THE EFFECT OF SWINE MANURE APPLICATION ON BACTERIAL QUALITY OF LEACHATE FROM INTACT SOIL COLUMNS

Elizabeth Warnemuende and Rameshwar Kanwar

ABSTRACT

Excessive application of swine manure on agricultural lands is likely to increase the potential of water pollution. The impact of swine manure management on bacterial contamination in subsurface drainage is often difficult to assess in the field. In this study, leachate from intact 20-cm (8-inch) diameter, 30-cm (12-in) long soil columns receiving fall and spring manure applications at 168 kg-N/ha (150 lb-N/ac) and 336 kg-N/ha (300 1b-N/ac) was analyzed for bacterial densities. The soil columns were collected in sterile galvanized tubing using a Giddings probe and 20-cm bit adapter. Fecal coliform, Escherichia coli (E. coli), and enterococci densities in leachate from the columns were determined for four weekly irrigation events following manure application. While a positive correlation between the manure application rate and bacterial densities in the leachate water was observed, this effect was not generally statistically significant at the 10% level. However, an interaction between the application rate and timing was observed, suggesting that an increase in application rate is more likely to cause greater bacterial contamination in subsurface drainage for spring application than for fall application. This contributed to significant differences between the spring 336 kg-N/ha treatment and other treatments. Therefore, manure applied at 336 kg-N/ha during the spring may contribute to bacterial contamination of ground water and tile drainage at a significantly higher level than fall and spring manure applications at 168 kg-N/ha and fall applications at 336 kg-N/ha. Additionally, more rapid bacterial die-off was observed in leachate from fall manure-applied columns, where soil columns were frozen for 7 weeks between manure application and irrigation, compared to the spring manure-applied columns. Bacterial densities in the leachate from fall manure-applied soil columns were significantly lower in comparison with bacterial densities in leachate from the spring manure-applied soil columns at the 10% level during the second, third, and fourth irrigation events.

INTRODUCTION

The total number of swine (*Sus* spp.) farms in the United States having at least 10,000 sows increased from 31 to 54 between 1994 and 1997 (Freese, 1994, 1997). The resultant manure, which is often land applied to cropland as fertilizer or soil conditioner, has been shown to effectively improve soil tilth and increase water holding capacity, resistance to crusting, and resistance to compaction (Letson and Gollehon, 1996). The shift towards larger hog confinement units has intensified the need for proper manure handling techniques. Appropriate manure application rates, timing, and methods are necessary maximize manure utility, while minimizing the pollution potential from the use of manure.

Potential pollutants, which may emanate from land-applied manure, include bacteria. Bacterial water quality determines suitability for drinking and recreational uses. Drinking water supplies must not contain more than 2,000 colony forming units per 100ml (cfu/100ml) fecal coliform prior to primary treatment, and recreational waters must not contain more than 200 cfu/100ml fecal coliform (limited contact). Typical swine manure contains 6,500,000 cfu/100ml fecal coliform. Under current manure application guidelines, leachate from manure-amended fields reaching subsurface tile drain often exceeds drinking water supply and recreational use standards. This paper will focus subsurface bacterial leaching, which may result in the movement of bacteria to receiving surface and ground waters. Specifically, the impacts of different manure management regimes on fecal coliform, Escherichia coli (E. coli), and enterococci densities in leachate from intact soil columns were examined.

The objective of this study was to identify the optimum swine manure application rate and timing, in order to minimize bacterial transport to receiving surface and ground waters via subsurface bacterial leaching.

REVIEW OF LITERATURE

Bacterial Pollution From Land Applied Manure

Bacteria in land-applied manure may pollute soil and vegetation, as well as surface and ground waters. This pollution threatens the environment and human health. Bacterial pollution may impair soil when nutrient cycling and decomposition rates are altered by competition of manure-borne bacteria with indigenous soil bacteria (Doran, 1979). Limiting the quantity of manure applied to a single site can reduce buildup of introduced bacteria in the soil. Bacterial pollution may additionally render vegetation unsuitable for grazing. The rate of bacterial die-off on vegetation is impacted by manure application timing (Brown et al.,1980), and by pasture management (Bell and Bole, 1976).

Surface waters are impacted by direct surface runoff, groundwater flow, and subsurface tile drainage that discharges to surface water. When surface waters that are used for drinking water or recreational uses become impaired by bacterial contamination,

a threat to human health exists. The greatest potential for bacterial losses to occur is associated with surface runoff. Implementing certain management practices can reduce this potential:

- Land application of manure should not take place during the 72 hours prior to a runoff event (Crane et al., 1978).
- Because bacteria are more likely to survive longer in cooler temperatures and to move from the field with runoff on frozen ground, manure application to frozen ground or snow cover should be avoided (Robbins et al., 1971).
- Greater manure storage capacity allows more flexibility in timing of application, and can increase bacterial decimation prior to land application by increasing storage time.
- Vegetative filter strips have been shown to be effective at substantially reducing fecal coliforms in overland flow reaching surface water (Larsen et al., 1994).
- In addition to flowing water, pathogenic bacteria from land-applied manure may also be transmitted by wind, insects, and rodents. For this reason, manure should not be land applied in densely populated areas (Morrison and Martin, 1977).
- Subsurface injection may greatly reduce, if not eliminate, bacterial losses with runoff, as compared to surface broadcast. However, this method reduces bacterial contact with surface soil, thereby increasing the likelihood of bacterial movement with drainage water.

Bacterial Transport to Subsurface Drainage Water

Bacterial movement to subsurface drainage water may contribute to surface water contamination via artificial tile drainage, or groundwater contamination via bacterial leaching. When bacteria are introduced to the soil through land application of manure, the rate at which they reach the depth of drain tile or aquifer is of great interest. The leaching of viable bacteria in the subsurface is a function of both their movement and their survival, is site and organism specific, and varies with atmospheric conditions and water and manure characteristics. This section addresses the factors that govern the transport of bacteria in the subsurface.

Soil characteristics

Texture and particle size distribution affect straining processes. A study by Jang et al. (1983) showed straining to contribute significantly to the removal of bacteria from leachate where the average bacteria cell size was greater than the size of at least 5% of particles. Pore size may contribute to filtration removal, sedimentation of bacteria in pores, and consequent reduction of permeability of the soil (Peterson and Ward, 1989).

Several soil characteristics influence bacterial sorption, and thus bacterial transport. Because bacteria sorb more readily to positively charged mineral surfaces than to negatively charged mineral surfaces (Scholl et al., 1990), mineral makeup of the soil impacts bacterial sorption. Organic matter can affect the surface charge and hydrophobicity characteristics of the base mineral (Harvey, 1991), and increase surface area and sorption sites. Soil pH influences the pH of infiltrating water. While the pH effects on bacterial sorption are dependent upon soil and organism characteristics, bacterial retention is generally higher in neutral to acidic conditions than in alkaline conditions (Goldschmidt et al., 1973).

Moisture properties

Physical moisture conditions such as soil water content, temperature and flux, as well as flow rate impact bacterial transport (McCoy and Hagedorn, 1979), (Yates and Yates, 1988). These factors influence the processes of advection and dispersion, as well as bacterial adsorption. High moisture content and flow rate contribute to bacterial leaching. The pH and ionic strength of infiltrating water impacts bacterial transport by the same mechanisms as the pH and ionic strength of the soil.

Bacterial characteristics

The density and dimensions of the microorganism affects the processes of straining and gravitational leaching. In saturated conditions, bacteria may become mobile through means of their own locomotion. This mobility depends on the type of microorganism, but has been shown to be a significant means of transport for motile strains of E. coli (Reynolds et al, 1989). A study by Huysman and Vertraete (1993) showed that cell surface hydrophobicity impacts bacterial transport. In this study, hydrophobic bacteria adhered to the soil more readily than hydrophillic bacteria. Cell surface charge may also play a role in bacterial transport (Sharma et al., 1985).

Bacterial Survival in the Subsurface

The survival rate of microorganisms introduced to soil is a function of many factors. Table 1 gives a summary of these factors.

Table 1. Factors influencing the survival of bacteria in the subsurface.

Physiochemical Characteristics of Soil
a) pH
b) porosity
c) organic matter
d) texture
e) temperature
f) moisture
g) adsorption/ filtration
I) nutrients

Table 1 continued. Factors influencing the survival of bacteria in the subsurface.

Atmospheric Conditions
- a) sunlight
- b) moisture
- c) temperature

Biological Interactions
- a) competition
- b) antibiotics
- c) toxic substances

Application Methods
- a) technique
- b) frequency
- c) organism density in waste material

MATERIALS AND METHODS

Eighteen soil columns were collected from the Iowa State University Agronomy and Agricultural Engineering Research center near Ames, IA in order to accommodate three replications of four manure treatments and two control treatments. Soil column treatments are listed in Table 2. The soil was a Clarion loam in annual corn and soybean rotation. Soil columns were extracted in late fall, after the 1999 soybean harvest, using a Giddings probe and a 20-cm bit adapter. The 30-cm columns were extracted in 38-cm sections of sterilized galvanized tubing that had been sharpened on the down - facing edge (Figure 12). In order to detect compaction, the vertical distance between the top edge of the column and the inside soil surface was measured and compared to the vertical distance between the top edge of the column and the outside soil surface, prior to extraction of each soil column (Figure 13). No compaction was detected.

Table 2. Experimental Treatments.

Spring Control	Not amended
Fall Control	Not amended
Spring Inject 1X	Manure application at a rate of 168kg-N/ha (150lb-N/ac)
Spring Inject 2X	Manure application at a rate of 336kg-N/ha (300lb-N/ac)
Fall Inject 1X	Manure application at a rate of 168kg-N/ha (150lb-N/ac)
Fall Inject 2X	Manure application at a rate of 336kg-N/ha (300lb-N/ac)

The soil columns were transported to a growth chamber, simulating the soil temperature at the 10 cm (4 inch) depth during the typical periods of fall and spring manure application. Autoclaved screen was installed on the bottom of each column in

order to prevent soil loss. The columns were then arranged in a random block design in a leachate collection apparatus consisting of 25-cm autoclaved funnels and a guide table that prevented the columns from deviating from the vertical position (Figure 14). They were saturated with 5000ml of water and allowed to drain for four days. After this period, manure was incorporated to the 10 cm depth. The manure was obtained from a finishing unit at Bilsland Memorial swine farm near Luther, IA and was less than 7 days aged. Bacterial analysis revealed a fecal coliform density of 2,000,000 cfu/100ml.

The spring soil columns remained under May conditions in the growth chamber following manure application. The growth chamber temperature was set to reflect the average daily minimum and maximum soil temperature fluctuations at the 10 cm depth, using a ten-year average from data collected at the experimental site from which the columns were extracted. The temperature regime is illustrated in Figure 1.

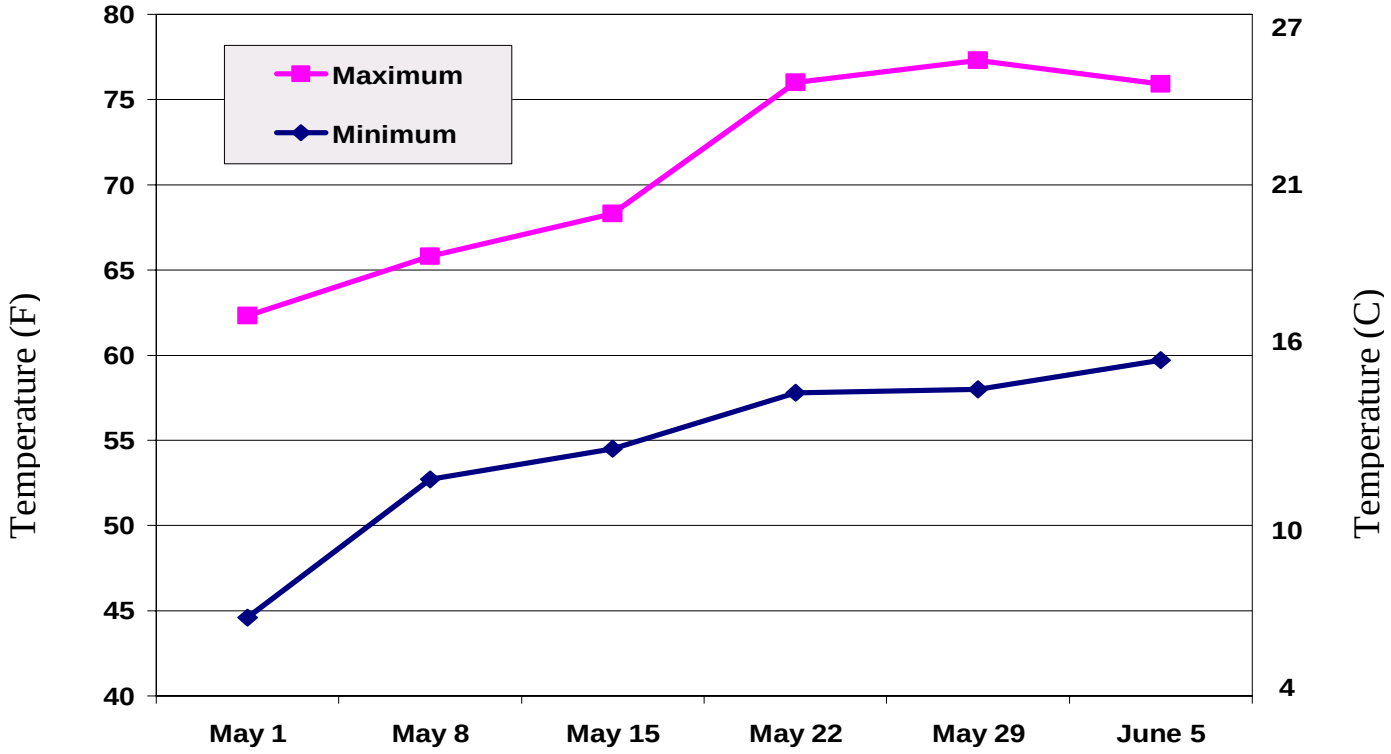

Figure 1. Average daily soil temperature at the 10 cm (4 inch) depth, Ames IA, 1990-1999.

Soil temperature was chosen over air temperature for the growth chamber program because of the semi-exposed condition of the soil columns, which is in contrast to the less exposed condition of a similar soil profile in situ. Buffering of air temperature fluctuations, which significantly affects soil temperature at depth, was built in to the growth chamber temperature program by setting the growth chamber air temperature equal to the average daily soil temperature at the 10 cm depth. In the growth chamber, the average daily minimum soil temperature occurred during 12 hours of darkness and was followed by 12 hours of the average daily maximum soil temperature during 12 hours of light.

Six days after manure application, the first of four irrigation events took place. Water was irrigated to a ponding depth of 5.3 cm (volume = 1700ml), which is a typical weekly rainfall amount for the first week in May. Weekly rainfall depths were based on weekly rainfall data and irrigated in a single event in order to produce the effects of macropore flow and yield enough leachate to perform bacterial analyses. The leachate was collected in sterile plastic sample bottles and analyzed for fecal coliform, E. coli, and enterococci using Standard Methods 9222D, 9222G, and 9230C, respectively. This process was repeated for the second, third, and fourth irrigation events. Ponding depth for these events was 3.7 cm (volume = 1200ml), 3.4 cm (volume = 1100ml), and 3.4 cm (volume = 1100ml), respectively. Outflow was quantified in order to provide data necessary to complete water budgets on each column, and confirm uniform moisture contents. Average outflows between treatments were similar.

A mass evaluation was performed on three representative soil columns. Prior to each irrigation event, these columns were weighed. The mass of outflow was monitored using volumetric analysis of leachate samples. The mass data were used in conjunction with moisture analysis of the columns after the completion of the study in order to model the water budget for each column. Mass data are given in Table 3.

Table 3. Mass balance for three representative fall soil columns.

	Fall control replicate 2 mass (kg)	Fall 2X replicate 1 mass (kg)	Fall 2X replicate 2 mass (kg)
Prior to Event 1	17.91	19.01	17.90
Irrigation	1.70	1.70	1.70
Drainage water	0.82	0.78	0.79
Evapotranspiration	0.67	0.77	0.64
Prior to Event 2	18.12	19.16	18.17
Irrigation	1.20	1.20	1.20
Drainage water	0.43	0.42	0.55
Evapotranspiration	0.71	1.00	0.83
Prior to Event 3	18.18	18.94	17.99
Irrigation	1.10	1.10	1.10
Drainage water	0.43	0.12	0.30
Evapotranspiration	1.06	1.62	0.71
Prior to Event 4	17.8	18.3	18.08
Irrigation	1.10	1.10	1.10
Drainage water	0.36	0.10	0.13

Six days after manure application, fall soil columns were sealed and transported to a freezer, where they remained for 7 weeks, to simulate over-winter conditions of below freezing temperatures and snow cover, and to produce the cell changes associated with freezing and thawing. After this period, they were transported to a growth chamber simulating the same time period as the spring columns. According to field data, this is the period during which bacterial leaching occurs on fall-manured plots as well as spring manured plots (Figures 2 & 3). Irrigation events on the fall soil columns began two days after transport to the growth chamber. The depth and timing of fall soil column irrigation events were the same as the depth and timing of spring column irrigation events.

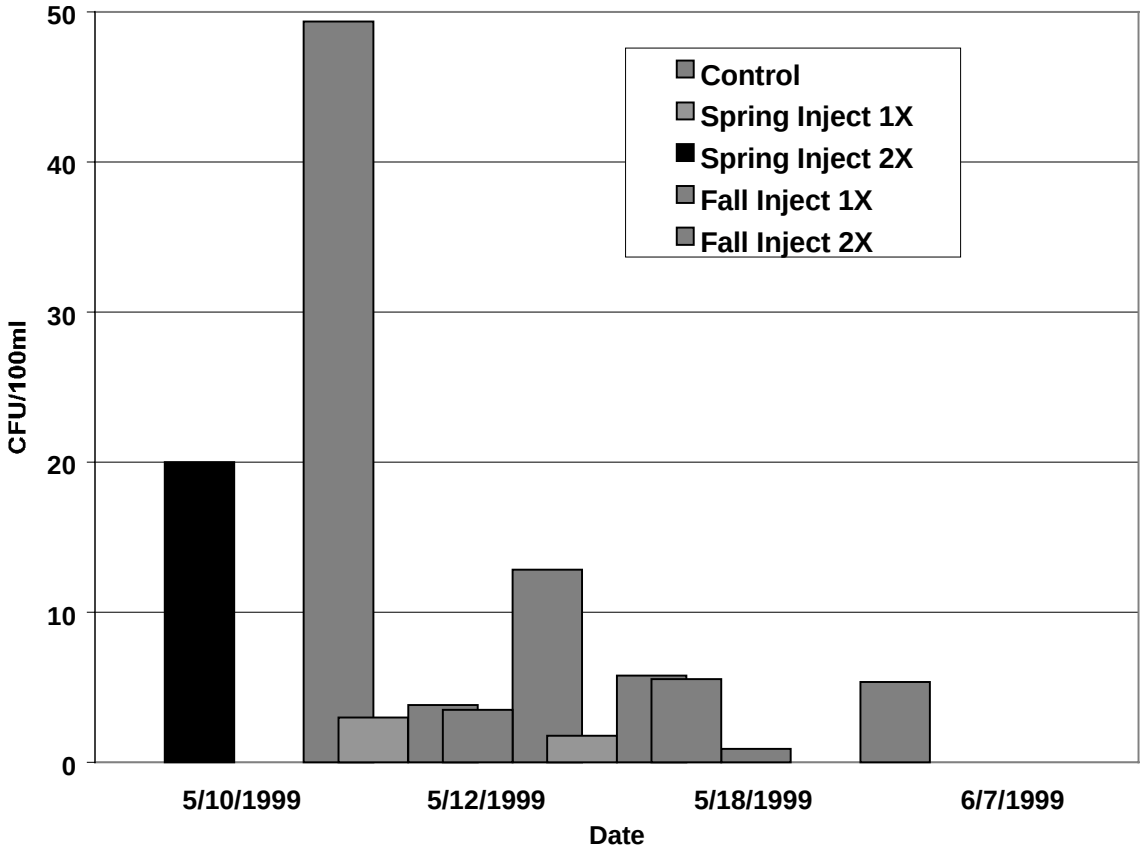

Figure 2. Fecal coliform densities in Subsurface Drainage from field plots.

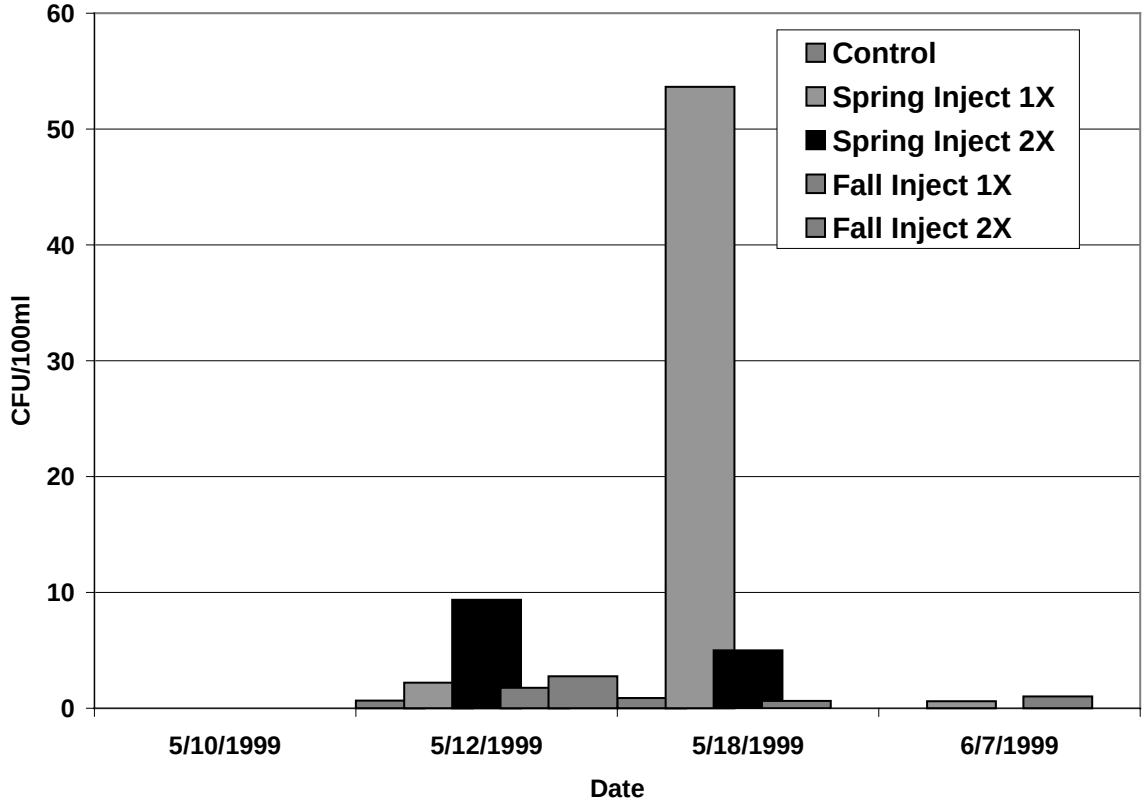

Figure 3. E. coli densities in Subsurface Drainage from field plots.

RESULTS AND DISCUSSION

Bacterial densities in soil column leachate from irrigation events 1 through 4 are given in Figures 4 through 7 and in Tables 4 through 11 of the Appendix. In general, the double rate manure treatment resulted in slightly higher bacterial densities in soil column leachate. This difference became more significant with successive irrigation events because of the higher organic matter present in double rate columns, which minimized the stress of between – event drying on bacteria. The application rate effect was statistically significant at the 10% level for enterococci during event 3. The fall columns yielded similar bacterial densities as the spring columns for event one, and lower bacterial densities for events 2, 3, and 4. The application timing effect was significant at the 10% level during events 3 and 4 for fecal coliform, during events 2 and 3 for E. coli, and during event 2 for enterococci. Fall bacteria survived the freeze-thaw cycle and over-winter conditions in a weakened state and experienced more rapid die-off than the spring columns. The higher organic matter available to bacteria in the double rate columns contributed positively to the survival of bacteria, particularly the fall bacteria. An interaction between rate and timing interaction was significant for fecal coliform during event 4 and E. coli during events 3 and 4.

While bacterial densities were higher in leachate from double rate manure columns during event 1, no significant differences between treatments were detected during this event. However, bacterial densities from control columns were significantly lower than in manured columns, with the exception of enterococci in leachate from the spring control column. Enterococci have a high degree of survivability in the soil. For this reason, the effects of wildlife activity or general farm operations on the soil column extraction site prior to soil column extraction, which may have caused bacterial contamination of control columns, would be most visible and most persistent in enterococci densities. With the exception of enterococci, bacteria were not detected in the control columns after the first irrigation event, and control columns were always significantly different from manure treated columns.

Event 2 resulted in higher bacterial densities in leachate from columns receiving double manure application rate, although this difference was not significant. The effect of timing was significant however, with E. coli and enterococci densities significantly lower in fall columns leachate than in spring column leachate. E. coli densities in leachate from spring columns were significantly lower than E. coli densities in leachate from the fall double rate columns.

Bacterial quality of leachate resulting from event 3 was significantly influenced by both timing and rate, and was poorest among the spring double rate columns. Spring double rate columns resulted in significantly higher E. coli densities than fall single and double rate columns. Spring single rate columns resulted in significantly higher E. coli densities in leachate than fall single rate columns. Other differences between treatments were evident, although not statistically significant at the 10% level.

During event 4, spring double rate columns continued to result in the poorest quality leachate. This treatment resulted in fecal coliform densities in leachate significantly higher than all other treatments, and E. coli densities higher than spring single rate and fall double rate treatments.

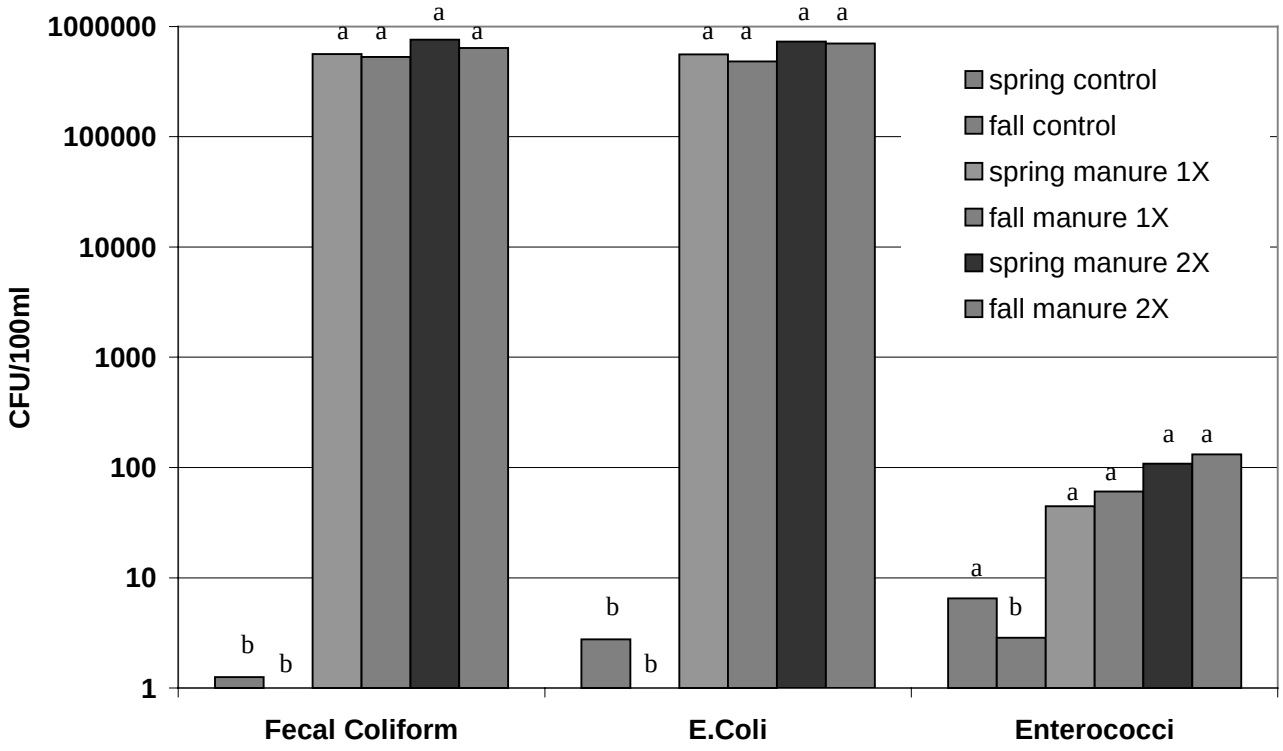

Figure 4. Bacterial densities in soil column leachate from event 1.

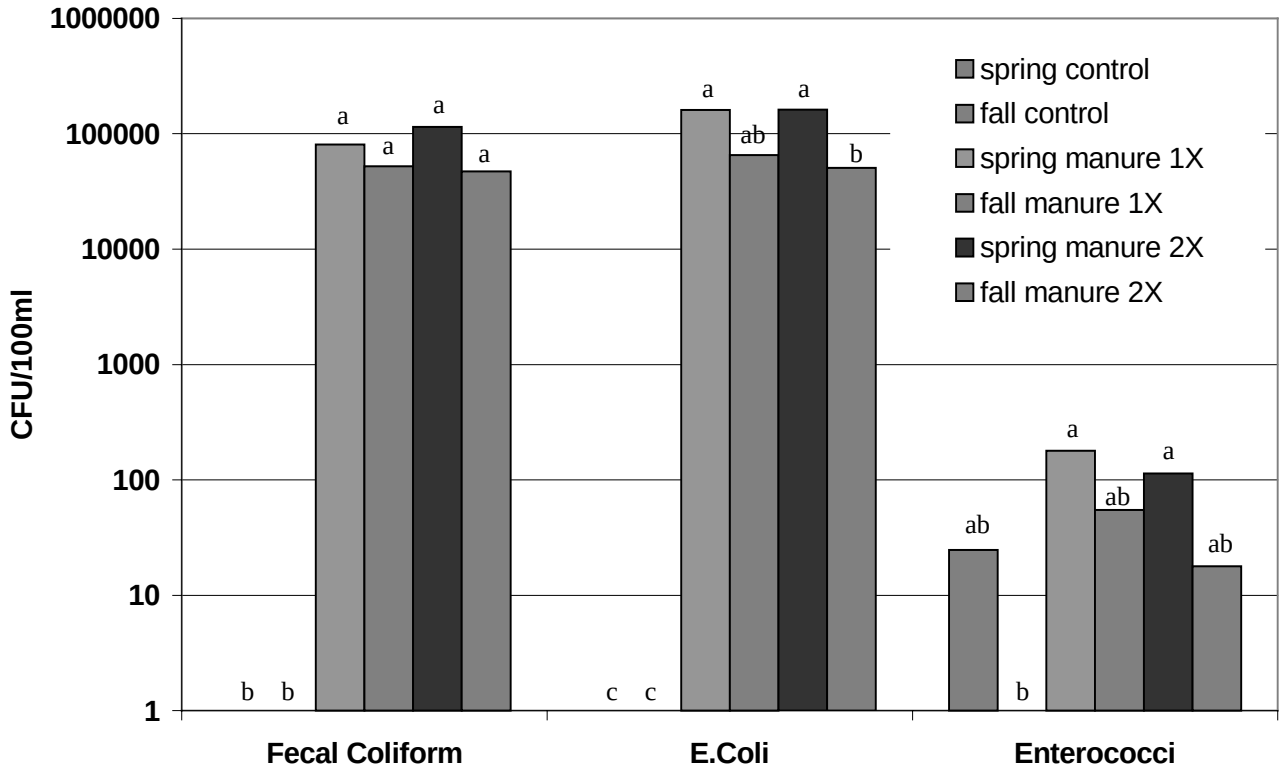

Figure 5. Bacterial densities in soil column leachate from event 2.

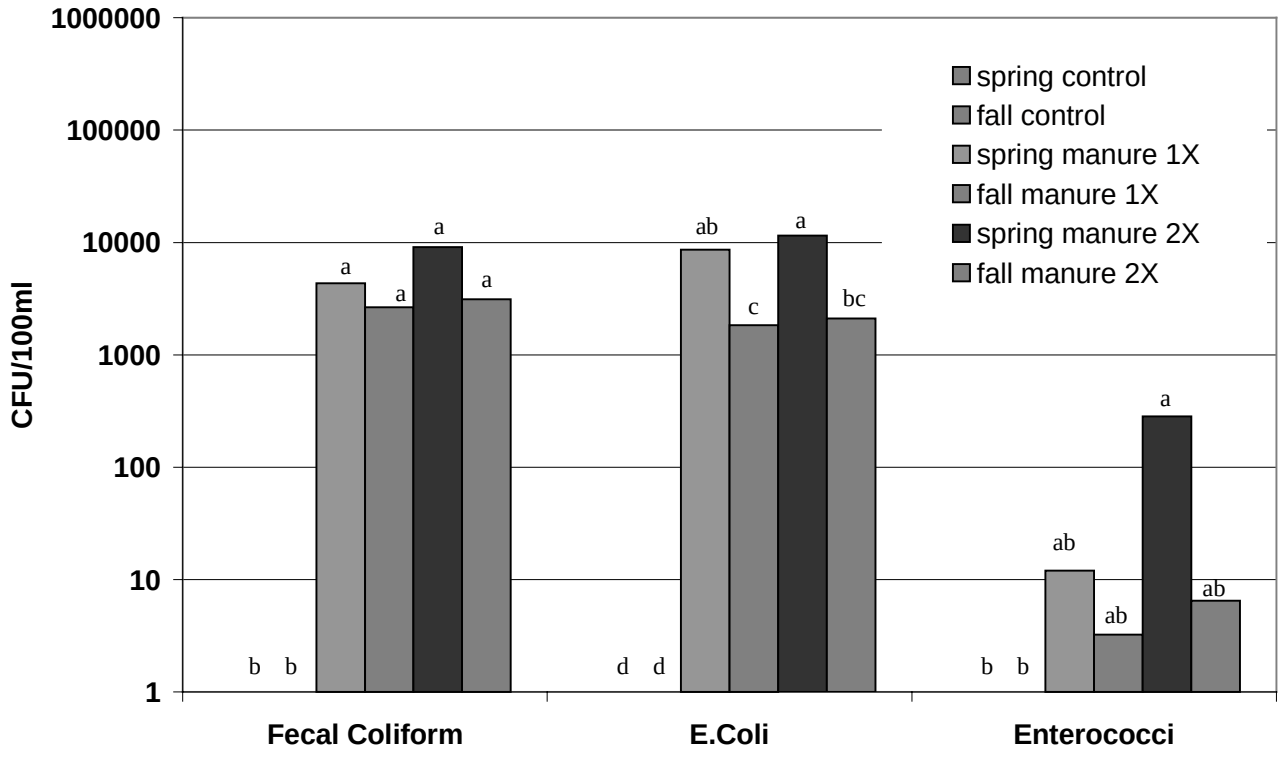

Figure 6. Bacterial densities in soil column leachate from event 3.

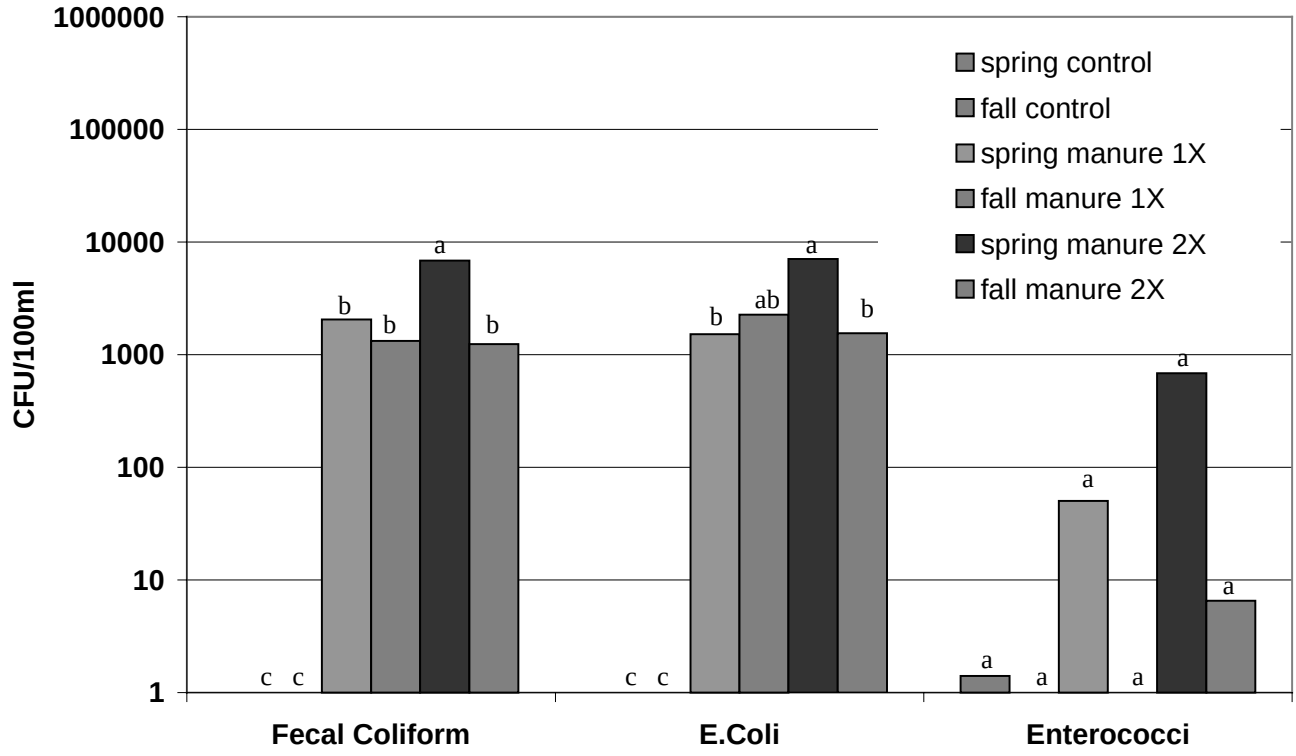

Figure 7. Bacterial densities in spring soil column leachate from event 4.

It is expected that fecal coliform densities follow a similar pattern to E. coli densities, since E. coli is a subset of fecal coliforms. Enterococci are unrelated enteric organisms however, with a higher degree of survivability in the soil. This may explain the different pattern of enterococci levels over time and background levels of enterococci in control columns, which received no manure application (Figures 8 - 10). Faster die off of bacteria in single rate treatments can be clearly observed in Figures 8 through 10.

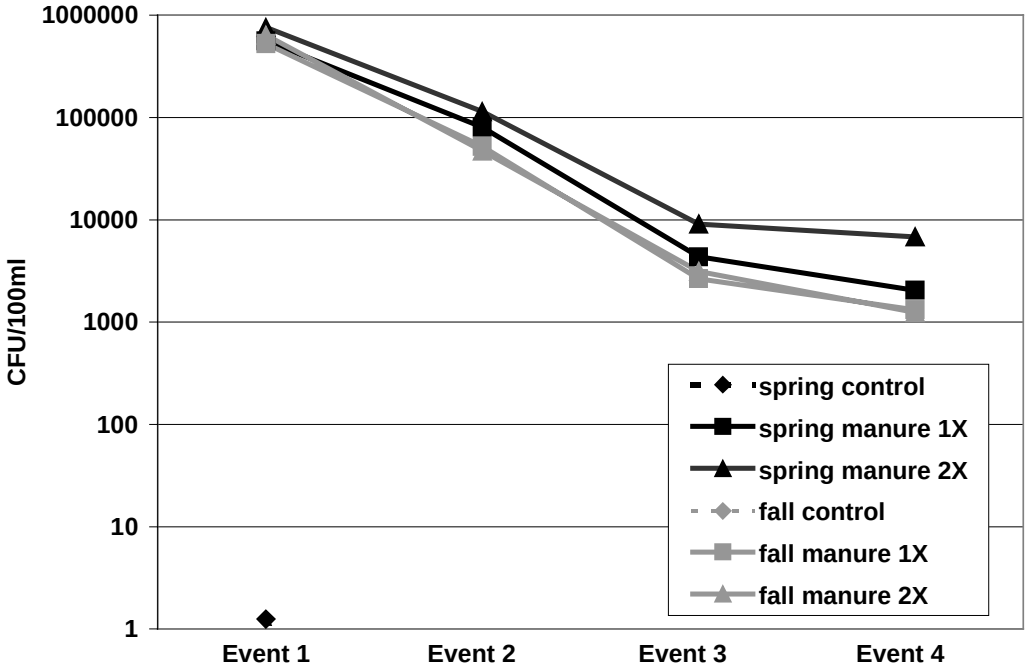

Figure 8. Fecal coliform density in soil column leachate.

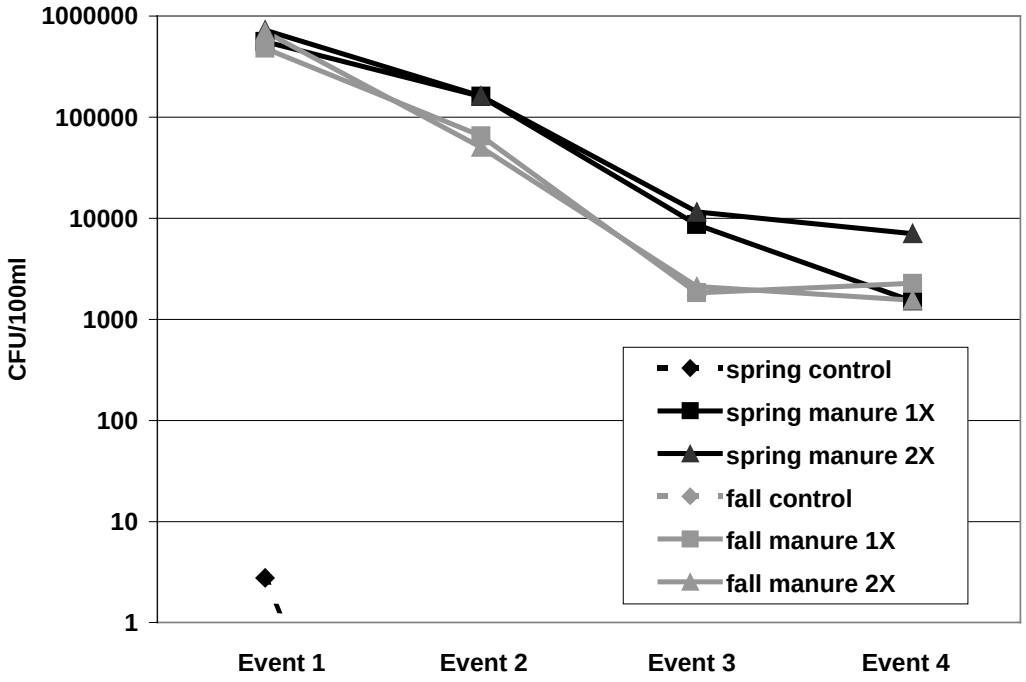

Figure 9. E. coli density in soil column leachate.

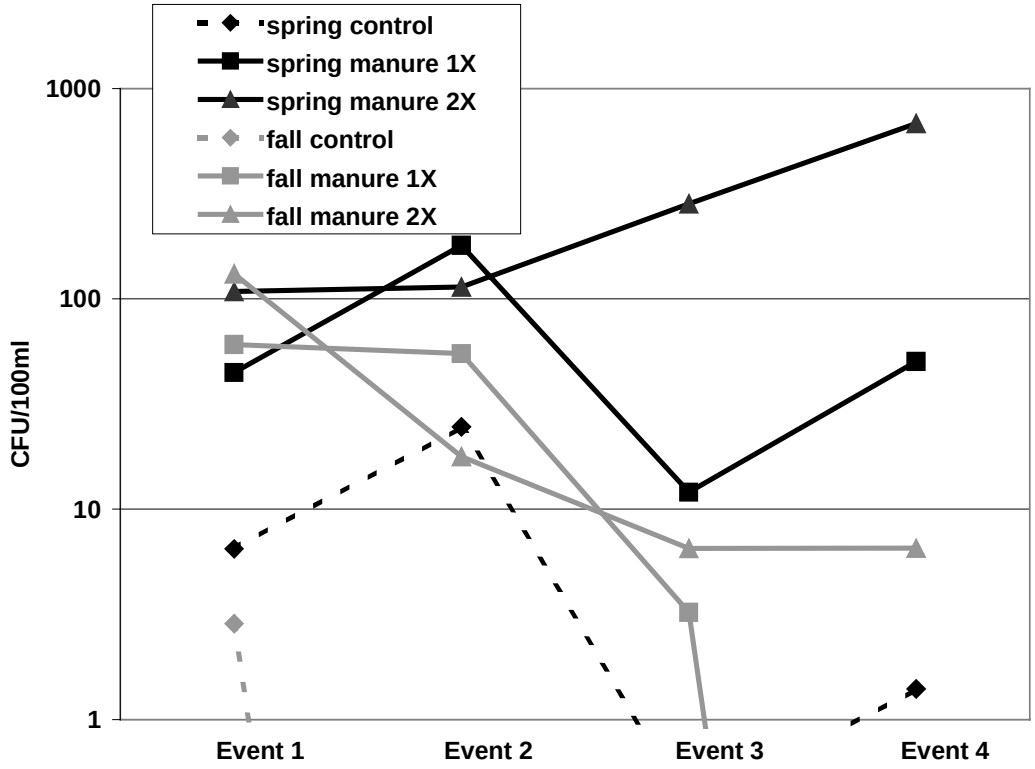

Figure 10. Enterococci density in soil column leachate.

Fluctuations in soil column gravimetric moisture content are believed to have been the major factor contributing to bacterial die-off in this study. These fluctuations can be observed in Figure 11. It is possible that more significant differences resulting from application timing and rate would be observed under more ideal moisture conditions.

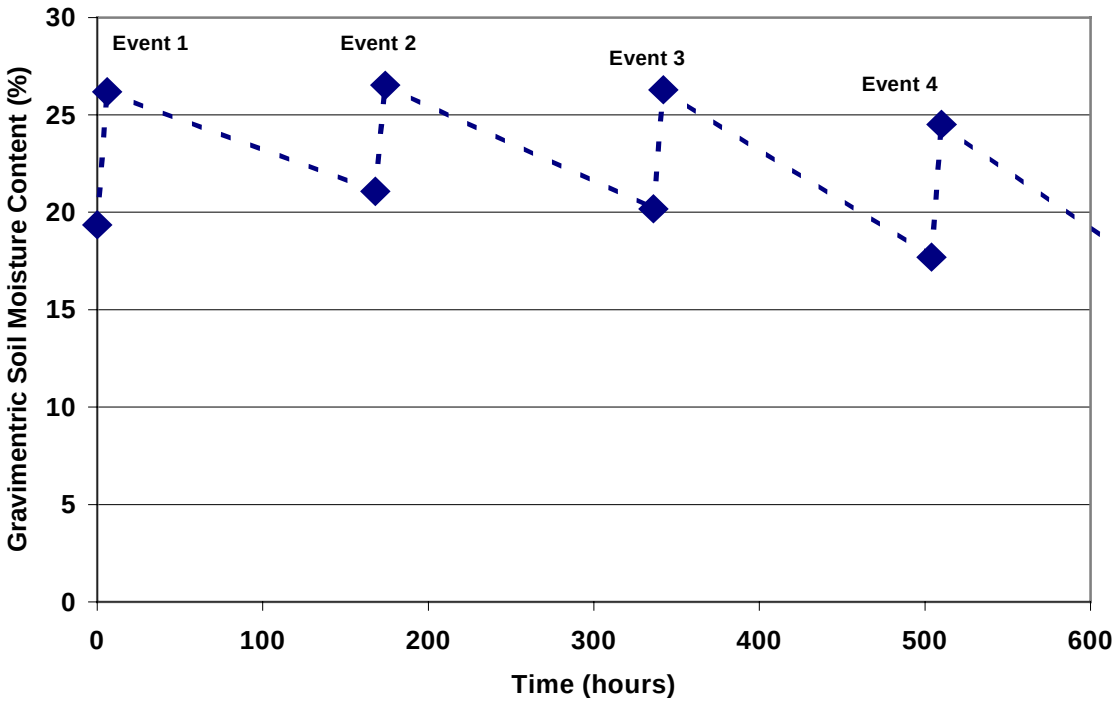

Figure 11. Average gravimetric moisture content of soil columns over time.

Statistical analysis of bacterial counts yielded similar results to statistical analysis of bacterial densities. There were no significant differences in drainage volume between treatments.

CONCLUSIONS

Intact soil columns were used to model the movement of bacteria to subsurface drainage following fall and spring swine manure applications at a rate of 168 kg-N/ha and a rate of 336 kg-N/ha. In almost every case, leachate from manured columns had significantly higher bacterial densities than leachate from non-manured control columns. This suggests that land application of swine manure is likely to cause bacterial contamination of subsurface drain water, even at the recommended application rate of 168 kg-N/ha.

Clear differences in bacterial densities were identified between treatments during the second, third, and fourth irrigation events following manure application. Spring application of swine manure resulted in higher bacterial densities in subsurface drainage than fall application during the five-week period following spring manure application. Specifically, the spring 336 kg-N/ha treatment yielded higher bacterial densities than other treatments during all but the first irrigation event. This suggests that manure applied to the field at a rate of 336 kg-N/ha during the spring may contribute significantly more bacterial contamination to ground water and tile drainage than fall and spring 168 kg-N/ha manure applications and fall 336 kg-N/ha applications.

Although few significant differences were detected between application rates, the columns that received 336 kg-N/ha swine manure almost always yielded higher bacterial densities in leachate than the columns that received 168 kg-N/ha swine manure during the same season. Additionally, an interaction between the application rate and timing was observed, suggesting that an increase in application rate is more likely to cause greater bacterial contamination in subsurface drainage for spring application than for fall application.

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APPENDIX

Figure 12. Positioning tubing for soil column extraction with the Giddings probe.

Figure 13. Measuring the soil column for compaction.

Figure 14. Spring soil columns in the leachate collection apparatus.

Table 4. Bacterial densities in spring soil column leachate for event 1.

Event 1 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Spring control 1	<1	<1	<10	300
Spring control 1	<1	<1	2	300
Spring control 2	<10	<10	1	400
Spring control 2	8	10	<1	300
Spring control 3	<1	<1	5	300
Spring control 3	<1	<1	31	300
Spring 1X 1	580,000	640,000	32	300
Spring 1X 1	600,000	610,000	48	300
Spring 1X 2	530,000	500,000	30	250
Spring 1X 2	520,000	570,000	24	300
Spring 1X 3	550,000	570,000	66	400
Spring 1X 3	590,000	460,000	58	300
Spring 2X 1	1,000,000	960,000	52	300
Spring 2X 1	1,000,000	990,000	70	300
Spring 2X 2	990,000	1,000,000	150	350
Spring 2X 2	700,000	680,000	86	300
Spring 2X 3	510,000	420,000	152	450
Spring 2X 3	460,000	440,000	110	300

Table 5. Bacterial densities in spring soil column leachate for event 2.

Event 2 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Spring control 1	<1	<1	83	325
Spring control 2	<1	<1	<2	330
Spring control 3	<1	<1	<10	440
Spring 1X 1	98,000	190,000	440	301
Spring 1X 2	85,000	210,000	100	480
Spring 1X 3	57,000	62,000	55	325
Spring 2X 1	220,000	240,000	150	500
Spring 2X 2	77,000	170,000	160	420
Spring 2X 3	22,000	57,000	20	400

*Leachate samples from spring columns were divided by time of collection for event 1 in order to detect bacterial changes between first and final flushes within the event. None were detected and samples were composited for the remaining events.

Table 6. Bacterial densities in spring soil column leachate for event 3.

Event 3 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Spring control 1	<2	<2	2	198
Spring control 2	<1	<1	<1	320
Spring control 3	<1	<1	<1	520
Spring 1X 1	1000	3000	<10	395
Spring 1X 2	6300	9900	27	475
Spring 1X 3	6400	17,000	<10	195
Spring 2X 1	2700	2700	650	500
Spring 2X 2	21,000	25,000	110	415
Spring 2X 3	5500	9100	50	480

Table 7. Bacterial densities in spring soil column leachate for event 4.

Event 4 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Spring control 1	<2	<2	7	180
Spring control 2	<1	<1	<1	280
Spring control 3	<1	<1	<1	440
Spring 1X 1	1300	1000	<10	170
Spring 1X 2	2500	1800	82	350
Spring 1X 3	1500	1300	<10	50
Spring 2X 1	2000	1900	80	360
Spring 2X 2	5200	5000	3400	140
Spring 2X 3	15,000	16,000	<10	240

Table 8. Bacterial densities in fall soil column leachate for event 1.

Event 1 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Fall control 1	<3	<3	<3	980
Fall control 2	<1	<1	<1	820
Fall control 3	<1	<1	10	720
Fall 1X 1**	4,800,000	3,700,000	1200	780
Fall 1X 2	690,000	650,000	60	740
Fall 1X 3	430,000	370,000	70	680
Fall 2X 1	890,000	900,000	310	780
Fall 2X 2	650,000	900,000	73	790
Fall 2X 3	420,000	360,000	30	920

Table 9. Bacterial densities in fall soil column leachate for event 2.

Event 2 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Fall control 1	<1	<1	<1	500
Fall control 2	<1	<1	<1	430
Fall control 3	<1	<1	<1	310
Fall 1X 1**	340,000	350,000	91	460
Fall 1X 2	32,000	45,000	<10	440
Fall 1X 3	110,000	130,000	80	300
Fall 2X 1	71,000	87,000	20	420
Fall 2X 2	34,000	35,000	30	550
Fall 2X 3	41,000	35,000	<10	430

Table 10. Bacterial densities in fall soil column leachate for event 3.

Event 3 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Fall control 1	<1	<1	<1	500
Fall control 2	<3	<3	<3	425
Fall control 3	<3	<3	<3	350
Fall 1X 1**	24,000	11,000	<10	320
Fall 1X 2	2300	1800	<10	335
Fall 1X 3	3400	1900	10	160
Fall 2X 1	5400	3900	20	120
Fall 2X 2	2000	2100	10	300
Fall 2X 3	3300	1600	<10	410

Table 11. Bacterial densities in fall soil column leachate for event 4.

Event 4 Column	Fecal Coliform (CFU/100ml)	E.coli (CFU/100ml)	Enterococci (CFU/100ml)	Volume (ml)
Fall control 1	<1	<1	<1	480
Fall control 2	<3	<3	<3	360
Fall control 3	<3	<3	<3	300
Fall 1X 1**	7500	7100	<10	320
Fall 1X 2	870	1700	<10	260
Fall 1X 3	2300	3500	<10	120
Fall 2X 1	730	1400	10	100
Fall 2X 2	1600	1900	10	125
Fall 2X 3	1300	1300	<10	120

** outlier