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Impact of liquid swine manure on nutrient loss and bacteria transport to subsurface drainage water under corn-soybean rotations

by

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Nonpoint source pollution continuously draws the attention of both scientist and the public. Nutrient and microbial pathogens from the application of liquid swine manure (LSM) have been implicated as the source of contamination and pollution to water and soil. The overall goal of this dissertation was to investigate the effects of LSM on nutrient loss and pathogenic bacteria transport to subsurface drainage water quality under corn and soybean crop rotation. A field experiment at the plot-scale was conducted to achieve this goal. Data collected from the 6-year experiment (2001-2006) at Nashua research site was used to study the effects of LSM applied to both corn and soybeans in comparison to manure applied to the corn phase only in term of NO_3-N and PO_4-P losses to subsurface drainage and its residual in the soil. Data used for investigation of directly connected macropores in transporting bacteria from soil surface to subsurface drainage were conducted in four different experiments in 2008 and 2009. Overall the results showed that LSM applied to soybean crop every year increased the NO_3-N in subsurface drainage water and accumulated a high concentration of phosphorus in soil profile, especially in the top 30 cm of soil depth. So, the changes of this LSM application should be modified in terms of application rate and/or adding a land cover crop to minimize the contamination of these nutrients. Also, transport of bacteria like *E. coli* from LSM through macropores, especially for the ones that are directly connected from soil surface to subsurface tile drainage are clearly shown. This is specially shown in the first three hours if rainfall occurred after manure application. For these reasons, it is suggested that weather conditions should be seriously taken into consideration in the timing of the LSM application in order to minimize the transportation not only of bacteria, but also nutrients to tile drainage.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Introduction

Liquid swine manure (LSM), produced by many confined livestock facilities in the US, has been applied to crop fields as an excellent source of nutrients for crop production. LSM provides essential nutrients such as nitrogen (N) in inorganic form (NO_3^- or NH_4^+), phosphorus (P) in a form of orthophosphate ion (PO_4^-) (Zhang et al., 1998) and potassium (K) (Gilley and Risse, 2000; Chen and Samson, 2002). LSM is usually applied to a corn crop but sometimes is also applied to a soybean crop as a way of manure disposal and not for the purpose of increasing soybean yield (Schmidt et al., 2000). LSM can be applied to crop land by different ways such as surface spreading, spray irrigation, and soil injection. Manure injection into the soil is highly recommended to minimize nutrient losses and reduce odor. Even with proper control of the LSM application rate, for example at the recommended application rates of 112 to 168 kg- N $ha^{-1}$to corn in Iowa (Blackmer et al., 1997), the LSM can possibly result in water quality problems because of higher concentrations of nitrate-nitrogen (NO_3-N), dissolved P, and bacteria in water. NO_3-N is soluble in water and can easily move to subsurface drainage water and groundwater, whereas P is mainly carried by soil and ends up in surface water through runoff. Many factors affect N and P losses from agricultural land including precipitation, tile drainage volume, soil characteristics, application timing and method, land use management.

Nitrogen used in agricultural watersheds is a major source of nonpoint-source pollution of surface waters via subsurface drainage transportation. The case of the world's third largest hypoxia development (where oxygen concentration in water is smaller than 2 mg L^{-1}) in the Gulf of Mexico was the evidence of N overloading received from the Mississippi river (Alexander et al., 1995; Baker et al., 2005; Kanwar et al., 1997; Mitsch et al., 2001). The amount of nitrate nitrogen in the Mississippi River has more than doubled since 1965 (Turner and Rabalais, 1991; Justic et al., 1995). A similar problem was also reported in other locations like the Baltic Sea (Larson et al., 1985) and the Black Sea (Tomazin, 1985).

Similar to N, a high level of P in fresh water also causes eutrophication conditions where algae and plants will grow excessively. Consequently, oxygen depletion happens and the level of available oxygen is not enough to provide the oxygen needs of aquatic life (Baker and Tidman, 2001). The impact of P on eutrophication can be found in more detail in the overall review conducted by Daniel and Correll (Daniel et al., 1998; Correll, 1998). Subsurface drainage normally has a lower concentration of sediment and phosphorus, bacteria and some pesticides in comparison with respective concentrations in the surface runoff. Because of the physical property of low mobility, P transport thorough subsurface drainage is considered very small or negligible. However, several researchers have stated that under some conditions P can predominantly transport through subsurface drainage in sandy loam soils in humid regions (Gilliam et al., 1999) or in areas with high P concentration and low soil sorption capacity in high organic matter soils (Sims et al., 1998). Several studies have reported P concentrations higher than the USEPA’s standard in water bodies. (Algoazany et al., 2007; Gaynor and Findlay, 1995; Hooda et al., 1999; Miller, 1979).

The N and P concentrations in subsurface drainage water are closely related to the amount of available N and P in the soil. Weed and Kanwar (1996) noticed that NO_3-N concentrations in tile drainage water were the highest at the time when NO_3-N amount was also highest in the soil. House et al. (1998) reported that the major source of P transported to surface water was from soil. Higher application rates of LSM to corn under corn and soybean rotation resulted in increased concentrations of both NO_3-N and PO_4-P in subsurface drainage water (Algoazany et al., 2007; Bakhsh et al., 2005; Bakhsh et al., 2009; Hoang et al., 2010).

Few studies have reported on the long term effect of LSM applied to soybean crops. Schmidt et al. (2000) in their study on soybean yield's response to LSM conducted for two years in Minnesota found that the amount of N in 0-120 cm soil depth ranged from 80 to 150 kg-N ha^{-1} if N application rates from either manure or fertilizer were more than N crop requirements. They concluded that the acceptable rates of LSM applied to soybeans were smaller or equal to the rate of crop accumulation to avoid agronomical and environmental problems. Bakhsh et al. (2009) reported that fall LSM applied to both corn and soybeans resulted in a 50% increase of NO_3-N concentrations and leaching losses with tile drainage water in comparison with LSM applied to corn crops only. Significant NO_3-N leaching loss was found in the fine sandy loam soil when manure was applied in the fall in comparison to spring application (Jayasundara et al., 2010).

Sims (2000b) stated that it was possible of soil with optimum or lower soil test causing water pollution due to the high amount of soil loss from erosion. High correlation ($r^2 = 0.96$) between P content in soil and soil loss by runoff was already reported by Vaithiyanathan and Correll (1992). The highest P adsorption happens in conditions of acidic soils or soils with high amounts of clay and/or Fe/Al oxides. Also, amounts of $CaCO_3$ and Fe oxides presented in soil with high pH also elevate P adsorption (Pierzynski et al., 2000). A few centimeters of top soil were found to be the most critical source of P concentration in runoff (Ahuja. L.R, 1986). Soil P at the topsoil might be above the optimum level in many crop lands as 52% of soil samples collected from an agricultural watershed in Pennsylvania had concentrations of soil P above the levels needed for optimum crop yield (Sharpley et al., 1999).

Water movement through preferential flow and/ or macropores in soil has been mentioned in soil physics for many years. In general, macropores are formed in many ways and can be categorized in two major groups: natural ways and biological ways. Natural ways of forming macropores include soil shrinkage by drying (Brewer, 1964; Blake et al., 1973), cycles of freezing and thawing (Beven and Germann, 1982), chemical weathering, or subsurface erosion channels (McMahon and Christy 2000). Biological ways of forming macropores consist of plant roots, tunneling insects, moving nematodes (McMahon and Christy, 2000; Priebe and Balckmer, 1989).

Number of macropores and the degree macroporosity vary with soil depth. Bouma et al. (1979) reported that the volume of macropores is around 1% of the total soil volume. Whereas, Douglas (1986) estimated the maximum volume of macropores in the soil was 5%. Munyankusi et al. (1994) found that the highest percentage of macroporosity (*e.g.* 2.5%) was observed at a depth of 2 cm in Seaton silt loam in Goohue County in Minnesota. The smoke technique was used in the experiment conducted by Shipitalo and Gibbs (2000) to determine the effect of burrows created by earthworms in transporting waste in tile line. However, to date not much research has been done on using the smoke test technique to quantify macroporosity of soils.

Continued application of LSM may contribute to the nonpoint source pollution by releasing microbial pathogens including bacteria, virus and protozoa, through runoff and subsurface drainage water to surface and ground water (Baxter-Porter and Gilliland, 1988; Thiagaranjan et al., 2007). *Escherichia coli* (*E. coli*), including a harmful pathogen O157:H7, have been detected in the range of 10^7 to 10^9 organisms per gram in feces (Gyles, 2004). *E. coli* can survive in the land for several weeks or up to 120 days in sandy loam soil (Hutchison et al., 2004; Nwachuku and Gerba, 2008; Sørensen et al., 1999). Survival of *E. coli* could be prolonged by the presence of manure in no-till soil (Gagliardi and Karns, 2000). In subsurface drainage water, *E. coli* survived up to two months after manure was applied to the soil (Gagliardi and Karns, 2002). For drinking water, USEPA has set a standard of zero presence of total coliforms (including fecal coliform and *E. coli*) in 100 ml (USEPA, 2003).

Many studies have been conducted in the laboratories and fields to understand the role of preferential flow through macropores in transporting pathogens to subsurface drainage water. Lobry de Bruyn and Conacher (1994) and McMahon and Christy (2000) reported that bio-pores with diameters bigger than 1 mm can quickly transport water, colloids, air, organic matter and microorganisms from the soil surface to deeper soils and to subsurface drainage systems. Alaoui and Helbling (2006) reported from two field experiments that about 74% to 100% of the infiltrated water moved through macropores which accounts for 0.23% to 2% of soil volume. Christiansen et al. (2004) also reported the significant effect of macropores on the leaching of pesticides to subsurface drainage and groundwater. *E. coli* could be transported to deeper soil depths in the soil columns if macropores were present(Abu-Ashour et al., 1998). Tillage practices have shown significant effects on the size and number of macropores in the field which then resulted in the transportation of pathogens and *E. coli* into the soil (Alaoui and Goetz, 2008; Miller et al., 1998; Schijønning and Rasmussen, 2000). Abu-Ashour et al. (1998) and Wang et al. (2001) found that *E. coli* could be more easily transported through undisturbed soil columns or in the soil under no-tillage practices. Gagliardi and Karns (2000) have reported the vertical transport of Rainfall intensity is one of the main factors that could affect pathogen transport to subsurface drainage water (Wang and Doyle, 1998). Besides that, the amount of manure applied to the field could influence the number of *E. coli* attached to the soil (Guber et al., 2005). More *E. coli* have been found to be attached to the soil under the condition of low flow velocity rather than high flow velocity. Joy et al. (1998) found that very little bacteria tracer moved to tile drainage under low rainfall intensity. There was no connection between volume of manure applied to soil or the concentration of the bacteria tracer in manure and effluent concentration of the bacteria tracer. Thiagarajan et al. (2007) reported that *E. coli* concentrations were higher in water if rainfall followed immediately after manure was applied to the soil.

The overall goal of this dissertation is to investigate the effects of liquid swine manure on nutrient loss and pathogen bacteria transport to subsurface drainage water under corn and soybean crop rotation. A field experiment was conducted to achieve the overall objectives of this project. Data collected from the six-year experiment (2001-2006) at Nashua research site were used to study the effect of liquid swine manure application to both corn and soybeans in comparison to manure applied to corn phase only on the loss of NO_3-N and PO_4-P to subsurface drainage water and NO_3-N and PO_4-P residual concentrations in the soil. Four different experiments were conducted in 2008 and 2009 to collect data to investigate the role of directly connected macropores in transporting bacteria from soil surface to subsurface drainage water.

1.2 Dissertation Overview

This dissertation is organized in five different chapters. The first chapter includes an introduction to the research and summary of dissertation organization. The next 3 chapters (2, 3 and 4) are manuscripts prepared for publication. Chapter 2 includes a manuscript entitled “Effects of liquid swine manure application on NO_3-N and PO_4-P leaching losses to subsurface drainage system”. Chapter 3 presents the manuscript entitled “Residual soil phosphorus and nitrate-nitrogen concentrations after long-term application of liquid swine manure to soybean crop under corn-soybean rotation”. These two manuscripts were prepared for publication in the Transactions of the ASABE. Chapter 4 is a paper entitled “The role of directly connected macrospores on *E. coli* transport to subsurface drainage water”. This manuscript is prepared for publication in the Journal of Environmental Quality. The final chapter, the fifth, summarizes the general conclusions of the research and presents recommendation for future research.

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CHAPTER 2. EFFECTS OF LIQUID SWINE MANURE APPLICATION ON NO_3-N AND PO_4-P LEACHING LOSSES TO SUBSURFACE DRAIN WATER

A paper to be submitted to the Transactions of the ASABE

2.1 Abstract

A field study was conducted at Iowa State University's Northeastern Research Center, near Nashua, Iowa, U.S.A to evaluate the effects of liquid swine manure (LSM) application to both corn *(Zea mays L.)* and soybean *(Glycine max L.)* plots compared with an LSM application to corn plots only in a corn-soybean production system. Liquid swine manure was injected in the experimental plots in the fall after harvesting corn and soybean crops. Six-year (2001-2006) data on crop yields and subsurface drain water quality were collected and analyzed in a randomized complete block design to determine the effects of swine manure treatments on NO_3-N and PO_4-P leaching losses and concentrations in tile drain water. Results of this study indicated that treatment effects on subsurface drainage volume were significant at $p=0.05$ and block and season effects were highly significant, showing the spatial and temporal variability effects. The effects of treatment and season were highly significant on NO_3-N leaching losses and concentrations in subsurface drain water ($p<0.01$). The NO_3-N concentrations in subsurface drain water were high in all treatments but they were twofold greater in the plots receiving LSM every year (to both corn and soybean plots). No significant effects of experimental treatments were observed on PO_4-P leaching losses and concentrations in subsurface drain water as well as on corn and soybean grain yields. Corn grain yields did show an increase of 1.8% when manure was applied to both corn and soybeans in the corn-soybean rotation. However, no significant increase in soybean yields were found between soybeans plots receiving LSM versus no LSM application.

Keywords: Corn and Soybeans, Nitrate nitrogen, Manure, Phosphorus, Subsurface drainage.

2.2 Introduction

Nitrogen (N) and phosphorus (P) are the main mineral nutrients that need to be supplied to plants to meet nutrient uptake needs to get profitable production. Excessive application of N and P to croplands can lead to nutrient leaching losses to subsurface and surface waters. Excessive amounts of these nutrients in surface water can accelerate growth of algae and aquatic plants and result in eutrophication problems (Baker and Tidman, 2001). Losses of these nutrients via subsurface drainage from agricultural fields have been recognized as the biggest nonpoint source pollution of surface water pollution (Carpenter et al., 1998). A part of excess rainfall is removed through an artificial subsurface drainage system in Iowa as well as in other Corn and Soybean Belt States in the Midwest to minimize its effect on crop productivity. About 25% of agricultural land in Iowa, and up to 30% of the agricultural land in other states in the Midwest, has had these artificial subsurface drainage systems to remove excessive amount of water from the root zone (Baker et al., 2004; Hatfield et al., 1998).

Subsurface drainage was one of the main pathways for contributing NO_3-N to the Gulf of Mexico (Mitsch et al., 2001). The world's third largest hypoxic zone in the Gulf of Mexico was a suspected link to NO_3-N overloading received from the Mississippi river (Alexander et al., 1995; Baker et al., 2005; Mitsch et al., 2001; Rabalais et al., 2001). The amount of NO_3-N in the Mississippi River has more than doubled since 1965 (Turner and

Rabalais, 1991; Justic et al., 1995). A similar problem was also reported in other locations like the Baltic Sea (Larson et al., 1985), and the Black Sea (Tomazin, 1985).

Subsurface drainage water normally has lower concentrations of sediment and phosphorus. Because of the physical property of low mobility, P transport thorough subsurface drainage water is considered very small or negligible. However, several researchers have stated that under some conditions P can be predominantly transported through subsurface drainage water in sandy loam soils in humid regions (Gilliam et al., 1999) or in an area with high P concentrations and low soil sorption capacity with high organic matter soils (Sims et al., 1998). The P concentrations in subsurface drainage water higher than the USEPA standards have been reported by several researchers (Algoazany et al., 2008; Gaynor and Findlay, 1995; Hooda et al., 1999; Miller, 1979; Smith et al., 2001). Algoazany et al. (2008) have also reported that soil type and tillage systems affected on the transportation of soluble P not only in surface runoff but also in subsurface drainage water. Several researchers have reported the important role of P in the occurrence of hypoxia in the Gulf of Mexico (Daniel et al., 1998; Correll, 1998, Sylvan et al., 2006).

Liquid swine manure (LSM) has been used widely in the United State as a source of nutrients for crop production. When LSM is applied to crop fields, plant will uptake nitrogen in inorganic forms (NO_3^- or NH_4^+) and phosphorus in a form of orthophosphate ion (PO_4^-) (Zhang et al., 1998). Similar to other N sources, LSM should be applied at the appropriate amount to meet the needs of plants uptake. Applying less nitrogen than the crop needs could negatively affect crop yields and grain quality. However, using excessive nitrogen does not benefit yields and crop quality but can result in significant NO_3-N leaching losses in subsurface drainage water and groundwater (De Vos et al., 200l; Dinnes et al., 2002; Kanwar et al., 1988; Schmidt et al., 2000). Even within the recommended application rates of N to corn phases under corn-soybeans rotation (*i.e.,*112 to 168 kg ha^{-1}), the NO_3-N concentration in subsurface drainage water was found still higher than the drinking water standard of 10 mg L^{-1} (Lawlor et al., 2008).

Traditionally, manure has been used only for corn production. Soybeans and alfalfa themselves as legume plants can fix nitrogen needed from atmosphere at the amount of 45 to 170 kg ha^{-1} per year, respectively (Mitsch et al., 2001). These plants do not require extra application of nitrogen for optimum growth. Few studies were conducted on the effect of manure application on soybean crop yield. Schmidt et al., (2000) mentioned that manure has been applied to soybean fields as a way of manure disposal rather than using manure to increase crop yields. Their experiments conducted in Minnesota, USA to study the effect of manure applications to soybean fields at different rates of 100, 200, 300, 400 and 500 kg ha^{-1} showed that the residual NO_3-N in the soil after crops harvested ranged between 39 to 67 kg N ha^{-1} and between 80 to 158 kg N ha^{-1} in plots receiving less than 260 and more than 260 kg N ha^{-1} of manure, respectively. They concluded that as long as N in manure application rate did not overload N accumulation in the crop, no environmental risk could be found. In other experiments conducted at three different locations in Iowa, Sawyer (2001) reported that an increase in soybean yields in response to swine manure applications was not consistent. It did improve soybean yields when tests of P and K in soil were deficient. However, when those nutrients were sufficient in the soil, manure application rate must be lower than N removal ability of soybean grain to reduce the effects on the environment.

Several studies have been conducted to find the effects of tillage, cropping systems, manure or fertilizer application timing, and their application rates to corn crops on NO_3-N and PO_4-P leaching losses with subsurface drainage (Algoazany et al., 2007; Baker et al., 1975; Bakhsh et al., 2002; Bakhsh et al., 2005; Lawlor et al, 2008; Washney et al., 1993). No study has been conducted to specifically examine the effects of swine manure application to both corn and soybeans every year, on nitrate-nitrogen and phosphorus losses to subsurface drainage water. Therefore, the objectives of this study were to evaluate the long-term effects of liquid swine manure applications to both a corn and soybeans on crop yields and nitrate and phosphorus leaching to subsurface drainage water under corn-soybean rotation system.

2.3 Materials and Methods

2.3.1 Study site description

Field experiments were conducted at Iowa State University's Northeast Research Center near Nashua, Iowa, U.S.A. The study site has thirty-six 0.4 ha plots (58.5 by 67 m), with fully documented tillage and cropping records for the past 28 years. The three dominant soils at this site are Floyd loam (fine-loamy, mixed, mesic Aquic Hapludolls), Kenyon loam (fine-loamy, mixed, mesic Typic Hapludolls), and Readlyn loam (fine-loamy, mixed, mesic Aquic Hapludolls) (Kanwar et al., 1997). These soils have organic matter ranging from about 3% to 4% and lie over highly fluctuating water table conditions, from 20 to 160 cm. Therefore, a subsurface drainage system was installed to maintain high crop productivity.

Subsurface drainage system was installed at this site in 1979, at 1.2-m depth and tile spacing of 28.5m apart. The drainage water from each plot was collected and measured using a flow meter installed in each independent sump. Subsurface drainage water samples were weekly collected for each plot when drainage water was flowing. More details on the subsurface drainage system installed and the water sampling process at this site can be found in Kanwar et al. (1999). 30-year long-term average precipitation data (1971-2000) presented in this paper was taken from the weather station in Charles city, Iowa, about 16 km from the research site.

2.3.2 Experimental treatment designs

Three different study phases were conducted at this research site from 1979-2006 under randomized complete block design. From 1979-1993, the effects of four tillage practices (chisel plow, ridge, moldboard plow, ridge-till, and no-till) and two crop rotations (continuous corn and corn-soybean rotation) on crop yields and water quality were examined (Kanwar et al., 1997). From 1993-1998, tillage systems were reduced to two (chisel plow and no-till). Effects of these two tillage systems and two crop rotations (continuous corn and corn-soybean rotation), in combination with nine nitrogen management systems (including the use of LSM as a nutrient), were examined on crop yields and water quality (Bakhsh et al., 2002). From 1999-2006, a new set of nutrient management treatments was established at this research site, including one corn-soybean rotation with an application of LSM to both corn and soybean crops. Data used in this study were from the six-year period from 2001 to 2006 as the previous two years (1999-2000) were considered as the transition time between two studies, from the last experimental study (1993-1998) and this experimental study (2001-2006). Twelve experimental plots from a total of 36 plots were used for this particular study. Four experimental treatments were established, each replicated three times in a randomized complete block design: (1) corn after soybeans- with liquid swine manure application in the fall at a rate of 168 kg-N/ha to the corn crop only (CNMS); (2) soybean after corn without the application of liquid swine manure to soybeans crop (SNM); (3) corn after soybeans with the liquid swine manure application to corn and soybeans every year in the fall at a rate of 168 kg-N ha^{-1} to corn crop (CWMS) and (4) soybeans after corn with the fall liquid swine manure application to the soybeans in the fall at a rate of 225 kg-N ha^{-1}.

The LSM application was taken from a finishing swine production facility. The LSM was injected in the fall of the prior year. A total targeted rate of 168 kg-N ha^{-1} yr^{-1} of manure was applied to the corn phase (CNMS and CWMS treatments) and the targeted rate of 225 kg-N ha^{-1} yr^{-1} to the SWM treatment. No manure was applied to soybean plots in the SNM treatment. An exact amount of N was not easily determined in LSM at the time of application, therefore the actual LSM application rates varied from year to year and ranged from 141 to 260 kg-N ha^{-1}. The six-year averages of actual LSM-N application rates were 176, 175 and 221 kg-N ha^{-1} for treatments CNMS, CWMS and SWM, respectively. More details on the actual LSM application rates to the field during the study period are given in table 2.1.

2.3.3 Analysis and calculation of NO_3-N and PO_4-P losses and concentration

Subsurface drain water samples were analyzed at the Water Quality Laboratory in the Department of Agricultural Biosystems Engineering at Iowa State University in Ames, Iowa. The subsurface drain water samples were preserved by adding a drop of 2% concentrated sulfuric acid and then stored at 4^{o}C until NO_3-N and PO_4-P concentrations were analyzed. The NO_3-N and PO_4-P concentration in subsurface drainage water samples was analyzed using the automated flow injection Cadmium reduction method and ascorbic acid methods in the Lachat Quickchem 8000 Automated Ion Analyzer system.

The NO_3-N and PO_4-P losses (kg-N ha^{-1}) with subsurface drainage water for each interval of sampling were calculated by multiplying those concentrations with drainage water volume. The annual flow weighted average NO_3-N and PO_4-P concentrations for each year were calculated based on the cumulative NO_3-N and PO_4-P loss and sum of drainage effluent for the entire monitoring season for that year (Bakhsh et al., 2005; Bjorneberg et al., 1998; Karlen et al., 2004;).

2.3.4 Statistical analysis

Statistical analysis was performed to test the effects of block, yearly conditions and liquid swine manure application rates on subsurface drainage volume, flow-weighted average NO_3-N and PO_4-P concentrations and leaching losses of NO_3-N and PO_4-P to subsurface drainage water. Crop yield data for corn and soybeans were analyzed separately. The general linear method procedure (GLM) using SAS version 9.1 for Windows (SAS Institute, 2003) was used to analyze the data. GLM procedure uses the least square method to find the relationship between dependent variables (subsurface drainage volume, NO_3-N and PO_4-P concentrations and losses and crop yields) and independent variables (blocks, treatments and seasons). Analysis of variance (ANOVA) tables were developed for dependent variables. Treatment means within the years and over the years were tested at alpha level of 0.05 for all statistical tests, using the least significant different (LSD) method.

2.4 Results and Discussion

Results of the analysis of variance for subsurface drainage volume, flow weighted average concentrations and leaching losses of NO_3-N and PO_4-P in subsurface drainage water across 6 years average and on yearly basis are given in table 2.3 and 2.4 respectively. Tables 2.5, 2.6, 2.7, 2.8 and 2.9 present treatment means on annual subsurface drainage flow volume; annual NO_3-N and PO_4-P leaching losses and concentrations of in tile drainage water. The

ANOVA table for corn and soybean yields and treatment means of corn-soybean yields are presented in tables from 2.10 to 2.12.

2.4.1 Precipitation and subsurface drainage

A summary of monthly precipitation and subsurface drainage flow from 2001 through 2006 are given in table 2.2. The 30-year long-term average precipitation (1971-2000) at the research site was 881 mm yr^{-1}. The six-year average annual precipitation at the research site was 745 mm yr^{-1}, or 15% below the long-term average. The driest year was 2003 (32% below the long-term average) and the wettest year was 2004, in which the annual precipitation exceeded the 30-year long-term annual precipitation.

The 30-year long-term average precipitation in the drainage season (March through November) was 808 mm yr^{-1}. Over the six years of the study, the precipitation in drainage season ranged from 602 to 884 mm yr^{-1} with the lowest in 2003 and the highest in 2004. Three of the six years received 100% of the annual precipitation within the drainage season (years 2002, 2003 and 2005). The other three years received more than 90% of the rainfall in the drainage season. The overall six-year average precipitation in the drainage season for this site was 728 mm yr^{-1}, or 10% below the 1971-2000 normal drainage season value. Two years, 2004 and 2005, drainage season precipitations were higher than the average value by 9% and 4%, respectively.

The six-year average growing season precipitation was 534mm yr^{-1}, or 4% below the long-term normal at the site (554 mm yr^{-1}). The growing season precipitation in years 2004 and 2005 were 643 mm and 729 mm yr^{-1}, higher than the long-term average value by 16% and 32%, respectively. October had the greatest average rainfall deficiency of 28 mm.

Rainfall in the months of June through September supported the high water uptake requirement of the growing crop and did not generate much to subsurface drainage flow.

The highest six-year average subsurface flow was recorded for the treatment SNM followed by CNMS, SWM and CWMS. Treatment SNM had the highest six-year average percentage of precipitation (15%) in drainage season removed by subsurface drainage, followed by treatments CNMS (11%), SWM (10.9%) and CWMS (8.7%), respectively. These changes in rainfall patterns during the drainage season and growing season resulted in mainly variable amounts of NO_3-N leaching losses and NO_3-N concentration to subsurface drainage water.

Tables 2.3 and 2.4 show the effects of different independent variables of block, treatments and seasons to subsurface drainage flow across the six-year average and on a yearly basis. Table 2.3 shows that block has a highly significant effect on tile flow volumes ($p<0.01$) when averaged across six years (2001-2006), which indicates that there were effects of spatial variability due to soil types and landscape attribute at this study site (Bakhsh and Kanwar, 2005). Topographic attributes have played an important role in the storage and movement of water up and down and under the soil surface (Kravchenko and Bullock, 2000; Iqbal et al., 2005). Similar to the block, yearly variable also showed a highly significant effect ($p<0.01$) while treatments showed significant effects at $p<0.05$. However, interaction between them did not show significant effects on tile flow volumes.

Comparisons among treatment means for subsurface drainage flow for each year were not significantly different from each other (table 2.5). In the six year study period, the lowest tile flow volumes were found in 2002 among all treatments, as rainfall for this year was the second lowest and mainly occurred in July and August (179 and 155 mm, respectively) when crops mostly required the highest uptake of water for meeting evapo-transpiration needs coupled with high temperature in the summer. The highest tile flow volume was found in 2001 (191 mm) from soybean plots receiving no manure. The spatio-temporal variability effects on tile flow drainage were recognized by looking at the volume of tile flow generated under the plots of treatments CNMS and SNM.

The overall six-year average means of tile flow volumes were significantly different among treatments (table 2.5). In general, corn plots receiving manure each year generated the lowest tile flow (63 mm) whereas soybeans plots with no manure application gave the highest tile flow volume of 108 mm. Differences were also observed in each crop. Soybean plots receiving manure gave lower tile flow volume in comparison with soybean plots receiving no manure e. Similar results were also observed in corn plots.

2.4.2 NO_3-N leaching losses to subsurface drainage

The NO_3-N leaching losses to subsurface drainage water were highly dependent on precipitation as well as nitrogen application rates (Lawlor et al., 2008). Table 2.3 shows the results of treatment, block, and yearly effects on NO_3-N leaching losses with subsurface drainage for the average six-year combined data. Effects of treatments, seasons and interaction between them were found to be highly significant ($p<0.01$) (table 2.3). Blocks showed significant effects on NO_3-N losses ($p<0.05$) which indicates the effects of spatial variability on NO_3-N losses as similar results have been reported by Bakhsh et al. (2002, 2005). On a yearly basis, blocks and treatments did not show significant effects among five of the six years of the study period. Year 2004 was an exceptional case when treatment effects were highly significant ($p<0.01$) because of high precipitation (884 mm) in the drainage season.

Table 2.6 presents the treatment means of NO_3-N leaching losses on a yearly basis and for a 6-year average. NO_3-N leaching losses to subsurface drainage water ranged from 1.8 to 64.1 kg-N ha^{-1} among all treatments. The highest NO_3-N leaching losses were found in 2004 which had the highest rainfall amount in the study period. In the following year 2005, NO_3-N leaching losses were found be the second highest. Rainfall in both year 2004 and 2005 were higher than the average long-term rainfall in th study area. The lowest NO_3-N leaching losses for all treatments were found in 2002 although it was not the driest year. This could be due to the fact that the highest rainfall amounts of 179 and 155 mm occurred in the months of July and August, respectively, the fastest crop growth period which then required a much higher amount of water to meet the evapotranspiration demand of the plants.

Soybean plots receiving manure application each year resulted in higher NO_3-N leaching losses compared with soybean plots with no manure application. This trend was found to be consistent for five of the six years in the study period (*e.g.,* 40.6 vs. 21.3 in 2003 kg-N ha^{-1} and 58.8 vs. 18kg-N ha^{-1} in 2004). Similar results were also found between corn plots in rotation with soybeans receiving manure in each year vs. soybean plots with no manure application. The highest NO_3-N leaching losses were also found in the soybean plots in three of the five years and for the overall six-year average. For the overall six-year average, soybean plots receiving manure each year gave about twofold increase in NO_3-N leaching losses compared with other soybean plots with no manure applied. Higher NO_3-N leaching losses were found in corn plots in rotation with soybean plots receiving manure each year. This clearly shows that higher nitrogen application rates can result in greater NO_3-N leaching losses to subsurface drainage water even through subsurface drainage flow may be lower in volume as shown for soybean plots in 2001, 2003 and 2005 (tables 2.5 and 2.6). In the comparison between two systems of corn-soybeans rotation combined, the system with manure applied to soybeans plots gave significantly higher NO_3-N leaching losses (59.4 vs. 38 kg-N ha^{-1}, respectively).

2.4.3 NO_3-N concentrations in subsurface drainage

Statistical effects and differences of flow-weighted NO_3-N concentrations in subsurface drainage are shown in tables 2.3, 2.4 and 2.7. Treatment and seasonal effects were observed to have highly significant effects on flow-weighed NO_3-N concentrations ($p<0.01$) when averaged across six years and on a yearly basis, except for year 2002 (table 2.4).The interaction between treatments and seasons was also examined with similar results.

Statistical differences ($LSD_{0.05}$) of flow-weighed NO_3-N concentrations in subsurface drainage water are presented in table 2.7. For the six-year average, flow-weighed NO_3-N concentrations ranged from 16.7 to 40.6 mg L^{-1} (Table 2.4), which are exceedingly higher than the drinking water standard of 10 mg L^{-1} set by USEPA (U.S. EPA, 1992). Significant differences were also found among plots receiving manure each year in comparison with plots receiving manure to the corn phase only. Corn and soybean plots receiving manure each year showed the highest value of NO_3-N concentrations (40.6 mg L^{-1}), that was also significantly different compared with other corn plots receiving no manure in the soybean phase. Significant differences were also noted when comparing the six-year average NO_3-N concentrations in subsurface drainage water from soybean plots receiving manure each year and plots that received no manure in the alternative year (Table 2.7).

The average NO_3-N concentration values in subsurface drainage for individual years ranged from 14.4 mg L^{-1} (on soybean plots receiving no manure in 2005) to 70.4 mg L^{-1} (on corn plots receiving manure each year in 2004). In five out of six years (from 2001 to 2005), corn plots receiving manure each year gave higher flow-weighed NO_3-N concentrations in comparison with the corn plots receiving manure in an alternate year to corn phase only. Similar results were also observed in the soybean phase (table 2.7). Means of flow-weighed NO_3-N concentrations under the corn phase showed significant differences in four out of six years (2002, 2004, 2005 and 2006). For the soybean phase, means of flow-weighed NO_3-N concentrations exhibited significant differences in all years. These results obviously showed that there is a direct relationship between manure application rate, which exceeds crop needs, and the flow-weighed NO_3-N concentrations in subsurface drainage water. In 2004 and 2006, all treatments means showed significantly different results. Maximum flow-weighed NO_3-N concentrations of 70.4 mg L^{-1} were measured for corn plots receiving manure each year. The second highest flow-weighed NO_3-N concentrations (50.1 mg L^{-1}) were found in the same year for soybean plots receiving manure each year. These results could be strongly affected by combination of getting higher precipitation and drainage volumes within this six-year study and the higher amount of manure applied in soybean plots (Lawlor et al., 2008; Bakhsh et al., 2007; Schimidt et al., 2001).

2.4.4 PO_4-P leaching losses and concentrations in subsurface drainage water

Unlike the NO_3-N ion which is highly soluble and dissolves in water, PO_4-P is mainly adsorbed to soil particles and will gradually accumulate in the soil and runoff would be the main pathway of PO_4-P losses to surface water. Dougherty et al. (2006), Chinkuyu et al. (2002), and Chikuyu (2000) reported that higher concentrations of PO_4-P were observed in surface runoff in comparison with PO_4-P concentrations in subsurface drainage water. Thus, leaching losses and concentrations of PO_4-P in subsurface drainage water were consequently much lower than that of NO_3-N.

Effects of blocks, treatments and seasons on PO_4-P leaching losses to subsurface drainage and subsurface flow-weighted PO_4-P concentrations are presented in table 2.4 and 2.5. No significant effects of these variables were found on overall the six-year average PO_4-P leaching losses and on a yearly basis.

PO_4-P leaching losses to subsurface drainage water for all treatments were calculated and are presented in table 2.9. PO_4-P leaching losses values ranged from 0.5 g ha^{-1} (year 2002) to 42.3 g ha^{-1} (year 2003). No significant differences in PO_4-P leaching losses were found among treatments. The PO_4-P leaching losses values ranged from 3.5 g ha^{-1} for corn plots receiving manure to 12.2 g ha^{-1} for soybean plots receiving manure. Those values are much lower in comparison with the amount of 36.8 kg ha^{-1} of dissolved PO_4-P leaching losses to subsurface drainage water in organic soil, found by Miller (1979) and much lower than the average amount of 0.38 kg ha^{-1} of PO_4-P loss to subsurface drainage flow from a clay loam soil (Gaynor and Findlay, 1995).

The overall six-year study average and yearly average flow-weighted PO_4-P concentrations in subsurface flow are presented in table 2.10. Average flow-weighted PO_4-P concentrations ranged from 1.3 µg L^{-1} to 40.6 µg L^{-1}. There are no significant differences among all treatments for year 2001. This trend seems to be consistent for almost all other years, except 2005. Overall six-year average, flow-weighted PO_4-P concentrations were highest (13.7 µg L^{-1}) in soybean plots receiving manure while the corn phase of the same system showed the lowest PO_4-P level of 6.3 µg L^{-1}. Flow-weighted PO_4-P concentrations in all treatments in every individual year and averaged across the six year period (2001-2006) were much lower than the EPA recommended total P level of 0.05 mg L^{-1} for stream water entering lakes and reservoirs to control the eutrophication (Muller and Helsel, 1999). Baker et al. (1975) reported that the PO_4-P concentrations in subsurface drainage water ranged from 0 to 38 µg L^{-1} whereas total phosphorus ranged from 7 to 182 µg L^{-1}. Randall et al. (2000) in his study in Minnesota also found that the concentrations of molybradte-reactive P, which is mostly exited as PO_4-P and highly bioavailable, were 16 µg L^{-1} for plots under manure application. However, some other findings have reported that the PO_4-P concentrations in subsurface drainage water in organic soil were very high at concentrations of 18200 µg L^{-1} (Miller, 1979); the flow-weighted molybradte-reactive P was at higher values, ranging from 160-380 µg L^{-1} (Hooda et al., 1999) and flow-weighed soluble-P concentrations in subsurface drainage water ranged from 86 to 100 µg L^{-1} in four different sites through a long-term study conducted by Algoazany et al. (2007). Four of the six years studied (2001, 2004, 2005, and 2006) showed that corn plots receiving manure in alternative years gave highest PO_4-P concentrations. In the other two years (2002 and 2003), soybean plots gave the highest PO_4-P concentrations. This shows that PO_4-P concentrations in subsurface drainage do not correspond to the manure application rate. Similar finding was also reported by Algoazany et al. (2007) that higher soluble P concentrations were found in the years without P application

2.4.5 Treatment effects on crop production

The results of statistical analysis of blocks, seasons and treatment effects on corn and soybean yield across six years are given in table 2.10. Block and treatments showed highly significant effects ($p<0.01$) on corn yield while they did not show the same effects on soybean yields. Seasons were found to be a highly significant for both corn and soybean yields. The interaction between treatment and year showed highly significant effect ($p<0.01$) on soybean yield but not on corn yield.

Results of statistical analysis on a yearly basis showed that treatment effects on corn grain yield were not significant for all years of the study. Treatment means for corn yield on a yearly basis and across the six-year averaged were also highly consistent with the treatment effects. In four of the six years of the study period, corn yields were higher in plots receiving manure each year (table 2.12). In the overall six-year average, corn plots receiving manure each year in soybean plots gave significantly higher corn grain yield in comparison with corn plots receiving the manure application in the alternative year (with no manure to soybeans) (11.93 vs. 11.72 Mg ha^{-1}, about 1.8% yield increase). Corn yields were lowest for both treatments CNMS and CWMS in the year 2003 which was the driest year compared with other years in this study period.

Table 2.11 shows a yearly analysis of block and treatment effects on soybean grain yields. Block did not show t effects for all years but significant effects of treatments were found for years 2001, 2003 and 2005. Three out of six years had soybean yields that were significantly different. Soybean plots receiving manure gave higher grain yields in comparison with soybean plots without manure application (*e.g.,* 4.97 vs. 4.62 Mg ha^{-1} in 2005). Similar to the corn crop, the lowest soybean yields were observed in 2003 for both SNM and SWM treatments in year 2003, which had the lowest rainfall and resulted in significant effects on crop yields.

2.5 Conclusions

Field experiments were conducted at Iowa State University's Northeast Research Center near Nashua, Iowa, U.S.A from 2001 through 2006, to evaluate the effects of liquid swine manure application to both corn and soybeans on subsurface drainage water quality, specifically on subsurface drainage volume, NO_3-N and PO_4-P leaching losses and concentrations in tile drainage water, and corn and soybean grain yields. Four treatments (CNMS, CWMS, SNM and SWM) were studied in this research. Fall manure at the average rate of 176 kg-N ha^{-1} was applied to treatment CNMS. Average rates of 175 and 221 kg-N ha^{-1} were also applied in the fall to CWMS and SWM treatments, respectively. No manure was applied to treatment SNM. The complete data set from this six-year study was used and analyzed as a randomized complete block design using GLM procedure of SAS version 9.1. This study resulted in the following conclusions:

1. For overall six-year averages, effects of block and season showed highly significant effects ($p<0.01$) on subsurface drainage volume. Interaction between treatment effects and seasonal effects was not found to be significant on subsurface drainage flow volumes. The highest subsurface drainage volume of 108 mm was found from soybean plots receiving manure whereas the lowest value of subsurface drainage volume of 63 mm was found in the corn year under corn-soybean rotation with liquid swine manure applied to both phases.
2. Highly significant effects ($p<0.01$) of treatment, season and interaction were found on NO_3-N leaching losses and concentrations in subsurface drainage water. On the average, corn and soybean plots receiving manure every year resulted in significantly higher NO_3-N leaching losses to subsurface drainage water in comparison with corn and soybean plots receiving manure to corn plots only. Similarly, flow-weighted NO_3-N concentrations were found to be significantly different between treatments. The highest NO_3-N concentration of 41 mg L^{-1} in subsurface drainage water was found in corn plots receiving manure each year followed by soybean plots in the same system. However, NO_3-N concentrations in subsurface drainage water for all

treatments were found to be higher than the USEPA water quality standard of 10 mg L^{-1}.

3. Seasonal effects on yearly basis were found to be significant on both NO_3-N leaching losses and concentrations in subsurface drain water. Manure applications to plots in both phases of corn-soybean rotation resulted in significantly higher NO_3-N concentrations in tile water comparing withplots receiving manure in the corn year only.
4. Inversely, the effects of all dependent variables (block, treatment and season) were not found to be significant on PO_4-P leaching losses and PO_4-P concentrations in subsurface drainage water. It showed that tile drainage water was not the major pathway to carry phosphorus under these soils and condition at the research site.
5. Treatment effects were significant on corn grain yields but not on soybean grain yields. Therefore, any difference in soybean grain yields on a yearly basis between soybean plots receiving manure every year and soybean plots receiving no manure application may be caused by temporal variability effects rather than by changes in manure application rates. Manure application to soybeans resulted in the highest NO_3-N concentrations in subsurface drain water. Therefore, the LSM application to soybeans at the rate of 225 kg-N ha^{-1} is not a very good practice. More research is needed to determine the appropriate application rates of LSM to soybeans without degrading the quality of subsurface drainage water.

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Table 2.1. Actual application rates of LSM (kg ha^{-1}) to corn-soybean plots

LSM	Years						Average (01-06)
	2001	2002	2003	2004	2005	2006	
Fall manure application to corn plots in corn-soybean rotation							
N[a]	171	220	141	173	182	172	176
P_2O_5	169	162	71	100	75	95	112
K_2O	180	175[b]	111	129	118	106	137
Fall manure application to both corn and soybean plots in corn-soybean rotation							
Corn plots							
N	173	212	148	154	183	177	175
P_2O_5	402	161	66	72	67	99	145
K_2O	178	173	114	128	119	110	137
Soybean plots							
N	223	260	176	227	211	233	221
P_2O_5	233	187	80	130	83	140	143
K_2O	238	214	154	161	138	125	172

[a]: Potentially available N from manure during first cropping season = 50% [TKN-NH_3-N] + NH_3-N.

[b]: 109 kg ha^{-1} of K_2O applied to soybean plots in this year.

Table 2.2. Precipitation data from 2001-2006 at Nashua research site (mm).

	Month											
Year	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Growing Season[a]	Drainage Season[a]	Annual Total
2001	41	63	148	64	70	73	149	40	26	504	674	714
2002	14	109	75	75	179	155	51	54	7	535	719	719
2003	30	97	99	155	76	13	48	15	69	391	602	603
2004	109	46	284	74	155	74	56	51	36	643	884	898
2005	13	58	109	201	99	152	168	8	30	729	838	838
2006	42	122	34	116	59	68	126	38	48	402	652	697
Average	42	82	125	114	106	89	100	34	36	534	728	745
Normal[b]	51	87	104	132	117	116	85	62	54	554	808	881

[a] Growing season was May through September, and drainage season was March through November.

[b] Source: National Climate Data Center for Charles city, Iowa, 1971-2000

Table 2.3. Analysis of variance for NO_3-N and PO_4-P leaching loss in subsurface drainage water when averaged across the years (2001-2006)

Source of variation	df	Subsurface drainage flow (p-values)	NO_3-N loss with sub-surface drainage water (p-values)	Flow weighted average NO_3-N concentrations (p-values)	PO_4-P loss with subsurface water (p-values)	Flow weighted average PO_4-P concentrations (p-values)
Block (blk)	2	<0.01	0.04	0.15	0.76	0.73
Treatments (trt)	3	0.03	<0.01	<0.01	0.51	0.62
Error (blk*trt)	6					
Year	5	<0.01	<0.01	<0.01	0.43	0.46
Trt*Year	15	0.12	<0.01	<0.01	0.62	0.82
Residual	40					

Table 2.4. ANOVA table for subsurface drainage flow, NO3-N and PO4-P leaching losses with drainage water on yearly basis.

Source of variation	df	Probability values (subsurface drainage flow)					
		2001	2002	2003	2004	2005	2006
Blocks (blk)	2	0.23	0.24	0.38	0.46	0.42	0.62
Treatments (trt)	3	0.28	0.26	0.22	0.32	0.2	0.52
Error (blkxtrt)	6						
		Probability values (NO_3-N loss in subsurface drainage water)					
Blocks (blk)	2	0.33	0.24	0.68	0.46	0.77	0.70
Treatments (trt)	3	0.23	0.30	0.12	<0.01	0.37	0.49
Error (blkxtrt)	6						
		Probability values (NO_3-N concentrations in subsurface drainage water)					
Blocks (blk)	2	0.04	0.36	0.11	0.21	0.18	0.27
Treatments (trt)	3	<0.01	<0.09	<0.01	<0.01	<0.01	<0.01
Error (blkxtrt)	6						
		Probability values (PO_4-P loss in subsurface drainage water)					
Blocks (blk)	2	0.73	0.26	0.52	0.53	0.15	0.22
Treatments (trt)	3	0.24	0.29	0.56	0.35	0.5	0.02
Error (blkxtrt)	6						
		Probability values (PO_4-P concentrations in subsurface drainage water)					
Blocks (blk)	2	0.85	0.67	0.51	0.51	0.2	0.65
Treatments (trt)	3	0.3	0.73	0.66	0.48	0.1	0.67
Error (blkxtrt)	6						

Table 2.5. Treatment means for annual subsurface drainage flow (mm)

Treatments	Years[a]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
CNMS	75.6a	46.1a	55.4a	142.6a	53.8a	107.4a	80.1ab
CWMS	84.3a	11.6a	61.1a	91.3a	73.1a	58.9a	63.4b
SNM	190.9a	10.8a	140.9a	98.6a	150.2a	54.7a	107.7a
SWM	132.5a	7.6a	90.7a	118.6a	70.9a	56.1a	79.4ab

[a] Means within years and on average followed by the same letters are not significantly different at *p*=0.05.

CNMS = corn after soybeans –fall manure applied to corn only.
CWMS = corn after soybeans – fall manure applied to corn and soybeans every year
SNM = soybeans after corn – no manure applied to soybeans
SWM = soybeans after corn – fall manure applied to soybeans

Table 2.6. Treatment means for annual subsurface NO_3-N loss (kg ha^{-1})

Treatments	Years[a]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
CNMS	19.4a	6.4a	15.7b	50.2a	14.0a	22.8a	21.4bc
CWMS	21.7a	3.7a	18.8b	64.1a	29.8a	26.0a	27.3ab
SNM	28.3a	2.0a	21.3ab	18.0b	21.1a	9.1a	16.6c
SWM	41.7a	1.8a	40.6a	58.8a	30.7a	19.0a	32.1a

[a] Means within years and on average followed by the same letters are not significantly different at p=0.05

CNMS = corn after soybeans –fall manure applied to corn only.

CWMS = corn after soybeans – fall manure applied to corn and soybeans every year

SNM = soybeans after corn – no manure applied to soybeans.

SWM = soybeans after corn – fall manure applied to soybeans.

Table 2.7. Treatment means for annual subsurface NO_3-N concentration (mg L^{-1})

Treatments	Years[a]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
CNMS	24.9a	16.9b	26.8b	36.6c	25.7b	22.5c	25.5b
CWMS	25.9a	31.8a	29.4b	70.4a	43.2a	16.1d	40.6a
SNM	15.8c	19.3b	16.1c	18.6d	14.4c	43.2a	16.7c
SWM	31.5a	20.7ab	44.6a	50.1b	43.5a	34.0b	37.4a

[a] Means within years and on average followed by the same letters are not significantly different at *p*=0.05

CNMS = corn after soybeans –fall manure applied to corn only.

CWMS = corn after soybeans – fall manure applied to corn and soybeans every year

SNM = soybeans after corn – no manure applied to soybeans

SWM = soybeans after corn – fall manure applied to soybeans.

Table 2.8. Treatment means for annual subsurface orthophosphate loss (g ha^{-1})

Treatments	Years[a]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
CNMS	7.6a	7.1a	6.0a	19.9a	7.4a	8.3a	9.4a
CWMS	3.4a	0.9a	1.7a	4.8a	7.8a	3.6b	3.5a
SNM	2.6a	1.9a	10.4a	4.3a	7.3a	2.7b	5.0a
SWM	12.8a	0.5a	42.3a	12.1a	2.5a	2.7b	12.2a

[a] Means within years and on average followed by the same letters are not significantly different at *p*=0.05

CNMS = corn after soybeans –fall manure applied to corn only.

CWMS = corn after soybeans – fall manure applied to corn and soybeans every year

SNM = soybeans after corn – no manure applied to soybeans

SWM = soybeans after corn – fall manure applied to soybeans

Table 2.9. Treatment means for annual subsurface orthophosphate concentration (µg L^{-1})

Treatments	Years[a]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
CNMS	12.2a	13.0a	14.2a	14.2a	13.7a	11.3a	13.1a
CWMS	3.7a	7.9a	6.5a	5.4a	9.2ab	4.9a	6.2a
SNM	1.3a	15.2a	9.1a	4.5a	4.7b	7.80a	7.1a
SWM	9.8a	13.3a	40.6a	8.8a	3.5b	6.3a	13.7a

[a] Means within years and on average followed by the same letters are not significantly different at *p*=0.05
CNMS = corn after soybeans –fall manure applied to corn only.
CWMS = corn after soybeans – fall manure applied to corn and soybeans every year
SNM = soybeans after corn – no manure applied to soybeans
SWM = soybeans after corn – fall manure applied to soybeans.

Table 2.10. ANOVA for corn-soybean yields over the years (2001-2006)

Source of variation	df	corn (p-values)	soybean (p-values)
Blocks (blk)	2	<0.01	0.58
Treatments (trt)	1	<0.01	0.07
Error (blk*trt)	2		
Year	5	<0.01	<0.01
Trt*year	5	0.28	<0.01
Residual	20		

Table 2.11. ANOVA for corn-soybean yields on an yearly basis

Source of variation	df	Probability values (corn)					
		2001	2002	2003	2004	2005	2006
Blocks (blk)	2	0.01	0.38	0.32	0.88	0.25	0.09
Treatments (trt)	1	0.05	0.75	0.50	0.29	0.10	0.16
Error (blkxtrt)	2						
		Probability values (soybean)					
Blocks (blk)	2	0.16	0.26	0.84	0.30	0.59	0.33
Treatments (trt)	1	0.01	<0.01	0.87	0.18	0.03	0.17
Error (blkxtrt)	2						

Table 2.12. Treatment means for corn-soybean yields (Mg ha^{-1}) on yearly basis

Treatments	Years[a]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
<u>Corn</u>							
CNMS	11.09b	12.21a	10.21a	12.29a	11.99a	12.54a	11.72a
CWMS	11.34a	12.17a	10.51a	12.74a	12.44a	12.36a	11.93a
<u>Soybean</u>							
SNM	3.45b	3.75b	1.92a	4.00a	4.62b	4.18a	3.65a
SWM	3.79a	3.99a	1.90a	3.76a	4.97a	4.38a	3.80a

[a] Means within years and on average followed by the same letters are not significantly different at p=0.05

CNMS = corn after soybeans –fall manure applied to corn only.

CWMS = corn after soybeans – fall manure applied to corn and soybeans every year

SNM = soybeans after corn – no manure applied to soybeans

SWM = soybeans after corn – fall manure applied to soybeans

CHAPTER 3. RESIDUAL SOIL PHOSPHORUS AND NITRATE-NITROGEN CONCENTRATIONS AFTER LONG-TERM APPLICATION OF LIQUID SWINE MANURE TO SOYBEAN CROP UNDER CORN-SOYBEAN ROTATION

A paper to be submitted to the Transactions of the ASABE.

3.1 Abstract

The effects of liquid swine manure (LSM) applied to both corn *(Zea mays L.)* and soybeans *(Glycine max L.)* every year and LSM applied to corn crops only at a different time (fall application vs. spring application), are not very clear on residual nitrogen and phosphorus concentrations in the soil profile under the corn and soybean production system. Therefore, a six-year field study was conducted, from 2001 to 2006, at the Iowa State University research center, Nashua, Iowa. The objectives of this study were: (1) to investigate the effects of liquid swine manure applied to both corn and soybean phases of the corn-soybean production system on residual soil nitrate and phosphorus concentrations; (2) to quantify the differences between spring and fall LSM applications on residual soil N and P concentrations in soil profile and (3) determine the effects on corn and soybean yields under different nutrient application rates at different application timing. A total of 18 plots, each of 0.4 ha, were designed as a complete randomized block design for this study. Three systems selected for this study included: (1) corn-soybean rotation with fall LSM was applied to corn plots only in a two year rotation (CSMF); 2) corn-soybean rotation with fall LSM applied to both corn and soybean plots (CSMB) and (3) corn-soybean rotation with LSM applied in the spring to corn plots only in a two year rotation (CSMS). Averaged nitrogen application rates for the LSM treatment in the CSMB system were 174 and 222 kg ha^{-1} for corn and soybean plots, respectively. Whereas, nitrogen application rates for the LSM treatment averaged 176 and 177 kg ha^{-1} to the corn crop only in CSMF and CSMS systems, respectively. Field data were collected on Bray-P_1 phosphorus (BP_1) and nitrate-nitrogen (NO_3-N) concentrations for the soil depths from top to 120 cm depths at different increment 0-15, 15-30, 30-60, 60-90 and 90-120 cm. The results of this study showed that manure applied to corn and soybean plots clearly had significant effects on both and BP_1 and NO_3-N concentrations in the top 30 cm of soil depth in comparison with manure applied to the corn crop only, regardless of fall or spring applications ($p<0.05$). On the average over the six year study period, a total amount of 289.8 kg ha^1 of BP_1 was accumulated in the 120 cm soil depth which was 55% more in comparison to plots where manure was applied to corn in the fall and 151% more with plots where manure was applied to corn in the spring. A similar pattern was found for residual soil NO_3-N content which accumulated to 129.8 kg ha^{-1} in 120 cm soil depth of CSMB, and was about 37% and 41% more than CSMF and CSMS, respectively. Fall manure application to the corn crop resulted in significantly higher BP_1 concentration in the top 15 cm of soil in comparison with spring manure application. No significant difference was found in residual soil NO_3-N concentrations between the fall and spring applications of swine manure. Also, no significant differences were found among the three treatments on corn and soybean yields. Though, the yields of the CSMB system were generally higher than that of the others, the soybean yields of CSMB treatment were about 4% and 8% higher than the CSMF and CSMS treatments systems.

Key Words: Corn-soybean crop, Liquid swine manure, NO_3-N and BP_1 concentration, Soil test.

3.2 Introduction

Liquid swine manure (LSM), produced by many confined livestock operations in the USA, has traditionally been applied to crop lands to replace chemical fertilizer since LSM provides essential nutrients such as nitrogen (N), phosphorus (P) and potassium (K) and some other micro nutrients (Gilley and Risse, 2000; Chen and Samson, 2002;Sims et al., 1998).In Iowa, the total number of pigs has been reported to increase from 15,486,531 in 2002 to 19,295,092 in 2007 (USDA, 2009). The amount of manure produced by growing/finishing swine is between 3.3-6.6 kg day^{-1} per head (MWPS, 2004). Thus, the higher numbers of swine produced in Iowa have resulted in the larger amount of animal waste produced, requiring good manure disposal and management practices on swine producing farms.

Corn and soybeans are the two major crops grown in Iowa which account for 85% of Iowa crop land use (ISU, 2011). The total area growing both crops has been continuously increasing from app. 8,872,005 to 8,982,036 ha from 2002 to 2007, respectively and over 9,112,000 ha in 2010 (USDA, 2009; ISU, 2011). Increasing of corn and soybean land eventually leads to a higher demand for nutrients from manure and/or commercial fertilizers. The LSM traditionally has been used on the corn crop only. Recently, its use has increased on the soybean crop as a way of animal waste disposal in nearby crop lands to avoid hauling costs, not for the purpose of increasing soybean yields (Daverede et al., 2004; Schmidt et al., 2000). As a result, long-term excessive application of LSM is likely increase the risk of N and P residual in soil, causing a potentially higher loss of N and P to tile drainage water.

Several studies have reported that higher application rates of LSM to corn under corn and soybean rotation resulted in increasing concentrations of both NO_3-N and PO_4-P in subsurface drainage water (Algoazany et al., 2007; Bakhsh et al., 2005; Bakhsh et al., 2009; Hoang et al., 2010). An overloading of nitrate-nitrogen (NO_3-N) and dissolved phosphorus (PO_4-P) from subsurface drainage water have been linked to surface water problems such as the hypoxia case in the Gulf of Mexico (Rabalais et al., 2001; Rabalais et al., 2002, Sylvan et al. 2006).The N and P concentrations in tile drainage water are closely linked to the amount of available N and P in the soil. A high P adsorption was found in soil with high amount of clay, Al and Fe oxides, $CaCO_3$, especially in the few centimeters of top soil (Ahuja, 1986; Pierzynski et al., 2000). Soil P in the top soil might be above the optimum level in many crop lands as 52% of soil samples collected from an agricultural watershed in Pennsylvania had concentrations of soil P above the levels sufficient for producing optimum crop yields (Sharpley et al., 1999). Weed and Kanwar (1996) found that NO_3-N concentrations in tile drainage water were at the highest values at the time when soil residual NO_3-N concentrations were also largest in the soil. House et al. (1998) reported that the major source of P transported to surface water was from the soil. Similarly, Sims (2000b) stated that there was still a possibility of water pollution due to a high amount of soil loss from erosion even when soils are at optimum or lower soil P concentrations. High correlation ($r^2 = 0.96$) between P content in the soil and soil loss by runoff was already reported by Vaithiyanathan and Correll (1992).

Smiths et al., (2000) in their study of soybean yields's response to LSM conducted for two years in Minnesota found that the amount of N in 0-120 cm of soil depth ranged from 80 to 150 kg-N ha^{-1} if application rates of either manure or fertilizer were more than N crop requirements. No findings were reported in terms of P residual in soil. Significant NO_3-N leaching losses were found in the fine sandy loam soil when manure was applied in the fall in comparison to spring applications (Jayasundara et al., 2010).

Few studies have reported the effects of LSM applied to soybean crop yields. About 1.4kg of soybean yield increased per 1 kg of N-manure application rate added at three of the seven areas studied in Minnesota (Smiths et al., 2001); 4% of yield increased in soybeans with manure applied vs. soybeans without manure applied (Bakhsh et al., 2009). Soil test P level was found to range between 30-50 mg kg^{-1} to obtain optimum relative yield (Sims, 2000b). In Iowa particularly, to obtain an optimum crop production of both corn and soybean, phosphorus concentrations in subsoil were recommended to be maintained at 16-20 mg kg^{-1} for Bray-P_1 (BP_1) and Melich-3P (M-3P) and 11-14 mg kg^{-1} for Olsen P (OP). These values are equivalent to the amounts of 61.6 and 44.8 kg ha^{-1} of P_2O_5 needed for corn and to soybean crops, respectively (Sawyer et al., 2002). Mallarino et al. (2010) showed that yields of corn and soybean crops increased when the soil test BP_1 was below a low level (<16 mg kg^{-1}) or at an optimum level. And, no higher response in yields was noted beyond those values.

In general, soybean yield did increase when manure was applied to the soybean crop, but this increase was not consistent and this yield increase depended on many other factors (Randall and Schmidt, 1998). But the potential risk of soil N residual and P buildup in the soil is there. So the interesting questions of what are the impacts on residual soil N and P values when long-term LSM was applied to both years of a corn-soybean rotation crop and between different LSM application timing (fall vs. spring) should be further investigated. Therefore, the specific objectives of this study conducted from 2001 to 2006 were: (1) to investigate the effect of liquid swine manure applied to both corn and soybeans on N and P residual in the soil; (2) to evaluate the differences between spring and fall LSM applications on N and P residual in the soil, and (3) to determine the impacts on corn and soybean yields under different LSM application rates at different application timings.

3.3 Materials and Methods

3.3.1 Site description

The field study was conducted at Iowa State University's northeastern research center near Nashua, Iowa, USA. Three dominant soil types across this study site are: Floyd loam (fine-loamy, mixed, mesic Aquic Hapludolls), Kenyon loam (fine-loamy, mixed, mesic Typic Hapludolls), and Readlyn loam (fine-loamy, mixed, mesic Aquic Hapludolls). These soils have 3 to 4 % organic matter and belong to the Kenyon-Clyde-Floyd soil association that is moderately well to poorly drained.

The study site has thirty-six 0.4 ha plots (58.5 by 67 m), with fully documented tillage and cropping records for more than 30 years. The site lies over a high fluctuation of water table conditions, from 20 to 160 cm, and therefore requires a subsurface drainage system to maintain high crop productivity. A subsurface drainage system was installed in 1979 at 1.2 m depth and with tile spacing of 28.5 m apart. Tile drain water from the central tile line of each plot was collected and measured for water quality purposes, using a flow meter installed in each independent sump at the end of the plot. Long-term weather conditions for this site were based on weather station in Charles City, Iowa, about 16km from the research site. More details of site description and subsurface drainage water monitoring systems were described by Kanwar et al. (1997, 1999).

The study was conducted from 1999 to 2006 using a randomized complete block design with three replications. Data used in this paper, however, covers only 6 years from

2001 through 2006 as we excluded data for years 1999 and 2000, which were considered a transition period due to previous treatment history (previous treatment included continuous corn and higher numbers of no-tillage plots in the research site). Eighteen out of thirty six plots at the research site were assigned in this study for three different systems, named: (1) corn-soybean rotation initiated with corn in 2006 with fall liquid swine manure applied to the corn year only (CSMF); (2) corn-soybean rotation with fall liquid swine manure applied to both corn and soybean plots (CSMB); and (3) corn-soybean with liquid swine manure applied in the spring to corn plots only (CSMS). Thus, each system included 6 plots, 3 for corn and 3 for soybeans, in a single year.

3.3.2 Agronomic management

Liquid swine manure, taken from a growing-finishing confinement facility, was applied to corn plots for treatments to achieve the N application rate of 168 kg-N ha^{-1} And, LSM was applied to soybeans to achieve the N application rate of 225 kg-N ha^{-1} for the CSMB treatment only. However, due to the variability of N in liquid swine manure, the actual N rates of LSM applied in the field varied from year to year (Karlen et al., 2004). Details on the amounts of manure applied to each system are presented in table 3.1. Corn plots of CSMS system was injected with LSM early in the spring (in late march or in early April) before corn planting. Corn variety of NK 45-T5[1] or DK C53-07 (Monsanto Corp., St. Louis, MO) and soybean variety of Asgrow 2105[1] (year 2002-2005); Kruger 2525 (year 2001) or DEKALB B22-22-52RR (year 2006) were planted at the rate of 80,160 and of 494, 000 seeds ha^{-1}, respectively. More detail on agronomic field activities during the entire sixyear period are presented in table 3.2.

3.3.3 Soil sampling

Soil samples were taken during the six years of the study, from 2001 to 2006. Every year after crop was harvested three soil cores were collected randomly from the inner quarter of each plot to minimize any effect of plot edges. Soil cores were 22 mm (0.875 inches) in diameter and 120-cm long, and were taken by using the hydraulic cylinder soil core machine (also known as the Gidding's probe). Those soil cores were then divided into 5 different sections, representing 0- to15-, 15- to 30-, 30- to 60-, 60- to 90-, and 90- to 120 cm depths. Three soil sections of the same depths within the same plot were then composited to make one final set of soil samples for each soil depth. These samples were immediately frozen and were kept in the freezer until analyzed to avoid the reduction of any N loss due to microbial activities.

3.3.4 Soil test P and N analyses

Soil tests for P of all samples at different depths were conducted in duplicate for Bray-P_1 in every year. In year 2006, the last year of the study period, the soil samples were analyzed for three types of agronomic soil test for P including Bray-P_1, Mehlich-3 extractable P and Olsen-P. Soil samples were analyzed for P concentrations by following the procedures recommended and described for the North-Central Region of the USA (Brown, 1998; Frank et al., 1998; M-3P, Mehlich, 1984).

For the BP test, 1 g of soil was extracted with 10 mL of ammonium fluoride (0.03 M NH_4F) and hydrochloric acid (0.025 M HCl). For the M-3P, 1 g of soil was extracted with 10 mL of acetic acid (0.2 M CH_3COOH), ammonium nitrate (0.25 M NH_4NO_3), 0.015 M NH_4F, nitric acid (0.013 M HNO_3), and 0.001 M EDTA. For the OP test, 1 g of soil was extracted with 20mL of sodium hydrocarbonic oxyltosion (0.5 M $NAHCO_3$ at pH = 8.5). The BP_1 and M-3P tests were shaken for 5 minutes whereas 30 minutes was required for the OP tests by using the shaker with a capability of 200 excursions per minute (Brown, 1998). All extracts were filtered through a Whatman No. 42 filter paper and P was determined colorimetrically by the Murphy and Riley method (1962).

For residual soil NO_3-N concentrations, 10 g of the soil was extracted with 50 mL of 2 M KCl solution by shaking for 5 minutes. The suspension was filtered through Whatman No. 40 filter paper and the extract analyzed for NO_3–N using a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI) (Gelderman and Beegle, 1998).

3.3.5 Soil water solution sampling

Data were analyzed for each individual year separately and the data for the entire six year study period was combined to test the significance of different LSM rates and timing. Differences in residual P and N concentrations in the soil were examined at different depths. PROC MIXED procedure in SAS package version 9.2.2 for windows was performed to analyze data (SAS Institute Inc., 2008) at the $p \leq 0.05$. Tukey-Kramar adjusted method was performed in SAS to compare the system means. Separate analysis of variance (ANOVA) tables were constructed for corn and soybean yields. Correlation and linear regression were performed for BP_1, M-3P and OP for the year 2006 to examine the relationship between them. The total BP_1 and NO_3-N concentrations at different soil depth layers were then also converted to units of kg ha^{-1} by using appropriate unit conversions based on the rule of thumb of assuming 2,025,000 kg (about 2 million kg) soil over 15cm soil depth (with a bulk soil density of 1.35 g cm^{-3}).

3.4 Results and Discussion

3.4.1 Effect of manure application rate and timing to soybean crop on residual soil P

Table 3.3 gives an overall summary of the effects of LSM application rates and timings to soybeans on the residual soil concentration of BP_1 at different soil depths. Table 3.4 gives the comparison between means of soil BP_1 concentrations (as only soil BP_1 concentrations were analyzed for all six years of the study) as affected by experimental treatments. Treatment effects were statistically significant on soil BP concentrations ($p<0.01$) for every single year from 2001 to 2006 and for the overall six year average BP soil concentrations for the top 15 cm of soil depth (table 3.3). Also, treatment effects were significant on soil BP concentrations for the top 30 cm of depth for five out of six years (except for year 2002 when p value was equal to 0.45). Below 30 cm of soil depth, the treatment effects did not show any consistent trends and treatment effects were not significant. These results show that it is very important to sample soils for the residual soil BP concentrations in the top 30 cm of soil profile for environmental and production sustainability of soils. Treatment effects were also significant on the sixyear average BP soil concentrations for the top 60 cm of soil depth ($p<0.01$). Treatment effects were not significant on soil BP concentrations below 60 cm of soil depth. The results of this long-term study showed that LSM application rates and timings to soybeans significantly affected the soil phosphorus concentration in the top 60 cm of soil depth. The 15 cm of this soil depth is considered the most critical layer for crop growth as well as the source of P loss with runoff to cause water pollution.

Table 3.4 gives the comparison between means of soil BP_1 concentrations (as only soil BP_1 concentrations were analyzed for all six years of the study) as affected by experimental treatments. In the top 15 cm of soil depth, the lowest BP_1 concentration was observed in the CSMS system, whereas the highest BP values were found in CSMB. Six year average soil BP concentrations for the top 15 cm soil depth ranged from 19.8 to 32.6 mg kg^{-1}; 42.3 to 56.0 mg kg^{-1}, and 74.5 to 97.6 mg kg^{-1} for CSMS, CSMF and CSMB treatments, respectively (table 3.4). In every single year and the overall six year average concentrations of soil BP_1 in the top 15 cm of depth were always the highest concentrations for all three treatments in comparison with soil BP concentrations for lower soil layers. The soil BP_1 concentrations for the CSMB treatments were almost twice the soil BP concentrations for the CSMF treatment and three times the soil BP concentrations for the CSMS treatment. These results clearly show that LSM applied to both corn and soybean crops each year resulted in significantly higher BP_1 concentrations in the soil in comparison with the treatment when LSM was applied to the corn crop only in every other year under the corn-soybean rotation system.

Also, the soil BP_1 concentrations found in this study for all three treatments are very high compared to the optimum value of P recommended for crop land soils in Iowa (Sawyer et al., 2002). The BP_1 soil concentrations in the CSMS treatment (when manure was applied in the spring to corn only) were slightly higher than the optimum BP value recommended for Iowa soils. However, for the other two treatments the soil BP concentrations were two to four times higher than the optimum BP soil concentration value for Iowa soils. These high values of soil BP concentrations are likely to cause water quality problems because of the potential loss of top soil with runoff water carrying soil P (Ahuja, 1986; Allen et al., 2006; Sims, 2000b; Vaithiyanathan and Correll, 1992).

Higher BP_1 soil concentrations found in the CSMF treatment in comparison to with CSMS treatment also indicated that manure applied in the fall, after the harvesting of crops, resulted in a much higher level of BP_1 accumulation in the top 15 cm of soil. Therefore, it is recommended that LSM should be applied in the spring to reduce the potential of higher soil P losses with runoff water.

The BP_1 concentrations in the soil decreased sharply in the 15 to 30 cm of soil depth for all three treatments and soil BP concentrations were lower by about 56.5-76 % in compared with the soil BP concentrations in the top 15 cm soil depth. The BP_1 soil concentrations in the CSMS treatment were below the optimum P value for Iowa soils. The BP_1 soil concentrations in the top 15 cm for the CSMF system ranged from 9.7 to 17.3 mg kg^{-1}, which meets the recommended requirement of P in the soil. From 30-cm to 90-cm soil depth, the BP_1 soil concentrations continuously decreased and ranged somewhere between approximately 2 to 6 mg kg^{-1}. Below the soil depth of 90 cm, the BP_1 concentrations in all systems did not show any significant differences and soil BP concentrations were very low. The majority of BP_1 concentration values for all years were between 5-6 mg kg^{-1}.

Figure 3.1 shows the changes in BP_1 accumulation at different soil depths as affected by treatment. Because the highest concentration of soil BP_1 was found in the CSMB treatment, the vertical BP_1 accumulation in soil for this system always got the highest value in every single year as well as averaged across six years. The estimated amounts of BP_1 accumulation in the top 15 cm of soil depth ranged from 150.9 to 197.6 kg ha^{-1}, 85.7 to 113.64 kg ha^{-1} and from 40.1 to 67.0 kg ha^{-1} for CSMB, CSMF, and CSMS treatments, respectively from 2001-2006 (figure 3.1). The total amounts of BP_1 content in 120-cm of soil depth ranged from 245.2 to 304.2 kg ha^{-1} for CSMB, from 176.4 to 201.9 kg ha^{-1} for CSMF and from 99.9 to 145.2 kg ha^{-1} for CSMS treatment during the six- year study period, when averaged over the six-year period (2001-2006), the total amount of BP_1 content in 120-cm of soil depth for the CSMB system was 289.8 kg ha^{-1}, which is 55.4 % and 151.2 % higher than the amounts of BP_1 content in the CSMF and CSMS systems, respectively. These results clearly showed that when liquid swine manure was applied to both corn and soybean crop every year, it resulted in significantly higher P residual in the soil in comparison with the treatment when manure was applied to the corn crop only. Difference amount of BP_1contents in the soil were also found between fall and spring manure application to corn but differences were not significant.

3.4.2 Relationship between three agronomic soil tests for P

All three types of agronomic soil tests, BP_1, M-3P and OP, were performed and soil samples were analyzed for phosphorus concentrations for the year 2006 only, the last year of the experimental study because the last year of the study gave an overall effect of experimental treatments on soil P accumulation in the top soil layers Thus, correlation and regression of these soil tests were performed for the top 15 cm of soil depth as this layer contains the highest amount of P and plays an important role in providing soil nutrients to crops and also P losses to surface water. These correlation analyses will help to predict the concentrations of other agronomic forms of P since the data were not available for the previous years of study at this research site. In fact, analysis of M-3P could be applied for broader soil ranges in Iowa. The OP and M-3P soil tests were recommended to be more suitable in predicting available P for Iowa soils where soils are in the acid to $CaCO_3$conditions (Mallarino, 1997; Saweyer et al., 2002).

The correlation analyses between amounts of BP_1, M-3P and OP in the soil are presented in figure 3.3. This figure shows strong correlations between the three soil tests for P in all three experimental treatments. Higher correlations were found in two systems with only corn receiving manure in the fall and spring. The R^2 values ranged from 0.93 to 0.96 for

CSMF and CSMS treatments whereas $0.74 < R^2 < 0.83$ was found for the CSMB treatment. This situation happened because pH values for plots having corn in rotation with soybeans receiving manure each year were lowest among all plots used for three treatment systems. As shown in table 3.7, a significant difference in pH values among three treatment systems was observed in 2006. The pH values were 6.1, 6.3 and 6.8 for CSMB, CSMS and CSMF, respectively. Similar results have been reported by Atia and Mallarino (2002).

3.4.3 Soil nitrate residual

A summary of the system effects and mean comparison of NO_3-N concentrations in the 120 cm of soil depth during six years of the study is presented in table 3.5 and table 3.6. The yearly analysis of variance on NO_3-N concentration in the soil showed that system effects varied at different depths and were not consistent over the years. Significant system effects ($p < 0.05$) were found between years 2003 and 2004 and between 2001 and 2004 at soil depths of 15 cm and 30 cm, respectively. The system effects again showed ($p<0.01$) at deeper depths, from 90 to 120 cm, except in 2005 (depth 90 cm) and in 2006 (at depth =120 cm). The differences were closely linked to the nitrate-nitrogen transported to subsurface drainage through tile drain. The NO_3-N flow-weighted concentration and leaching losses in subsurface drainage was found to be increasing by 50% in soybean plots receiving LSM (Bakhsh et al., 2009). Only in year 2004, the system effect was found in all depth layers (table 3.5). This might be the results of a wet condition caused by nearly 900 mm received in 2004, which was the highest among six years of the studies (Hoang et al., 2010). The overall six-year average showed the significant system effects within the 30 cm of the top soil and a soil depth from 90-120 cm.

The concentrations of NO_3-N in the top 15 cm of soil ranged from 11.1 to 30.7; 10.1 to 31.9 and from 15.5 to 32.8 mg kg^{-1} for CSMF, CSMS and CSMB systems, respectively. These are higher than the EPA maximum contaminant level of 10 mg L^{-1} NO_3-N allowable in drinking water (EPA, 1992). Under these concentrations, the amounts of NO_3-N residual in the top 15 cm of were estimated at 22.5 to 62.2 kg ha^{-1} for CSMF; 20.5 to 64.6 kg ha^{-1} for CSMS and 31.4 -66.4 kg $ha^{-1}$for CSMB systems. Those were the largest amounts among five soil layers. The concentrations of NO_3-N gradually declined with increasing of soil depth. At a soil depth of 30 cm, the NO_3-N concentration was reduced from 34 to 69% for CSMF, 31-72% for CSMB and 41-75% for CSMS in comparison with that at the depth of 15 cm(table 3.6). Similar to the situation which happened with P concentrations in the soil; the NO_3-N concentration of the system with manure application each year got the highest values among three systems in almost all depths and in all years of the study period. This is not surprising because higher amounts of manure were applied to the corn and soybeans of this system. These results showed that if manure is applied to soybean plots in every year for the long-term, it probably accelerates the NO_3-N concentrations in soil. Overall across six years of studies, the concentration of NO_3-N showed significantly higher in the CSMB system, which had manure application to both corn and soybean crop in every year. No significant differences of NO_3-N concentration were found between systems had manure applied in the fall vs. in the spring. System receiving manure application to the soil in the fall season, however, likely resulted in a higher concentration of NO_3-N when compared with plots with manure applied in the spring.

The NO_3-N content for the studied depth (0-120 cm) varied from year to year, ranging from 40.7 to 155.1; 35.2 to 156.9 and 70.5 to 178.8 kg ha^{-1} for CSFM, CSMS and

CSMB systems, respectively. Overall the six- year period of study, the NO_3-N content at the soil depth of 120 cm for CSMB was 129.8 kg ha^{-1}; that was 41.5% and 37.6 % higher than that for CSMS and SBMF, respectively. There was not a big difference of NO_3-N residual in 120 cm soil depth between CSMS and CSMF (91.7 vs. 94. 4 kg ha^{-1}, respectively).

3.4.4 Corn and soybean yields

Mean comparisons for corn and soybean yields were performed separately on a yearly basis and across the six years of the study period. These results are presented in table 3.6. The yearly analysis showed that the system effect on corn yields was obtained for year 2006 only. The highest value of corn yield, however, was measured in corn plots with manure applied to corn in the fall season. The yields were 11.33, 12.36 and 12.54 Mg ha^{-1} for CSMS, CSMB and CSMF, respectively. In the year 2004, significant differences in corn yields were observed between CSMS vs. CSMF and CSMB. For all other years, no significant difference was found for the yield of corn plots among the three systems (table 3.8). Overall, six years of the study showed no significant differences on corn grain yield among the three systems. However, corn yield from plots with manure application to both corn and soybeans gave the highest corn yield, followed by corn plots receiving manure in the fall and the lowest corn yield measured in plots receiving manure in the spring.

For soybean crop yield, the treatment effects were not consistent over the six years. The yearly analysis of variance showed the significant system effects for only three out of six years (2001, 2002 and 2005). No significant differences in soybean yields were found for the rest years. However, the soybean plots receiving manure each year always produced the highest yield. The second highest yield was observed in the soybean plot with manure application in the fall and the lowest yield was found for soybean plot receiving manure in the spring. The six year average soybean yields were 3.8; 3.65 and 3.51 Ma ha^{-1} for CSMB, CSMF and CSMS treatments, respectively. Manure application to both corn and soybeans each year showed an increase of 4.1% and 8.2 % in comparison with manure applications to corn only in the the fall and in the spring, respectively. The results of this study clearly shows that even when we applied more liquid swine manure to soybean crop, no significant yield could be obtained while it increased significantly the amount of P and N accumulated in the soil. This potentially could result in a higher loss of dissolved P to runoff water.

3.5 Conclusions

A six-year field experimental study was conducted from 2001 to 2006 at Iowa State University's northeastern research center near Nashua, Iowa to investigate the effects of LSM on corn and soybean yields and the accumulation of nitrogen and phosphorus in the soil. Experimental treatments included both N application rates and timings of applications of liquid swine manure to corn and soybeans. In this study, treatment of LSM was applied to both corn and soybeans each year was compared to when LSM was applied to corn crop only in the corn-soybean rotation system. And, the effects of LSM application to corn only in the fall and in the spring were investigated on corn yields and residual soil N and P accumulations. Data on BP_1 and NO_3-N concentrations in soil depths of 0-120 cm were collected for these treatments and analyzed for each of the six years of this study. The following conclusions were drawn from this study.

Corn and soybean crops receiving LSM in the fall of each year resulted in significantly higher concentration of BP_1 and NO_3-N in the top 0-15 cm and 15-30 cm of soil depths as a result of increased applications of N and P from manure compared to LSM applications to the corn crop only every other year in the corn-soybean production system.

Total accumulation of BP_1 contents in the top 15 cm of soil from the CSMB treatment (when LSM was applied to both corn and soybeans) were 55 % higher in comparison with CSMF (when LSM was applied to corn only in the fall) and 151% higher in comparison with CSMS treatment (when LSM was applied to corn only in the spring). The BP_1 concentrations in the top 15 cm of soil depth were 3 to 4 times higher than optimum BP_1 values recommended for Iowa soils.

Liquid swine manure applied to the corn crop in the spring resulted in significantly lower BP_1 concentration in the top 15 cm of soil depth in comparison with the fall manure application. No significant differences were found in the residual NO_3-N concentrations in the soil between fall and spring manure application at any soil depths although spring applications resulted in lower soil NO_3-N concentrations in comparison with fall manure applications.

The LSM applications to soybeans crop did not result in significantly higher crop yields in comparison with fall and spring manure applications to the corn crop only. At the same time, LSM applications to soybeans increased residual P and N concentrations in the soil, therefore, fall application of LSM at the rate of 225 kg-N/ha is not a good practice because of the potential of increased N and P losses to surface and ground water systems.

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Table 3.1. Actual liquid swine manure application rates at research site (kg ha^{-1}) for six years

Systems		Years						
		2001	2002	2003	2004	2005	2006	Average
CSMF	PAN	171	220	141	172	181	172	176
	P_2O_5	169	162	71	100	75	95	112
	K_2O	180	175	111	129	118	105	136
CSMB (corn phase)	PAN	172	212	148	153	183	177	174
	P_2O_5	402	161	66	72	67	99	144
	K_2O	178	172	114	128	119	110	137
CSMB (soybean phase)	PAN	223	260	176	227	211	233	222
	P_2O_5	233	187	80	130	83	140	142
	K_2O	237	214	153	161	138	125	172
CSMS	PAN	123	224	213	_ _	148	_ _	177
	P_2O_5	101	95	129	_ _	147	_ _	118
	K_2O	146	153	136	_ _	110	_ _	136

PAN= potentially available nitrogen
CSMF: Corn and soybeans rotation with fall application of LSM to corn phase only.
CSMB: Corn and soybeans rotation with fall application of LSM to both corn and soybean phases
CSMS: Corn-soybean rotation with spring application of LSM to corn phase only.

Table 3.2. Timings of various agronomic activities (planting, harvesting, LSM applications etc.) at the research site for six years

Agronomic management	2001	2002	2003	2004	2005	2006
Spring liquid swine manure application	27-Apr	29-Mar	02-Apr	5-Apr	5-May	5-Apr
Field cultivation for corn plots	22-Jun	__	17-Jun	15-Jun	5-May	21-Apr
Field cultivation for soybean plots	18-May	14-May	25-Apr	6-May	5-May	9-May
Corn planting	19-May	07-May	26-Apr	24-Apr	28-Apr	23-Apr
Soybean planting	18-May	15-May	20-May	7-May	5-May	10-May
Corn harvesting date	12-Oct	07-Oct	23-Sep	15-Oct	6-Oct	20-Oct
Soybean harvesting date	03-Oct	10-Oct	25-Sep	25-Sep	20-Sep	3-Oct
Soil sampling	31-Oct	28-Oct	13-Oct	25-Oct	18-Oct	25-Oct
Fall liquid swine manure application[a]	1-Nov-00	7-Nov-01	5-Nov-02	21-Oct-03	9-Nov-04	30-Oct-05
Primary chisel plow for cornstalk plots	__	13-Nov	07-Nov	17-Nov	11-Nov	__
Corn variety	NK45-T5	NK45-T5	NK45-T5	NK45-T5	NK45-T5	DK C53-07
Soybean variety	KRUGER 2525	ASGROW 2103	ASGROW 2105	ASGROW 2105	ASGROW 2106	DEKALB B22-52RR

[a] LSM was applied in the previous fall preparing for the next crop year.

Table 3.3. ANOVA for soil phosphorus test on yearly basis and average across years

Sources of variation	df	Probability values						
		2001	2002	2003	2004	2005	2006	Average
15 cm depth								
Block	2	0.74	0.90	0.73	0.62	0.07	0.34	0.53
Sys	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Rot	1	0.48	0.30	0.31	<0.01	0.50	<0.01	<0.01
Sys*Rot	2	0.04	0.25	0.04	0.07	0.13	0.04	0.19
30 cm depth								
Block	2	0.71	0.63	0.46	0.23	0.23	0.70	0.61
Sys	2	0.03	0.45	<0.01	0.02	<0.01	<0.01	<0.01
Rot	1	0.10	0.17	<0.01	<0.01	0.04	<0.01	0.53
Sys*Rot	2	0.55	0.88	<0.01	<0.03	0.04	0.02	0.6
60 cm depth								
Block	2	0.70	0.08	0.47	0.41	0.52	0.80	0.29
Sys	2	0.27	0.04	<0.01	0.59	0.26	<0.01	<0.01
Rot	1	0.16	0.90	0.16	0.63	0.88	0.69	0.87
Sys*Rot	2	0.02	0.47	<0.01	0.34	0.27	0.69	0.24
90 cm depth								
Block	2	0.71	0.64	0.22	0.46	0.68	0.51	0.3
Sys	2	0.27	0.03	<0.01	0.42	0.81	0.35	0.09
Rot	1	0.16	0.93	0.35	0.33	0.17	0.59	0.58
Sys*Rot	2	0.02	<0.01	<0.01	0.55	0.41	0.20	0.47
120 cm depth								
Block	2	0.51	0.21	0.30	0.34	0.92	0.24	0.51
Sys	2	0.34	0.24	0.60	0.58	0.37	0.97	0.15
Rot	1	0.25	0.08	0.41	0.42	0.21	0.90	0.98
Sys*Rot	2	<0.01	0.20	<0.01	0.90	0.11	<0.01	0.21

Table 3.4. Means comparison of Bray-P_1 concentrations for individual year and averaged across six years (mg kg^{-1})

Systems	Years*						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
------15 cm depth------							
CSMF	51.3 b	42.3 b	56.0 b	49.0 b	45.1 b	51.8 b	49.3 b
CSMB	75.5 a	96.8 a	91.8 a	74.5 a	90.9 a	97.6 a	88.1 a
CSMS	19.8 c	25.1 b	25.5 c	25.7 b	32.6 c	33.1 c	27.0 c
------30 cm depth------							
CSMF	9.7 b	21.9 a	17.3 b	13.0 ab	15.6 b	11.9 b	14.8 b
CSMB	18.0 a	20.3 a	26.5 a	19.8 a	28.2 a	23.4 a	22.8 a
CSMS	8.7 b	9.3 a	9.2 b	9.5 b	11.1 b	9.0 b	9.5 b
------60 cm depth------							
CSMF	3.5 a	3.6 a	3.5 a	3.2 a	5.5 a	3.3 b	3.8 a
CSMB	4.2 a	3.4 a	4.3 a	2.9 a	6.0 a	4.8 a	4.3 a
CSMS	2.3 a	2.1 b	2.8 b	2.3 a	3.8 a	2.7 b	2.6 b
------90 cm depth------							
CSMF	4.3 a	3.0 ab	3.6 ab	3.5 a	4.4 a	3.1 a	3.6 a
CSMB	4.6 a	4.3 a	4.8 a	13.3 a	3.8 a	4.8 a	6.0 a
CSMS	2.9 a	1.9 b	2.6 b	2.1 a	4.0 a	3.2 a	2.8 a
------120 cm depth------							
CSMF	6.5 a	6.7 a	5.9 a	5.9 a	9.6 a	5.3 a	6.6 a
CSMB	5.0 a	7.9 a	6.2 a	5.9 a	5.0 a	5.0 a	5.8 a
CSMS	5.2 a	4.2 a	5.1 a	3.3 a	6.2 a	5.1 a	4.8 a

* Means within years and on average (i.e., within column) followed the same letters are not significantly different at *p*=0.05

Table 3.5. ANOVA for residual soil NO_3-N on an yearly basis and averaged across 6 years

Source of variation	df	Probability values						
		2001	2002	2003	2004	2005	2006	Average
--------15 cm depth--------								
Block	2	0.27	0.63	0.06	0.56	0.51	0.61	0.86
Sys	2	0.16	0.08	<0.01	0.02	0.22	0.93	0.02
Rot	1	0.37	0.02	<0.01	<0.01	0.68	0.03	<0.01
Sys*Rot	2	0.64	0.31	0.03	0.10	0.16	0.86	0.75
--------30 cm depth---------								
Block	2	0.02	0.06	0.27	0.22	0.54	0.81	0.73
Sys	2	<0.01	0.12	0.14	<0.01	0.54	0.07	0.02
Rot	1	0.01	0.34	0.53	<0.01	0.09	0.40	0.46
Sys*Rot	2	0.40	0.74	<0.01	0.19	0.57	0.99	0.36
--------60 cm depth---------								
Block	2	0.77	0.38	0.33	0.37	0.84	0.25	0.95
Sys	2	0.12	0.08	0.88	0.01	0.59	0.40	0.63
Rot	1	0.77	0.94	0.40	<0.01	0.38	0.16	0.87
Sys*Rot	2	0.98	0.29	0.04	0.01	0.27	0.01	0.37
--------90 cm depth---------								
Block	2	0.76	0.36	0.10	0.61	0.55	0.73	0.27
Sys	2	<0.01	0.02	<0.01	<0.01	0.16	0.04	<0.01
Rot	1	0.64	0.29	0.13	<0.01	0.41	0.11	0.95
Sys*Rot	2	0.52	0.35	<0.01	0.09	0.38	0.25	0.76
--------120 cm depth---------								
Block	2	0.19	0.41	0.46	0.30	0.93	0.78	0.15
Sys	2	0.01	<0.01	<0.01	<0.01	<0.01	0.41	<0.01
Rot	1	0.70	0.46	0.78	0.90	0.08	0.77	0.36
Sys*Rot	2	0.46	0.79	0.08	0.83	0.32	0.44	0.94

Table 3.6. Means comparison of NO_3-N concentration for individual years and average across six years (mg kg^{-1})

Systems	Years[*]						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
----15 cm depth----							
CSMF	16.5 a	14.3 a	18.4 b	11.1 b	19.2 a	30.7 a	18.4 b
CSMB	18.8 a	20.4 a	27.1 a	15.5 a	27.1 a	32.8 a	23.9 a
CSMS	14.9 a	22.0 a	15.4 b	10.1 b	24.5 a	31.9 a	19.9 ab
----30 cm depth----							
CSMF	8.4 ab	9.4 a	6.1 a	3.4 ab	11.1 a	12.1 a	8.4 ab
CSMB	10.7 a	14.1 a	7.7 a	4.3 a	11.9 a	14.7 a	10.6 a
CSMS	6.8 b	11.2 a	5.5 a	2.5 b	10.4 a	11.6 a	8.0 b
----60 cm depth----							
CSMF	4.7 a	6.2 a	2.0 a	1.0 b	6.3 a	8.2 a	4.7 a
CSMB	5.1 a	7.2 a	2.2 a	1.4 a	8.1 a	8.7 a	5.5 a
CSMS	3.4 a	4.5 a	1.9 a	0.8 b	10.0 a	7.5 a	4.7 a
----90 cm depth----							
CSMF	3.4 b	3.4 b	1.3 b	0.5 b	3.9 a	4.9 ab	2.9 b
CSMB	4.3 a	7.1 a	2.3 a	1.7 a	6.0 a	6.9 a	4.7 a
CSMS	1.5 b	2.9 b	1.1 b	0.5 b	3.3 a	4.4 b	2.3 b
----120 cm depth----							
CSMF	1.3 a	2.4 b	1.9 b	1.3 b	3.0 b	3.8 a	2.3 b
CSMB	2.3 a	5.8 a	4.2 a	4.4 a	5.8 a	4.8 a	4.6 a
CSMS	0.0 b	1.5 b	1.4 b	1.1 b	2.8 b	5.1 a	1.7 b

[*] Means within years and on average (*i.e.*, within column) followed the same letters are not significantly different at p=0.05

Table 3.7. Means comparison of pH for annual year and averaged across 6 years

Systems	Years*						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
---15 cm depth---							
CSMF	6.9 b	6.9 a	6.7 a	6.8 a	6.5 a	6.6 a	6.8 a
CSMB	6.2 a	6.3 b	6.0 b	6.1 b	5.9 ab	5.9 b	6.1 c
CSMS	6.4 b	6.6 b	6.2 b	6.3 b	6.3 b	6.2 b	6.3 b
---30 cm depth---							
CSMF	6.5 a	6.5 a	6.6 a	6.5 a	6.3 a	6.6 a	6.5 a
CSMB	6.1 a	6.2 ab	6.0 b	6.2 b	6.1 a	6.2 b	6.1 b
CSMS	6.5 a	6.2 b	6.2 b	6.2 b	6.3 a	6.2 b	6.3 b
---60 cm depth---							
CSMF	6.5 a	6.2 a	6.7 a	6.7 a	6.1 a	6.6 a	6.5 a
CSMB	6.3 a	6.2 a	6.4 a	6.4 ab	6.3 a	6.4 a	6.3 a
CSMS	6.5 a	6.3 a	6.4 a	6.3 b	6.3 a	6.4 a	6.3 a
---90 cm depth---							
CSMF	6.6 a	6.4 a	6.6 a	6.9 a	6.3 a	6.9 a	6.6 a
CSMB	6.5 a	6.5 a	6.6 a	6.5 b	6.5 a	6.6 ab	6.5 a
CSMS	6.7 a	6.5 a	6.6 a	6.7 ab	6.5 a	6.7 b	6.6 a
---120 cm depth---							
CSMF	7.2 a	7.2 a	7.1 a	7.4 a	6.9 a	7.6 a	7.2 a
CSMB	7.6 a	7.6 a	7.6 a	7.5 a	7.0 a	7.6 a	7.5 a
CSMS	7.5 a	7.4 a	7.2 a	7.2 a	7.2 a	7.4 a	7.3 a

* Means within years and on average (*i.e.*, within column) followed the same letters are not significantly different at p=0.05

Table 3.8. Means of corn-soybean yields (Mg ha^{-1}) for six years

Systems	Years*						Average
	2001	2002	2003	2004	2005	2006	(2001-2006)
Corn yield							
CSMF	11.09 a	12.21 a	10.21 a	12.29 ab	11.99 a	12.54 a	11.72 a
CSMB	11.34 a	12.17 a	10.51 a	12.74 a	12.44 a	12.36 b	11.93 a
CSMS	10.59 a	12.04 a	9.86 a	11.61 b	12.12 a	11.78 c	11.33 a
Soybean yield							
CSMF	3.45 b	3.75 b	1.92 a	4.00 a	4.62 b	4.18 a	3.65 a
CSMB	3.79 a	3.99 a	1.90 a	3.76 a	4.97 a	4.38 a	3.80 a
CSMS	2.98 c	3.55 c	1.91 a	3.74 a	4.65 b	4.23 a	3.51 a

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[*] Means within years and on average (i.e., within column) followed the same letters are not significantly different at *p*=0.05

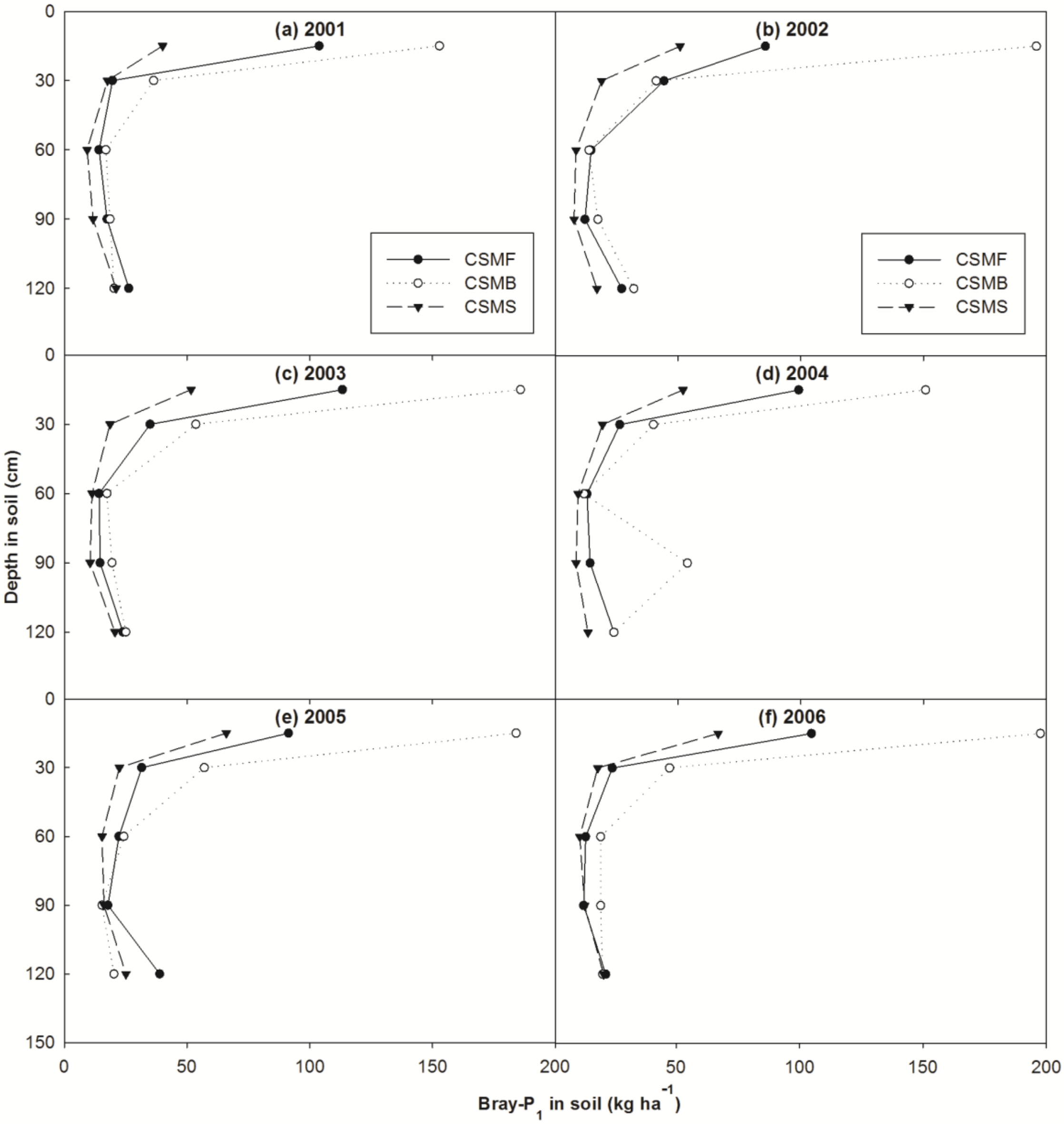

Figure 3.1. Vertical Bray-P1 accumulation as a function of soil depth at the time of harvest for six years.

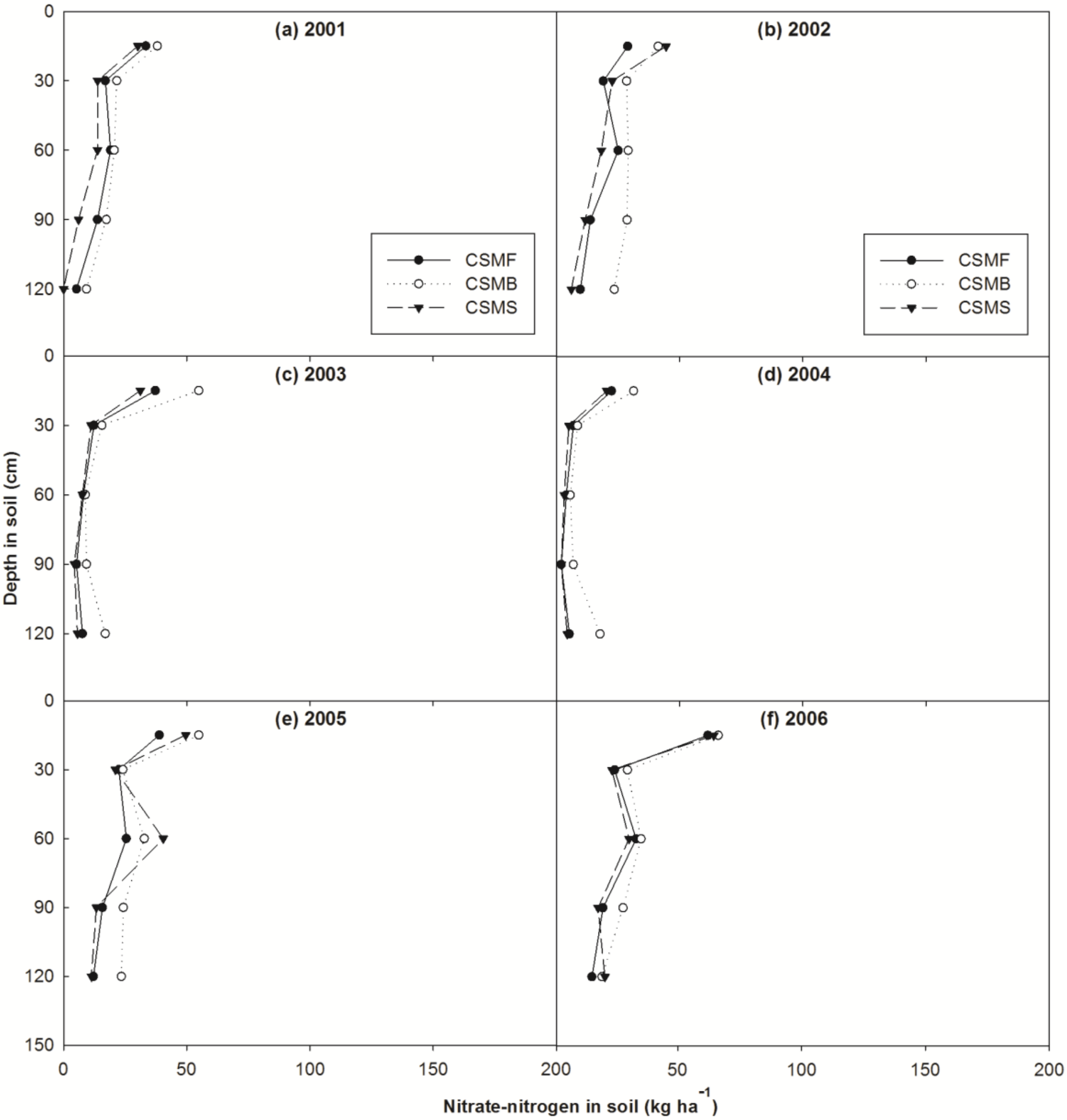

Figure 3.2. Vertical soil residual of NO3-N in the different depths after crop harvested.

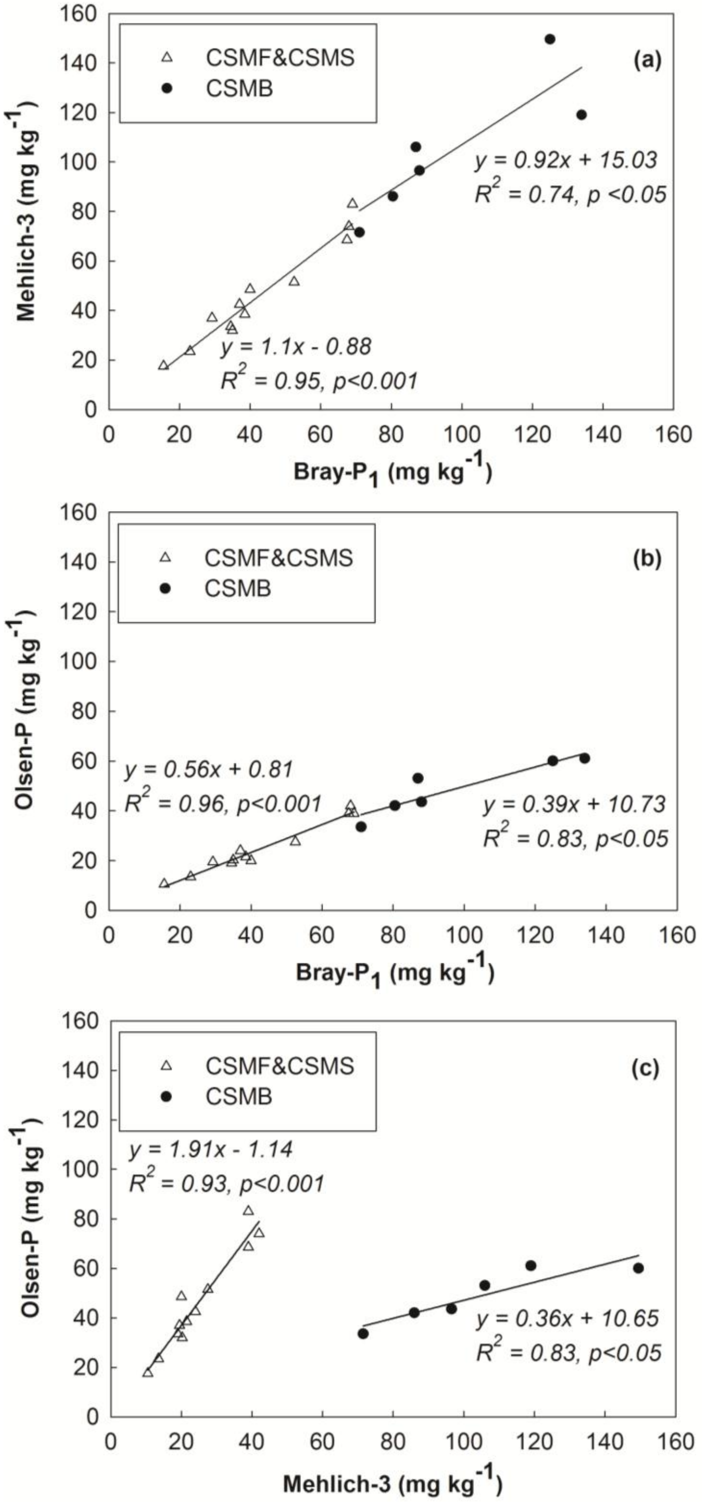

Figure 3.3. Regression correlations between (a) Bray-P$_1$ and Mehlich-3 P, (b) Bray-P$_1$ and Olsen P, and (c) Mehlich-3e and Olsen-P.

CHAPTER 4. THE ROLE OF DIRECTLY CONNECTED MACROPORES ON *E. coli* TRANSPORT TO SUBSURFACE DRAINAGE WATER

A paper to be submitted to the Journal of Environmental Quality

4.1. Abstract

Better understanding is needed on the mechanism of pathogen transport through soils to shallow groundwater for minimizing water pollution. The prevalence of directly connected macropores (DCM) in the soil can significantly increase pathogen concentration in subsurface drainage water immediately after the application of liquid swine manure (LSM) to the croplands. Four field experiments conducted at Iowa State University's Northeast Research Center near Nashua, Iowa with specific objectives were: (1) to identify and quantify the number of macropores between no-till and chisel plow practices by using the smoke test; and (2) to investigate the role of directly connected macropores in transporting *E. coli* to subsurface drainage water under no-till and chisel plow condition after the application of LSM. Two field experiments were conducted in each 2008 and 2009 under no-till and chisel plow plots. A smoke test was conducted at the beginning of each experiment to identify and quantify a number of macropores in the field. LSM was applied at the rate of 169 kg ha^{-1} and artificial rainfall was simulated after that. The overall results of this study indicated that no significant differences were observed in the total number of macropores found between no-till and chisel plow tillage practice at level of p=0.05. However, the results from smoke tests did show that higher numbers of macropores were present in no-till plots where soil was not disturbed during planting and no field cultivation was done. Time to get peak *E. coli* concentration in tile water were 1.62 vs. 2.66 hr. after rainfall simulation started in plot with DCM and without DCM, respectively, in experiments conducted in spring 2008.

In fall 2009, the times for peak *E. coli* concentration were 0.74 for plot with DCM and 0.97 hr. for plot without DCM. The results showed a relationship between the DCM and rapid transportation of water and *E. coli* from soil surface to subsurface drainage water through those macropores.

Keywords: Breakthrough curve, *E. coli,* Liquid swine manure, Macropores, Rainfall, Subsurface drainage water.

4.2. Introduction

Water movement through preferential flow and macropores in soil is an interesting research topic for soil physicists and soil and water engineers. Macropores are formed in many ways and can be categorized in two major groups: natural ways (*i.e.*, soil shrinkage by drying, cycles of freezing and thawing, chemical weathering, and subsurface erosion channels) and biological ways (*i.e.*, worm holes, decaying of plant roots, tunneling insects, and moving nematodes) (Beven and Germann, 1982; Brewer, 1964; Blake et al., 1973; McMahon and Christy 2000; Priebe and Balckmer, 1989). Luxmoore (1981) quantitatively classified soil porosity based on its capillary potential (ψ), in which macropores were defined at ψ > -0.3 kPa. McMahon and Christy (2000) categorized the sizes of soil pores into three main groups based on diameter: macropores > 100 µm, mesopores 30-100 µm and micropores <60 µm. Other researchers found that the size of macropores could be as small as 60 µm or up to 3000 µm depending on the method used for quantifications (Beven and Germann, 1981; Bullock and Thomasson, 1979; Edwards et al., 1988; and FitzPatrick et al., 1985).

A number of macropores and soil macroporosity vary with soil types and soil depths. The estimated volume of macropores varies from 0.23% to 5% of total soil volume (Alaoui and Helbling. 2006; Bouma et al., 1979; Douglas. 1986; Munyankusi. 1994). Proportions of large and small pores in different layers of the soil were at an inversion with soil depth (Assouline et al., 1997).Tillage practices have shown significant effects on sizes and numbers of macropores in the field. (Alaoui and Goetz, 2008; Miller et al., 1998; Schijønning and Rasmussen. 2000). Singh et al. (1991), by using the resin impregnation and image analysis technique to measure the area occupied by macropores, found that lower areas of macropores were estimated in deeper layers of Nicollet loam soil (fine-loamy, mixed, mesic Aquic Hapludoll) under both no-till and conventional tillage systems. And, the tillage systems (no-till vs. conventional tillage) did not show significant different volumes of macropores.

Several techniques have been developed to quantify a number of macropores and to estimate the size of macropores. Those techniques are mainly divided in two groups: (1) indirect approach and (2) direct approach. Indirect techniques are basically based on measuring flow characteristics like breakthrough curves and the use of a tension infiltrometer or infiltration rate to determine soil hydraulic properties of the pores (Everts and Kanwar, 1990; Schmidt and Lin, 2008; Smith et al., 1985). The direct approach estimates the number and size of macropores by using a resin impregnated through the soil column, photographs and image analysis, computed tomography (CT) X-ray, and digital radiography for different soil conditions (Alaoui and Goetz, 2008; Hurbet et al., 2007; Luo et al., 2008; Luo et al., 2010; Ranchman et al, 2005; Singh et al., 1991; Warner et al., 1989). Blowing smoke through sewage pipes for detecting leaks or faulty connections were used in municipalities. Shipitalo and Gibbs (2000) use this smoke technique to identify the burrow, created by earthworm, and to determine the effect of those in transporting waste in tile line. To date few studies have reported the use of smoke test technique to identify and quantify the number of macropores in the fields having subsurface drainage system installed.

Liquid swine manure (LSM) application to agricultural land contributes to the nonpoint source pollution by releasing microbial pathogens including bacteria, virus and protozoa, through runoff and subsurface drainage water.(Baxter-Porter and Gilliland, 1988; Thiagaranjan et al., 2007). *Escherichia coli* (*E. coli*) has been detected at concentrations of 10^7 to 10^9 organisms per gram in feces (Gyles, 2004). *E. coli* can survive in the soil from several weeks to 120 days, and prolonged if manure was present (Gagliardi ad Karns, 2000; Hutchison et al., 2004; Nwachuku and Gerba, 2008; Sørensen et al., 1999). In subsurface drainage water, *E. coli* survived up to two months since the application of manure to the soil (Gagliardi and Karns, 2002). Recreational lake waters were found as the primary sources that caused waterborne disease outbreaks due to *E. coli* O157:H7 (Bruce et al., 2003). Up to five percent of those outbreaks occurred in the period 1991-2002(Chapman et al., 1997; Craun, 1991; Craun et al., 2006; Goss et al., 1998; Lee et al., 2002). For safety, the total coliforms (including fecal coliform and *E. coli*) were set at the goal of zero presence for drinking water (USEPA, 2003).Several studies have shown that bacteria could be more easily transported through undisturbed soil and/or no-tillage than in the soil with tillage practices (Abu-Ashour et al., 1998; Gagliardi and Karns. 2000; Stoddard et al., 1998; Thiagaranjan et al., 2007; Wang et al., 2000). Field conditions, topography, soil moistures, time of first rainfall event after manure application to the land and rainfall intensity are the main factors that affect the transport of *E. coli* to subsurface drainage water. Leaching of *E. coli* from the surface to subsurface drainage water was considerably influenced by soil structure (Artz et al., 2005; Smith et al., 1985). Rainfall intensity and manure application method could affect pathogen transport to subsurface drainage water (Joy et al., 1998; Wang and Doyle, 1998). Guber et al. (2005) concluded that the amount of manure applied to the land could influence the numbers of *E. coli* attached to the soil. *E. coli* concentrations were higher in water if rainfall followed immediately after manure was applied to the soil (Thiagarajan et al., 2007). Table 4.1, adapted from Abu-Ashour et al. (1994), presents a brief summary of various factors that affect the movement of microorganisms in soil.

Several studies have been conducted in the laboratories and fields to understand the preferential flow in the soil. Preferential flow through macropores was found as the main pathway for immigrating microorganisms into the soil (Abu-Ashour et al., 1994; Christiansen et al., 2004; Wollum and Cassel, 1978). Large pores could govern the saturated flow in the compacted soil (Lin et al., 1996; Lipiec et al., 1998). Biopores with diameters bigger than 1 mm can quickly transport water, colloids, air, organic matter and microorganisms from soil surface to deeper layers and to subsurface drainage water systems (Lobry de Bruyn and Conacher. 1994; McMahon and Christy, 2000). Alaoui and Helbling (2006) reported that about 74% to 100% of total water flow could be transported through macropores which accounts for only 0.23% to 2% of soil volume. Higher concentrations of microbial pathogens were found on the wall of macropores (Parkin and Berry, 1999; Tiunov and Scheu, 1999; Vinther et al., 1999). *E. coli* could be transported to deeper soil depths in the soil columns if macropores were present (Abu-Ashour et al., 1998).

Shipitalo and Gibbs (2000) reported that there were open surface biopores that were directly connected from soil surface to artificial subsurface drainage systems. They observed the macropores created by deep burrowing (*Anecic*) species of earthworms in a silt loam soil that allow transferring water from soil surface directly to subsurface drains. Dyed water was traced in the tile drainage through macropores emitting smoke. Immediate breakthrough of solutes and contaminants in subsurface drainage was proficiently transported through directly connected macropores (Fox et al., 2004; Villholth et al., 1998). Akay and Fox (2007) verified this "direct connectivity" by conducting infiltration experiments in a laboratory soil columns with surface connected and buried macropores. Faster breakthrough was observed in soil columns with macropores connected to the drain pipe. No laboratory and field experiments have been conducted to examine pathogen breakthrough with directly connected macropores.

The overall objective of this study was to identify the role of directly connected macropores in transporting pathogen from soil surface to subsurface drainage water. Specific objectives were to: (1) Identify and quantify number of macropores between no-till and chisel plow practices by using the smoke test and (2) Investigate the role of directly connected macropores in transporting *E. coli* to subsurface drainage water under no-till and chisel plow condition after the application of LSM.

4.3 Materials and Methods

4.3.1 Field site description

Field experiments were conducted at the Iowa State University's Northeast Research Center near Nashua, Iowa. The study site has a total of thirty six 0.4 ha plots (58.5 by 67 m), with fully documented tillage and cropping records for the past 32 years (1979-2011). The dominant soils at this site are Floyd loam (fine-loamy, mixed, mesic Aquic Hapludolls), Kenyon loam (fine-loamy, mixed, mesic Typic Hapludolls), and Readlyn loam (fine-loamy, mixed, mesic Aquic Hapludolls) (Kanwar et al., 1999). These soils are moderately well to poorly drained and lie over the fluctuation of water level of about 20 to 160 cm. Thus, subsurface drainage system was installed at 1.2 m depth and at tile spacing of 28.5 m apart in 1979 to drain excess water for maintaining high crop productivity. The central tile line of each plot was intercepted and connected to an independent sump at the end of each plot for measuring subsurface drain flow and collecting water samples for analysis (Kanwar et al., 1997; 1999).

Two field experiments were conducted in each 2008 and 2009, one in the spring before planting and one in the fall after harvesting crop. Plot number 20 (P20) under no-till tillage and plot 30 (P30) under chisel plow were used for conducting field experiments in the spring 2008 and the fall 2009. Plot number 25 (P25) under no-till and plot number 23 (P23) under chisel plow s were used in fall 2008 and spring 2009 (table 4.2). Those plots were chosen because they were going to receive LSM to grow corn in next season. The sub-area of 30.5 m by 30.5 m located over the central tile line at the bottom slop, near the border of each plot, was chosen to conduct the experiment. The specific soil types of sub-area were Floyd for P20 and P30; Kenyon and Readlyn for P25 and P23, respectively (table 4.2). Data on bulk density, particle density as well as porosity on Floyd, Kenyon and Readlyn soils at different soil layers (0-8, 38-59, 64-77 and 94-120 cm) were taken from field measured data reported in Ma et al. (2007). Soil properties of other layers (18-38, 59-64 and 77-94 cm) were interpolated by taking the average values of available properties of the soil layers above and below that interpolated layer. More details on management practices, timings and rates of manure application, and other data on field experiments and soil properties are given in tables 4.2 and 4.3.

4.3.2 Macropores identification and quantification

Identification and quantification of directly connected macropores in no-till and chisel plow plots by using the smoke test technique was the first activity to be conducted in the field experiments. Smoke was produced by using a non-explosive smoke bomb (W3C, Superior Signal Company, Spotswood, NJ). Each smoke bomb could last for 3 minutes and generated the smoke volume of approximately 1100 m^3. Smoke air was injected at the outlet of the subsurface drain and then was pumped through the central tile line of each plot by high-pressure air generated from liquid manure application wagon (honey wagon).This smoke started to emerge out of the soil surface at different locations along the length of central line of each plot through well-structured and established macropores within 5 to 10 minutes after pumping the smoke into the tile line. Each location of the smoke emitting macropore was identified by putting a flag next to the location and numbers of macropores were counted for each plot. Conservative tracer (Rhodamine WT) was used to test the macropores that were considered as directly connected macropores from soil surface to subsurface drainage. Infiltration rates of some selected flagged macropores were measured by using Mariotte-type infiltrometer (Shipitalo et al., 2004). The volume of the infiltrometers was ranged from 6.7 L. to 13 L. Water level in the infiltrometer was recorded until the infiltration rate reached at steady-state.

4.3.3 Manure and bromide application

LSM was injected to each plot by using the injection technique at the rate of 168 kg ha^{-1} before simulated rain was applied in each plot. In the first experiment (spring 2008), manure was applied in parallel to plant rows. This application, however, caused lot of odor problems and a little amount of LSM was lost as it flowed through crop rows for lack of adsorption to soil after injection. To avoid this problem, LSM was injected perpendicularly to crop rows in experiments 2 to 4 (fall 2008, spring 2009 and fall 2009). In addition to that, manure injectors were equipped with soil mulching disks to cover the area immediately after manure injection. This manure injection equipment allowed much less manure odor and most of the LSM got absorbed or attached to the soil.

Bromide (Br) in granular form as a tracer was surface applied on the soil at the rate of 20 kg per experiment area (30.5 m by 30.5 m), before the application of manure and artificial rainfall simulations. Bromide has been used as a tracer for many purposes including plant uptake, nitrogen transport, and solute transport and recycling (Magarian et al., 1998; Paramasivam et al., 2002; Xu et al., 2004). Bromide was used in this study to find the time of peak bromide concentration in subsurface drainage water and to develop breakthrough curves for this chemical compound.

4.3.4 Rainfall simulation at the field experiment

To transport bromide and bacteria from soil surface to subsurface drainages, artificial rain was applied on each experimental plot using a boom, linear-movement irrigation system installed at the research site. All nozzles (3TN#44, Nelson Irrigation Corporation, Walla Walla, WA) were rotated by water carried through the boom of the irrigation system. Nozzles, having orifice diameters of approximate 8.73 mm (44/128th inches), were assembled vertically downward every 3 m along the boom. The irrigation system moved roughly at a speed of 29 m h^{-1} under 25% speed designed. Water from nozzles sprayed within 9 m wetted radius. Under a stationary state, the simulated rainfall intensity of 5.57 cm h^{-1},which was considered adequate for this study, could be obtained. However, the actual amount of applied irrigation water on each plot varied with wind velocity as well as application efficiency. In addition to that, initial soil moisture and conditions of water table level required the different amounts of rainfall needed to generate subsurface drainage flow.

For the first experiment in the spring 2008, the irrigation system was run at minimum speed of 25% for two passes. For the second experiment in the fall 2008, the irrigation system was run three times: at 25% speed for the first run and at 100% speed for the last two passes in both plots. One week before the third experiment in the spring 2009, the field was pre-irrigated to raise water table level and soil moisture. The irrigation system was run at 25% speed for the first run and 50% of the speed the second paths in plot 25. Three runs were needed (at 25% speed for first run and at 50% of the speed for the last two runs) in plot 23. The reason for giving one extra pass of irrigation in plot 23 was that there was no tile flow occurring after the first two runs of rainfall simulation.

Rain gages were placed randomly over the experimental area to measure amount of application artificial rainfall for each experiment. The x, y coordinates of the rain gage points were recorded from hand held GPS receiver. These coordinate data then were used to create the map of water application rates over the experimental area using ArcGIS® v.9.1 to visually show the variations in the amount of water received on the soil surface in each plot.

4.3.5 Soil, water sampling and analysis

Subsurface drainage water samples were taken during and after the rain to analyze the concentrations of *E. coli* and bromide. Background concentrations of *E. coli* and bromide in the subsurface drainage water were determined to check their presences in the flow at the beginning of the experiments. Water samples were collected in 15 minute increment for the first three hours of rainfall and in three-hour increment after that until the end of experiment in 24 hours. Soil cores were also taken and sampled at depths of 2.5 cm and 20 cm to determine background concentrations of *E. coli* in the soil. Water samples were taken after rainfall simulation for 24 consecutive hours.

Soil and water samples for *E. coli* enumeration were refrigerated and analyzed on-site within 8 hours of collection, using semi-automated quantification methods based on standard Methods Most Probable Number (MPN) model. A QuantriTray 2000 test (IDEXX, Westbrook, ME) can count from 1 to 2,419.6 MPN per 100 mL. Water samples for bromide and Rhodamine WT analysis were stored at 4^{o}C until analyzed at the Water Quality Lab at Iowa State University by using method 4500-Br-C for bromide and fluorescent method for Rhodamine WT (APHA, 1998).

4.3.6 *Statistical analysis*

Mean comparison of average amount of water received from rainfall simulation between two plots of each experiment was performed using PROC ANOVA in SAS® software v.9.2.2 (SAS, 2008). Other procedures in SAS (i.e., PROC NLIN, PROC MODEL and PROC REG) were used for non-linear regression analyses to predict the breakthrough times and peak concentrations of *E. coli* and bromide in the experiments conducted in the spring 2008 and in the fall 2009. Different rational polynomial models (e.g., Gammas, Gompert, logistic and rational factional polynomial), were attempted to analyze data. The initial values for those models were obtained by fitting a simple unweighted parabola. We compared the descriptive statistical results of parameters (*e.g.*, the coefficient of determination (R^2); the coefficient of determination with the predictive residual sum of squares (R^2_p); Akaike's Information Criterion-corrected (AIC_c) acquired from those functions and chose the one that most properly fit the data. The chosen models used for this experiment data are shown in table 4.4. All statistical runs of non linear regressions met the convergence criterion. More details of analysis method are described in Meek et al. (2011).

4.4 Results and Discussion

4.4.1 Artificial rainfall simulations

The maps generated by GIS showing the amount of irrigation water applied to the soil surface from irrigation are showed in the Fig. 4.1 to 4.4. Table 4.5 gives details of statistical descriptive data of all irrigation events. All four experiments showed that water applied from each irrigation event was not universally distributed on the soil surface. Figures 4.1 to 4.4 show that more water was received surrounding the center of the experimental areas among all plots. Besides that, there might be slight difference of water pressure at each of the individual nozzles along the linear move irrigation system that may have contributed to these variations in rainfall amounts. The average means of amount of water applied between two plots were not the same in each individual experiment. Those differences in water application, however, were not significantly different from each other at p=0.05.There were large variations between the lowest and the highest amount of water measured in the raingages. These differences varied from more than 2 times and up to almost 10 times, 12.7 vs. 127 mm in P25-S09 (table 4.5). This clearly shows that it is not easy to control the distribution of irrigation water applied on the soil surface under rainfall simulation conditions. For experiments conducted in spring 2008, the average amount of water received on soil surface in plot P20 was higher than the amount of water applied in plot P30. The amount of water applied measured from raingages was 53.6 and 42.0 mm for plots P20 and P30, respectively (table 4.5). These amounts varied from 35.6 mm to 76.2 mm for plot P20 whereas water amounts ranged from 12.7 to 63.5 mm for plot P30.

The average amounts of water applied were 41.6 and 53.8 mm to pP25 and P23, respectively for the experiments conducted in the spring 2009. Thus, higher amount of water was applied to plot 23 as one more extra path of irrigation was run for this plot to help build the water table in this plot. In the fall 2009, the water received for each plot was very close to each other, 48.2 and 47.1 mm (table 4.5).

4.4.2 Macropores quantification by using the smoke test in field experiments

Smoke tests were conducted in the beginning of every field experiment to identify the number and size of the macropores above the tile line of the experimental plot. Two sets of smoke tests were conducted in fall and spring seasons of year 2008 and 2009. Macropores where smoke emitted from the soil surface were flagged and counted as a single macropore for each flag. Big macropores where concentrated smoke emitted as a flame or plume and can be visually observed were counted as directly connected macropore.

The conductivity of directly connected macropores from soil surface to subsurface tile drain was successfully measured by using Rhodamine WT dye in the Marriot-type infiltration test. Figure 4.5 shows the Rhodamine WT and *E. coli* concentrations observed at the outlet of tile drainage for two different DCM. The peak concentrations were observed between 35 to 50 minutes after the application of Rhodamine WT or *E. coli.* After that, the concentrations of Rhodamine WT and *E. coli* gradually tailed off with time.

Results of the comparison of means of macropores found between different tillage practices and between fall and spring seasons are presented in table 4.6. These data on macropores showed that there were no statistically significant differences in the numbers of macropores between no-till plot the chisel plot (*p*=0.05). However, total numbers of macropores found in no-till plot always were higher than that the chisel plot, 66.3 versus 40.5 macropores, respectively (table 4.6). Observations from all smoke tests in these field experiments indicated that larger numbers of macropores were found closer to the outlet of tile drain. This shows that earthworms probably are more activate closer to tile outlets. Comparisons of means of macropores between spring and fall seasons were also conducted. These comparisons also do not show any statistically significant differences between seasons at *p*=0.05, but showed significant difference at *p*=0.l. The average numbers of macropores were 32 and 74.7 in spring and fall, respectively. In reality, both regular macropore and DCM did show higher numbers of macropores in the fall compared to spring season. These results clearly indicate that fall season might create more macropores than spring reason as a result of the dry conditions in the fall and frozen conditions in the winter of each year.

4.4.3 E. coli transported to subsurface drainage through directly connected macropores

4.4.3.1 E. coli transport to subsurface drainage in experiment conducted in spring 2008.

Total numbers of macropores counted from the smoke test in this experiment were 49 and 31 in P20 and P30, respectively, which were approximately equivalent to 0.8 and 0.5 macropores per meter length along tile drain line. The experimental area of both plots had the same soil type of Floyd (table 4.2). The total calculated volume of soil space is approximately 457.2 m^3 per total soil volume of 1114.8 m^3 in experimental area (table 4.3). Initial *E. coli* tests in soil background and in tile water samples for both plots were taken on the day of conducting the experiment. No initial level of *E. coli* was found in both plots. Total coliform, which used to be an indicator of bacteria in water but was not a subject focused in this study, was always detected by quantri-tray test in both soil and tile flow water samples.

Figure 4.6 shows the breakthrough curves of observed and predicted concentrations of *E. coli* and bromide as a tracer in P20 and P30. *E. coli* was detected in the tile flow samples taken from both plots about 15 minutes after irrigation started, and these concentrations were found to be at 9.7 and 4.1 MPN per 100mL for P20 and P30, respectively. The statistically analyzed results of breakthrough curves of *E. coli* and bromide are showed in table 4.7. The functions generated by using SAS software to predict concentrations are shown by Equations [5] and [6] for *E. coli* and [7] and [8] for bromide for no-till-P20 and chisel plow-P30 plots, respectively. Relatively good coefficient of determination (R^2) were observed from these predicted functions and ranged from 0.73 to 0.88 (table 4.6). This suggests that predicted curves fit nicely with observed data in the field and could also serve for other purposes, e.g. modeling the concentrations of these parameters during that time.

$$Y(E.coli) = \frac{X}{0.003 - 0.003X + 0.001X^2}, (MPN) \qquad [5]$$

$$Y(E.coli) = \left\{ \begin{array}{ll} 17.9, & 0 < X \le 1.5 \\ 17.9 + \dfrac{(X - 1.5)}{0.009 - 0.015\sqrt{(X-1.5)} + 0.007(X-1.5)}, & 1.5 < X \end{array} \right\} (MPN) \qquad [6]$$

$$Y(Br.) = \frac{X}{0.13 - 0.21X + 0.13X^2}, (mgL^{-1}) \qquad [7]$$

$$Y(Br.) = \left\{ \begin{array}{ll} 6.22X - 3.38X^2, & 0 < X \le 1.5 \\ -1.72 + \dfrac{(X - 1.5)}{2.24 - 3.92\sqrt{(X-1.5)} + 1.83(X-1.5)}, & 1.5 < X \end{array} \right\} (MPN) \qquad [8]$$

(Where X is time (hr.) since irrigation started and Y is concentration of *E. coli* bromide).

The statistical time series analysis showed that estimated times to get peak concentration of *E. coli* were about 1.62 and 2.66 hr. after irrigation started for P20 and P30, respectively (Table 4.7 and Fig. 4.6). Similarly, the estimated peak times for bromide tracer were 1.5 hr. for P20 and 2.78 hr. for P30. Times to get peak concentrations of both *E. coli* and bromide tracer was significantly delayed in plot 30, where macropores were covered, in comparison with the plot 20. It obviously shows that breakthrough times of both *E. coli* and bromide curves in observed and predicted condition came at least one hour earlier in P20, where macropores exposed directly to irrigation water. These results showed the important functions of open macropores, especially directly connected macropores in transporting *E. coli* as well as bromide tracer from soil surface to subsurface tile drainage. It also means plastic tarp used to cover macropores on top of tile lines in plot 30 functioned as a physical barrier to disconnect the mechanism of transporting water from soil surface to tile drainage via directly connected macropores in soil.

The trends of breakthrough curves of *E. coli* and bromide tracer for each plot look similar. It means that time to get peak concentration of *E. coli* and bromide happened approximately at the same time. Overlapping time was observed at 90% CI of time to get peak concentration between *E. coli* and bromide within plot (table 4.7). The concentrations gradually tailed off when subsurface drain flow declined. This means that use of bromide could be used as a very good tracer for bacteria, especially for determining *E. coli* trends and travel time for conditions similar to this experiment.

Higher *E. coli* concentrations were continuously observed in all water samples taken from plot P20 in the first two hours after irrigation started. This shows that rapid transport of *E. coli* occurred with water from soil surface to subsurface drain depth through DCM in no-till P20 after irrigation, while it took more time for water to move through chisel plow plot P30 where water infiltrated through soil matrix and then went to tile drainage. The estimated highest concentration of *E. coli* from plots P20 and P30 were 749 and 2565 MPN per 100 mL, respectively. The tile flow in plot P30 was higher in comparison to plot P20 at most of the time despite the fact that plot P20 received more rainfall water. The highest tile flow rates were 470 ml s^{-1} and 640 ml s^{-1} in plots P20 and P30, respectively. Therefore, the explanation of having higher concentration of *E. coli* in tile flow from plot P30 was possibly due to higher tile flows in this plot. Another possible explanation for higher *E. coli* concentrations in tile flow in P30 was because of the overland flow that had accumulated in P20 after 1.5 hr. during irrigation. That water may have had a chance to go through the large macropores, initially covered up by the plastic tarp.

4.4.3.2 E. coli transport to subsurface drainage: Field experiment 2 in fall 2008 and 3 in spring 2009

A set of two other plots under no-till (P25) and chisel plow (P23) tillage practices were used for experiments conducted in fall 2008 and spring 2009. Plastic tarp was used to cover macropores found on top of tile line in P23. Water table in both plots was low and below the tile line. Therefore, none of the tile lines was flowing in both plots at the beginning of the experiments. And, background concentration of *E. coli* and bromide in drainage water could not be measured before irrigation started. To learn from the experiences of experiment conducted in fall 2008, we decided to pre-irrigate these two plots the previous-day before conducting experiment in spring 2009 to increase soil moisture and possiblely increase the water table depth. In fall 2008 experiment, approximately 1.9 and 0.75 macropores per meter length were counted in plots P25 and P23, respectively. Earlier tile flow did appear at the drain outlet in plot 25 and first set of water samples, were collected at 1.2 hr. and 1.78 hr. after the beginning of rainfall in plots P25 and P23, respectively (Fig. 4.7). However, only 12 water samples could be collected and analyzed for *E. coli* concentration in P25 within about one and a half hours of tile flow. Similarly, only 8 water samples were collected from P23. The limited number of water samples was because of low initial soil moisture and lower water tables in fall 2008. The first two water samples taken from P25 did not give any presence of *E. coli*, indirectly indicating that no initial *E. coli* was present in the tile flow. The next two set of water samples showed *E. coli* concentrations at the highest level observed of more than 24691 MPN per 100 mL or about 10% of initial *E. coli* concentration presented in LSM. *E. coli* concentrations peaked in P25 about 1 hour after the first peak concentration was observed. This shows possibly the functioning of additional number of macropores which resulted in the second peak. None of water samples collected from plot P23 was detected for *E. coli* which shows that chisel plow plots did not have functioning directly connected macropores.

In the spring 2009 experiments, although we could collect tile water samples but much lower volume of tile drainage water was observed in P23 compared to previous experiments conducted in 2008. The first tile water sample collected arrived at 0.88 hr. from plot P25 and 1.97 hr. from plot P23, after the beginning of irrigation. Both *E. coli* and bromide were found in tile water samples taken from P25. Only a few tile water samples collected from P23 were detected for *E. coli* at very low concentrations, between 1-4 MPN per 100mL, while most of the tile water samples could not be detected for any *E. coli,* but bromide was present in all water samples. Peak concentrations of *E. coli* as well as bromide were observed right during the first hour after irrigation started in P25 whereas it took 2.17 hr. for bromide tracer concentration to peak in P23 (Fig. 4.8).

The results from these two experiments, when there was no base-flow in tile drainage at the beginning of rainfall in both plots, clearly show the importance of macropores in transporting water from soil surface to subsurface tile drains. Plot 25, where all macropores were freely connected with the tile flow did show a faster transport time for rainwater to reach the tile line. This finding is very similar to what Alaoui and Helbling (2006) found in their earlier study that 70% to 100% of total water flow could be transported through macropores. No *E. coli* was found in tile drainage in P23 suggested that water discharged at tile drainage outlet mainly moved through soil matrix and *E. coli* might be held by soil particles because filtration capacity of soil.

4.4.3.3 E. coli transport to subsurface drainage: Field experiment 4 in fall 2009

The last set of experiments was conducted again in no-till, plot 20, with directly connected macropores from surface to subsurface drainage, and chisel plow-plot 30, where macropores were covered by thick plastic tarp (table 4.2). Tile flow was observed at the beginning of experiment in both plots because base flow of about 8.3 mL s^{-1} for plot 20 and 17.7 mL s^{-1} for plot 30 was observed before the beginning of rainfall. Therefore, tile water samples were collected before starting the irrigation event. Table 4.8 and figure 4, gives data on *E. coli* and bromide tracer concentrations for plot 20 and plot 30. Equations [9] and [10] give the trend model for *E. coli* concentration and Equations [11] and [12] give trend model for bromide concentrations for plot 20 and plot 30, respectively. High value of R^2 for predicted concentrations of *E. coli* and bromide were obtained, ranging from 0.86 to 0.94

(table 4.8) which shows that these trend models were developed giving best fit of observed data on *E. coli* and bromide concentrations.

$$Y(E.coli) = \left\{ \begin{array}{ll} 946.8 + 930.3[\exp(\frac{-X}{0.09}) - 1], & 0 < X \le 0.67 \\ 17.2 + \frac{\sqrt{(X-0.67)}}{-0.001\sqrt{(X-0.67)} + 0.003(X-0.67)}, & 0.67 < X \end{array} \right\} (MPN) \qquad [9]$$

$$Y(E.coli) = \left\{ \begin{array}{ll} 3.45, & 0 < X \le 0.72 \\ 3.45 + \frac{(X-0.72)}{0.006 - 0.04(X-0.72) + 0.09(X-0.72)^2}, & 0.72 < X \le 1.83 \\ 3.45 + \frac{(X-1.83)}{-0.007(X-1.83) + 0.01(X-1.83)^2}, & 1.83 < X \end{array} \right\} (MPN) \qquad [10]$$

$$Y(Br) = \left\{ \begin{array}{ll} 1.30, & 0 < X \le 0.67 \\ 1.3 + \frac{1}{0.1 - 0.38\sqrt{(X-0.67)} + 0.4(X-0.67)}, & 0.67 < X \le 1.83 \\ 7.99 + \frac{1}{0.16 - 0.41\sqrt{(X-1.83)} + 0.4(X-1.83)}, & 1.83 < X \end{array} \right\} (mgL^{-1}) \qquad [11]$$

$$Y(Br) = \left\{ \begin{array}{ll} 3.17, & 0 < X \le 0.83 \\ 3.17 + \frac{1}{1.95 - 6.0\sqrt{(X-0.83)} + 5.78(X-0.83)}, & 0.83 < X \le 1.83 \\ 3.75 + \frac{1}{1.22 - 3.54\sqrt{(X-1.83)} + 3.16(X-1.83)}, & 1.83 < X \end{array} \right\} (mgL^{-1}) \qquad [12]$$

(Where X is time (hr.) since irrigation started and Y is concentration of *E. coli* / bromide).

In general, peak concentrations of *E. coli* and bromide in tile water from both plots give similar trends as observed in previous experiments. In plot 20, the concentrations of both *E. coli* and bromide peaked at the time earlier than that in plot 30. *E. coli* concentration in tile water in plot 20 peaked at 0.74 hr. and at 0.97 hr. in plot 30, after irrigation started.

This is statistically significant time difference when there were no overlap of 90% CI obtained, between 0.56 to 0.89 hr. in plot 20 and 0.94 to 0.99 in plot 30 (table 4.8). Similar trends in peak bromide concentration were observed in these two plots.

For each plot, bromide tracer still showed a good match of getting time peak time closer to *E. coli* peak concentration at either 90% or 95% CI of time. Therefore, it can be concluded with confidence that bromide tracer could be used in predicting the transport of *E. coli* to subsurface drainage water.

Plot 30 also showed the second peak concentrations for both *E. coli* and bromide. The highest concentrations of both *E. coli* and bromide usually occurred in the first set of water samples collected after application of irrigation water and rapidly declined after about 1 hour. These results indicate that the first rainfall event right after the manure application in the field has a significant impact on transporting *E. coli* to subsurface drain water through directly connected macropores and should be extensively monitored to understand the water impacts of manure applications. From a practical point of view, manure applications should be made much earlier before the rainy season begins to minimize negative water quality impacts of manure applications, especially in fields with the presence of directly connected macropores.

4.5 Conclusions

Field experiments were conducted in 2008 and 2009 to investigate the role of directly connected macropores in transporting *E. coli* from soil surface to subsurface drainage water from LSM applications in crop lands. Four separate experiments were conducted in both fall and spring seasons under no-till and chisel plow plots. Smoke tests were performed at the beginning of the experiment to identify and quantify macropores located above the tile drainage line. LSM was applied to experimental plots followed by artificial rainfall by using the linear move irrigation system. Tile flow water samples were collected during and after the rain and were analyzed for *E. coli* and bromide concentrations by using quantri-tray method within 24 hours after irrigation started. The overall results of this study indicated that no significant differences were observed in the total number of macropores found between no-till and chisel plow systems at level of p=0.05. However, experiments did show that higher numbers of macropores were present in no-till plots where soil was not disturbed during planting and no field cultivation was done. Total number of macropores was found to be significantly different between fall and spring season at level p=0.01. Fall seasons showed a higher number of macropores that might be the results of activities of earth worms in spring, summer, and fall after a long period of frozen conditions during winter months.

Directly connected macropores from soil surface to subsurface drainage were successfully tested by using Rhodamine WT. Four experiments were conducted at different field conditions which clearly showed a relationship between the directly connected macropores and transportation of water and *E. coli* from soil surface to subsurface drainage water. Times to peak *E. coli* concentration in tile water were 1.62 and 2.66 hr. after rainfall simulation started in plot with DCM and without DCM, respectively for experiments conducted in the spring 2008. In fall 2009, the times to peak *E. coli* concentration were 0.74 for plot with DCM and 0.97 hr. for plot without DCM. Similar findings were also observed in other two experiments where there was no base-flow at the beginning. Bromide tracer worked very well in tracing the path of *E. coli* concentrations in tile flow. The overall results of this study clearly indicate that bromide can be used as a good tracer and predictor of *E. coli* concentrations in subsurface drain water.

4.6 References

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Table 4.1. Factors affecting the movement of microorganisms in the soil (adapted from Abu-Ashour et al., 1998).

1.	Soil physical characteristics
	- Texture
	- Particle size distribution
	- Clay type and content
	- Organic matter type and content
	- pH
	- Pore size distribution
	- Bulk density
2.	Soil environment and chemical factors
	- Temperature
	- Soil water content
	- Soil water flux
3.	Chemical and Microbial factors
	- Ionic strength of soil solution
	- pH of infiltrating water
	- Nature of organic matter in waste
	- Type of microorganism
	- Density and dimensions of the microorganism
	- Presence of larger organisms
4.	Application method
	- Soil drying between application
	- Time of application (winter, spring)

Table 4.2. General information on field experiments including tillage practices and manure application rates.

Plot No.	Tillage	Soil type	Manure	Plastic tap covered	Field experiments conducted	
			(kg ha^{-1})	(Width 1.2m)	Spring	Fall
P20	No-till	Floyd	168	No	2008	2009
P30	Chisel plow	Floyd	168	Yes	2008	2009
P25	No-till	Kenyon	168	No	2009	2008
P23	Chisel plow	Readlyn	168	Yes	2009	2008

Table 4.3. Soil properties at the field site experiments.

Soil types	Soil depth (cm)	Bulk density (g cm^{-3})	Particle density (g cm^{-3})	Porosity (cm^{3} cm^{-3})	Volume of pore space (m^{3})
Floyd	0-8	1.42	2.60	0.45	33.7
	18-38	1.46	2.65	0.45	124.9
	38-59	1.50	2.69	0.44	86.3
	59-64	1.60	2.69	0.41	56.7
	64-77	1.69	2.69	0.37	44.9
	77-94	1.70	2.68	0.36	57.6
	94-120	1.71	2.66	0.36	53.1
		Total volume of pore space			**457.2**
Kenyon	0-8	1.42	2.60	0.45	33.7
	18-38	1.52	2.66	0.43	119.1
	38-59	1.62	2.71	0.40	78.5
	59-64	1.60	2.70	0.41	56.8
	64-77	1.58	2.69	0.41	49.8
	77-94	1.62	2.70	0.40	63.5
	94-120	1.65	2.71	0.39	58.1
		Total volume of pore space			**459.6**
Readlyn	0-8	1.45	2.60	0.44	32.9
	8--38	1.44	2.64	0.45	126.4
	38-59	1.43	2.67	0.46	90.6
	59-64	1.47	2.68	0.45	63.0
	64-77	1.50	2.68	0.44	53.2
	77-94	1.61	2.67	0.40	62.8
	94-120	1.71	2.65	0.35	52.7
		Total volume of pore space			**481.6**

Table 4.4. Equations for general models used in predicting of *E. coli* and bromide concentrations in subsurface drain water.

RPEs	Equations		Conditions
RPE0	$Y = \dfrac{1}{a + b\sqrt{X} + cX}$	[1]	a, c >0 and b<0 $X_{ymax} = (-b/2c)^2$
RPE1	$Y = \dfrac{\sqrt{X}}{a + b\sqrt{X} + cX}$	[2]	a, c >0 $X_{ymax} = a/c$
RPE2 (Gunary model)	$Y = \dfrac{X}{a + b\sqrt{X} + cX}$	[3]	a, c >0 and b<0 $X_{ymax} = (-2a/b)^2$
RPE3	$Y = \dfrac{X}{a + bX + cX^2}$	[4]	a, c >0 $X_{ymax} = \pm (a/c)^{1/2}$

* Rational polynomial equation.

Where: X is time (hr) and Y is the concentration of *E. coli* (MPN) or bromide (mgL^{-1}) depending on each run and model run. Letter a, b, c represent parameters whose values are to be estimated in order to get the best fitting of model for the data.

Table 4.5. Statistical descriptive data of irrigation events

Plot & Season/Year	# of rain gages	Minimum (mm)	Maximum (mm)	Mean (mm)	Median (mm)	SD
P20-S08	10	35.6	76.2	53.6a*	50.8	11.9
P30-S08	15	12.7	63.5	42.0a	48.3	17.3
P25-F08	20	26.2	83.8	51.7a	50.5	15.3
P23-F08	18	24.1	63.5	47.1a	45.1	10.0
P25-S09	19	12.7	127.0	41.6a	33	25.1
P23-S09	19	35.6	83.8	53.8a	49.5	14.7
P20-F09	15	40.6	59.4	48.2a	49.5	5.5
P30-F09	16	25.4	63.5	47.1a	49.5	10.4

S= Spring and F = Fall

SD= Standard Deviation

* Means with the same letter are not significantly different from at p =0.05.

Table 4.6. Statistical descriptive of macropores analysis

Type of Macropore	Parameters	Tillages		Seasons	
		No-till	Chisel plow	Spring	Fall
Total number of macropores	Mean	66.3a*	40.5a	32a**	74.7a**
	SD	44.5	11.1	13.1	34.0
	SE	22.2	5.5	6.6	17.0
	CI 95%	4.6-137	22.9-58	11.1-52.9	20.6-128.9
Directly connected Macropores	Mean	24.0a	12.0a	9.3a	26.8a
	SD	20.4	7.3	3.3	18.7
	SE	10.2	3.7	1.7	9.4
	CI 95%	8.4-56.0	0.3-23.7	4.0-14.5	3.1-56.5

* The same letter meaning no significant different at *p*=0.05;

** Significant different at *p*=0.1;

SD = Standard Deviation; SE = Standard Error; CI = Confidence Interval.

Table 4.7. Summary of breakthrough curve results of the field experiment conducted in the spring 2008.

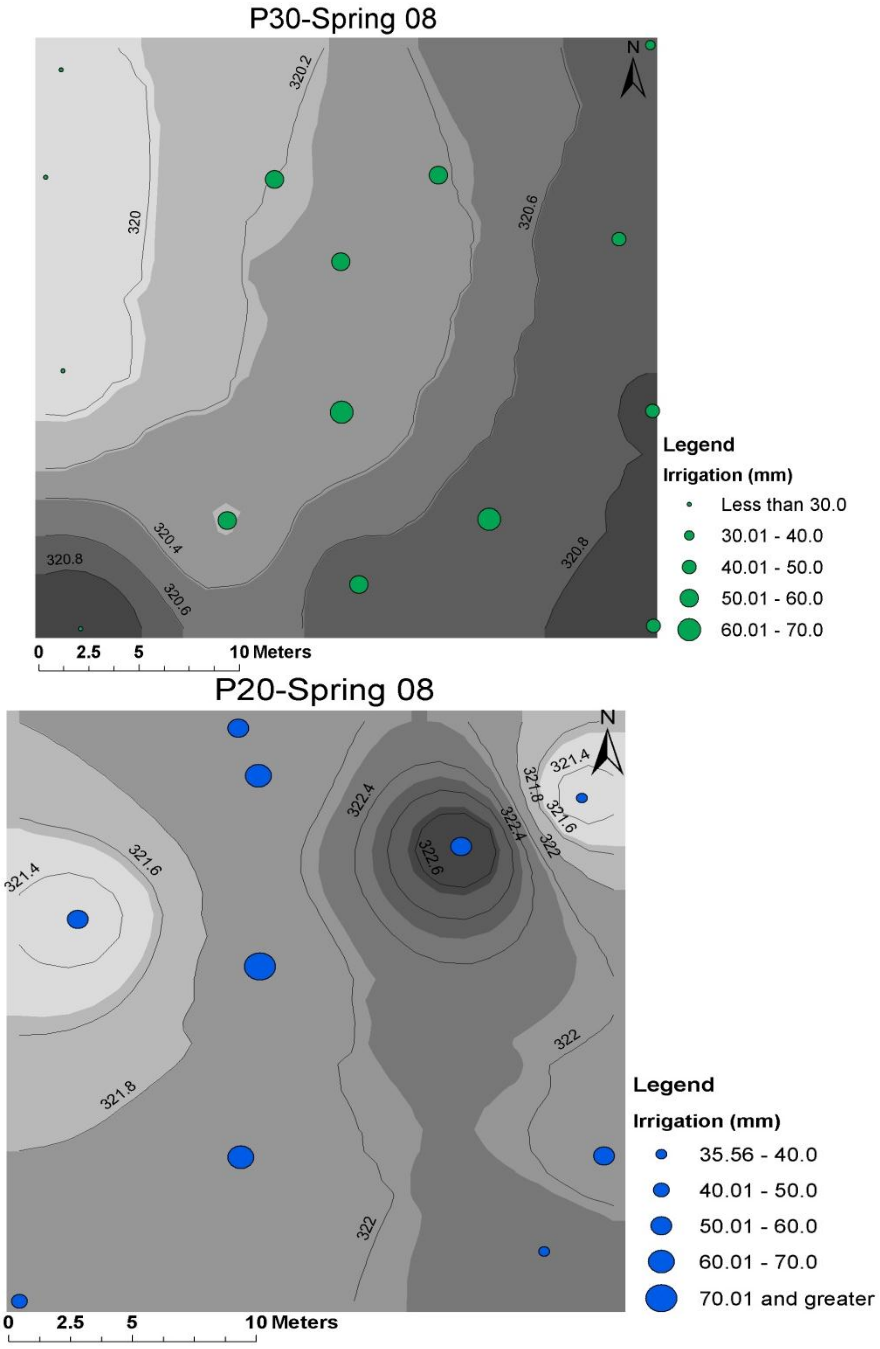
Models & Parameters	Field experiment conducted in spring 2008	
	Plot 20	**Plot 30**
	E. coli	
Models	Eq. [5]	Eq. [6]
Time to peak concentration [90%CI] (hr.)	1.62 [1.31-1.93]	2.66 [2.56-2.76]
Peak concentration [95%CI] (MPN)	749.3 [174.2-1324.5]	2565 [1423-3704]
R^2	0.82	0.91
AICc	178.5	126.2
	Bromide	
Models	Eq. [7]	Eq. [8]
Time to peak concentration [90%CI] (hr.)	1.5 [1.31-1.69]	2.81 [2.72-2.89]
Peak concentration [95%CI] (mgL^{-1})	26.3 [18.5-34.1]	9.3 [6.8-11.8]
R^2	0.88	0.93
AIC_c	51.3	15.7

Eq., Equation; CI, Confidence intervals; R^2, the coefficient of determination; AIC_c, Akaike's Information Criterion-corrected.

Table 4.8. Summary of breakthrough curve results of the field experiment conducted in the fall 2009

Models & Parameters	Field experiment conducted in Fall 2009	
	Plot 20	**Plot 30**
E. Coli		
Models	Eq. [9]	Eq. [10]
Time to first peak concentration [90%CI] (hr.)	0.74 [0.56-0.89]	0.97 [0.94-0.99]
First peak concentration [95%CI] (MPN)	3458.6 [-5215.2-12131.4]	363.8 [195.4-532.2]
Time to second peak concentration [90%CI] (hr.)	----	1.84 [1.84-1.85]
Second peak concentration [95%CI] (MPN)	----	144.3 [68.2-220.3]
R^2	0.89	0.93
AICc	193.3	138.9
Bromide		
Models	Eq. [11]	Eq. [12]
Time to first peak concentration [90%CI] (hr.)	0.9 [0.87-0.91]	1.10 [0.99-1.2]
First peak concentration [95%CI] (mgL^{-1})	119.9 [80.2-159.5]	5.7 [4.1-7.3]
Time to second peak concentration [90%CI] (hr.)	2.10 [1.99-2.21]	2.15 [2.07-2.22]
Second peak concentration [95%CI] (mgL^{-1})	26.7 [12.2-41.1]	7.9 [6.0-9.8]
R^2	0.94	0.86
AICc	86.50	12.40

Eq., Equation; CI, Confidence intervals; R^2, the coefficient of determination; AIC_c, Akaike's Information Criterion-corrected.


P30-Spring 08

P20-Spring 08

Figure 4.1. Raingage locations and amount of rainwater water measured by raingages in the field experiment conducted in the spring 2008. (Contours show the elevation in meters above mean sea level).

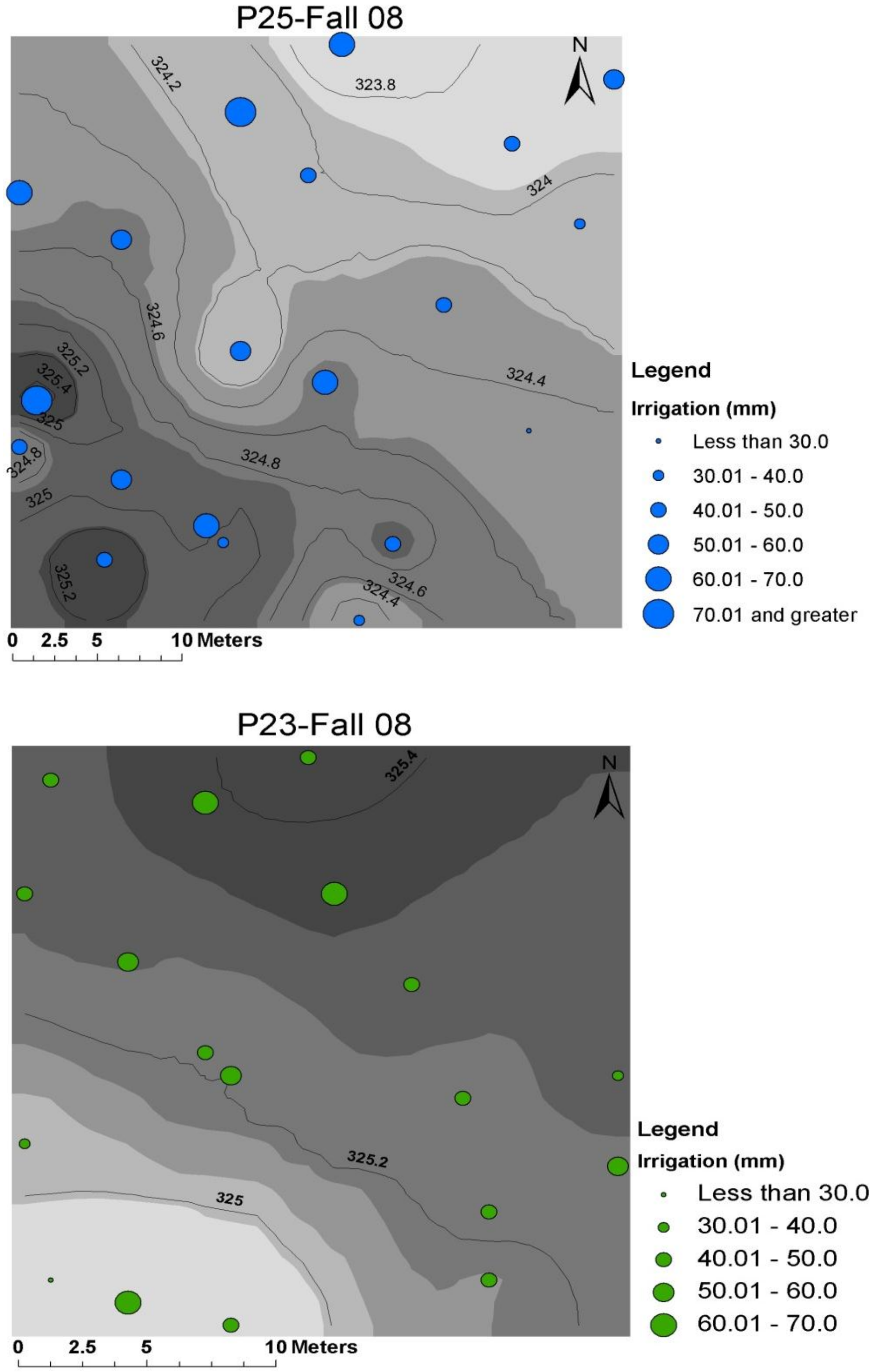
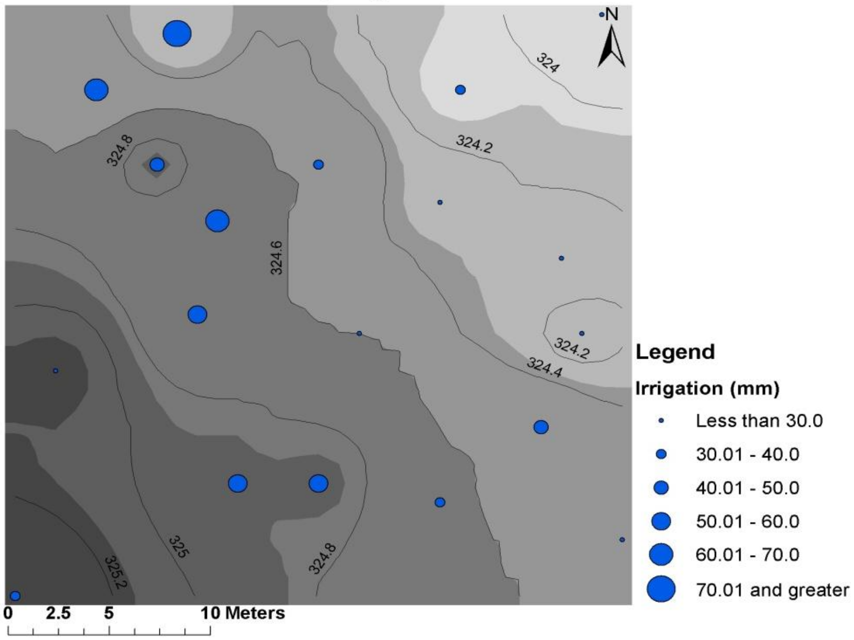


Figure 4.2. Raingage locations and amount of water measured by raingages in the field experiment conducted in the fall 2008. (Contours show the elevation in meters above mean sea level).

P25-Spring 09



P23-Spring 09

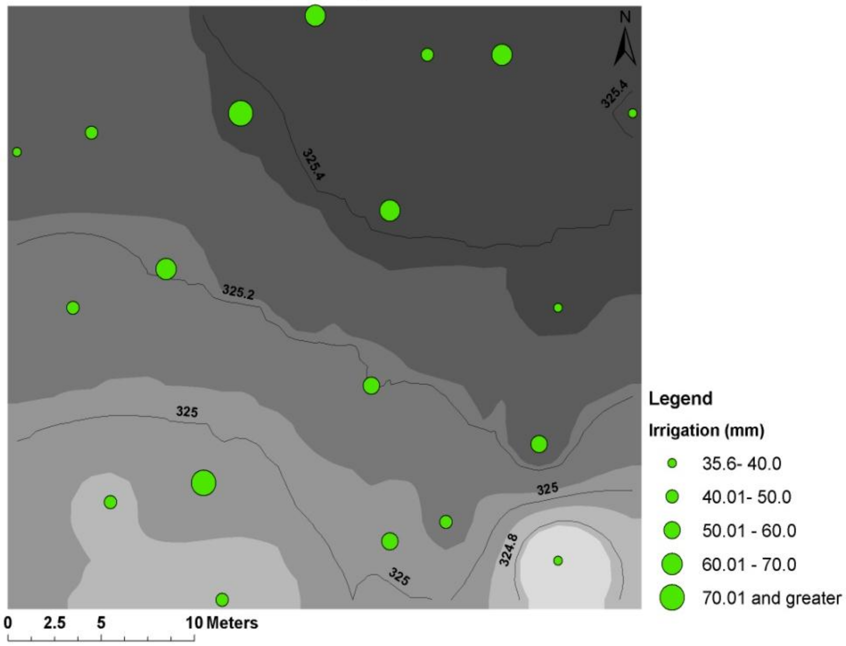


Figure 4.3. Raingage locations and amount of water measured by raingages in the field experiment conducted in the spring 2009. (Contours show the elevation in meters above mean sea level).

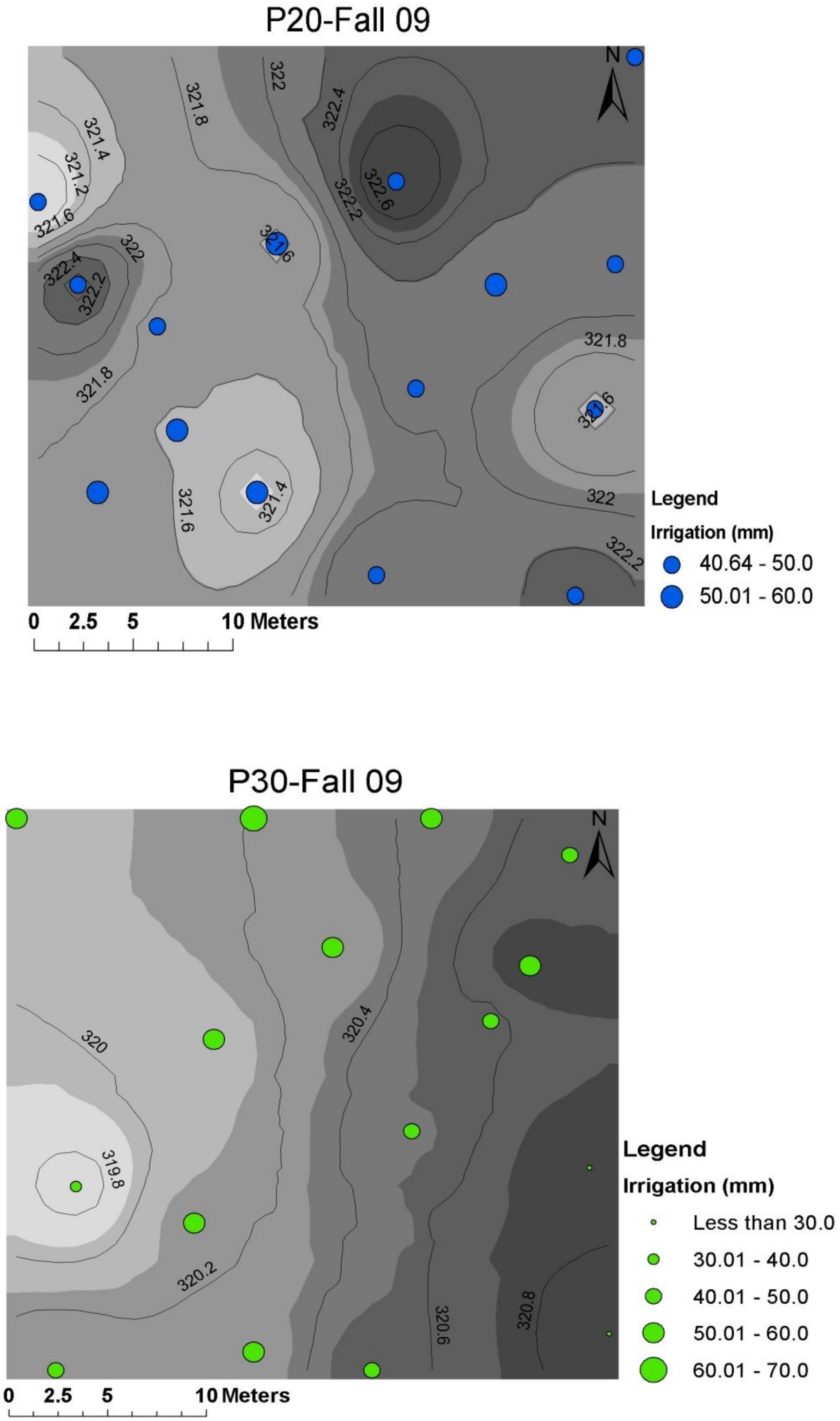


Figure 4.4. Raingage locations and amount of water measured by raingages in the field experiment conducted in the fall 2009. (Contours show the elevation in meters above mean sea level).

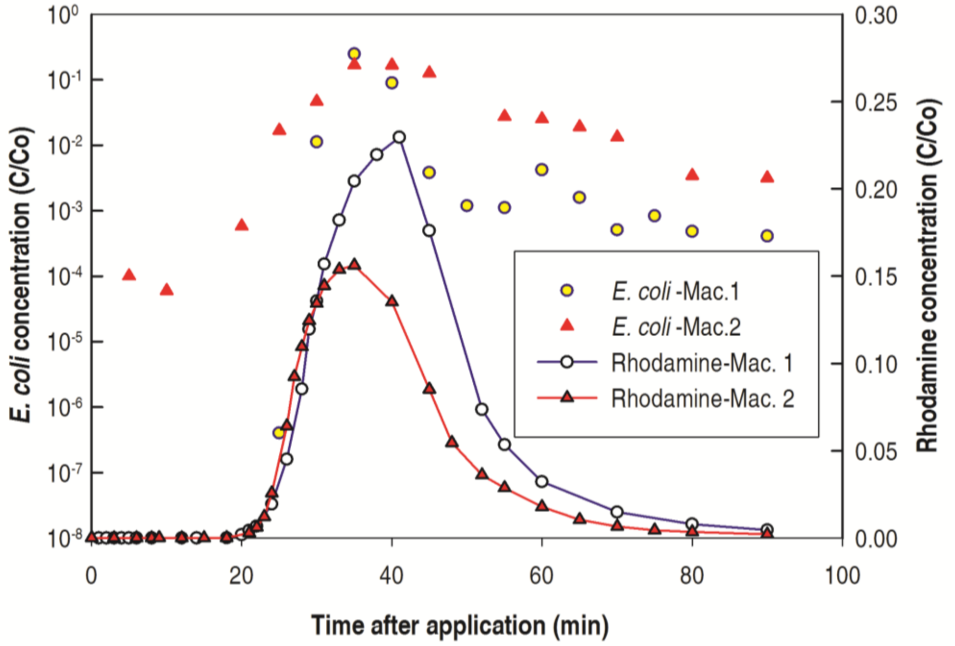


Figure 4.5. Rhodamine WT and *E. coli* breakthrough curves for the test of directly connected macropores.

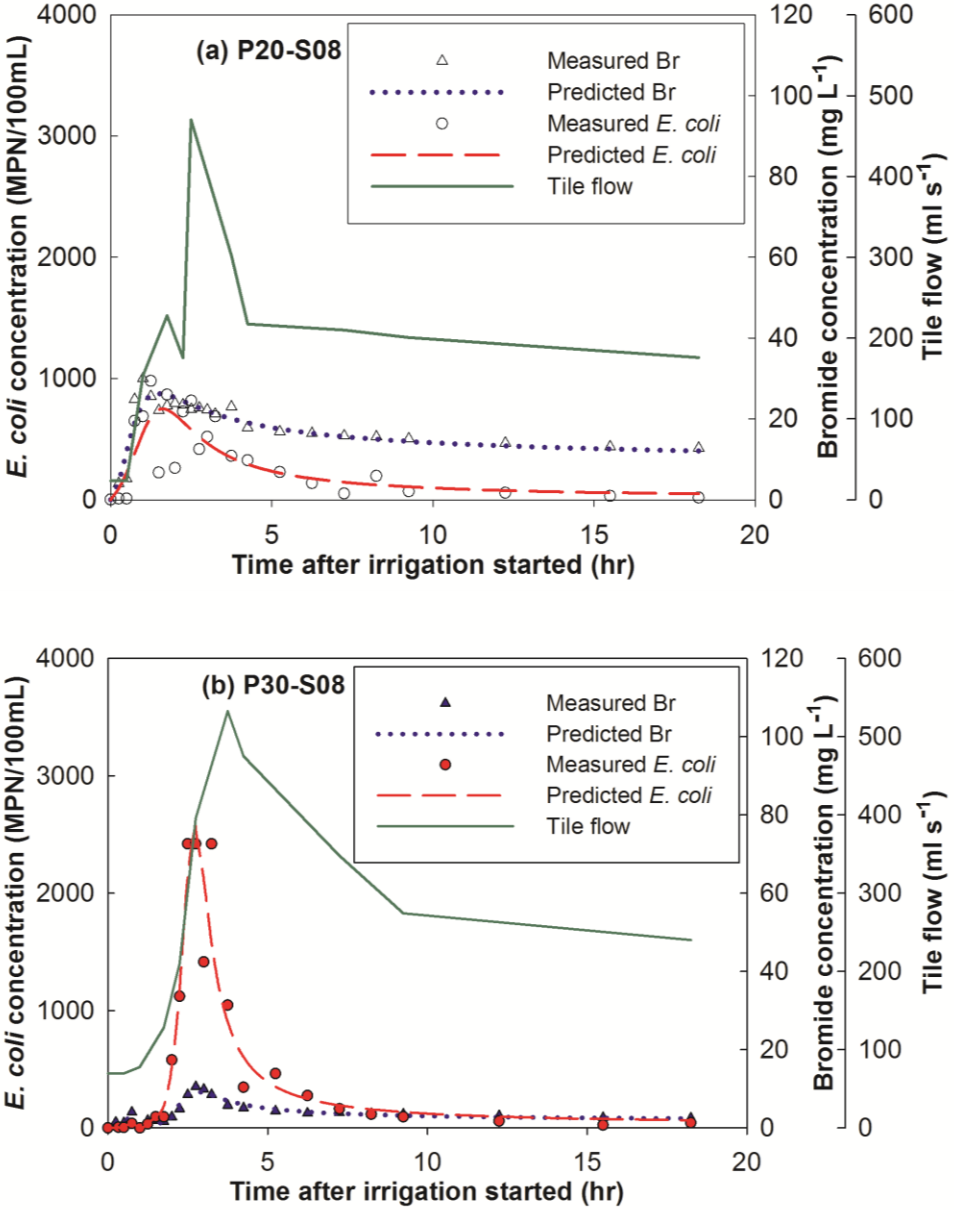


Figure 4.6. The breakthrough curves of *E. coli*, bromide tracer, their predicted concentrations and the tile hydrograph in the spring 2008 experiment.

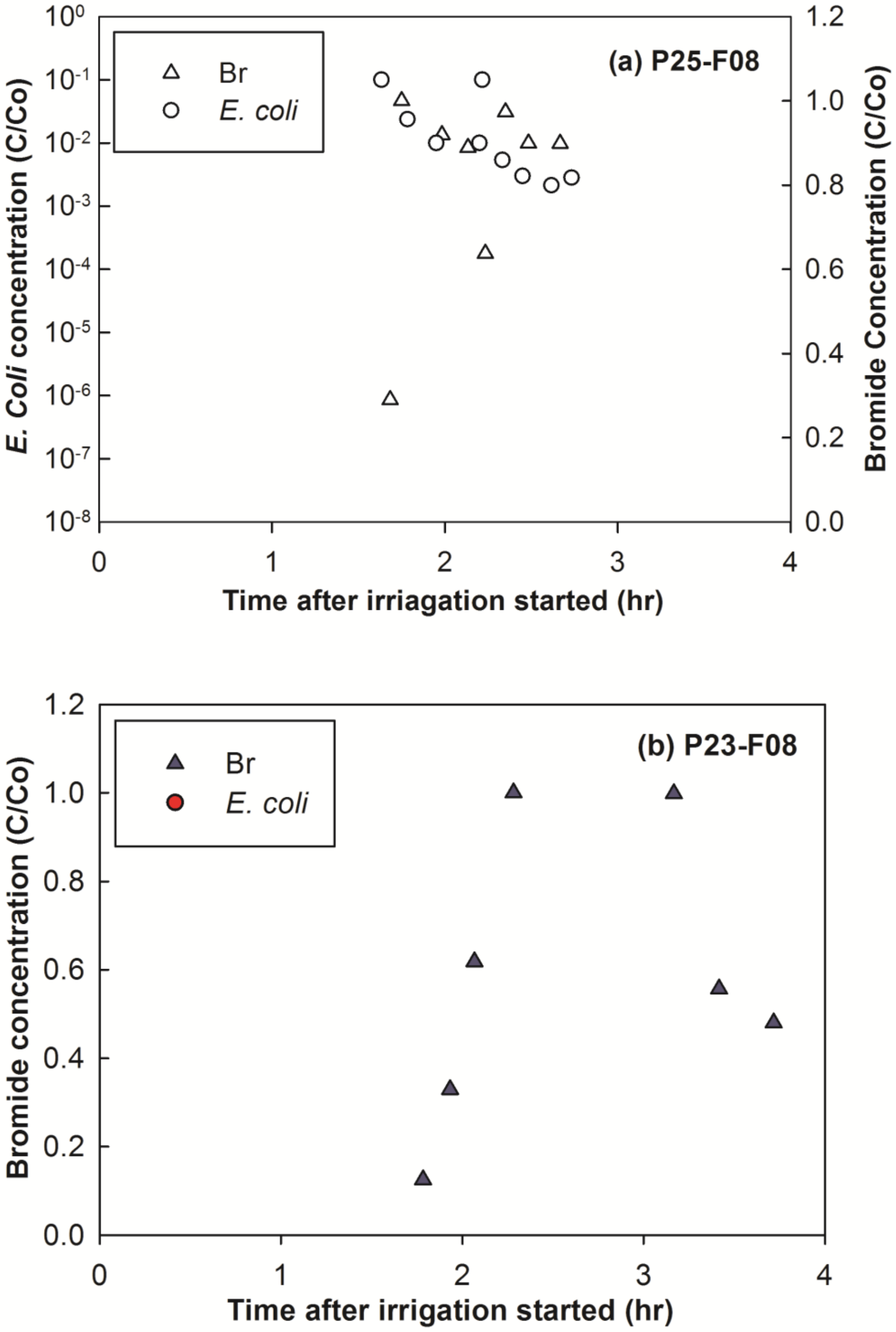


Figure 4.7. The breakthrough curves of *E. coli* and bromide tracer in the fall 2008 experiment.

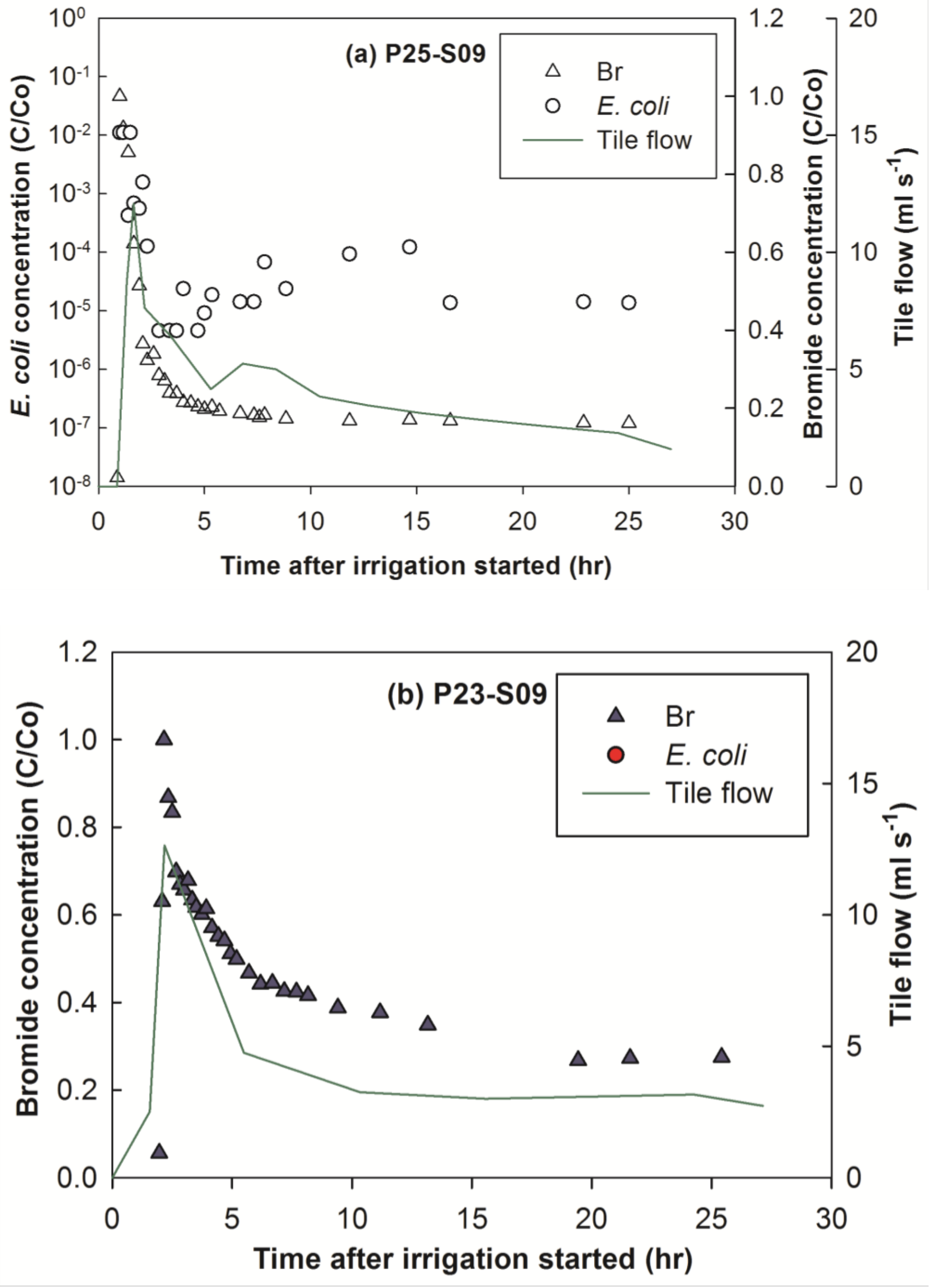


Figure 4.8. The breakthrough curves of *E. coli*, bromide tracer and the tile hydrograph in the spring 2009 experiment.

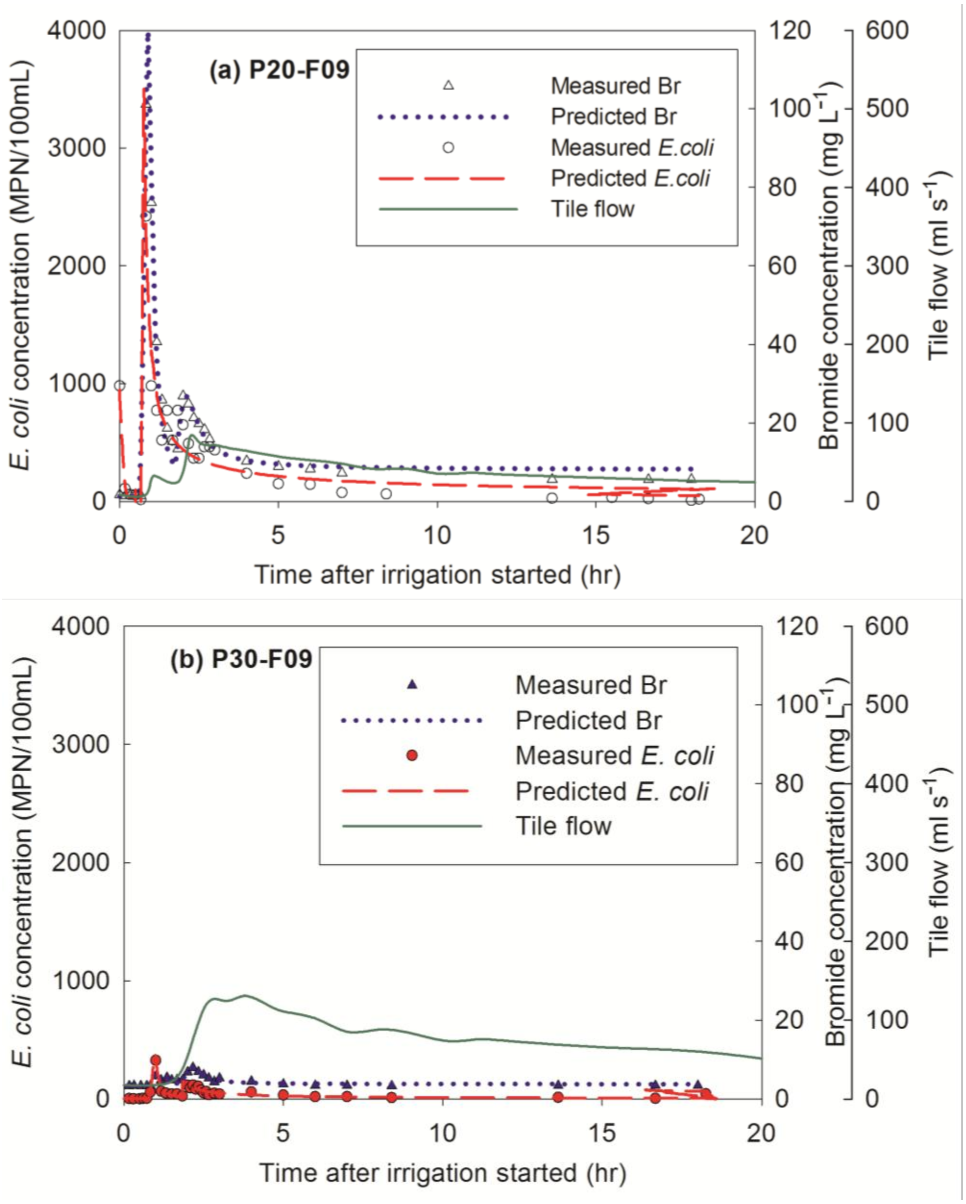


Figure 4.9. The breakthrough curves of *E. coli*, bromide tracer, their predicted concentrations and the tile hydrograph in the fall 2009 experiment.

CHAPTER 5. GENERAL CONCLUSIONS

5.1 Conclusions

Liquid swine manure applied to the land growing corn and soybeans crop rotation has been studied in two different field experiments at plot-scale, conducted from 2001 to 2006 and from 2008 to 2009. Transport of both nutrient and pathogen to subsurface drainage water and nutrient residual in the soil were examined. The primary conclusions from field experiments are reported in the following sections:

5.1.1 Nutrient in subsurface drainage water

Liquid swine manure applied to both corn and soybean crops under corn and soybean rotation significantly increased the nitrate nitrogen leaching losses and concentrations to subsurface drainage water when compared with the only corn crop receiving liquid swine manure. This trend showed in every year and especially high values in wet year. Although the phosphorus concentrations and losses in subsurface drainage water were not significantly increased, liquid swine manure applied to soybean crops every year did raise the concentration of phosphorus in tile drainage water.

5.1.2. Nutrient residual in soil

Inversely to the findings in subsurface drainage waters, the residual of phosphorus concentrations in soil were found highly significant difference in the top 30 cm soil of corn and soybean plot receiving liquid swine manure each year. The concentrations were 3 to 4 times higher than the optimal values recommended for land growing corn and soybean crop in Iowa. The nitrate nitrogen concentrations in top 15 cm-depth soil also showed higher values in the soybean plots that receiving liquid swine manure, and higher than EPA's MCL level of NO_3-N for safe drinking water. The high residual of these nutrient substances wasclosely linked to the amount leaching losses to subsurface drainage water and present of that in the environment.

5.1.3. Pathogen transported to subsurface tile drainage through macropores

Significance of the role of directly connected macropores in transporting *E. coli* from soil surface to subsurface drainage was well recognized after four field experiments, regardless of base flow condition of tile drainage at the beginning of experiment. The concentrations and time to get the peak of *E. coli* concentration should be mainly focused on approximately first three hours since liquid swine manure applied and following rainfall. Bromide could be used as effective tracer in tracing the path and time of travel for *E. coli* concentrations in subsurface drain water.

5.2 Prospects for future research

The impacts of applying liquid swine manure to a soybean crop at the level of 225kg ha^{-1} in fall on the quality of subsurface drainage water and soil were evident. The yield, however, did not clearly show significant increase. Directly connected macropores clearly shows the important roles in transporting bacteria from soil surface to subsurface drain water. Therefore, a future study could be suggested to investigate.

1. A further study should be incorporative of using other crop as land cover to the soybean plots that receiving manure in order to decrease the nitrate nitrogen and phosphorus remained in soil and reduces leaching losses of these nutrient substances.
2. A comprehensive qualitative of cost and benefit of applying liquid swine manure to soybean crop should also be examined carefully in attempting of solving the environmental problem of animal waste disposal.

3. The investigations of the role of macropores to transport microorganisms like virus would be also promising studies. In addition, the empirical equations generated from these studies should be assessed in the simulation of modeling where macropores component is available and/or cooperative and under the similar field condition.

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