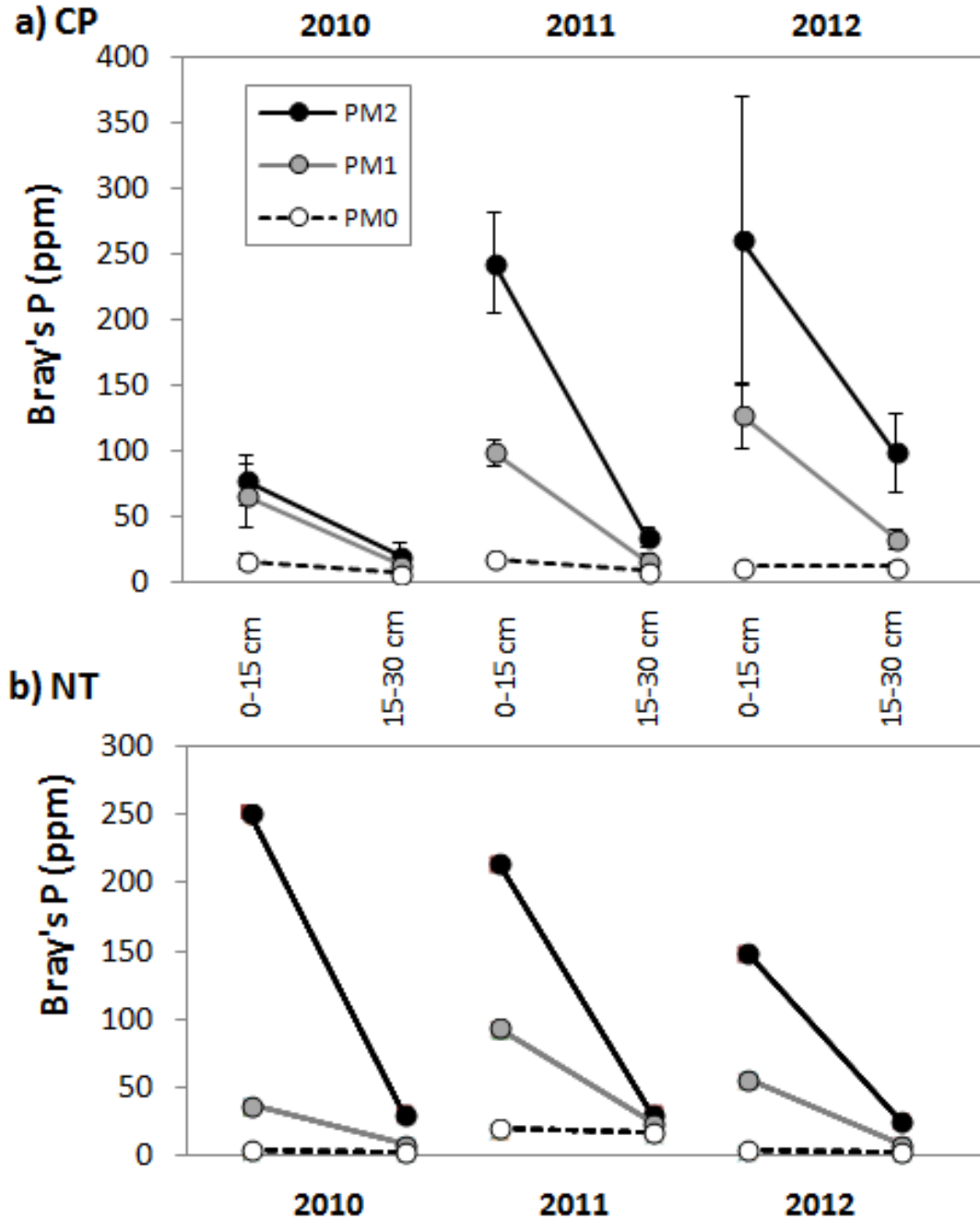*Figure 4.2 Shallow (0-15 cm) and deep (15-30 cm) soil test phosphorus (STP) results for samples collected each spring (2010-2012) from control plots (PM0), and plots with low (PM1) and high (PM2) manure application rates. A) Average STP concentrations are displayed for replicate chisel-plowed plots with error bars representing one standard deviation. B) Individual STP values for no-till samples are displayed.*

The effect of PM application is significant, as illustrated in Figure 4.3. Increased PM application was correlated to higher shallow soil STP results for CP plots, with statistical differences between all 3 levels of treatment (PM0, PM1, and PM2). For NT plots, PM2 treatments resulted in higher shallow STP results than PM1 and PM0 treatments, but no statistical difference between PM0 and PM1 treatments was observed. All STP values for manure-amended plots are considered "very high" with respect to corn needs as defined by Mallarino and Sawyer (2013) for the top 15 cm soil. Additional application of manure would not be recommended on these plots, until the shallow STP values dropped to optimum levels or below (<26 ppm – Bray's P).
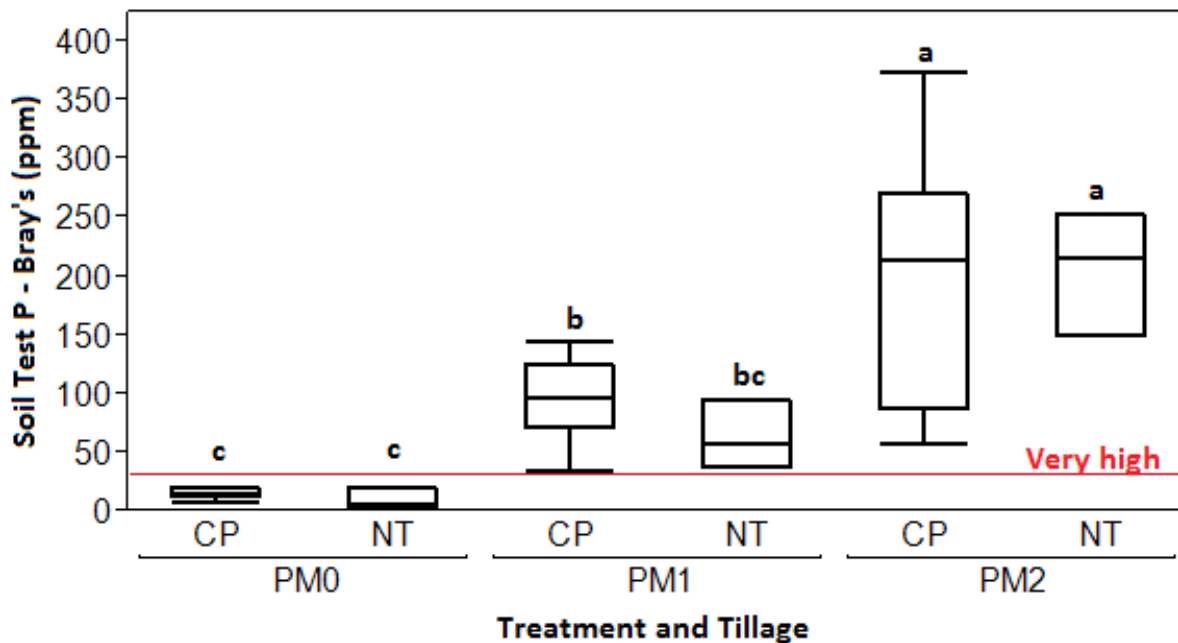

Figure 4.3 Distributions of shallow (0-15 cm) soil test phosphorus values by treatment and tillage. Lettering indicates statistical differences between distributions (p < 0.05). The red line represents 31 ppm (Bray's P). STP concentrations above 31 ppm (Bray's P) are defined as "very high" based on corn needs (Mallarino and Sawyer, 2013).

While the STP results observed in these plots are not surprising given the imbalance between N and P in poultry manure, these conditions represent frequent over-application of P, and are not representative of the majority of manured fields in this region. The average STP level in Iowa in 2001 and 2005 was 25 ppm (Bray's 1 P) as reported by Snyder (2006). Mean STP values in Iowa ranged from 8 – 131 ppm P (Bray's-1) by county based on analysis of 12,772 farm samples submitted to ISU between 2006-2010, with 40% of samples statewide falling into the "very high" STP category (>30 ppm) (Mallarino et al. 2011).

For deep soil samples from both CP and NT plots, mean STP levels increased with increased PM application, but statistically higher STP levels were only seen for CP PM2 samples (Figure 4.4). The wide range of values in deep CP PM2 samples may be the result of increased leaching of P from corresponding surface soils, followed by readsorption to deeper soils. However, further study would be necessary to evaluate the level of P saturation in these soils. Alternatively, the increase in STP at depth in the CP PM2 samples could be the simple result of mixing during tillage. When data from all three years are compared; however, no statistical differences were observed between STP concentrations in deep soil samples from manure-amended CP and NT plots ($p = 0.05$) (Figure 4.5). This implies that tillage was not the primary mechanism for elevated STP concentrations in deep CP soils.

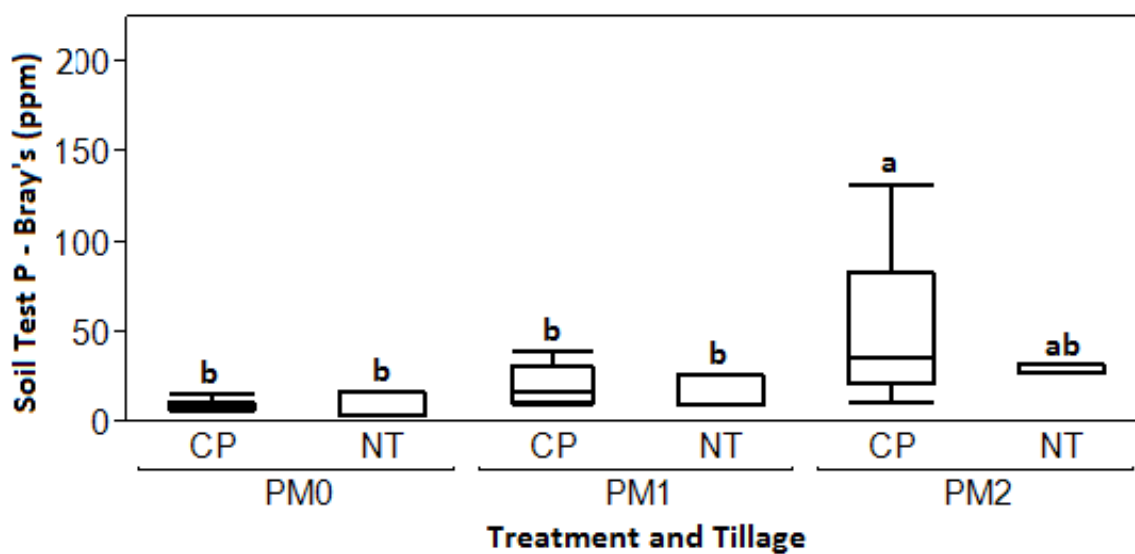*Figure 4.4 Distributions of deep (15-30 cm) soil test phosphorus values by treatment and tillage. Lettering indicates statistical differences between distributions ($p < 0.05$).*
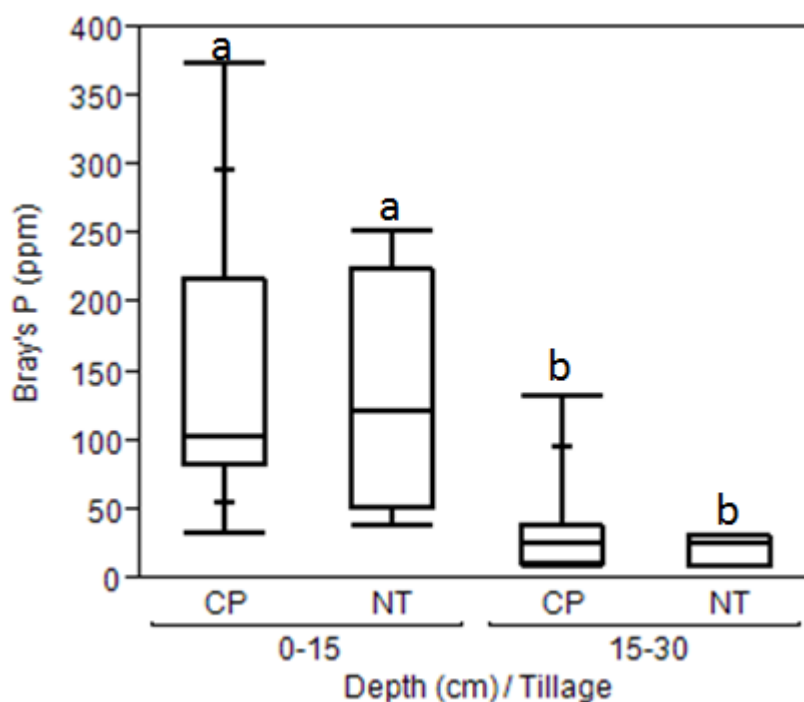

Figure 4.5 Boxplot distributions of spring soil test phosphorus for manure-amended plots by sample depth and tillage. Lettering indicates differences between distributions as determined from comparison of the means using the Student's t-test.

Linear analysis of STP levels over time, indicates that manure application resulted in increased STP levels from 2010 to 2012 in both shallow and deep samples from CP plots (Figure 4.6). For CP PM1 plots, shallow STP levels increased by 34 ppm per year ($r^2 = 0.71$, RMSE = 20, and $p = 0.0041$). Shallow STP levels in CP PM2 plots increased by 92 ppm per year ($r^2 = 0.56$, RMSE = 75, and $p = 0.0197$). STP levels in deep CP PM1 plots increased 10.5 ppm per year ($r^2 = 0.65$, RMSE = 7, and $p = 0.0084$). For CP PM2 plots, deep STP levels increased 40 ppm per year ($r^2 = 0.74$, RMSE = 22, and $p = 0.0030$). Increasing STP levels in manure-amended CP plots over these 3 years continued the long-term trend (1998-2006) of increasing STP concentrations (0 - 30 cm) (Hoover et al. in press) under a corn-soybean rotation. Increasing trends were also observed at several other sites in Iowa after long-term manure application (Allen and Mallarino 2006). Increasing trends were not observed for the NT plots over the three years. This may have been the result of extremely high initial STP concentrations, variability in test results due to soil pH, or plant uptake dynamics.

Bray's method for STP analyses has been shown to underestimate STP levels in calcareous soils with pH levels above 7.4 (Mallarino 1997). Eight out of nine shallow NT soil samples had pH levels at or above 7.4, but none exceeded 7.6. Two of the nine deep NT soil samples had pH levels at 7.4, and these were samples from the control plots (PM0) from 2010 and 2012. None of the shallow CP samples from 2010 or 2012 measured at or above a pH of 7.4. In 2011, one of the three shallow CP PM1 replicates, and two of the shallow CP PM2 replicates had pH values at or above 7.4, but none exceeded 7.65, and the corresponding STP values were either within or above the levels measured for the other replicates. Therefore, while STP concentrations theoretically may have been underestimated in 14% of soil samples, we can find no evidence to suggest that STP levels were systematically lowered in these samples.
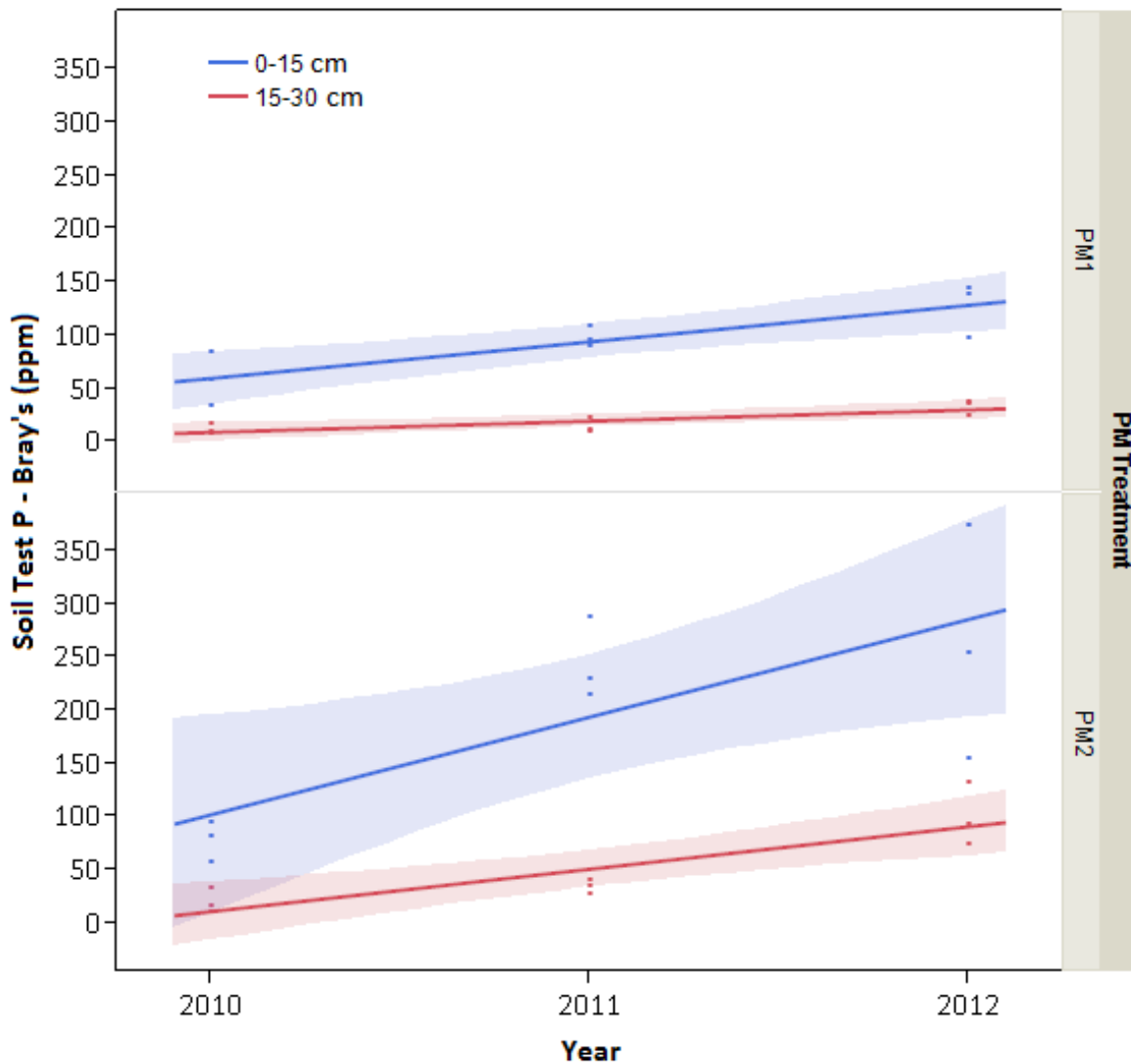
Figure 4.6 Best fit lines for shallow (0-15 cm) and deep (15-30 cm) spring STP values in chisel-plowed plots from 2010 to 2012 under low (PM1) and high (PM2) rates of manure application. Shaded regions represent 95% confidence intervals.

4.4.3 Effects of tillage and treatment on orthophosphate in drainage tile-waters

Analyses of 2010 and 2011 grab-sample tile-water reveal that NT PM2 plots had significantly higher PO_4 concentrations than all other combinations of tillage and treatment (Figure 4.7). Table 4.3 displays summary statistics for PO_4-P concentrations in grab samples

collected from tiles in 2010 and 2011. Median and mean PO_4-P values for NT PM2 (0.122 and

0.163 mg/L PO_4-P, respectively) are above the US EPA's suggested criteria for total phosphorus

(TP) of 0.118 mg/L P for rivers and streams in sub-ecoregion 47, the "Western Corn Belt Plains"

(US EPA, 2010). Median and mean values for all other combinations of tillage and treatment

were at or below 0.04 mg/L PO_4-P. The mean concentration of orthophosphate in NT PM2 tile

waters during this study period is higher than concentrations of orthophosphate in nearby surface

waters that drain predominantly agricultural areas. For example, the Skunk River north of Ames

(upstream of the wastewater treatment outfall) averaged 0.112, 0.085, and 0.058 mg/L PO_4-P in

2010, 2011, and 2012, respectively (Iowa Department of Natural Resources 2015). This

suggests that tile drainage could contribute significantly to surface water quality in this region,

although a much broader watershed study would be necessary to quantify this impact.

In addition to exceeding recommended nutrient standard concentrations for

orthophosphorus in surface waters, it is useful to look at these data with respect to potential load

contributions, which is the basis for most P-index calculations. Orthophosphate loading from

tiles can be estimated using median tile-water concentrations and tile discharge records. For

example, discharge from the lower NT PM2 tile during the 2010 growing season (March-

September), a relatively wet year, and the 2011 growing season, a relatively dry year, totaled

approximately 85,400 and 19,200 liters, respectively. At the median concentration of 0.122

mg/L, total P loading for the growing season is 260 g/ha (0.23 lb/acre) in 2010, and 59 g/ha (0.05

lb/acre) in 2011. These values represent the potential range of subsurface drainage contributions

from NT fields that receive repeated application of manure at N-based rates.

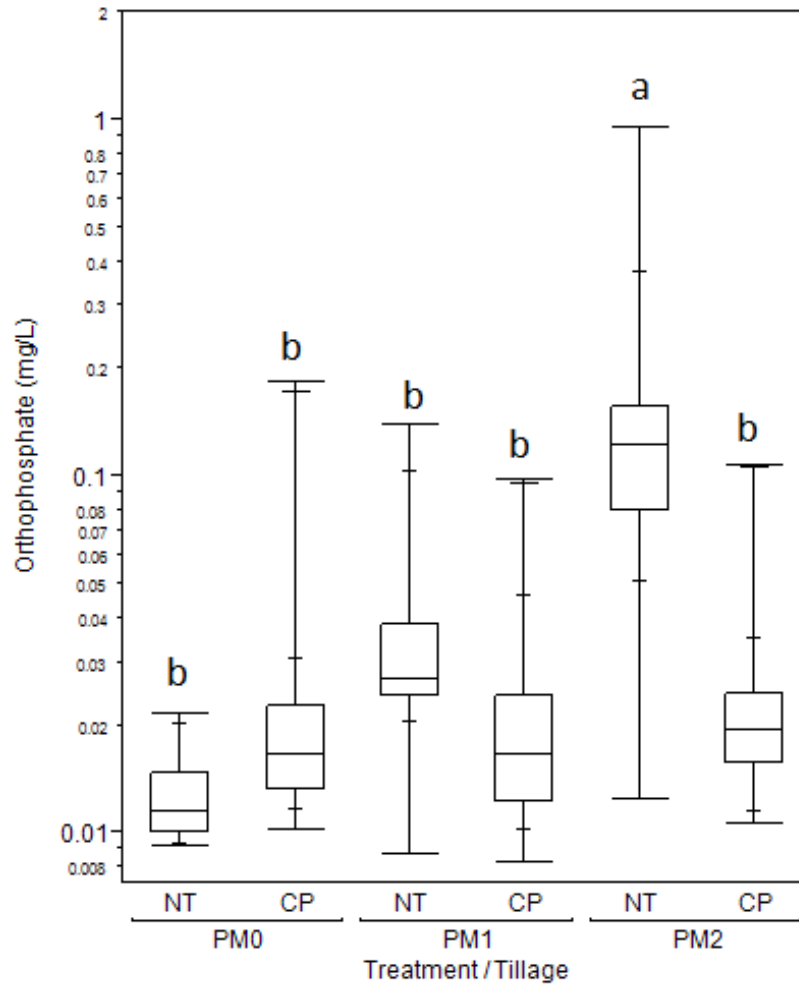*Figure 4.7 Boxplot distributions of tile-water orthophosphate (PO_4-P) concentrations under combinations of no-till (NT) and chisel-plowed (CP) plots with no manure (PM0), low manure rate (PM1), and double manure rate (PM2) treatments. Data from grab samples obtained in 2010 and 2011. Letters indicate significant differences ($p<0.05$ as determined by the Student's t-test.*

Table 4.3 Summary statistics for grab sample tile-water orthophosphate concentration under combinations of no-till (NT) and chisel-plowed (CP) plots with no manure (PM0), the low manure rate (PM1), and the high manure rate (PM2) treatments.

Tillage Treatment	NT PM0	CP PM0	NT PM1	CP PM1	NT PM2	CP PM2
N	13	50	35	41	27	48
	Orthophosphate as P (mg/L)					
Min	0.009	0.010	0.009	0.008	0.012	0.011
Med	0.011	0.017	0.027	0.017	0.122	0.019
Mean	0.013	0.025	0.040	0.022	0.163	0.024
Max	0.021	0.184	0.122	0.097	0.950	0.107

4.4.4 Relationship between spring soil test P and tile-water concentrations

Despite the observed similarity of STP concentrations under CP PM2 and NT PM2 plots, tile-water PO_4 concentrations were significantly higher in NT plots. To further our understanding of the relationship between STP and tile-water concentrations, linear models were evaluated relating STP values to median annual tile-water concentrations (Figure 4.8). Results indicate little correlation between tile-water PO_4 concentrations and STP values from CP plots. In contrast, tile-water PO_4 concentrations increased significantly with STP concentrations from NT plots above a STP value of 100 ppm (Bray's P). For NT plots tile-water PO_4-P was estimated to increase in 0.007 mg/L for every 10 ppm increase in STP (Bray's-1 P). Root mean squared values for both best-fit lines from 2010 and 2011 data are 0.01. Given that there were no samples with STP concentrations between 100-200 ppm (Bray's P), it was difficult to assess the saturation point (break in slope). If we assume a break in slope between 100 ppm STP and 120 ppm STP as observed by Klatt et al. (2003) and Heckrath et al. (1995), respectively, the increase in PO_4 per unit STP would be increased.
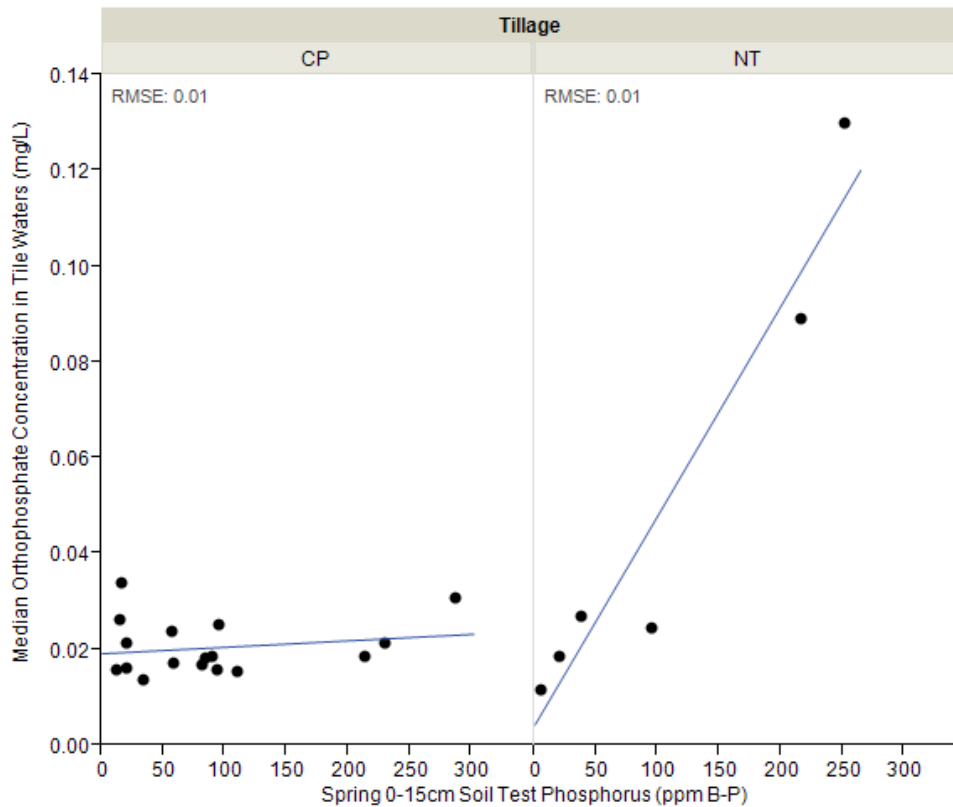
Figure 4.8 Linear relationships between concentration of phosphorus (Bray's P) in 0 - 15 cm spring (pre-manure) soil samples and median tile-water orthophosphate concentrations from 2010 and 2011 by tillage practice (CP = chisel-plowed and NT = no-till).

Application of organic materials, such as poultry manure, has been shown to decrease P sorption (Singh and Jones 1976; Reddy et al. 1980). Variations in soil mineral and organic matter content, pH, and redox conditions are also known to influence phosphorus sorption (Hemwall 1957; Iyamuremye and Dick 1996; Devau et al. 2009). Repeated application of manure and a lack of tillage can cause significant increases in pH levels (Sharpley et al. 1984; Abdi et al. 2014), and reduce oxygen levels (Iyamuremye and Dick 1996). These conditions may be less favorable to P sorption due to reduction in adsorption sites and potential for mineral precipitation. Mixing of topsoil, via tillage, increases the available oxygen in soils. Because soil types and manure treatments were the same for CP and NT plots, chemical conditions within the

undisturbed NT soils, such as pH and redox conditions, may explain differences between

phosphorus cycling in these plots. pH values in spring NT soil samples were consistently

measured above 7, and were significantly higher than in CP soils (Figure 4.9). Dissolved oxygen

or oxidation-reduction potential (Eh) were not measured as part of this study.
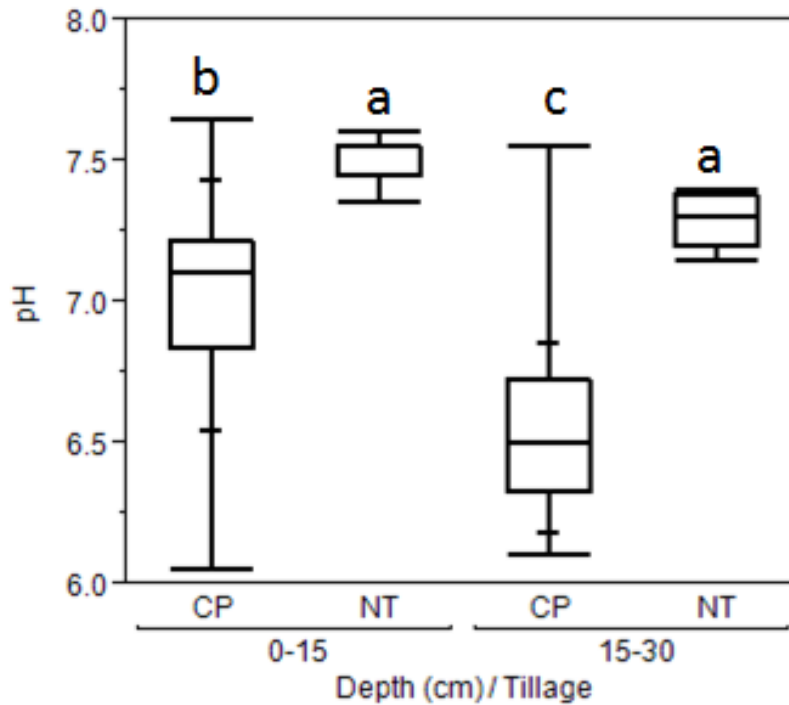
Figure 4.9 Boxplot distributions of pH values for spring soil samples from 2010 - 2012. Lettering indicates significant differences at (p < 0.05) from Student's t-test analyses.

Soil saturation and temperature variations can also influence redox conditions

(Iyamuremye and Dick 1996). Our data represent typical ranges in ambient air temperature

during growing seasons in the Upper Midwest. While these upland soils are not frequently

flooded, standing water was observed at this site three times in 2010 after heavy precipitation.

While subsurface redox conditions were not directly assessed, it is likely that our data are

representative of the characteristic ranges of redox conditions in these upland soils.

Preferential flow through macropores has also been proposed as an important pathway for

phosphorus transport to drainage tiles (Geohring et al. 2001; Madison et al. 2014). Phosphorus

concentrated at the soil surface (Abdi et al. 2014), could become entrained in rainwater and transported directly to tile lines via macropores. Comparisons between CP and NT plots reveal significantly greater macropore densities above tiles lines in NT plots used for this study (Chapter 2) and at other locations (Endale et al. 2010; Hoang et al. 2013). Macropore transport was determined to account for less than 2% of total drain flow, and between 9-20% of nitrate on a mass basis, for a conventionally tilled plot, adjacent to the test plots used for this experiment (Everts and Kanwar 1990).

4.4.5 Event data

 To further our understanding of PO_4 transport to tile lines, we evaluated data collected in response to precipitation events in 2011 and 2012. Flow rates during the first 40 DAM in 2011 were higher than in 2012. Also, tile flow rates were considerably lower for NT plots than CP plots as a result of the different plot lengths. Despite the lower flow rates, most PO_4 concentrations in tile-waters under manure-amended NT plots were generally an order of magnitude higher than under CP plots (Figure 4.10). PO_4 concentrations varied over an order of magnitude in response to significant precipitation events (> 2 cm total rainfall), with peak concentrations generally occurring immediately after rainfall began, and then falling to baseline levels within 24 hours. It should be noted that for some events in 2011 samples collected immediately (1 hour) after precipitation from CP PM2 plots reached the same concentration as NT PM2 samples. This suggests that macropore transport may play a role in high concentrations of PO_4 immediately following storm events, however, macropore transport does not appear to explain the differences between orthophosphate concentrations between CP and NT plots during the remainder of the hydrograph. Low flow in 2011 and 2012 limited data collection; however, in the future, hydrograph separation could be used to determine the proportion of orthophosphate

in tile-water discharges that comes from macropore and matrix flow, as described by Everts and
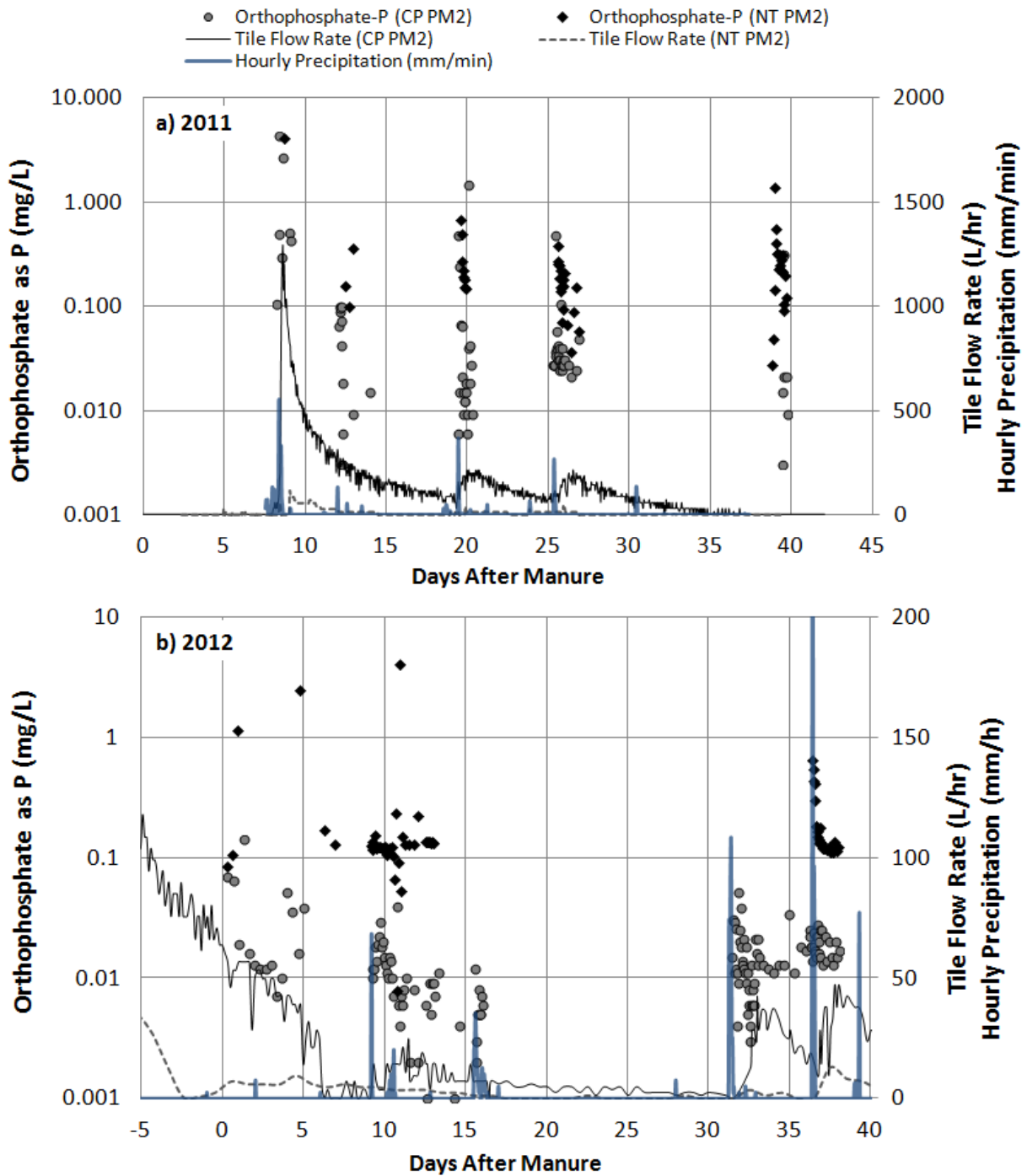
Kanwar (1990).

Figure 4.10 Orthophosphate data collected from CP PM2 and NT PM2 plots following precipitation events in a) 2011 and b) 2012. Tile flow rates and hourly precipitation data are presented on the secondary axes.

4.4.6 Iowa P-Index

The Iowa P-Index is composed of three components, erosion, runoff, and tile/subsurface P losses. In general, P losses in tile drainage are thought to be small. In the worst-case scenario, with high STP values and maximum transport factors available, the maximum annual "tile/subsurface recharge" factor in the Iowa P-Index is 0.15. Although this subsurface factor does not necessarily equate to lb/acre/year, it accounts for only 3% of the P-Index value of 5, at which producers would be required to apply based on P needs rather than N requirements. A review of a small set of manure management plans in Iowa DNR records, indicates that tile-drained fields with exceptionally high STP values, averaging over 200 ppm (Bray's-1 P), can achieve a P-Index value of 2 by employing soil conservation measures, such as terracing and buffer strips. Repeated application at N-based rates could continue on these fields, unrestricted, despite the potential for additional increases in STP, and the potential for subsurface P losses. In one P-Index based manure management plan that was reviewed, soil pH levels were highly variable throughout the tiled field, with only a small portion of the field (30 acres) having pH levels above 7. Depending on the tillage practices and soil composition, P saturation may not have been exceeded; however, if manure application at the N-based rate continues, P saturation is likely to occur, at which point, the potential for transport of PO_4 via tile drainage waters will increase. If P-based indices continue to be the primary method for reducing phosphorus contributions from manure application areas, they should be modified to address the potential for PO_4 losses to drainage tiles once the capacity of soils to hold phosphorus has been exceeded.

4.5 Conclusions

Annual application of poultry manure at rates calculated to meet maximum agronomic nitrogen needs for corn, results in signficant increases in soil test phosphorus (STP) levels in

both CP and NT fields. In NT plots, with alkaline pH, drainage tile-waters from plots with high

(>200) STP levels contain signficantly higher concentrations of orthophosphate than CP plots

with similar STP concentrations. These observations support the hypothesis that P sorbtion

capacity of NT soils is decreased relative to CP soils. Additional analysis of variations in

drainage tile-water concentrations reveals that peak concentrations associated with macropore

transport are short-lived and do not contribute significantly to P loading, while elevated PO_4

concentrations in matrix waters can result in P loading during the growing season that exceed

reference levels for streams in this region.

Review of the current Iowa P-Index, shows that P losses to drainage tiles may not fully

represent situations where soils are likely to be saturated with respect to P. Future research

should determine whether a significant portion of high STP fields under manure management

plans currently have P-index values that allow application at N-based rates, especially those

fields that are likely to be tile drained and use NT practices. Modification of P-indices, based on

the potential for P saturation, may be necessary to achieve phosphorus reduction goals.

Modifications could include estimations of P saturation based on information already collected,

including STP levels, soil types, and pH values. In addition, practices that increase the soil's

capactity to hold phosphorus, such as pH control, occasional tillage, biochar application, or other

techniques may reduce losses to tile drains. Alternatively, practices that reduce excess P in soils

could be incentivized, such as cover crops, controlled drainage, and wetlands.

4.6 Acknowledgements

material are those of the authors and do not necessarily reflect the views of the IEC. Carl

Pederson provided valuable assistance with planning, pump maintenance, manure application,

and crop management. Numerous graduate and undergraduate students at Iowa State University,

and volunteers assisted with sample collection and analyses, including Ross Tuttle, Kendal Agee,

Amanda Homan, Jason Garder, Nick Terhall, and Conrad Brendel.

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CHAPTER 5. CONCLUSIONS

5.1 Summary of Research Outcomes

Several important conclusions are supported by the three studies presented here:

- Poultry manure application can contribute bacteria to tile-drains, and ultimately surface waters, especially in years with above-average precipitation.

- Even in dry years, when little transport occurs, bacteria can persist in soils for months and even up to a full year after application.

- Higher densities of macropores were quantified in no-till plots compared to chisel-plowed plots, and these are likely to be responsible for the significantly higher concentrations of bacteria observed in tiles draining no-till plots.

- Enterococci are better indicators of the pathogen, *Salmonella*, than *E. coli* in a loamy calcareous tile-drained field in the Upper Midwest. However, *Salmonella* was present even in samples that contain fecal indicator bacteria at or below current recommended recreational water quality standards.

- Despite repeated annual applications of poultry manure at nitrogen-based rates, only the combination of high soil test phosphorus and no-till practices significantly increased the potential for orthophosphate losses to tiles.

- For both bacteria and phosphorus studies, the largest differences were between control plots with no manure and plots with the high rate of poultry manure application. Often, differences between control plots and plots receiving poultry manure at the low rate of application based nitrogen needs of corn were insignificant.

5.2 Implications for Manure and Watershed Management

Producers and watershed managers can take away several important lessons from this research. This study confirms what is already widely accepted, that using lower application rates for poultry manure, and using fertilizers with lower levels of P to balance crop needs, would reduce contaminant losses, benefitting both producers and the environment. An increase in tillage, on the other hand, increases the risk of runoff and soil compaction, and may have other drawbacks; therefore, more modest changes to tillage, such as occasional disking, may be recommended to reduce bacterial water quality impacts by disconnecting macropores and helping to distribute phosphorus within the soil column. Control of pH, and other techniques for increasing the ability of soils to retain phosphorus, may prevent losses to tiles as well as surface-runoff. Although additional research is necessary, the results also suggest that adaptation of the Iowa P-Index to account for the potential for greater losses of P via the subsurface should be considered. Furthermore, transport of bacteria and phosphorus via tile drainage should be considered when designing watershed models and water quality improvement plans.

5.3 Future Research

During the course of these investigations, many questions arose that deserve further investigation, including the following:

- Is re-growth of bacteria occurring in soils? If so, how is re-growth affected by soil moisture and other variables? Are certain strains of bacteria more likely to re-grow than others? Are the strains of bacteria found in poultry manure, the same as those found in soils days, months, and years after application?
- Are bacteria transitioning between culturable and viable-but-not-culturable states in the field? Would more frequent sampling and comparisons between bacteria quantified

by culturation methods and qPCR, be an effective method for evaluating the
parameters that control these transitions?

• What is the fate of *Salmonella* released to surface waters via tile outlets? Are these
pathogens found in recreational waters downstream of manure application fields, and
are concentrations of *Salmonella* sufficient to cause infections.

• How common are no-till fields that contain soils saturated with respect to phosphorus?
Does tillage, pH adjustment, or other techniques improve the soil's capacity to hold
phosphorus? What is the relative contribution of tile-waters to surface water
orthophosphate loads? What modifications could be made to the Iowa Phosphorus
Index to better reflect the increased risk of orthophosphate losses to tile-waters when
the capacity of soils to hold phosphorus is exceeded?

5.4 New Solutions

Significant research has been devoted to determining practical and effective methods for
reducing bacteria in poultry manure. Stockpiling poultry manure, and avoiding addition of fresh
manure prior to field application, will help reduce risks of pathogen transport to water resources;
however, there is variation in the length of time and temperatures needed to achieve sufficient
reduction in bacteria concentrations. Guan and Holley (2003) recommend holding manure at 25
°C for 90 days to reduce bacteria to acceptable levels, far more conservative than the 7 days at
10 °C recommended by Brooks et al. (2009) for the reduction of *Salmonella* and *Campylobacter*
in poultry litter, or the > 8 days recommended by Hartel et al. (2000) for broiler litter. Graham et
al. (2009), however, warn that antibiotic resistant bacteria, and resistance genes, can survive 120
days of stockpiling. Alternatively, several authors show that composting can significantly reduce

Salmonella and other pathogens (Macklin et al. 2008; Erickson et al. 2014b). Anaerobic digestion also shows potential for reduction of pathogens in animal wastes, with the added benefit of energy generation (Popova et al. 2009; Goberna et al. 2011).

In addition to changes in storage practices, numerous other promising solutions have been proposed. Recently, a vaccine for *Salmonella* in chickens has been proposed (Nandre and Lee 2014), which would help to eliminate the pathogen at the source. Numerous in-field practices have also been proposed for minimizing the risks of pathogen and phosphorus transport to water, including application of biochar (Abit et al. 2014; Zhao et al. 2014) and introduction of cover-crops (Adeli et al. 2011; Rothrock et al. 2012). Edge-of-field practices, including grass and prairie filter strips have been shown to decrease risks from runoff (Coyne et al. 1998; Sharpley et al. 2004; Guber et al. 2009), and may have benefits for subsurface drainage as well. Finally, watershed scale efforts, such as strategically placed wetlands, could help reduce impacts of upstream manure application (Hill and Sobsey 2001; Ibekwe et al. 2002).

5.5 Farming for the Future

There is a growing awareness of issues of sustainability in agriculture in Iowa and around the world (Davis et al. 2012; Sutherland et al. 2015), and growing interest in alternative methods of farming that do not require close confinement of animals or large-scale monocropping (Rissing 2013). However, social, economic, and political forces cause widespread adoption of alternative methods to be unlikely in the short-term (Gelfand et al. 2013; Sharpley and Wang 2014). Hopefully, careful manure management and tillage, combined with promising new technologies, will help mitigate the impacts of manure application and protect our valuable water resources, while allowing farming to be a sustainable source of income for generations to come.

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