Fecal Indicator Bacteria in Subsurface Drain Water Following Swine Manure Application

E. A. Pappas, R. S. Kanwar, J. L. Baker, J. C. Lorimor, S. Mickelson

Abstract. *Appropriate manure application parameters are necessary to maximize nutrient utilization by plants from manure while minimizing water pollution potential. This study focused on the movement of bacteria to receiving tile drains following swine manure application. Specifically, the impacts of different manure application regimes on fecal coliform (FC), Enterococcus (EN), and Escherichia coli (EC) densities in subsurface tile drain water were examined for three years. Manure treatments, including fall, spring, and late winter application at a recommended rate of 168 kg N ha^{-1} (1X) and at 336 kg N ha^{-1} (2X) were compared with a non-manure treatment where commercial urea-ammonium nitrate (UAN) was applied. Results indicate that flow-weighted average and maximum observed EN and EC levels in tile water were significantly higher where manure had been applied during late winter at the 2X rate versus the UAN and fall treatments. Levels of FC were highly variable, and the spring injection 1X treatment yielded the highest flow-weighted average and maximum tile water FC levels. Results of this study suggest that manure broadcast onto frozen ground may lead to significantly elevated EN and EC levels in tile water in similar environments, especially when applied in excess of crop nutrient requirements.*

Keywords. E. coli*, Fecal bacteria, Manure application, Subsurface drainage.*

The application of livestock manure on cropland utilizes nutrients and organic matter in the manure to increase soil fertility and tilth, while allowing producers to void manure storage facilities, but it is often associated with fecal contamination of receiving waters. In artificially drained fields, where subsurface drain tiles discharge to surface waters, tile drainage water may become a pathway for bacterial contamination of the receiving surface water, as well as the groundwater (Hunter et al., 2000; Monaghan and Smith, 2004). Current manure application guidelines do not explicitly prevent the introduction of pathogenic microorganisms to surface and ground waters. Therefore, it is important to identify manure application procedures that can minimize bacterial pollution from land application while maintaining crop yield. Specific manure application parameters include application timing and rate. In the absence of firm application timing and rate guidelines in many areas, manure application timing and rate are often functionally dictated by manure storage limitations. These application parameters must be optimized to maximize manure benefit, while minimizing the pollution potential from the use of manure.

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Livestock manure is associated with several pathogens that pose a health risk to humans. Fecal pathogens that may cause human waterborne illness include: *Escherichia coli* O157:H7, *Salmonella* sp., *Campylobacter* sp., *Shigella* sp., *Giardia lamblia*, and *Cryptosporidium parvum*. Because it is often difficult and expensive to detect these pathogenic organisms within reasonable detection limits, indicator organisms are used to detect fecal contamination and predict the likelihood of the presence of pathogenic organisms. Microbial water quality is usually described in terms of common indicator bacteria, such as fecal coliforms (FC), *Enterococcus* (EN), and/or *Escherichia coli* (EC).

Bacterial water quality determines the suitability of a water body for both drinking and recreational uses. In the U.S., the Ambient Water Quality Criteria (adopted 1986) set maximum geometric mean levels of EC and EN in recreational waters of 126 and/or 33 colony forming units (cfu) in a 100 mL sample, respectively (USEPA, 1986). States rely on these criteria in the development of state water quality standards. Public drinking water systems are expected to have zero total coliform (including FC and EC) in 100 mL (USEPA, 2003).

Several previous studies have examined bacterial transport in the subsurface using soil columns. Warnemuende and Kanwar (2002) found that bacterial densities in soil column leachate were decreased in fall manure-applied columns versus the spring manure-applied columns, especially when comparing the application timings at higher manure application rates. Another study revealed that the application rate of EC suspension to intact soil columns was the dominant factor influencing EC leaching (Smith et al., 1985). Other soil column studies examined transport mechanisms as a function of soil properties (Abu-Ashour et al., 1998; Fontes et al., 1991; Tan et al., 1992) and tillage or macropore effects on bacterial leaching (McMurry et al., 1998; Gagliardi and Karns, 2000).

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Several field studies have documented the potential for land application of manure to cause fecal bacterial contamination in subsurface drainage. Evans and Owens (1972) reported a 30- to 900-fold increase in fecal bacteria concentrations in subsurface drain water 2 h after land application of swine manure, and Dean and Foran (1992) found that fecal bacterial levels in subsurface drain water were elevated within 20 min to 6 h following land application, except where the soil was recently tilled. Joy et al. (1998) found that liquid dairy manure application resulted in high levels of tracer bacteria in drainage ditches downstream of drain tile discharge points.

Survival of bacterial populations in the environment is dependent on many variables, including temperature (McFeters and Stuart, 1972; Zibilske and Weaver, 1978), pH (McFeters and Stuart, 1972; Sjorgen, 1994), moisture (Tate, 1978; Hagedorn et al., 1978; Entry et al., 2000a; Entry et al., 2000b), nutrient availability (Klein and Casida, 1967), presence of competitor or predatory organisms (Reddy et al., 1981; Andrews et al., 2004), and freeze-thaw cycling (Kibbey et al., 1978; Stoddard et al., 1998; Warnemuende and Kanwar, 2002; Walker et al., 2006). The relative importance of these factors to bacterial survival is species and strain specific, so while some environmental conditions may favor the survival of one species over another, others may favor the survival of a different organism. Lau and Ingham (2001) found that EC and EN may survive at least 19 weeks in soil-incorporated manure in Wisconsin. The same study found that survival rates were higher for EC than EN. Another study found that EC survived better than EN in sandy soil, while EN survived better than EC in loamy soil (Cools et al., 2001). Probable survival times of EC have been previously estimated to be over 100 days (Filip et al., 1988), over 4 months (Evans and Owens, 1972), and 20.7 to 23.3 months (Sjogren, 1994). Difficulty in detecting treatment impacts to FC densities have been attributed to extended survival of FC in soil and tile water (Howell et al., 1996). Variability in treatment effects between different bacterial indicator organisms are attributed to a combination of the above factors.

This article examines the plot-scale impacts of different manure management regimes on three fecal indicator populations (FC, EC, and EN) in subsurface field drain water. The objective of this study was to evaluate swine manure application timing and rate in order to minimize impacts to the bacterial quality of subsurface drain water, without diminishing crop yield.

Methodology

Experimental Site Description

The experimental site was located at the Iowa State University's Agronomy and Agricultural Engineering Research Center west of Ames, Iowa, on Clarion loam soil in the Clarion-Nicollet-Webster Soil Association (USDA, 2007). The soil is generally well drained and suited to cultivated crops. The bulk density of on-site Clarion loam was approximately 1.4 g cm^{-3}. The area receives an annual average of 82.5 cm of precipitation, with about 55.0 cm occurring during the spring and summer months.

Experimental Treatments

Six manure treatments were compared with a commercial N treatment of 168 kg N ha^{-1} as liquid urea-ammonium nitrate (UAN). Because a producer is likely to fertilize regardless of manure supply, this experimental design was chosen in order to provide the basis for comparison between manure treatments and the alternative commercial N treatment. Experimental treatments were divided into three application schedules: fall, late winter, and spring. Manure was injected in the fall and spring using standard injection methods. In late winter, injection was not possible due to frozen soil, so manure was broadcast onto frozen ground after snowmelt, a practice for winter manure storage voiding. For each manure application, a recommended application rate of 168 kg N ha^{-1} (1X) was compared to a double application rate of 336 kg N ha^{-1} (2X). Liquid UAN was incorporated on the commercial plots in the spring at the time of planting.

Experimental Plot Layout

The study site was divided into three experimental blocks, each having seven treatment plots, to accommodate three replications of the commercial fertilizer treatment and six manure treatments. The resulting 21 individual plots were arranged in a randomized block design. Data were collected from three replicate plots of each treatment for three years, resulting in three spatial replications and three temporal replications, or nine total replications for each of seven treatments. Each experimental plot was 7.5 m wide, to accommodate an annual rotation of five rows of corn (*Zea mays*) in half the plot and five rows of soybeans (*Glycine max*) in the other half, and 22.5 m (74 ft) long. Since corn and soybean annual rotation is common, and only corn would receive manure, it is important to represent both crops equally. Each plot was equipped with a subsurface drain water collection system consisting of PVC drain tile, located 1.2 m deep, draining by gravity to a vertical 37.5 cm diameter

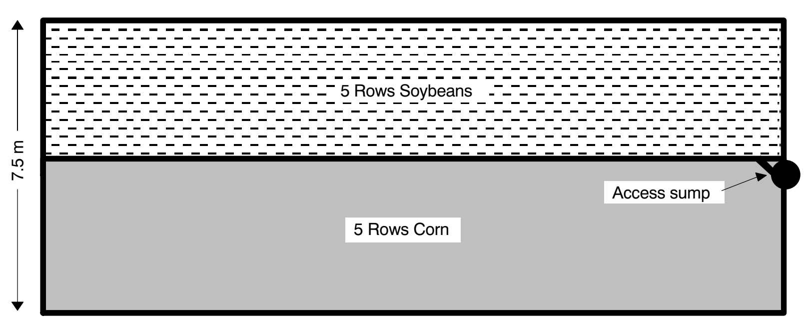

Figure 1. Plot design schematic.

PVC access sump (fig. 1). All plots were surrounded by earthen berms to prevent overland flow and subsequent cross-contamination between plots.

Manure Application

Manure was obtained from the Iowa State University Swine Nutrition Farm. The manure application dates for fall, late winter, and spring were November 12, March 8, and March 27, respectively. Because this research required a degree of application uniformity and accuracy above that which could be provided by manure applicators currently on the market, the research team designed and built a precision manure applicator for this study. Manure was applied at 53,500 and 107,000 L ha^{-1} to achieve the desired 168 and 336 kg N ha^{-1}. Average total N content of the manure was 3137 mg L^{-1}, while average phosphorus content was 1100 mg L^{-1} as P_2O, and average K content was 1578 mg L^{-1}. Average solids content of manure was 3.7%. Error in manure application volume was less than 5%. Grab samples of the liquid (composited over the total volume of application) were also taken as the manure was being applied. One composite sample was collected for each replication of each treatment. Manure had average initial FC, EC, and EN densities of 2,500,000, 770,000, and 1,100,000 cfu/100 mL, respectively.

Sampling and Analysis

All subsurface drainage samples taken for bacterial analysis were taken from flowing tile at the outlet to the access sump and collected in sterile plastic sample bags. Samples were stored at 4°C until they were analyzed, and were analyzed within 24 h. Grab samples were taken weekly starting with the onset of flow in the spring or summer and continuing through the flow season ending mid-fall. Additional samples were taken when a runoff event occurred. Analyses for FC, EC, and EN were done according to the membrane filtration technique (APHA, 1992), plating on m-FC, m-*coli* blue, and m-*Enterococcus* agars for FC, EC, and EN, respectively. All bacterial analyses were conducted in triplicate with one blank sample in every ten. In cases where bacterial colonies were too numerous to count after incubation, sterile water dilutions were used.

Subsurface flow was determined by using an electric sump pump, which operated automatically on a float mechanism, to pump drainage water from each plot through orifice plates that diverted 0.2% of the total tile flow into 3.78 L (1 gal) glass sampling bottles. This volume was used to calculate flow rates and total flow volumes necessary in conjunction with bacterial data for the calculation of flow-weighted average bacterial densities. Rainfall was measured with a tipping-bucket rain gauge located adjacent to the study site. All statistical analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, N.C.). Non-normally distributed data were log-transformed according to Neter et al. (1996). Significant effects of application rate, timing, and treatment were determined by analysis of variance with $P \leq 0.05$.

Results and Discussion

Flow-weighted average bacterial densities according to treatment are given in table 1. All three of the measured indicator bacteria were detected in tile water from all plots, including UAN plots where no manure had been applied.

Table 1. Bacterial densities (cfu/100 mL) in subsurface drain water, according to treatment, during the period of tile flow 1997-1999.[a]

	Fecal Coliform		*E. coli*		*Enterococci*	
Treatment	Avg.	Max.	Avg.	Max.	Avg.	Max.
UAN[b]						
168 kg N ha^{-1}	10 ab	83	<1 b	3	52 b	278
Fall						
168 kg N ha^{-1}	2 b	12	<1 b	2	27 b	156
336 kg N ha^{-1}	4 b	27	1 b	6	37 b	237
Late winter						
168 kg N ha^{-1}	6 b	174	2 ab	53	76 b	1191
336 kg N ha^{-1}	24 ab	307	6 a	82	206 a	1025
Spring						
168 kg N ha^{-1}	45 a	967	4 ab	54	52 b	416
336 kg N ha^{-1}	13 ab	124	3 ab	45	98 b	694

[a] Values followed by the same lowercase letters are not significantly different (α = 0.05.); Avg. = flow-weighted average, and Max. = maximum observed value.
[b] No manure applied.

Indicator bacteria present in tile water from non-manured plots most likely originated from non-agricultural sources such as wildlife. Other researchers have also found indicator bacteria in drainage water from non-manured areas (Patni et al., 1985; Entry et al., 2000a, 2000b). Patni et al. (1984) found little difference in the bacterial quality of subsurface drainage from manured and non-manured fields. The maximum observed level of FC was actually higher in the non-manured field than from manured areas, while geometric mean FC levels were marginally lower in non-manured versus manured areas. In another study, FC were almost always present in runoff from non-manured cropland (Patni et al., 1985). Other studies have found higher bacterial losses with subsurface drain water for a few hours to 2 days following manure application, but this did not persist over the longer time scale (Cook and Baker, 2001).

Flow-weighted average densities of EC were significantly higher in tile water from late winter 2X manured plots than from UAN and fall manured plots, while flow-weighted average densities of EN were significantly higher in tile water from late winter 2X manured plots than from all other treatments. Maximum observed EC and EN densities occurred in the late winter 1X and 2X treatments, respectively. Levels of FC were highly variable, and relatively high levels were observed in the non-manured UAN plots. Flow-weighted average FC densities were statistically greater in the spring 1X plots than the fall manured and late winter 1X plots. Non-manure sources of FC are believed to have affected FC densities in tile water, and this has been observed by other researchers as well (Medrek and Litski, 1960; Patni et al., 1984).

Application Timing Effects

In general, bacterial densities were somewhat lower in fall-applied plots than in late winter and spring-applied plots (table 2). However, significant differences due to timing of application were observed only in EN densities, and not in FC and EC. Because winter survival rates of EN are much greater than FC (Von Donsel et al., 1967), it is expected to exhibit the most significant timing differences. Flow-weighted average levels of EN were highest associated with late winter manure application versus fall, spring, and UAN treatments.

Table 2. Flowweighted average bacterial densities (cfu/100 mL) in subsurface drain water, according to time of application.[a]

Timing	Fecal Coliform	*E. coli*	*Enterococci*
UAN[b]	10 a	<1 a	52 b
Fall	3 a	1 a	32 b
Late winter	15 a	4 a	141 a
Spring	29 a	4 a	75 b

[a] Values followed by the same lowercase letters are not significantly different ($\alpha = 0.05$).
[b] No manure applied.

Research by Fenlon et al. (2000) also emphasizes the need for manure storage sufficient to allow for application when soil conditions are suitable.

One factor contributing to timing differences may be the temperature dependence of bacterial survival rates. Above 4°C, lower temperatures are generally more favorable to bacterial survival than higher temperatures (Zibilske and Weaver, 1978). Between 5°C and 30°C, the die-off rate of fecal bacteria generally doubles with each 10°C increase in temperature (McFeters and Stuart, 1972). Lower temperatures may have favored bacterial survival in late winter and fall manure-applied plots. However, the freeze-thaw cycle is detrimental to the survival of indicator bacteria (Kibbey et al., 1978; Stoddard et al., 1998; Warnemuende and Kanwar, 2002; Walker et al., 2006), so bacterial densities in fall-applied manure were expected to be greatly diminished by the time of onset of tile flow in spring.

Manure application timing effects were likely further influenced by the timing of applications relative to timings of greatest tile flow and rainfall. Average tile flow and rainfall are given in figure 2. The onset of tile flow occurred between the middle of February and the beginning of April, and the highest flow rates occurred during April, most directly following spring and late winter manure applications. Tile flow generally declined throughout the growing season, as plant moisture demands and evapotranspiration rates increased. Tile flow ceased by the end of September. Tile were not flowing during the time of fall manure applications, but were during late winter and spring applications (fig. 2). Previous research has shown that bacterial densities in running tile water following application were higher when manure was applied while tile were actively flowing than when manure was applied while tile were dry (Joy et al., 1998). The greatest average weekly rainfall occurred from the middle of June to the middle of July (fig. 2). High densities of EN were also observed during this period (fig. 3). The two main periods of highest tile water bacterial densities occurred during the second half of May (fig. 3), when the first post-application rainfalls having typically high intensity generally occurred, and during the second half of June, when high weekly rainfall totals occurred. Elevated bacterial densities in tile water were also closely associated with rainfall events in a previous study (Joy et al., 1998). As illustrated in figure 2, seasonal distribution of tile water bacterial density was not correlated with timing of manure application timing, although magnitude of bacterial density was.

Disruption of macropore flow by fall and spring injections may have also contributed to observed timing differences. Because soil is often frozen at the time of winter or late winter manure applications, manure is usually broadcast instead of injected, as was the case in this study. Macropore flow has been found to be a dominant pathway for bacterial leaching (Smith et al., 1985; Abu-Ashour et al., 1998; Scott et al., 1998). Through the injection process, macropores may be sheared or otherwise disturbed, while the broadcasting process leaves macropores essentially intact. Maximum macropore flow in response to ponding following snowmelt may have further amplified this effect in late winter plots.

Application Rate Effects

Flow-weighted average tile water bacterial densities according to rate of application are given in table 3. No significant differences in FC or EC densities were observed. However, the 336 kg N ha^{-1} rate resulted in significantly higher tile water EN densities as compared to the UAN treatment and 168 kg N ha^{-1} manure rate. Application rate of an *E. coli* suspension has been found to influence leaching of EC through intact soil columns (Smith et al., 1985), while manure application rate was found not to influence EC densities in tile drains (Joy et al., 1998). Another study found

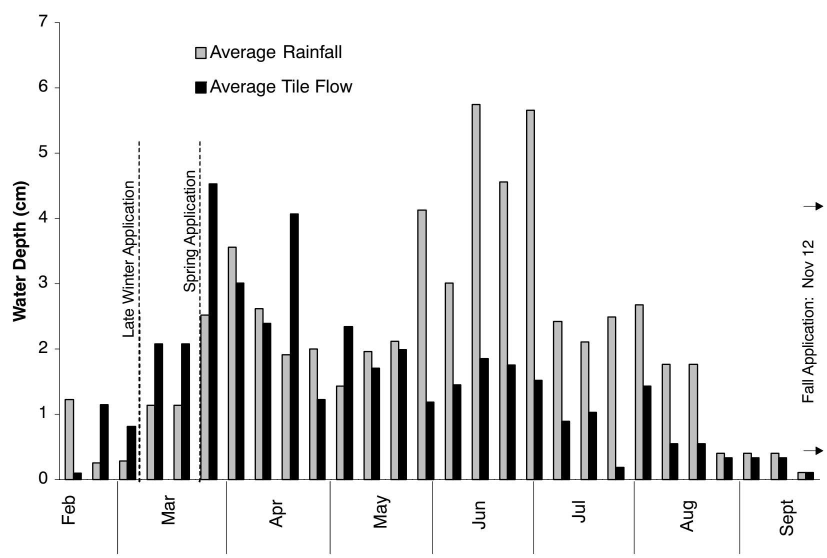

Figure 2. Average weekly rainfall and tile flow at study plots during the period of tile flow, 1997-1999.

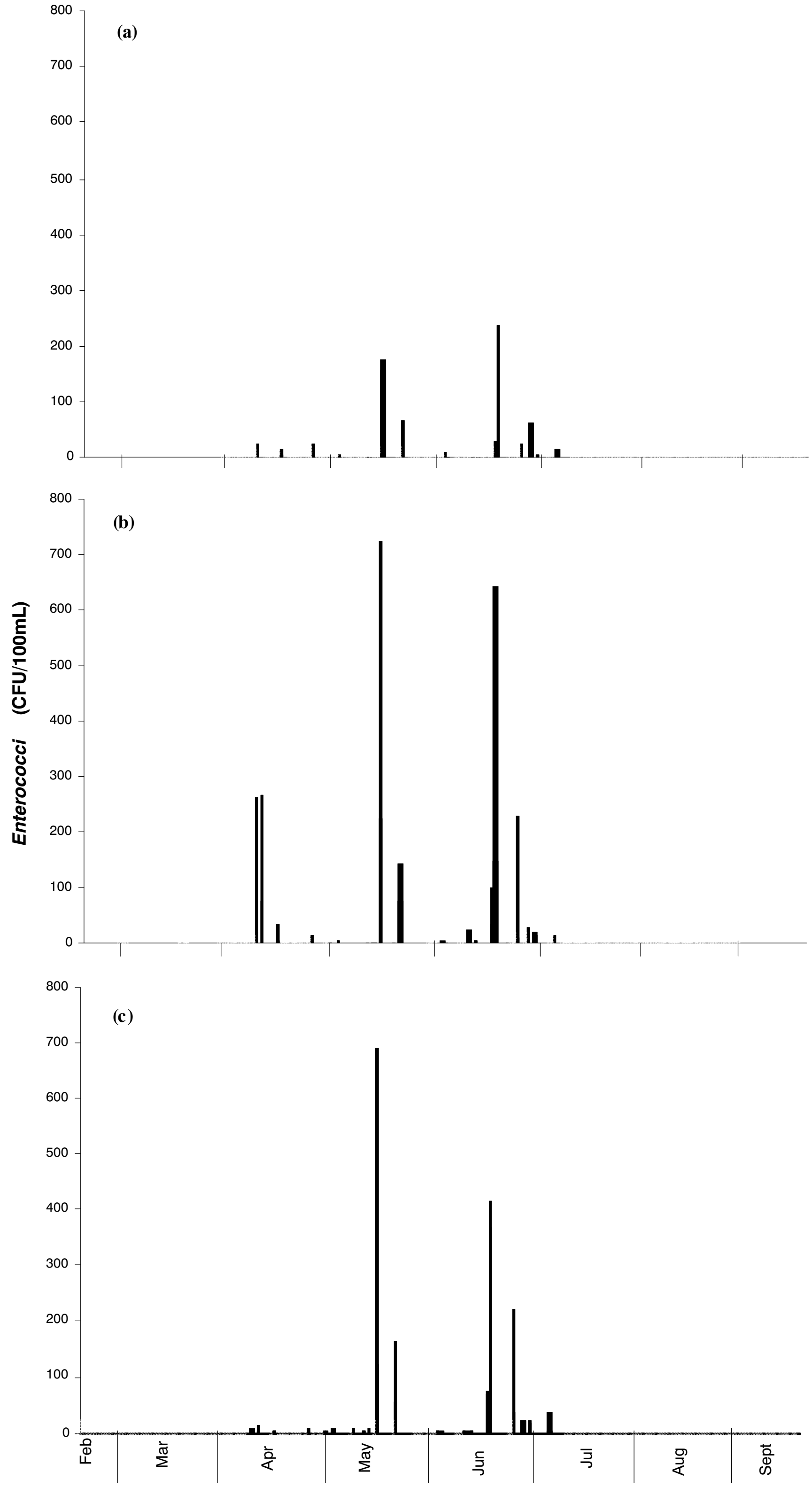

Figure 3. Seasonal bacterial incidence in tile water, 1997-1999 for plots treated with manure application in (a) fall, (b) late winter, and (c) spring.

that while application rate effects were not significant, they did interact with manure application timing effects such that timing effects were intensified at higher application rates (Warnemuende and Kanwar, 2002). It was expected that the higher manure application rate would lead to more indicator bacteria available for leaching and ultimately result in high tile water bacterial densities. This effect was observed only for EN.

Table 3. Flow-weighted average bacterial densities (cfu/100 mL) in subsurface drain water, according to rate of application.[a]

Treatment	Fecal Coliform	*E. coli*	*Enterococci*
UAN[b]	10 a	<1 a	52 b
168 kg N ha^{-1}	18 a	2 a	52 b
336 kg N ha^{-1}	14 a	3 a	114 a

[a] Values followed by the same lowercase letters are not significantly different ($\alpha = 0.05$).
[b] No manure applied.

Conclusions

Tile water EN densities were significantly higher in plots where manure had been applied in late winter versus fall, spring, and UAN treatments. This was attributed to higher macropore flow due to the necessity that manure be broadcast rather than injected when applied to frozen ground and increased surface ponding related to recent snowmelt at the time of late winter applications. Since fall and spring applied manure was injected, some disruption of macropores, which provide a major pathway for bacterial leaching, was expected. Levels of EN were also significantly higher where manure had been applied at double the N-based recommended rate (336 kg N ha^{-1}) than where manure or UAN had been applied at the recommended N rate (168 kg N ha^{-1}). This was expected due to the higher availability of EN for leaching associated with higher manure application rate. Application timing and rate effects led to an overall significant elevation of EN levels in late winter 2X tile water versus all other treatments.

Fewer significant differences were observed in FC and EC densities. This was attributed to an overall difference in organism survival rates and responses to non-treatment-related environmental variables such as soil moisture conditions, temperature, and freezing and thawing. Levels of EC were significantly higher in tile water where manure had been late winter broadcast at the double rate than for all fall treatments.

Fecal coliform results were unexpected in that the spring 1X treatment yielded significantly higher FC levels than the fall manure and winter broadcast 1X treatments. We attribute this in part to previously documented prevalence of FC in the natural soil environment. However, the effects of application rate and timing had no individual significant effect on EC or FC levels. Further, doubling the manure application rate had no significant impact on bacterial densities in tile water where manure had been injected. This is partially attributed to the disturbance of macropores that is likely to result from the injection process. The overall results suggest that manure should not be applied to frozen ground, especially in excess of crop nutrient requirements, as this may lead to significantly higher levels of EN and EC in tile water that ultimately drains to surface water bodies.

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Nomenclature

EC = *Escherichia coli*
EN = *Enterococcus*
FC = fecal coliform
UAN = urea-ammonium nitrate
1X = 168 kg N ha^{-1}
2X = 336 kg N ha^{-1}