In cooperation with the U.S. Department of Agriculture, Natural Resources Conservation Service and the Lenawee Conservation District

Chemical and Microbiological Water Quality of Subsurface Agricultural Drains during a Field Trial of Liquid Dairy Manure Effluent Application Rate and Varying Tillage Practices, Upper Tiffin Watershed, Southeastern Michigan

By Sheridan Kidd Haack and Joseph W. Duris

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Conversion Factors and Abbreviated Water-Quality Units

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
ft (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
acre	0.4047	hectare (ha)
	Volume	
gallon (gal)	3.785	liter (L)
milliliter (mL)	0.03381	ounce, fluid (fl. oz)
microliter (µL)	0.0001	milliliter (mL)
	Flow rate	
gallon per acre (gal/acre)	9.354	liter per hectare (L/ha)
gallon per acre per year [(gal/acre/)yr]	9.354	liter per hectare per year [(L/ha/)yr]
	Mass	
pound (lb)	0.4536	kilogram (kg)
pound per gallon (lb/gal)	119.826	gram per liter (g/L)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
°F=(1.8×°C)+32

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Small volumes are reported in milliliters (mL) and microliters (µL). Very small masses are given in nanograms (ng; a nanogram is one billionth of a gram). Pore sizes of filters are given in micrometers (µm).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L), micrograms per liter (µg/L), millimolar (mM) or micromolar (µM).

Bacteria concentrations are given in colony-forming units per 100 milliliters (CFU/100 mL).

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Abstract

A field trial was done in the Upper Tiffin River Watershed, in southeastern Michigan, to determine the influence of liquid dairy manure effluent (LDME) management practices on the quality of agricultural subsurface-drain water. Samples from subsurface drains were analyzed for nutrients, fecal-coliform and *Escherichia coli* (*E. coli*) bacteria, antibiotics, chemicals typically detected in wastewater, and the occurrence of genes indicating the presence of shiga-toxin-producing *E. coli*, or of bovine-specific *Bacteroidetes* bacteria. Samples were collected from November 2, 2006, to March 20, 2007, from eight subsurface drains under field plots that received no LDME and no tillage (controls) or received 4,000 or 8,000 gallons per acre (gal/acre) of LDME and either no tillage or two different types of tillage. The two types of tillage tested were (1) ground-driven, rotary, subsurface cultivation and (2) rolling-tine aeration. Samples were collected before LDME application and at 4 hours, and 1, 2, 6, 7, and 14 days post-application.

Nutrient concentrations were high in subsurface-drain water throughout the field-trial period and could not be attributed to the field-trial LDME application. Of the 59 drain-water samples, including those collected before LDME application and control samples for each date, 56 had concentrations greater than the U.S. Environmental Protection Agency (USEPA), Ecoregion VI recommended surface-water criterion for total phosphorus, and all samples had concentrations greater than the recommended total nitrogen criterion. Nitrate + nitrite nitrogen concentration exceeded 20 milligrams per liter for every sample and contributed most to the total nitrogen concentrations. Substantial increases in drain-water concentrations of organic and ammonia nitrogen and total phosphorus were found for all treatments, including controls, at 14 days post-application after 0.84 inch of rainfall over 2 days.

E. coli concentrations exceeded the USEPA recreational-water-quality single-sample criterion of 235 colony forming units per 100 milliliters in only 3 of 56 samples. Of these three samples, two were collected within 1 day post-LDME application from the treatment receiving 8,000 gal/acre LDME with no tillage (NT8000). The third sample was from the rolling-tine aerator treatment with 4,000 gal/acre LDME application rate after the first significant rainfall.

Two wastewater chemicals and two bacterial genes (*eae*A and *stx*1) detected in the LDME, but absent in field blank or pre-application samples, were detected in the 4-hour or 1-day post-application NT8000 samples. No LDME-associated chemicals were detected in later samples from the NT8000 treatment, and none were detected in samples from other treatments after the first significant rainfall.

Results of this field trial were somewhat equivocal with respect to the influence of LDME concentration and tillage practices on subsurface-drain water quality, both immediately after LDME application and in the longer term, after significant rainfall. Interpretation of study findings is limited by the fact that treatments were not replicated, and flow rate or discharge from the subsurface drains was not measured. Nevertheless, study results provide useful information about nutrient and bacteria concentrations in subsurface drains during the non-growing season. In addition, study results demonstrate some potential for the use of chemical and microbiological indicators of LDME transport to subsurface drains.

Introduction

The lower parts of the Bean Creek Watershed (Hydrologic Unit Code 04100006106) and the Lime Creek Watershed (Hydrologic Unit Code 04100006107), in southern Hillsdale and Lenawee Counties, Michigan, are part of the headwaters of the Tiffin River (fig. 1). Land use in these watersheds is predominantly agricultural: approximately 75 percent in the Bean Creek Watershed and approximately 91 percent in the Lime Creek Watershed. Soils are fine textured and require subsurface drainage for agricultural use. Historically, subsurface drainage systems were formed with clay tiles installed at depth in a field. Currently, various types of plastic piping are used, but the term “tile drains” or “tiles” is still in use. Manure from livestock operations is land-applied to cropland. In soils of the region, liquid manure has the potential to move through the soil profile and into subsurface tile drains through soil cracks and wormholes, a process referred to as “preferential flow” (Shipitalo and Gibbs, 2000; Hoorman and Shipitalo, 2006). Thus, manure and manure-associated chemicals and bacteria have the potential of moving offsite to surface waters through subsurface drainage tiles.

In the past 5 years, the watersheds in the Tiffin River headwaters have been subject to establishment and expansion of livestock operations, particularly large dairies. Parts of Bean Creek and Lime Creek fail to meet Michigan Water-Quality Standards for *Escherichia coli* (*E. coli*) bacteria. The Michigan Department of Environmental Quality (MDEQ) has established Total Maximum Daily Loads (TMDLs) for *E. coli* for parts of these watersheds (Michigan Department of Environmental Quality 2003a,b) and has identified agricultural tile drains as one potential contributor of *E. coli* to surface water in these watersheds. MDEQ identified stormwater runoff, sewage outfalls, and possible connections of sewage or septic-system waste to storm or agricultural drains (referred to in the TMDL documents as “illicit connections”), as other potential sources of *E. coli*. A volunteer monitoring group called the Environmentally Concerned Citizens of South Central Michigan has also documented instances of discharge of manure and nutrients through tile lines into surface waters of Bean and Lime Creeks. The Medina Drain, which flows into Bean Creek, has been placed on Michigan’s 303(d) list of impaired water bodies; nuisance odors, phosphorous, nuisance plant growth, and organic enrichment are identified as specific impairments.

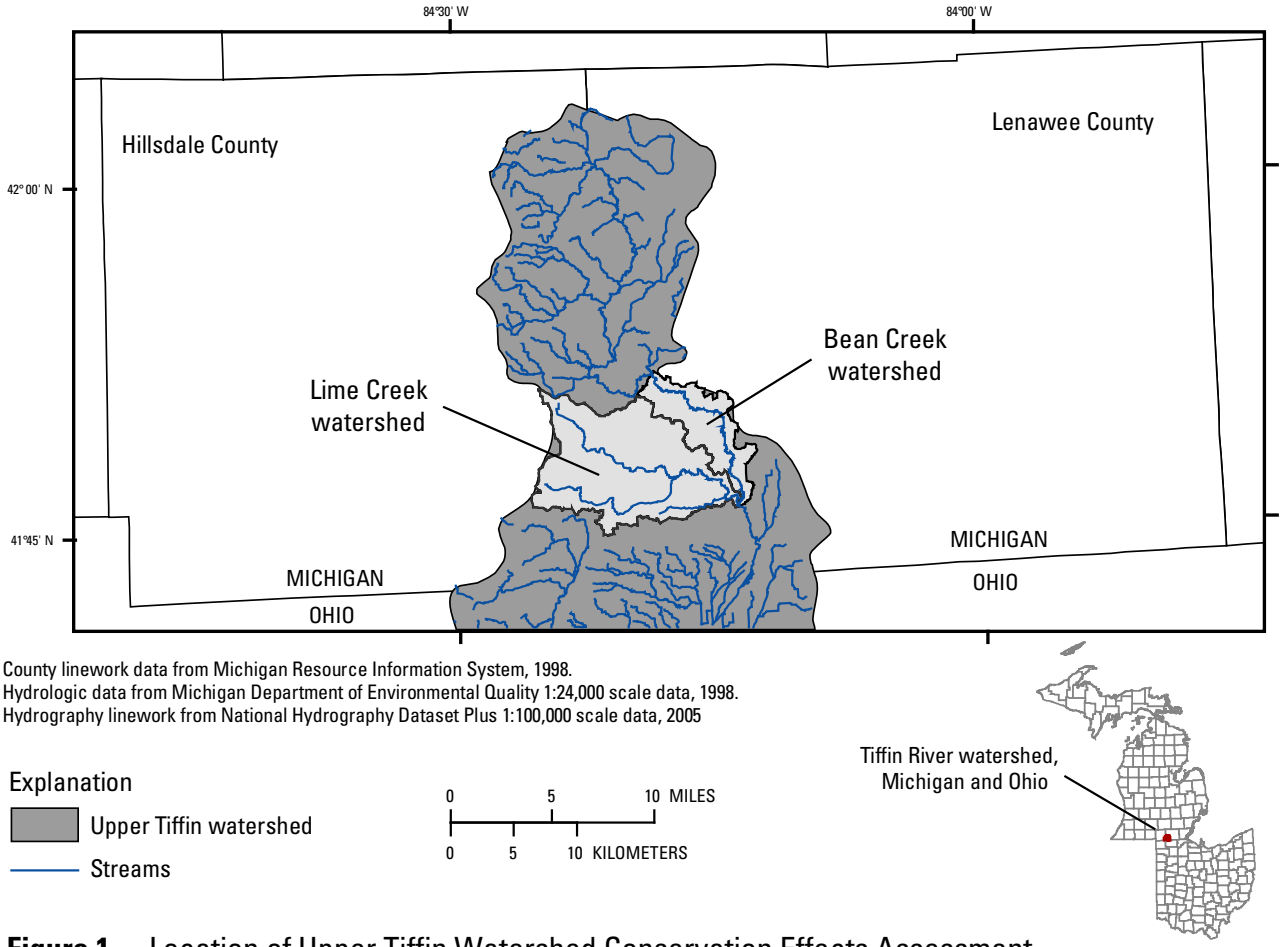

Figure 1. Location of Upper Tiffin Watershed Conservation Effects Assessment Project area.

In 2004, the Natural Resources Conservation Service (NRCS) began a 3-year project in the Lime and Bean Creek Watersheds, collectively called the Upper Tiffin Watershed. The Upper Tiffin Watershed was designated a Special Emphasis watershed, funded under the National U.S. Department of Agriculture Conservation Effects Assessment Project (CEAP; *http://www.nrcs.usda.gov/Technical/nri/ceap/*). The NRCS established a partnership agreement with the Lenawee Conservation District (LCD) to assist with implementation of the watershed project. The Upper Tiffin Watershed Project seeks to determine which combination of manure management practices will mitigate the risk of offsite movement of nutrients and bacteria from subsurface discharge. Manure management practices that were evaluated included three types of tillage and three rates of manure application. A field trial was established in 2006.

To help identify the potential for offsite movement of nutrients and bacteria through subsurface drainage under various manure management practices, the U.S. Geological Survey (USGS), in cooperation with the NRCS and the LCD, evaluated concentrations of nutrients, fecal-coliform bacteria, and *E. coli* bacteria in liquid dairy manure effluent (LDME) and subsurface-drain-water samples for the Upper Tiffin Watershed Project field trial. In addition, to help identify potential indicators of dairy manure contamination of water, the USGS analyzed LDME and selected subsurface-drain-water samples for chemicals found in many wastewaters, for antibiotics used by humans and in animal agriculture, and for genes of bacteria often associated with cattle.

Drain-water samples analyzed for these constituents were collected both immediately after LDME application and on subsequent dates, especially after a significant rainfall event (for the purposes of this study, a storm total greater than 0.5 in.).

Purpose and Scope

The primary purpose of this report is to describe the concentration of nutrients and fecal indicator bacteria in subsurface agricultural drainage water over a period of 2 weeks after various combinations of LDME application rate and tillage practices. A secondary purpose is to describe, in a subset of these samples, the occurrence of chemicals and microbiological constituents that might indicate LDME transport to subsurface drains.

The field trial investigated three different management practices within a field subdivided into eight plots. The first was application to soil with no tillage (NT). The second was tillage with a ground-driven, rotary, subsurface cultivator that disturbed the soil profile to a depth of 2 to 3 in. The third was tillage with a rolling-tine aerator that disrupts the top 6 to 8 in. of soil. In each case, the plot was tilled immediately before the LDME application. In this field trial, LDME either was not applied, was applied at the rate of 8,000 gal/acre, or was applied at half that rate. Samples of LDME were collected from September 27, 2006, to November 2, 2006. Samples of tile water were collected after the growing season, from November 2, 2006, to March 20, 2007.

This report includes (1) concentrations of organic, ammonia, and nitrate + nitrite nitrogen and total and orthophosphorus, (2) concentrations of antibiotics and of other chemicals typically detected in wastewater, (3) concentrations of *Escherichia coli* (*E. coli*) bacteria, (4) data on the occurrence of genes indicating the presence of shiga-toxin-producing *E. coli*, and (5) data on the occurrence of a genetic marker indicating the presence of bovine-specific *Bacteroidetes* bacteria.

Management Practices

Factors that might influence manure movement to subsurface drains include the amount or rate of manure applied and tillage practices that influence the degree to which preferential flowpaths are established or disrupted. The amount of manure applied under agricultural best management practices is governed by factors such as the water and nutrient content of the manure, the water-holding capacity of the soil, and existing levels of nutrients in the soil (Michigan Department of Agriculture, 2007).

Manure contains substantial amounts of nitrogen, mostly in organic forms. Some of this organic nitrogen is converted to ammonia nitrogen during storage. When applied to soil, some of this ammonia nitrogen volatilizes to air immediately, but some moves into the soil; the latter is bound by soil particles and taken up by plants for growth. The organic form of nitrogen also tends to remain bound to the soil; however, bacteria may degrade the organic forms of nitrogen and may convert ammonia nitrogen to nitrate and nitrite, which are very soluble in water and which may be lost to subsurface drains. How much nitrogen may be lost by these various processes depends on the methods of manure application, weather conditions, the type of soil, the type of crop, and the season (Bakhsh and others, 2005). During the growing season, plants may take up ammonia nitrogen, but during fallow seasons, nitrate may be lost to subsurface drainage.

Manure also typically contains substantial amounts of phosphorus, again much of it in organic forms. Phosphorus transformations and interactions with soils are complex (Sharpley and others, 2003), and the exact forms of phosphorus in soils are difficult to determine. Historically, phosphorus leaching from soil to the subsurface was considered negligible, owing to the affinity of phosphorus for the soil matrix. In the past, therefore, concern for water contamination with

phosphorus focused primarily on overland runoff of phosphorus attached to soil particles or of soluble phosphorus in surface-runoff water (Sharpley and others, 2003). Agricultural management practices (conservation tillage; see below) designed to reduce soil losses by runoff appear to have been effective in reducing phosphorus concentrations in the Maumee River (to which the Tiffin River is a tributary) during 1976–95. However, recent research indicates that newer agricultural practices, including the growth of animal feeding operations, have contributed to phosphorus surplus at the farm and watershed scale (Sharpley and others, 2003). Calhoun and others (2002) found that soluble phosphorus (Bray P1 test) measured from 1996 to 1998 in soils of the Maumee and Sandusky River Watersheds was significantly greater than concentrations determined from 1953 to 1982. Nationally, soil phosphorus has increased in many parts of the country (Sharpley and others, 2003). The relation between soil phosphorus measurements and the potential for loss of phosphorus by surface runoff, or to subsurface flow, is complex, and depends on soil properties, the forms of phosphorus applied, weather conditions, plant growth, and management practices (Sharpley and others, 2003). Michigan Generally Accepted Agricultural Management Practices (GAAMPs; Michigan Department of Agriculture, 2007) for manure management and use give specific recommendations for the levels of plant-available nitrogen and phosphorus that should be applied to soils in any form (fertilizer or manure or both) and recommend manure and soil analyses regularly to avoid exceeding these levels.

Conservation tillage consists of a variety of no-till or reduced-tillage approaches that were designed to reduce soil and nutrient runoff to surface water and retain water and nutrients for plant growth (Lemunyon and Gross, [n.d.]). However, earthworm burrows and cracks (macropores) are more prevalent in land that is subject to conservation tillage (Shipitalo and others, 2000; Shipitalo and Gibbs, 2000). Earthworm burrows or cracks in soil may serve as direct transport routes for rapid movement of land-applied pesticides, nutrients, bacteria, or manure to subsurface drainage tiles (Shipitalo and others, 2000; Shipitalo and Gibbs, 2000). Rapid transport may take place within minutes or hours of application, and it is particularly an issue when liquid manure is applied to already wet soils or when significant rainfall occurs soon after application. Several studies have shown that chemicals that might normally bind tightly to the soil matrix (for example, ammonia nitrogen, phosphorus, or pesticides) move rapidly through preferential flow pathways without interacting with the soil (Sims and others, 1998; Shipitalo and others, 2000; Simard and others, 2000; Geohring and others, 2001; Hodgkinson and others, 2002; Stamm and others, 2002; de Jonge and others, 2004; Toor and others, 2004; Schelde and others, 2006; Ball Coehlo and others, 2007). Likewise, research also indicates rapid bacteria movement to tile lines by macropores after liquid manure application (Joy and others, 1998; Cook and Baker, 2001; Jamieson and others, 2002; Ball Coelho and others, 2007). Chemicals such as nitrate, which are readily soluble, are less affected by preferential flow pathways. Very recently, several studies have documented particle-associated phosphorus movement through macropores, especially during significant rainfall after extended dry periods (Simard and others, 2000; de Jonge and others, 2004; Schelde and others, 2006; Gentry and others, 2007). Even if transport of chemicals to subsurface drainage lines does not occur immediately by preferential flow pathways, this transport mechanism still permits rainfall or irrigation to move chemicals below the root zone—where they are not available for plant uptake during the growing season—and remain available for movement to tile drains when the growing season ends and soils become more saturated (Shipitalo and others, 2000). Manure application with equipment that disrupts earthworm burrows and cracks may prevent direct transport to the subsurface and provide more time for plant uptake or for diffusion of manure and

manure-associated chemicals and bacteria into the soil matrix (Goehring and others, 2001; Jamieson and others, 2002; Shipitalo and Gibbs, 2000; Thiagarajan and others, 2007).

Chemical and Microbiological Indicators of LDME Transport to Subsurface Drains

Distinguishing manure sources of nutrients or bacteria from other sources, such as sewage or septic systems, would be helpful in applying the appropriate practices to those source areas and more efficiently achieving water-quality standards and reducing water-quality impairments in the Upper Tiffin Watershed. Several water constituents and microbiological indicators could potentially be used to distinguish manure pollution from pollution by other sources. As reviewed in Cimenti and others (2007), these include physical or genetic characterization of fecal-coliform, *E. coli*, or enterococci bacteria; detection of nonpathogenic but host-specific microbes or their genes; direct testing for host-specific pathogens or their genes; and testing for chemicals associated with specific types of fecal pollution or with animal-specific waste streams. The best approach to source determination involves several methods and a multiple-lines-of-evidence approach (Cimenti and others, 2007).

One means of distinguishing pollution sources is to evaluate chemicals unique to those sources. Chemicals used every day in homes, industry, and agriculture can enter the environment in wastewater. A 1999 study by the USGS (*http://toxics.usgs.gov/regional/emc/index.html*) showed that a broad range of chemicals found in residential, industrial, and agricultural wastewaters commonly occurs in mixtures at low concentrations (typically less than 1 µg/L) in surface waters of the United States. The chemicals include human and veterinary drugs (including antibiotics), detergent metabolites, cleaning-product fragrances, plasticizers, agricultural and urban-use insecticides or herbicides, fecal sterols, and fire retardants. In Huron County, Mich., a USGS study detected both human-use and veterinary-use antibiotics in stream water but not in ground water (Duris and Haack, 2004). Other chemicals indicating domestic, industrial, and agricultural influences on water quality also were detected in the Huron County study. Some of these chemicals may be good indicators of sources of pollution (Cimenti and others, 2007), but little is known about their general occurrence in manure or their transport through soil. However, because antibiotics are used widely in animal agriculture, and because they have been detected in manure, several studies of antibiotic fate and transport in manured soils have been done (Burkhardt and others, 2005; Kay and others, 2004, 2005a, b; Hamscher and others, 2005). In addition, the sorption of commonly used veterinary antibiotics to soils under various conditions has been investigated (Rabolle and Spliid, 2000; Boxall and others, 2002; Schlüsener and Bester, 2006; Kahle and Stamm, 2007). In general, tetracycline antibiotics bind strongly to soil (Rabolle and Spliid, 2000; Hamscher and others, 2005) but sulfonamide antibiotics exhibit more complex transport, which is influenced by the pH, ionic strength, and nature of soils (Boxall and others, 2002; Kay and others, 2004, 2005a, b; Thiele-Bruhn and others, 2004; Hamscher and others, 2005; Burkhardt and others, 2005; Kahle and Stamm, 2007). Field studies indicate that the addition of antibiotics with manure may change the antibiotics' transport properties (Burkhardt and others, 2005), that sulfonamide antibiotics may accumulate in, and subsequently leach from, manured soils (Hamscher and others, 2005), and that tillage may decrease leaching of sulfonamide antibiotics by preferential flow pathways (Kay and others, 2004, 2005a, b). At least one study has indicated that soil-bound tetracycline antibiotics may retain their antibacterial activity (Chandar and others, 2005).

Another means of distinguishing pollution sources is to evaluate microorganisms or microbial genes unique to those sources. For example, shiga-toxin producing EC (STEC) may potentially indicate nonhuman sources. STEC are a major cause of gastrointestinal disease in

humans, and some strains may lead to severe disease such as hemolytic uremic syndrome (HUS) or hemorrhagic colitis (Nataro and Kaper, 1998). STEC include *E. coli* O157:H7, for which the dominant reservoir is cattle (Boerlin and others, 1999; Bach and others, 2002). However, other STEC may come from other animals. Transmission between wild and domestic animals and humans may occur through contaminated food or water. The health effects of STEC are owing to several genes, and variations in shiga-toxin genes may indicate the potential animal source of contamination as well as the potential severity of disease (Beutin and others, 1993). In addition, *Bacteroidetes* bacteria may indicate different animal sources. The *Bacteroidetes* are more numerous than *E. coli* in intestinal samples, and a gene-based test that is specific for the *Bacteroidetes* associated with cattle was recently developed (Bernhard and Field, 2000).

Conditions at the Study Area

The study area is within the Upper Tiffin Watershed in Lenawee County, Mich. (fig. 1). Soils of the field site are primarily Blount loam soils (fine, illitic, mesic, Aeric Epiaqualfs) on a 3–7 percent slope. Eight field plots were established overlying 8, 200-ft-long, 4-in. diameter PVC tiles, installed 32 in. below land surface at 50-ft horizontal intervals. Tiles were installed in 1993 and were connected to a larger drainage system for the entire field. Each PVC tile was modified in 2006 by connecting a 4-in. PVC riser at the terminal end, approximately 2 months before field trial initiation, to allow for water-sample collection.

The field site is on an active agricultural field that for the last 8 years had received liquid dairy manure effluent (LDME) at a rate of 8,000 gal/acre/yr. The field had been planted with typical rotations of corn and soybeans during that time and had received no tillage. The field had been in crop production for approximately 50 years and had received inorganic fertilizers before receiving LDME. Silage corn had been grown on the field before the field trial, and corn stubble (less than 15 percent cover) was present on the field plots during the field trial. In addition, manure application had taken place approximately 3 months before the field trial, in accordance with the management plan for the farm. Tile-drain flow for these plots was not measured. Tile-drain flow is a function of antecedent moisture, crop status, and soil characteristics (Baker and others, 2007). Occasional field observations indicated no water in these tile drains from September 27 to October 13, during which time a cumulative total of 1.6 in. of rain fell. From October 14 until the beginning of the field trial on November 2, water was generally present in the tiles. In this latter interval, removal of the summer's corn crop, as well as two rainfall events with greater than 0.5 in. of rain in a 24-hour period, likely contributed to the presence of water in the tiles. There was no rainfall in the 5 days preceding LDME application. Calculations that assume the LDME is 100 percent liquid indicate that application of LDME at 4,000 gal/acre is roughly equivalent to 0.15 in. of rain per acre.

The LDME applied to this field is analyzed on a routine basis in accordance with Michigan Generally Accepted Agricultural and Management Practices for Manure Management and Utilization (Michigan Department of Agriculture, 2007). The LDME typically has around 1.0 percent solids, 10 lb/gal (1.2 g/L) of total nitrogen, 8.8 lb/gal (1.1 g/L) of ammonia nitrogen, and 0.2 lb/gal (24 mg/L) of orthophosphorus.

This field trial investigated three management practices. The first was applying to soil without tillage (NT). The second was tillage with a ground-driven, rotary, subsurface cultivator that disturbed the soil profile to a depth of 2 to 3 in. (Dyna-Drive; DD). The third was tillage with a rolling-tine aerator that disrupted the top 6 to 8 in. of soil (AerWay; AW). Tillage immediately preceded LDME application. In this field trial LDME either was not applied at all, or was applied

at the rate of 8,000 gal/acre or at half that rate. The eight plots with individual drainage tiles were assigned these treatments as indicated in table 1.

Table 1. Treatment definitions and abbreviations.

[gal/acre, gallons per acre]

Plot/Tile number	Tillage method	Application rate (gal/acre)	Abbreviation
1	Dyna Drive	8,000	DD8000
2	Dyna Drive	4,000	DD4000
3	Control	None	C1
4	Aerway	8,000	AW8000
5	Aerway	4,000	AW4000
6	Not tilled	8,000	NT8000
7	Not tilled	4,000	NT4000
8	Control	None	C2

Sampling and Analytical Methods

Samples of the applied LDME, as well as tile-water samples, were collected on November 2, 2006 before the application of LDME (pre-application), then after the application on the same date (4 hours). Similarly, samples were collected from all tiles on November 3 (1 day), November 4 (2 days), November 8 (6 days), November 9 (7 days), and November 16 (14 days). Some sample dates were chosen specifically to evaluate the effects of rainfall on subsurface drain water quality. Rainfall amounts during the field trial are listed in table 2. The NT8000 treatment tile (tile 6) was additionally sampled on December 21, 2006, and both the NT8000 treatment tile (tile 6) and the Control 1 treatment tile (tile 3) were sampled on March 20, 2007, to gain further temporal perspective on nutrient concentrations.

Table 2. Rainfall during the course of the field trial.

[All results reported in inches; days with no rainfall not reported]

Date	Rainfall amount
10/26/2006	0.07
10/27/2006	.58
10/28/2006	.06
11/7/2006	.55
11/8/2006	.01
11/10/2006	.17
11/11/2006	.24
11/15/2006	.24
11/16/2006	.60
11/24/2006	.01
11/30/2006	.88
12/1/2006	.90
12/12/2006	.63
12/14/2006	.01
12/17/2006	.01
12/21/2006	.33

Sampling Procedures

Tile-water samples were collected in accordance with procedures in the USGS National Field Manual (NFM) (U.S. Geological Survey, variously dated). For nutrients and bacteria analyses, a peristaltic pump and polyethylene tubing were used to draw water from the sampling port on each subsurface tile. For other chemical analyses, silicon tubing was used. Tubing was cleaned between samples with successive rinses of distilled water, 1 percent soap solution, 0.005 percent bleach solution, distilled water, and at least 500 mL of water from the next tile. LDME samples were collected as grabs from the effluent access on the slurry spreading tank. Samples for chemical analysis were filtered and/or preserved in the field as per procedures described in the USGS NFM, and, for nutrients, in the references listed in table 3.

Table 3. Methods of nutrient analysis and reporting limits.

[mg/L, milligrams per liter; ASF, automated segmented flow]

Nutrient	Method	Reporting limit (mg/L)	Reference
Ammonia + organic N	Colorimetry, ASF, microkjeldahl	0.10	Patton and Truitt, 2000
Ammonia, as N	Colorimetry, salicylate-hypochlorite	.02	Fishman, 1993
Nitrite + nitrate, as N	Colorimetry, ASF, cadmium reduction – diazotization	.06	Fishman, 1993
Phosphorus as P	Colorimetry, ASF, microkjeldahl	.04	Patton and Truitt, 1992
Orthophosphate as P	Colorimetry, phosphomolybdate	.006	Fishman, 1993

Chemical Analyses

All samples were analyzed at the USGS National Water Quality Laboratory (NWQL), Denver, Colo., for the nutrients listed in table 3. Organic + ammonia nitrogen and total phosphorus were analyzed on whole (unfiltered) water samples. Ammonia nitrogen, nitrate + nitrite nitrogen, and orthophosphorus were analyzed on filtered water samples. Field blank samples for nutrient analyses were collected on November 16, 2006. Field blanks were processed by passing inorganic blank water through the entire sampling apparatus, in the field, after a between-sample cleaning per established protocols in the USGS National Field Manual. Selected samples were sent to the USGS NWQL for Schedule 1433 (filtered)/8033 (unfiltered, for LDME samples only) “Wastewater Analysis” (table 1–1) and the USGS Kansas Organic Research lab for Schedule LCAN, antibiotics analysis (table 1–2). The following samples were tested: LDME (November 2, 2006); field blank (November 16, 2006); pre-application (Control 2, November 2, 2006); all treatments except Control 1 on November 8, 2006; and NT8000 additionally on November 2, 3, 4, and 9, and December 21, 2006.

Microbiological Analyses

Tile-water samples were analyzed by use of standard membrane-filtration methods (APHA and others, 1998) for detection of fecal-coliform (FC) bacteria (mFC medium) and *E. coli* (NA-MUG medium). In addition, two gene-based assays were done to evaluate the presence of shiga-toxin-producing *E. coli* or the presence of bovine-specific *Bacteroidetes*.

Bacteria Enumeration and Preservation

All media was prepared according to manufacturer's instructions. For each water sample 50-, 5-, and 0.5-mL volumes were filtered though a 0.45-µm nylon membrane filter that was transferred to mFC medium and incubated at 44.5ºC for 24 hours. If growth was uncountable at these dilutions, further ten-fold serial dilutions were made to obtain countable growth. Because the second set of ten-fold dilutions of necessity took place on the second day, after evaluating overnight growth on the previous day's filters, such samples exceed typical holding times and concentrations are thus indicated as estimated (E) in table 4. Bacteria enumeration was based on preparations with 20 to 80 colonies or was calculated from multiple dilutions in the case of non-ideal counts. After enumeration of FC colonies, the filter with the appropriate range of colonies was transferred to NA-MUG medium and incubated at 37ºC for 4 hours. Fluorescent colonies were counted as *Escherichia coli*. Growth from the 50-mL mFC filter was transferred to 1 mL phosphate buffered saline (PBS) with a final concentration of 20 percent glycerol, and was frozen at -70ºC for further analysis; this set of processed samples is hereafter referred to as the "FC stock." In addition, samples from all treatments on November 2 and 8, 2006, and from the NT8000 treatment additionally on November 3, 4, 9, and 16 were directly preserved by centrifuging 10 mL of the sample for 15 minutes at 4,500 revolutions per minute. The supernatant was decanted and the pellet was resuspended in 1 mL of phosphate buffered saline with 20 percent glycerol. Stocks of the LDME were made by adding 20 mL of LDME to sterile 50-mL polyethylene tubes and mixing 1:1 with 40 percent glycerol to create a final concentration of 20 percent glycerol. All stocks were frozen and stored at -70ºC until analysis.

Microbiological Indicators of LDME Transport to Subsurface Drains

These microbiological assays were done to help identify whether fecal bacteria present in the tile drains could be from a cattle source. One set of tests was done to evaluate whether shiga-toxin-producing *E. coli* (STEC) were present. Tests targeted the *E. coli* O157 serotype by two different assays. Tests also targeted a suite of toxin genes carried by typical STEC. Positive results might indicate a human-health concern and a bovine source. A second test analyzed for bovine-source *Bacteroidetes.* Positive results would indicate a bovine source.

Immunological Test for O157 Antigen

For every sample, 100 µL of FC stock was inoculated into Reveal for *E. coli* O157:H7 medium and the 8-hour test was performed according to manufacturer's instructions (Neogen, Lansing, Mich.). This test uses anti-O157 antibodies and detects all H serotypes of *E. coli* O157; therefore, all positive results are classified as detection of *E. coli* O157.

Table 4. Water-quality field data and bacteria concentrations for LDME on date of application and for all tile-water samples.

[LDME, liquid dairy manure effluent; µS/cm, microsiemens per centimeter at 25 degrees Celsius; CFU, colony forming units; mL, milliliters; ND, not detected; E, estimated because of non-ideal holding times]

Sample identification	Sample date	Specific conductance (µS/cm)	pH	Fecal-coliform bacteria (CFU/100 mL)	*Escherichia coli* (CFU/100 mL)
LDME	11/2/2006	4,000	6.8	E1,030,000	E600,000
Pre-application samples					
Tile 1 - DD8000	11/2/2006	1,230	7.7	1	1
Tile 2 - DD4000	11/2/2006	1,250	7.6	130	150
Tile 3 - C1	11/2/2006	1,180	7.6	100	10
Tile 4 - AW8000	11/2/2006	1,040	8.3	ND	ND
Tile 5 - AW4000	11/2/2006	1,040	7.5	6,900	100
Tile 6 - NT8000	11/2/2006	1,280	7.5	6	1
Tile 7 - NT4000	11/2/2006	1,220	8.4	19	19
Tile 8 - C2	11/2/2006	1,360	7.8	260	180
4 hours post-application					
Tile 1 - DD8000	11/2/2006	1,250	7.6	ND	ND
Tile 2 - DD4000	11/2/2006	1,290	7.6	ND	ND
Tile 3 - C1	11/2/2006	1,210	7.5	2	2
Tile 4 - AW8000	11/2/2006	1,090	7.5	ND	ND
Tile 5 - AW4000	11/2/2006	1,060	7.5	3	ND
Tile 6 - NT8000	11/2/2006	1,370	7.4	E35,000	7,000
Tile 7 - NT4000	11/2/2006	1,250	7.5	ND	ND
Tile 8 - C2	11/2/2006	1,370	7.5	ND	ND
1 day post-application					
Tile 1 - DD8000	11/3/2006	1,190	7.5	ND	ND
Tile 2 - DD4000	11/3/2006	1,230	7.8	ND	ND
Tile 3 - C1	11/3/2006	1,190	7.8	ND	ND
Tile 4 - AW8000	11/3/2006	1,070	7.7	ND	ND
Tile 5 - AW4000	11/3/2006	999	7.8	ND	6
Tile 6 - NT8000	11/3/2006	1,270	7.5	1,290	400
Tile 7 - NT4000	11/3/2006	1,200	7.6	ND	ND
Tile 8 - C2	11/3/2006	1,310	7.5	ND	ND

Table 4. Water-quality field data and bacteria concentrations for LDME on date of application and for all tile-water samples.—Continued

[LDME, liquid dairy manure effluent; µS/cm, microsiemens per centimeter at 25 degrees Celsius; CFU, colony forming units; mL, milliliters; ND, not detected; E, estimated because of non-ideal holding times]

Sample identification	Sample date	Specific conductance (µS/cm)	pH	Fecal-coliform bacteria (CFU/100 mL)	*Escherichia coli* (CFU/100 mL)
2 days post-application					
Tile 1 - DD8000	11/4/2006	1,190	7.6	7	5
Tile 2 - DD4000	11/4/2006	1,230	7.7	2	2
Tile 3 – C1	11/4/2006	1,160	7.6	2	2
Tile 4 - AW8000	11/4/2006	1,080	7.5	ND	ND
Tile 5 - AW4000	11/4/2006	988	7.5	2	2
Tile 6 - NT8000	11/4/2006	1,280	7.5	127	80
Tile 7 - NT4000	11/4/2006	1,220	7.6	ND	ND
Tile 8 - C2	11/4/2006	1,380	7.5	8	8
6 days post-application – Rainfall event					
Tile 1 - DD8000	11/8/2006	1,220	7.6	26	13
Tile 2 - DD4000	11/8/2006	1,280	7.3	29	14
Tile 3 - C1	11/8/2006	1,140	7.5	15	6
Tile 4 - AW8000	11/8/2006	982	7.4	838	520
Tile 5 - AW4000	11/8/2006	945	7.4	35	21
Tile 6 - NT8000	11/8/2006	1,230	7.3	145	120
Tile 7 - NT4000	11/8/2006	1,200	7.5	18	17
Tile 8 - C2	11/8/2006	1,310	7.5	3	2
7 days post-application					
Tile 1 - DD8000	11/9/2006	1,250	7.4	ND	ND
Tile 2 - DD4000	11/9/2006	1,280	7.5	ND	ND
Tile 3 - C1	11/9/2006	1,150	7.6	ND	ND
Tile 4 - AW8000	11/9/2006	1,020	7.7	700	30
Tile 5 - AW4000	11/9/2006	992	7.7	118	40
Tile 6 - NT8000	11/9/2006	1,250	7.5	100	ND
Tile 7 - NT4000	11/9/2006	1,200	7.6	ND	ND
Tile 8 - C2	11/9/2006	1,280	7.6	12	ND

Table 4. Water-quality field data and bacteria concentrations for LDME on date of application and for all tile-water samples.—Continued

[LDME, liquid dairy manure effluent; µS/cm, microsiemens per centimeter at 25 degrees Celsius; CFU, colony forming units; mL, milliliters; ND, not detected; E, estimated because of non-ideal holding times]

Sample identification	Sample date	Specific conductance (µS/cm)	pH	Fecal-coliform bacteria (CFU/100 mL)	*Escherichia coli* (CFU/100 mL)
14 days post-application – Rainfall event					
Tile 1 - DD8000	11/16/2006	599	8.1	50	25
Tile 2 - DD4000	11/16/2006	640	8.0	74	44
Tile 3 - C1	11/16/2006	654	7.9	20	16
Tile 4 - AW8000	11/16/2006	680	7.9	82	70
Tile 5 - AW4000	11/16/2006	697	7.9	90	70
Tile 6 - NT8000	11/16/2006	709	7.8	90	50
Tile 7 - NT4000	11/16/2006	680	7.8	62	34
Tile 8 - C2	11/16/2006	624	7.8	82	50

DNA Extraction

DNA was extracted from 200 µL of frozen source stock (representing 2 mL of original water from the tile, 10 mL of the 50-mL mFC culture, or 100 µL of LDME stock). DNA was extracted by use of the QIAamp DNA stool mini kit (Qiagen, Valencia, Calif.) according to manufacturer's instructions.

Polymerase Chain Reaction (PCR)

PCR was done by use of a PerkinElmer GeneAmp PCR System 2400 Thermal Cycler (Perkin Elmer, Boston, Mass.). All reaction mixtures were 25 µL final volume. PCR fragments were separated on 2 percent agarose gels in Tris Acetate EDTA (TAE) buffer and stained for 15 minutes in 0.5 µg/mL ethidium bromide solution. Fragments were visualized by use of a Foto/prep transilluminator (Fotodyne, Hartland, Wis.), imaged with a Kodak EDAS 290 Zoom digital camera and analyzed with the Kodak 1D-gel image analysis software.

Multiplex PCR for Shiga-Toxin-Producing *E. coli*

This analysis was designed to detect four genes: *eae*A, *stx*1, *stx*2 (Gannon and others, 1992; Fagan and others, 1999) and the 16S rDNA of *E. coli* (Sabat and others, 2000). The latter gene was used as an internal positive control. All reagents were from Applied Biosystems (Foster City, Calif.). Each 15-µL PCR reaction mixture contained (final concentration) 1× Buffer II PCR reaction buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µg/µL bovine serum albumin (BSA), *eae*A, *stx*2 and *stx*1 primers (0.05 µM), *E. coli* 16S rDNA primers (0.025 µM), AmpliTaq Gold Polymerase (0.1 unit/µL), and from 1 to 100 ng of DNA. A separate PCR assay was used to detect the *rfb*O157 gene for the *E. coli* O157 serotype (Maurer and others, 1999). This is a confirmation assay for the Reveal test. All reagents were from Applied Biosystems (Foster City, Calif.). Each 15-µL reaction mixture contained (final concentration) 1 × Gold Buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µg/µL

BSA, 0.1 µM of forward and reverse primers, 0.1 unit/µL of AmpliTaq Gold polymerase, and from 1 to 100 ng of template DNA. All reagents were from Promega (Madison, Wis.).

PCR for Bovine *Bacteroidetes* Gene

Extracted DNA was amplified with primers described in Bernhard and Field (2000). PCR was performed on 1–10 ng of DNA. Each 15-µL reaction contained the after (final concentrations): 1× colored GoTaq buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µg/µL bovine serum albumin, 0.3 µM of CF128F primer, 0.3 µM of BAC708R primer, and 0.1 unit/µL of GoTaq Polymerase (Promega, Madison, Wis.). A touchdown DNA amplification was used with the after temperature cycles as per Fogarty and Voytek (2005): 94ºC for 3 minutes; 10 cycles of 94ºC for 30 seconds, 63ºC for 30 seconds (decreasing by 1ºC each cycle), and 72ºC for 30 seconds; and 20 cycles of 94ºC for 30 seconds, 53ºC for 30 seconds, and 72ºC for 1 minute and 30 seconds.

Quality Assurance/Quality Control

Several steps were taken to assure the quality of each PCR reaction, after recommendations of the USEPA (2004). For approximately every 20 samples of any given PCR reaction, PCR positive controls (extra reaction with 10 ng of DNA from the positive control strain), and PCR negative controls (blank water replaced template DNA) were included. Environmental matrix issues were addressed by including a matrix spike (addition of positive DNA to a previously analyzed negative sample) approximately every 40 reactions for the STEC multiplex and bovine *Bacteroidetes* gene assays. PCR inhibition controls (internal controls) were run with every reaction of the STEC multiplex PCR.

Results of Chemical and Microbiological Analyses

As described previously, LDME might move to tile drains by rapid transport through preferential flow pathways or by slower leaching through the soil matrix. In studies by others, if preferential flow pathways existed, transport typically occurred within a few hours to a day after LDME application. Subsequent leaching could occur at any time that the water-holding capacity of the soil was exceeded, usually after a rainfall event. Therefore, sample collection was designed to evaluate tile-drain water quality before LDME application and in both the short term (4 hours to 1 day) and in the longer term, especially after rainfall events. On each sampling date, tile-drain water was analyzed in the field for specific conductance (SC) and pH. Specific conductance is a measure of the concentration of ions in a solution, and pH indicates the degree of acidity or alkalinity of the sample. Both measures were chosen to possibly indicate the movement of LDME from the surface application to the tile drains. In addition, fecal-coliform and *E. coli* bacteria were enumerated in subsurface drain water, and concentrations of these bacteria were compared to various water-quality standards. Various forms of nitrogen and phosphorus were determined, patterns in nutrient concentrations with respect to LDME application and rainfall were evaluated, and nutrient concentrations were compared to USEPA-recommended water-quality criteria. The presence of chemical and microbiological indicators of LDME transport to subsurface drains was evaluated in one tile drain over the duration of the field trail and in all tile drains after the first significant rainfall event.

Specific Conductance and pH

Neither specific conductance nor pH gave a clear indication of short-term or longer term LDME movement to subsurface tiles under any treatment (table 4). The LDME on the day of application had an SC of 4,000 µS/cm. The SC of subsurface drain water before LDME application ranged from 1,034 to 1,361 µS/cm. For most of the sampling dates, changes in SC were within the range of accuracy for the SC probes (± 3–5 percent). On November 16, 2006, however, subsurface drains were full to overflowing, and SC values ranged from 599 to 709 µS/cm for all treatments. These SC values are approximately half of pre-application SC and most likely indicate the dilution of subsurface water with rainwater.

The LDME on the day of application had a pH of 6.8. The pre-application pH for subsurface drain water for all treatments ranged from 7.5 to 8.4. After application of LDME, there was no obvious trend in pH with respect to treatment. After heavy rainfall on November 16, 2006, pH increased by about 0.5 unit for all treatments.

Bacteria Concentrations

Both fecal-coliform and *E. coli* bacteria were enumerated. *E. coli* are members of the fecal-coliform group. In the LDME, *E. coli* made up 59 percent of fecal coliforms. Among the tile samples, *E. coli* ranged from less than 1 percent of fecal coliforms to (more typically) 60–100 percent of fecal coliforms. The LDME contained around 1,000,000 fecal coliforms, and 600,000 *E. coli*, per 100 mL.

One pre-application drain (tile 5, table 4) exhibited a relatively high concentration of fecal-coliform, but not *E. coli*, bacteria. Some fecal-coliform bacteria may occur naturally in the environment, and fecal-coliform bacteria and *E. coli* may persist from prior manure applications (Jamieson and others, 2002). This information may explain the presence of these bacteria in pre-application tile samples.

The only subsurface drain for which fecal-coliform and *E. coli* bacteria concentrations increased after LDME application was tile 6 (NT8000 treatment, table 4). The results for the NT8000 treatment appear to indicate rapid movement of bacteria to tile-drain water after LDME application. At 4 hours post-application, both fecal-coliform and *E. coli* concentrations were very high for the NT8000 treatment in comparison to pre-application concentrations and to concentrations in other treatment tile drains.

There was only one indication of the potential for rainfall to promote the movement of LDME bacteria to subsurface drains. Bacteria concentrations in tile 4 (AW4000 treatment, table 4) increased above prior levels 6 days after LDME application, after a rainfall event. For this treatment, fecal-coliform concentrations, but not *E. coli* concentrations, remained elevated on the seventh day.

In Michigan, surface waters are required to meet recreational water-quality criteria during the recreational season (May through September). For recreational waters, the concentration of *E. coli* permitted for full-body contact during the recreational season is 235 CFU/100 mL in a single water sample (USEPA) or, for the State of Michigan, 300 CFU/100 mL as a geometric mean of three samples. In this field trial, sampling was not done in such a way that the geometric mean could be calculated as required by the State of Michigan. In addition, sampling was done during the nonrecreational season. Therefore, recreational water-quality standards serve only as a reference against which bacteria concentrations can be compared. Two treatments had samples that exceeded the USEPA single-sample standard: NT8000 at 4 hours and 1 day post-application, and AW8000 at

6 days post-application, after a rainfall event (table 4). No sample from a control tile exceeded these standards.

Under the National Pollutant Discharge Elimination System (NPDES) the State of Michigan has issued a general permit for large, concentrated animal-feeding operations and for other animal-feeding operations that request coverage (*http://www.deq.state.mi.us/documents/deq-water-npdes-generalpermit-MIG019000.pdf*). Discharges that violate or that contribute to violation of Michigan Water Quality Standards are prohibited. The data from this field trial indicate that LDME application, combined with some management practices, may influence bacteria concentrations in subsurface drains. Bacteria leaching may occur immediately after application or subsequently in association with rainfall events, as others have observed (Joy and others, 1998; Geohring and others, 2001; Jamieson and others, 2002; Ball Coelho and others, 2007). However, in this field trial, subsurface-drain samples were not collected at a point of outflow to surface water. Concentrations of bacteria or other measured constituents might be different at the point where tile drains discharge to surface water.

Nutrient Concentrations

The LDME was analyzed on three dates to evaluate variability in nutrient concentrations (table 5). Concentrations of nutrients in LDME or in any type of manure may vary with holding time, frequency of application, number of animals contributing, relative solids percentage, and a wide array of environmental factors. As noted previously, the LDME is analyzed routinely and typically has around 1.0 percent solids, 10 lb/gal (1.2 g/L) of total nitrogen, 8.8 lb/gal (1.1 g/L) of ammonia nitrogen, and 0.2 lb/gal (24 mg/L) of orthophosphorus. For the three samples reported in table 5, concentrations of organic + ammonia nitrogen (roughly equivalent to total nitrogen), ammonia nitrogen, and orthophosphorus were consistent with those previous analyses by other laboratories, indicating that sampling the LDME from the applicator did not result in obvious bias in nutrient concentrations. Most nutrients in manure are in organic forms, and nitrate is not usually present in any abundance. The data in table 5 are consistent with this distribution of the various forms of nutrients.

A field blank sample was analyzed for all nutrients (table 5). No nitrate + nitrite nitrogen, total phosphorus, or orthophosphorus were detected in the field blank, but organic + ammonia nitrogen and ammonia nitrogen were detected. These detections might indicate that a contaminant such as a soil particle may have been introduced into the sample bottle by handling. These detections could also indicate that cleaning of the sampling apparatus did not remove all contamination from the previous sample. However, ammonia nitrogen was rarely detected in subsequent samples; and because all samples were collected by means of the same between-sample cleaning procedure as for the field blank, it would appear that there was no routine contamination with ammonia nitrogen due to the sampling procedure. The tile 3 Control 1 treatment exhibited anomalously high pre-application concentrations of organic + ammonia nitrogen and of total phosphorous, indicative of possible sample contamination. However, because both these analyses were done on unfiltered water samples, the relatively high concentrations of organic + ammonia nitrogen and total phosphorous may simply reflect the presence of naturally occurring particulate material in the sample. Nevertheless, the tile 3 C1 pre-application sample is marked in table 5 as possibly subject to contamination. In the tables and charts that follow, the concentrations of organic + ammonia nitrogen and ammonia nitrogen detected in the field blank have not been subtracted from the reported values.

Table 5. Nutrient concentrations in LDME, field blank, and tile-water samples.

[LDME, liquid dairy manure effluent; ND, not detected; C, sample possibly contaminated; E, estimated concentration; all concentrations in milligrams per liter]

Site identification	Sample date	Organic + ammonia nitrogen	Ammonia nitrogen	Nitrate + nitrite nitrogen	Ortho-phosphorus	Total phosphorus
LDME	9/27/2006	1,300	1,320	0.07	0.562	30
LDME	10/11/2006	930	860	.07	.058	25.8
LDME	11/2/2006	800	519	ND	195	195
Field Blank	11/16/2006	.5	.04	ND	ND	ND
Pre-application samples						
Tile 1 - DD8000	11/2/2006	.61	ND	44.0	.038	.06
Tile 2 - DD4000	11/2/2006	9.8	ND	39.3	.102	3.53
Tile 3 - C1	11/2/2006	C120	ND	52.6	.084	C49.3
Tile 4 - AW8000	11/2/2006	.93	ND	47.3	.093	.22
Tile 5 - AW4000	11/2/2006	1.3	ND	46.2	.092	.29
Tile 6 - NT8000	11/2/2006	1.3	ND	53.8	.061	.58
Tile 7 - NT4000	11/2/2006	.67	ND	41.0	.104	.12
Tile 8 - C2	11/2/2006	1.1	ND	48.8	.232	.36
4 hours post-application						
Tile 1 - DD8000	11/2/2006	.53	ND	44.1	.035	ND
Tile 2 - DD4000	11/2/2006	.78	ND	38	.084	.09
Tile 3 - C1	11/2/2006	4.2	ND	52.5	.068	2.15
Tile 4 - AW8000	11/2/2006	.98	ND	46.3	.089	.27
Tile 5 - AW4000	11/2/2006	1.6	ND	45	.094	.34
Tile 6 - NT8000	11/2/2006	3.2	.05	52.5	.008	.53
Tile 7 - NT4000	11/2/2006	.63	ND	41.1	.119	.12
Tile 8 - C2	11/2/2006	.63	ND	44.6	.126	.15
1 day post-application						
Tile 1 - DD8000	11/3/2006	.53	ND	43.6	.036	.06
Tile 2 - DD4000	11/3/2006	.6	ND	38.1	.091	.09
Tile 3 - C1	11/3/2006	.53	ND	43.7	.035	.06
Tile 4 - AW8000	11/3/2006	.84	ND	48.3	.099	.16
Tile 5 - AW4000	11/3/2006	.73	ND	43.7	.103	.13
Tile 6 - NT8000	11/3/2006	.66	.16	55.1	.072	.09
Tile 7 - NT4000	11/3/2006	.61	ND	40.3	.12	.1
Tile 8 - C2	11/3/2006	.86	ND	46.2	.138	.2

Table 5. Nutrient concentrations in LDME, field blank, and tile-water samples.—Continued

[LDME, liquid dairy manure effluent; ND, not detected; C, sample possibly contaminated; E, estimated concentration; all concentrations in milligrams per liter]

Site identification	Sample date	Organic + ammonia nitrogen	Ammonia nitrogen	Nitrate + nitrite nitrogen	Ortho-phosphorus	Total phosphorus
2 days post-application						
Tile 1 - DD8000	11/4/2006	0.68	ND	53.8	0.05	0.16
Tile 2 - DD4000	11/4/2006	.69	ND	38.6	.095	.34
Tile 3 - C1	11/4/2006	.82	ND	54.8	.05	.19
Tile 4 - AW8000	11/4/2006	.64	ND	47.5	.102	.12
Tile 5 - AW4000	11/4/2006	.68	ND	42.8	.106	.12
Tile 6 - NT8000	11/4/2006	.6	.09	55.3	.057	.17
Tile 7 - NT4000	11/4/2006	.86	ND	40.7	.131	.2
Tile 8 - C2	11/4/2006	.97	ND	49.9	.22	.28
6 days post-application – Rainfall Event						
Tile 1 - DD8000	11/8/2006	.93	ND	43.5	.024	.09
Tile 2 - DD4000	11/8/2006	1.5	.03	40	.059	.19
Tile 3 - C1	11/8/2006	.77	ND	49.8	.042	.15
Tile 4 - AW8000	11/8/2006	1.5	ND	41.1	.081	.25
Tile 5 - AW4000	11/8/2006	1.9	ND	42.5	.095	.41
Tile 6 - NT8000	11/8/2006	1	ND	54.4	.057	.2
Tile 7 - NT4000	11/8/2006	.71	ND	41.7	.053	.08
Tile 8 - C2	11/8/2006	.78	ND	44.3	.052	.08
7 days post-application						
Tile 1 - DD8000	11/9/2006	.51	ND	43.2	.024	ND
Tile 2 - DD4000	11/9/2006	1.4	ND	38.5	.056	.19
Tile 3 - C1	11/9/2006	3.8	ND	50.6	.035	2.31
Tile 4 - AW8000	11/9/2006	.79	ND	42.8	.081	.08
Tile 5 - AW4000	11/9/2006	.92	ND	43.7	.093	.11
Tile 6 - NT8000	11/9/2006	.58	ND	53.2	.049	.11
Tile 7 - NT4000	11/9/2006	.73	.62	39.9	.149	.12
Tile 8 - C2	11/9/2006	1.5	ND	40.8	.067	.39

Table 5. Nutrient concentrations in LDME, field blank, and tile-water samples.—Continued

[LDME, liquid dairy manure effluent; ND, not detected; C, sample possibly contaminated; E, estimated concentration; all concentrations in milligrams per liter]

Site identification	Sample date	Organic + ammonia nitrogen	Ammonia nitrogen	Nitrate + nitrite nitrogen	Ortho-phosphorus	Total phosphorus
14 days post-application – Rainfall Event						
Tile 1 - DD8000	11/16/2006	3.5	0.12	25.5	1.54	2.48
Tile 2 - DD4000	11/16/2006	4.9	.12	29.4	1.4	2.9
Tile 3 - C1	11/16/2006	3.7	ND	33.9	.67	1.63
Tile 4 - AW8000	11/16/2006	4.2	E.12	27.7	.582	1.93
Tile 5 - AW4000	11/16/2006	3.9	.04	21	.738	1.8
Tile 6 - NT8000	11/16/2006	3.6	.08	28	.735	1.46
Tile 7 - NT4000	11/16/2006	4.1	.05	29.5	.936	1.83
Tile 8 - C2	11/16/2006	3.6	.05	36.8	1.23	1.98
51 days post-application						
Tile 6 - NT8000	12/21/2006	1.6	ND	52.3	.037	.47
171 days post-application						
Tile 3 - C1	3/20/2007	5.4	.41	43.9	.079	1.63
Tile 6 - NT8000	3/20/2007	1.9	.03	43.5	.033	.57

Nutrient concentrations in tile water did not conclusively indicate short-term preferential flow of LDME to tile drains. Concentrations of organic + ammonia nitrogen, nitrite + nitrate nitrogen, and ortho- and total phosphorus measured within 4 hours to 1 day after LDME application were within the range of pre-application values for all treatments (table 5), indicating no immediate influence of LDME application on tile-drain water quality for these constituents. Ammonia nitrogen was detected in the NT8000 samples immediately after, and for 2 days after, LDME application. This was the only treatment to exhibit this result. With only this exception, concentrations of all forms of nutrients measured within 4 hours to 1 day after LDME application were generally similar among all treatments, including control treatment plots, to which no LDME was applied. Therefore, antecedent conditions appeared to have more influence on short-term nutrient concentrations than did the LDME application.

Nutrient concentrations also did not indicate an effect of LDME application or management practice on tile water quality after rainfall events. After the rainfall event at 6 days post-application (approximately 0.56 in. of rain within 24 hours), there was little change in tile-water nutrient concentrations with respect to previous samples nor any obvious pattern in nutrient concentrations among treatments receiving LDME in comparison to those receiving no LDME. However, after the rainfall event at 14 days post-application (approximately 0.84 in. of rain within 24 hours), organic + ammonia nitrogen, orthophosphorus, and total phosphorus concentrations increased above average concentrations for all prior sampling dates in every tile. In addition, ammonia nitrogen was present in every tile, including Controls, whereas it was not typically present in every tile on prior sampling dates. Because increases in the concentrations of these constituents also occurred in tiles draining Control plots to which no LDME was applied, this effect cannot be attributed to LDME

application or management practice. Finally, nitrate + nitrite nitrogen concentrations decreased in every tile, including Controls. This result may reflect dilution by rainwater.

Very recently, several studies have documented particle-associated phosphorus movement to subsurface drains, especially during significant rainfall events after extended dry periods (Simard and others, 2000; de Jonge and others, 2004; Schelde and others, 2006; Gentry and others, 2007). This recent research indicates that particles may be released from soil when the ionic strength of the soil water decreases or when pH increases (de Jonge and others, 2004; Schelde and others, 2006). Both water-quality changes were observed for the 14-day samples for all treatments (table 4), and are likely due to the influx of rainwater. It is common to estimate particulate phosphorus as the difference between phosphorous measured in unfiltered samples (in this study, total phosphorous) and phosphorous measured in filtered samples (in this study, orthophosphorus) (Simard and others, 2000; Toor and others, 2004). If this calculation is done for the data reported in table 5, the median particulate phosphorous for all samples preceding the 14 days post-application rainfall event is 0.062 mg/L. In contrast, for the samples collected at 14 days after application, the median particulate phosphorus concentration estimated by this method is 0.95 mg/L.

Additional samples were collected from the NT8000 treatment at 51 days and 171 days post-LDME application to provide some longer term perspective on nutrient concentrations (table 5). A sample was also collected from the Control 1 treatment at 171 days post-application. No further LDME was applied during this time. Nevertheless, for both the NT8000 and Control 1 treatments, concentrations for all constituents in March 2007 were similar to those noted during the field trial.

The USEPA has established recommended total phosphorus and total nitrogen water-quality criteria for streams and rivers (United States Environmental Protection Agency, 2000). These criteria contain USEPA's recommendations to States and authorized Tribes for use in establishing their water-quality standards consistent with section 303(c) of Clean Water Act. These values are not at this time incorporated into water-quality standards by the State of Michigan. At this time, these values serve only as a reference against which stream-water quality may be compared. Table 6 lists these values for Aggregate Ecoregion VI (Corn Belt and Northern Great Plains), into which the Maumee River Watershed falls; however, this ecoregion covers several states, including parts of South Dakota, Nebraska, and Minnesota. The 25^{th}-percentile values for the entire Region VI were taken as the recommended criteria for total nitrogen and total phosphorus. There are currently no recommended criteria for organic + ammonia nitrogen or nitrate + nitrite nitrogen. If criteria were to be recommended, it is possible the 25^{th}-percentile values would be used, so these are reported in table 6. In addition, the USEPA lists 25^{th}-percentile values for total phosphorous, total nitrogen, organic + ammonia nitrogen, and nitrate + nitrite nitrogen for the subregion that includes specifically the Maumee River Watershed and a region surrounding Saginaw Bay (Level III Ecoregion # 57, Huron/Erie Lake Plain). Although the USEPA has not recommended criteria for nutrients at the subregional level, the 25^{th}-percentile values for the Level III Ecoregion #57 more likely reflect specific water-quality conditions in the Maumee River Watershed.

Table 6. U.S. Environmental Protection Agency Ambient Water-Quality Criteria and 25th percentiles for nutrients in Ecoregion VI (Corn Belt) and Level III Ecoregion 57 (Huron/Erie Lake Plain).

[All concentrations in milligrams per liter; --, not applicable]

Region	Water-quality criteria		25th percentiles			
	Total phosphorus	Total nitrogen	Organic + ammonia nitrogen	Nitrate + nitrite nitrogen	Total nitrogen	Total phosphorus
Ecoregion VI	0.0763	2.18	0.591	0.633	2.18	0.0763
Level III Ecoregion 57	--	--	.65	.897	1.91	.070

Field-trial results indicate potential for water-quality degradation, based on USEPA nutrient water-quality criteria, if concentrations of nutrients in subsurface drain outfalls to surface water are similar to those measured. Nutrient concentrations in tile-drain water were greater than the USEPA criteria for most samples (tables 5 and 6). Total nitrogen (organic+ ammonia nitrogen + nitrate + nitrite nitrogen) was greater than the recommended water-quality criterion for Ecoregion VI and the 25th percentiles for all nitrogen forms for Level III Ecoregion #57 in all samples. Nitrate + nitrite nitrogen concentrations were the major contributor to the relatively high nitrogen concentrations. The median nitrate + nitrite nitrogen concentration for all samples was 43.55 mg/L (range, 21–55.3 mg/L). The median organic + ammonia nitrogen concentration for all samples was 0.89 mg/L (range, 0.51–9.8 mg/L). Among all samples and dates, only four did not have concentrations greater than the USEPA Ecoregion VI criterion, or the Level III Ecoregion # 57 25th percentiles for total phosphorus. The median total phosphorus concentration for all samples and all dates was 0.195 mg/L (range, 0.03–3.53 mg/L). The median orthophosphorus concentration for all samples and dates was 0.091 mg/L (range, 0.024–1.54 mg/L).

Although the field-trial results were inconclusive about the influence of LDME on tile-drain nutrient water quality in either the short or long term, field-trial results indicated significant concentrations of nutrients in tile water during the non-growing season, similar to previous studies (Gentry and others, 2007; Owens and Shipitalo, 2006). The field trial demonstrated that antecedent nutrient concentrations were relatively high in tile water and that a rainfall event exceeding 0.5 in. may result in increases in organic + ammonia nitrogen, ammonia nitrogen, orthophosphorus, and total phosphorous concentrations and decreases in nitrate + nitrite nitrogen concentrations in subsurface drains. Nitrate + nitrite nitrogen accounted for most of the nitrogen in tile water during the field trial. Nitrate concentrations in tile water are influenced by flow conditions and cropping strategies (Bakhsh and others, 2005). It is possible that nitrate + nitrite nitrogen concentrations do not remain as high throughout the year as those measured during the field trial, especially during the growing season, as noted by Bakhsh and others (2005). Flow rate or discharge of water from the subsurface drains was not measured, and nutrient concentrations were not determined at an outfall to surface water. It is therefore not possible to estimate the potential load of nutrients delivered to receiving waters. Loading rate for various nutrients may vary with tillage, season, and cropping strategy (Bakhsh and others, 2005; Schelde and others, 2006), and this type of treatment effect could not be determined by the field-trial design. Nevertheless, the field trial yielded important information about nutrient concentrations in tile drains during the non-growing season.

Future studies of nutrient concentrations in tile drains would benefit from treatment replication, measurement of tile-drain discharge, and analysis of seasonal or crop effects.

Chemical Indicators of LDME Transport to Subsurface Drains

Various pharmaceutical, antibiotic, and wastewater chemical compounds (tables 1–1 and 1–2) were analyzed for in a blank sample, in the LDME, in a pre-application tile-water sample, and in two categories of treatment tile-water samples. First, the occurrence of these chemicals was evaluated over time after LDME application for the NT8000 treatment. Second, the occurrence of the chemicals in tile water from all treatment plots and one control plot was evaluated at 6 days post-LDME application, after the first significant rainfall event.

In all, 1 pharmaceutical, 4 antibiotics, and 12 wastewater chemicals (WWCs) were detected and quantified in the LDME (table 7). The potential sources of the antibiotics and WWCs detected in the LDME are listed in tables 1–1 and 1–2. The four antibiotics detected are commonly used in animal agriculture, so their detection is not unexpected. Analgesics such as ibuprofen may be used for animal comfort and to reduce inflammation. The WWCs detected in the LDME (table 7) could readily come from typical on-farm practices and sources. Specifically, the cleaning products might come from any type of cleaning procedure for which wash water is added to the LDME. The detergent degradates may also be included directly in a variety of cleaning fluids. The fecal sterols are products of animal digestion.

None of the antibiotics detected in the LDME was detected in a treatment subsurface drain sample. Among the antibiotics detected in the LDME, previous research by others would indicate that oxytetracycline would bind tightly to the soil matrix, and that the sulfonamide antibiotics (sulfadimethoxine and sulfamethazine) would bind less strongly. The concentrations of antibiotics detected in the LDME are lower than concentrations observed, or artificially prepared, in other studies (Kay and others, 2004, 2005a, b; Burkhardt and others, 2005). Therefore, it is not surprising that the applied antibiotics were not detected in the subsurface drain samples. Tylosin, another antibiotic widely used in animal agriculture, binds tightly to soil (Boxall and others, 2002) and degrades rapidly (Schlüsener and Bester, 2006). Nevertheless, it was detected in the pre-application sample. The detection of tylosin in the pre-application sample may be the result of previous LDME applications to the field.

No WWC detected in the LDME was quantified in NT8000 tile water at a concentration exceeding the reporting level. However, the analysis method can confirm the identity of a given compound at concentrations less than the reporting level, even if the quantification is uncertain (Zaugg and others, 2001). Several WWCs were detected in the NT8000 tile water at concentrations less than the reporting level. These are indicated by a “+” in table 7.

One chemical detected in the LDME (phenol) was also detected in the field blank (table 8). Three chemicals (phenol, diethoxynonylphenol, and diethoxyoctylphenol) detected in the LDME also were detected in the pre-application sample, possibly reflecting previous LDME applications. Of the 12 WWCs detected in the LDME 2 chemicals (the fecal sterols cholesterol and indole) were detected in the NT8000 sample collected 1 day after LDME application, but not in a blank or pre-application sample. These chemicals might be inferred to indicate LDME transport from the current application to tile water. Subsequent NT8000 samples did not indicate clear evidence of any additional LDME-chemical transport.

Table 7. Results of antibiotic and wastewater chemical analyses for LDME, pre-application sample, and NT8000 treatment (Tile 6) over time.

[LDME, liquid dairy manure effluent; PA, pre-application sample; ND, not detected; +, detected but not quantified at a concentration greater than the reporting level; concentrations in micrograms per liter]

Compound name	LDME	PA	Numbers of days post-LDME application for NT8000 treatment – Tile 6				
			1	2	6	7	51
Pharmaceuticals							
Ibuprofen	4.5	ND	ND	ND	ND	ND	ND
Antibiotics							
Sulfadimethoxine	1.9	ND	ND	ND	ND	ND	ND
Sulfamethazine	1.2	ND	ND	ND	ND	ND	ND
Oxytetracycline	1.6	ND	ND	ND	ND	ND	ND
Lincomycin	1.2	ND	ND	ND	ND	ND	ND
Tylosin	ND	0.2	ND	ND	ND	ND	ND
Wastewater chemicals							
Cleaning products							
Phenol	649	+	+	ND	ND	ND	+
Detergent degradates							
Monoethoxy-nonylphenol	329	ND	ND	ND	ND	ND	ND
Diethoxy-nonylphenol	507	+	+	ND	ND	ND	ND
Monoethoxy-octylphenol	18	ND	ND	ND	ND	ND	ND
Diethoxy-octylphenol	26	+	ND	ND	ND	ND	ND
4-nonylphenol	ND	+	+	ND	ND	ND	+
Fecal sterols							
Cholesterol	381	ND	+	ND	ND	ND	ND
Coprostanol	456	ND	ND	ND	ND	ND	ND
Indole	5.2	ND	+	ND	ND	ND	ND
Methyl indole	230	ND	ND	ND	ND	ND	ND
β-sitosterol	692	ND	ND	ND	ND	ND	ND
β-stigmastanol	1,070	ND	ND	ND	ND	ND	ND
Polycyclic aromatic hydrocarbons							
Anthracene	ND	ND	ND	ND	ND	ND	+
Benzo[*a*]pyrene	ND	ND	ND	ND	ND	ND	+
Fluoranthene	ND	ND	ND	ND	ND	ND	+
Phenanthrene	ND	ND	ND	ND	ND	ND	+
Pyrene	ND	ND	ND	ND	ND	ND	+

Table 7. Results of antibiotic and wastewater chemical analyses for LDME, pre-application sample, and NT8000 treatment (Tile 6) over time.—Continued

[LDME, liquid dairy manure effluent; PA, pre-application sample; ND, not detected; +, detected but not quantified at a concentration greater than the reporting level; concentrations in micrograms per liter]

Compound name	LDME	PA	Numbers of days post-LDME application for NT8000 treatment – Tile 6				
			1	2	6	7	51
Wastewater chemicals—Continued							
Other							
1,4 dichlorobenzene	ND	+	ND	+	+	+	+
Benzophenone	ND	ND	ND	+	ND	ND	+
DEET	ND	ND	ND	ND	ND	ND	+
Carbazole	ND	ND	ND	ND	ND	ND	+
HHCB	ND	ND	ND	ND	ND	ND	+
Isophorone	ND	ND	ND	ND	ND	ND	+
Menthol	ND	ND	ND	ND	ND	ND	+
Methyl salicylate	1.8	ND	ND	ND	ND	ND	+
Metolachlor	ND	ND	+	+	+	+	ND
Tributyl phosphate	ND	ND	ND	ND	ND	ND	+
Triphenyl phosphate	ND	ND	ND	ND	ND	+	ND

Table 8. Results of wastewater-chemical analyses for field blank and for all treatments after the first significant rainfall event.

[Concentrations in micrograms per liter; NT, not tested; ND, not detected; +, detected but not quantified at a concentration greater than the reporting level]

Compound name	Field blank	DD8000 Tile 1	DD4000 Tile 2	AW8000 Tile 4	AW4000 Tile 5	NT8000 Tile 6	NT4000 Tile 7	C2 Tile 8
Pharmaceuticals								
Ibuprofen	NT	ND	ND	ND	ND	ND	ND	ND
Antibiotics								
Sulfadimethoxine	NT	ND	ND	ND	ND	ND	ND	ND
Sulfamethazine	NT	ND	ND	ND	ND	ND	ND	ND
Oxytetracycline	NT	ND	ND	ND	ND	ND	ND	ND
Lincomycin	NT	ND	ND	ND	ND	ND	ND	ND
Tylosin	NT	ND	ND	ND	ND	ND	ND	ND
Wastewater chemicals								
Cleaning products								
Phenol	+	+	ND	ND	ND	ND	ND	ND
Detergent degradates								
Mononethoxy-nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND
Diethoxy-nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND
Monoethoxy-octylphenol	ND	ND	ND	ND	ND	ND	ND	ND
Diethoxy-octylphenol	ND	ND	ND	ND	ND	ND	ND	ND
4-Nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND
Fecal sterols								
Cholesterol	ND	ND	ND	ND	ND	ND	ND	ND
Coprostanol	ND	ND	ND	ND	ND	ND	ND	ND
Indole	ND	ND	ND	ND	ND	ND	ND	ND
Methyl indole	ND	ND	ND	ND	ND	ND	ND	ND
β-Sitosterol	ND	ND	ND	ND	ND	ND	ND	ND
β-Stigmastanol	ND	ND	ND	ND	ND	ND	ND	ND
Other Compounds								
1,4-dichlorobenzene	+	+	+	+	+	+	+	+
Acetophenone	ND	+	ND	ND	ND	ND	ND	ND
Benzophenone	ND	+	+	ND	+	ND	ND	ND
Methyl salicylate	+	ND	ND	ND	ND	ND	ND	ND
Metolachlor	ND	ND	ND	+	+	+	ND	ND
Triphenyl phosphate	ND	ND	+	ND	ND	ND	ND	ND

A variety of other chemicals not detected in the applied LDME were detected in some subsequent NT8000 samples. Two of these additional chemicals (1,4-dichlorobenzene and DEET) also were detected in the field blank (table 8), again indicating potential contamination for these constituents. The other chemicals detected could feasibly come from some typical on-farm practice. In particular, the herbicide metolachlor is used on corn and soybeans, and its detection in subsurface drains would not be unexpected. Most of the other chemicals could be associated with cleaning products or possibly fuels.

To evaluate whether rainfall might influence chemical transport from LDME to subsurface drains, the occurrence of antibiotics and WWCs was tested for all treatments at 6 days post-application on November 8, 2006 (table 8). This sample date followed the first significant rainfall event. No LDME chemical except phenol (also detected in the field blank sample) was detected in any of the November 8 samples from any treatment. Additional WWCs not detected in the LDME were detected in some treatment samples. Of these, two also were detected in the field blank sample.

The antibiotic and WWC results do not indicate a substantive contribution of the LDME to subsurface drain water over the course of the field trial, either through short-term preferential flow pathways or after the first significant rainfall event. This result is consistent with the nutrient results presented previously. It should be noted that the second rainfall event did influence nutrient concentrations (table 5); however, no antibiotics or WWCs were analyzed for that sample date. In addition, it is possible that the 1-day post-application sampling (for treatment NT8000, table 7) was not soon enough to detect immediate transport by preferential flow. Finally, many of these chemicals may bind tightly to soil particles, but tile-water analyses were done on filtered water samples. Nevertheless, field-trial results demonstrated that a variety of chemicals associated with LDME, or with other farming practices, may be present in subsurface drains and might serve as indicators of transport from the surface to tile drains. Further study of the factors affecting transport behavior of these chemicals including their persistence in soil and the conditions under which they are transported to subsurface drains is needed.

Microbiological Indicators of LDME Transport to Subsurface Drains

The LDME on the date of application was analyzed for microbiological indicators of LDME transport to subsurface drains. All indicators except the *stx*2 gene were detected in the LDME (table 9). The *E. coli* O157 serotype was detected in bacteria from the LDME by both an immunological analysis and a DNA-based analysis. The *stx*1 and *eae*A genes also were detected in bacteria from the LDME. The *stx*1 gene has been associated with bovine sources but has rarely been associated with *E. coli* isolated from humans with gastrointestinal illness (Beutin and others, 1993; Boerlin and others, 1999). The *stx*2 gene is frequently associated *E. coli* causing human illness. The *stx*2 gene was not detected in bacteria from the LDME. The *eae*A gene is often found in *E. coli* bacteria in association with several other genes responsible for the symptoms of human gastrointestinal illness (Nataro and Kaper, 1998). The *eae*A gene was detected in bacteria from the LDME. The *Bacteroidetes* bovine-specific genetic marker was also detected in DNA from the LDME. These results indicate that bacteria and genes often detected in cattle were present in the LDME. The presence of the most pathogenic form of *E. coli*, *E. coli* O157:H7 carrying the *stx*2 gene, is not indicated by these data.

Table 9. Results of analyses for microbiological indicators in subsurface drain water for NT8000 treatment before and after addition of liquid dairy manure effluent.

[LDME, liquid dairy manure effluent; PA, pre-application sample; ND, not detected; +, detected; IT, immunological test; DT, DNA-based test; NT, not tested – no *E. coli* were present]

	LDME	PA	Time elapsed since LDME application for NT8000 Treatment–Tile 6					
			4 hours	1 day	2 days	6 days	7 days	14 days
E. coli O157 - IT	+	ND	NT	NT	NT	NT	NT	NT
E. coli O157 - DT	+	ND	ND	ND	ND	ND	ND	ND
*eae*A gene	+	ND	+	+	+	+	NT	+
*stx*1 gene	+	ND	+	ND	ND	ND	ND	ND
*stx*2 gene	ND	ND	ND	ND	ND	ND	ND	ND
Bacteroidetes marker	+	ND	ND	ND	ND	ND	ND	ND

Microbiological analyses indicate a shift in the types of *E. coli* present in the NT8000 tile after LDME application (table 9). Subsurface drain water for the NT8000 treatment was analyzed before LDME application and at 4 hours, and 1, 2, 6, 7 and 14 days post-application (table 9). The *eae*A gene was detected in every NT8000 post-application sample in which *E. coli* were detected. The *eae*A gene was not detected in pre-application tile water for the NT8000 treatment, even though *E. coli* were present in pre-application tile water. In addition, the *stx*1 gene, often found in STEC *E. coli* from cattle and present in the LDME, was detected at 4 hours post-application in NT8000 tile water but not in NT8000 tile water before application. The NT8000 tile also contained greater concentrations of *E. coli* after LDME application, than before application (table 4). However, the bovine-*Bacteroidetes* marker was not detected in any NT8000 sample. The test for the *Bacteroidetes* marker is done on DNA isolated directly from the water sample and is less sensitive than the test for *E. coli* genes. The test for the *E. coli* genes is done on bacteria grown from the water sample. The growth process creates greater numbers of the bacteria carrying the genes, so the potential for detecting the genes is enhanced over the direct-DNA-extraction method. In addition, *Bacteroidetes* are believed to die off very quickly in the environment. These field-trial results indicate that large numbers of *Bacteroidetes* bacteria from LDME application were not transported to subsurface drain water for the NT8000 treatment, either within 4 hours post-application or over longer time periods.

To evaluate whether rainfall might influence LDME transport to subsurface drains, tile water from all treatments was analyzed for all indicators before LDME application, and at 6 days post-application, after the first rainfall event (table 10). Among the pre-application drain-water samples, only the *eae*A gene was detected, and only for one treatment (DD4000, table 10). However, at 6 days after application and the rainfall event, the *eae*A gene was detected in bacteria from subsurface drain water from 4 of the 8 treatment plots: DD8000, DD4000, AW8000, and NT8000. The *eae*A gene was absent in bacteria from subsurface drain water for each of the Control plots. These results might indicate transport of bacteria associated with the applied LDME to the subsurface drains after the rainfall event at 6 days post-application; however, no other indicators of LDME bacteria were detected. The bovine-*Bacteroidetes* marker was not detected in any sample. However, even though the *stx*2 gene was not detected in the LDME, it was detected in tile water from the AW8000 treatment at 6 days post-application, after the rainfall event. Taken together, these results do not clearly indicate whether the microbiological indicators of LDME contamination detected in drain water were from the current LDME application. As noted previously, *E. coli* may

persist from prior manure applications (Jamieson and others, 2002). The detection of the *eae*A gene in a pre-application tile-water sample and the detection of the *stx*2 gene in tile water when it was not detected in the LDME could indicate a persistent population of *E. coli* in soils of the treatment plots. Nevertheless, tile water did contain some microbiological indicators of bacteria from probable bovine sources. These indicators may prove useful in future studies, but much more information needs to be obtained regarding bacteria and gene persistence and transport through these soils.

Table 10. Results of analyses for microbiological indicators in LDME and all treatments before LDME application and at 6 days post-application.

[LDME, liquid dairy manure effluent; ND, not detected; +, detected; DT, DNA-based test]

	Treatment							
	DD8000	**DD4000**	**AW8000**	**AW4000**	**NT8000**	**NT4000**	**C1**	**C2**
	Before application							
E. coli O157 - DT	ND	ND	ND	ND	ND	ND	ND	ND
*eae*A gene	ND	+	ND	ND	ND	ND	ND	ND
*stx*1 gene	ND	ND	ND	ND	ND	ND	ND	ND
*stx*2 gene	ND	ND	ND	ND	ND	ND	ND	ND
Bacteroidetes marker	ND	ND	ND	ND	ND	ND	ND	ND
	6 days post-application							
E. coli O157 - DT	ND	ND	ND	ND	ND	ND	ND	ND
*eae*A gene	+	+	+	ND	+	ND	ND	ND
*stx*1 gene	ND	ND	ND	ND	ND	ND	ND	ND
*stx*2 gene	ND	ND	+	ND	ND	ND	ND	ND
Bacteroidetes marker	ND	ND	ND	ND	ND	ND	ND	ND

Study Limitations

This field trial was done only during the non-growing season; nutrient concentrations might be different during the agricultural growing season, which also would be the time when algal growth in receiving streams would be most readily influenced by soluble nutrients. In addition, flow rate or discharge of water from the subsurface drains was not measured. It is therefore impossible to estimate the potential load of nutrients or bacteria delivered to receiving waters. Loading rate for various nutrients may vary with tillage, season, and cropping strategy (Bakhsh and others, 2005; Schelde and others, 2006), and this type of treatment effect could not be determined by the field-trial design. This field trial did not involve replication of treatments, so caution in interpreting results is warranted. In addition, the first post-application samples were collected at about 4 hours, and this may have been too long to observe very rapid losses to tile drains, if these did indeed occur. Chemical and microbiological indicators of potential LDME transport to subsurface drains were analyzed only on selected samples. A more complete analysis of all samples, which was not economically practical for this field trial, might have clarified subtle patterns of occurrence in the selected sample set.

Future studies would benefit from treatment replication so that statistical significance of differences in concentrations of nutrients or bacteria could be evaluated. In addition, measurement of tile-drain discharge and enhanced sampling in the initial time period after LDME application would be beneficial. Future studies might benefit from the addition of dyes or inorganic tracers,

such as bromide, to the LDME before application to help separate the influence of residual chemicals or bacteria surviving from previous applications from the influence of the newly applied LDME. Finally, continuations of similar studies over multiple seasons and crop rotations would help to determine the importance of these variables.

Summary and Conclusions

A field trial was done in the Upper Tiffin River Watershed, in southeastern Michigan, to determine the influence of liquid dairy manure effluent (LDME) management practices on the quality of agricultural subsurface-drain water. Samples from subsurface drains were analyzed for nutrients, fecal-coliform and *Escherichia coli* (*E. coli*) bacteria, antibiotics, chemicals typically detected in wastewater, and the occurrence of genes indicating the presence of shiga-toxin-producing *E. coli*, or of bovine-specific *Bacteroidetes* bacteria. Samples were collected from November 2, 2006, to March 20, 2007, from subsurface drains under eight field plots that received no LDME and no tillage (two Control plots), or received 4,000 or 8,000 gallons per acre (gal/acre) of LDME after no tillage, or after either of two different types of tillage (six Treatment plots). The two types of tillage tested were (1) ground-driven, rotary, subsurface cultivation, and (2) rolling-tine aeration.

The purpose of the field trial was to obtain initial information on whether manure management practices can affect the transport of nutrients and bacteria from LDME to subsurface drains. Water samples were collected from the eight subsurface drains before LDME application and at 4 hours, and 1, 2, 6, 7, and 14 days post-application. Samples collected at 4 hours and 1 day post-application were evaluated for evidence of immediate transport of nutrients and bacteria by potential preferential flow pathways. Samples at 6 and 14 days post-application were timed to follow rainfall. In addition, the potential of wastewater chemicals and bacterial genes as indicators of LDME transport to subsurface drains was evaluated. The occurrence of antibiotics, wastewater chemicals, and bacterial genes was evaluated at 4 hours, and 1, 2, 6, 7, and 14 days post-application in subsurface-drain water from the plot receiving 8,000 gal/acre of LDME with no tillage (NT8000). In addition, all drains were analyzed for these constituents both before LDME application and at 6 days post-application, after the first significant rainfall event.

Nutrient concentrations were high in tile-drain water throughout the field-trial period. In all, 56 drain-water samples were analyzed. Of these, 53 samples exceeded the U.S. Environmental Protection Agency (USEPA) water-quality criteria for total phosphorus, and all samples exceeded the total nitrogen criterion for Ecoregion VI (Corn Belt). Nitrate + nitrite nitrogen concentrations exceeded 20 mg/L for every sample and contributed most to the total nitrogen concentrations. These results include eight samples collected before LDME application as well as two control samples for each date, representing drain water from plots that received no LDME and no tillage. Therefore, the high nutrient concentrations measured were not a result of the LDME application during the field trial and reflect antecedent conditions and prior management practices. The management practices tested during the field trial did not affect the nutrient concentrations in subsurface drain water during the field-trial period. The field trial was done during the non-growing season only. As shown by others (Bakhsh and others, 2005; Schelde and others, 2006) nutrient concentrations in subsurface drains may vary with season and crop.

Nutrient concentrations did not indicate an effect of LDME application on tile-water quality either immediately after application or after subsequent rainfall events. Significant rainfall did, however, influence nutrient concentrations in tile water. After approximately 0.84 in. of rainfall in 24 hours, organic + ammonia nitrogen, orthophosphorus, and total phosphorus concentrations

increased above average levels for all prior sampling dates in every treatment tile. In addition, after this rainfall event, ammonia nitrogen was present in every tile, including Controls, whereas it was not typically present in every tile on prior sampling dates. Finally, nitrate + nitrite nitrogen concentrations decreased in every tile, including Controls. Because similar changes occurred in tiles draining plots to which LDME was, or was not applied, nutrient patterns after rainfall cannot be attributed to the LDME application.

E. coli concentrations exceeded the USEPA recreational-water-quality single-sample criterion of 235 CFU/100 mL in only 3 of 56 samples. Of these three samples, two were from the NT8000 treatment at 4 hours and 1 day post-LDME application. The NT8000 treatment was the only treatment for which post-application *E. coli* concentrations increased to levels exceeding water-quality standards immediately after LDME application. The NT8000 treatment also was the only treatment for which ammonia was detected at 4 hours, 1 day, and 2 days post-application. The changes in subsurface-drain-water microbiology and nutrient chemistry immediately after the LDME application may indicate transport of LDME to the tile drain for this treatment. No other treatment indicated any immediate change in nutrient or bacteria concentrations after LDME application. The third sample for which *E. coli* concentrations exceeded the single-sample standard was the AW8000 treatment after the first significant rainfall event. There was no additional evidence of rainfall-mediated movement of *E. coli* bacteria to any tile drain.

Both chemical and microbiological indicators of possible LDME transport to subsurface drains were analyzed in the LDME, in a pre-application tile-water sample and in two categories of treatment tile-water samples. First, the occurrence of these indicators was evaluated over time after LDME application for the NT8000 treatment. Two bacterial genes (*eae*A and *stx*1) and two fecal sterol chemicals, found in the LDME, but not detected in the pre-application NT8000 tile-water sample or in a field blank, were detected in water samples from the 4-hour or 1-day post-application NT8000 treatment. These findings may indicate rapid transport of the LDME to tile water for this treatment, and they are consistent with a large increase in bacteria concentrations after LDME application for this treatment. Only the NT8000 treatment was analyzed for chemical or microbiological indicators immediately after LDME application; therefore, general patterns of chemical or microbiological indicator occurrence in the short term after LDME application cannot be evaluated. Nevertheless, results for the NT8000 treatment indicate some potential for the use of chemical or microbiological indicators to track preferential flow of LDME to subsurface drains. In addition, other chemicals not found in the LDME but possibly associated with farming practices (such as the herbicide metolachlor) were detected in some samples. These chemicals also indicate transport from surface application to subsurface drains, even though they may not specifically indicate LDME.

The influence of rainfall on the occurrence of these chemical and microbiological indicators in tile water also was evaluated. Tile water from all treatment plots and one control plot was evaluated pre-LDME application and at 6 days post-LDME application, after the first significant rainfall event. No chemical present in the LDME but not in the blank or pre-application samples was detected. However, after the rainfall event, the *eae*A gene, present in the LDME, was detected in tiles draining three plots to which LDME was applied but was not detected in these tiles before application. The *eae*A gene was not detected in tiles draining control plots.

Two chemicals and one microbiological indicator of LDME transport also were present in tile-water samples collected before the LDME was applied. For one treatment (DD4000), the *eae*A gene was present both pre-application and after the rainfall event. In addition, the *stx*2 gene, not present in the LDME, was detected in drain water from the AW8000 treatment after the rainfall

event. Chemicals may bind tightly to, or leach slowly from, soil. *E. coli* may persist from prior manure applications (Jamieson and others, 2002). This information may explain the presence of LDME chemicals or of *E. coli* genes in pre-application tile samples or the presence of the genes or chemicals in tile-water samples when they were not detected in current-application LDME. Although chemical and microbiological indicators of LDME transport to subsurface drains were somewhat equivocal with respect to the influence of LDME in tile-drain water quality, the detection of indicators in tile water confirms that they may be useful in future studies. However, much more needs to be known about the factors that influence their persistence and fate in soil and subsurface drainage.

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Appendix 1

Table 1–1. Wastewater-method compound names, U.S. Geological Survey National Water Quality Laboratory reporting limits, and possible compound uses or sources.

[RL, Laboratory reporting limit; reporting limits in micrograms per liter; PAH, polycyclic aromatic hydrocarbon; possible use or sources from Zaugg and others, 2001]

Analyte	Synonym	RL	Possible use or sources
1,4-Dichlorobenzene		0.5	Moth Repellant, fumigant, deodorant
1-Methylnaphthalene		.5	2-5 percent of gasoline, diesel fuel or crude oil, pesticide adjuvant
2,6-Dimethylnaphthalene		.5	Present in diesel/kerosene (trace in gasoline)
2-Methylnaphthalene		.5	2-5 percent of gasoline, diesel fuel or crude oil, pesticide adjuvant
3-*beta*-Coprostanol		2	Carnivore fecal indicator
3-Methyl-1(H)-indole	Skatole	1	Fragrance, stench in feces and coal tar
3-*tert*-Butyl-4-hydroxy anisole	BHA	5	Antioxidant, general preservative
4-Cumylphenol		1	Nonionic detergent metabolite
4-n-Octylphenol		1	Nonionic detergent metabolite
4-*tert*-Octylphenol		1	Nonionic detergent metabolite
5-Methyl-1H-benzotriazole		2	Antioxidant in antifreeze and deicers
Acetophenone		.5	Fragrance in detergent and tobacco, flavor in beverages
Acetyl hexamethyl tetrahydronaphthalene	AHTN	.5	Musk fragrance (widespread)
Anthracene		.5	Wood preservative, component of tar, diesel or crude oil
9,10-Anthraquinone		.5	Manufacturing of dye/textiles, seed treatment, bird repellant
Benzo[*a*]pyrene	Benz[a]pyrene	.5	Regulated PAH, used in cancer research
Benzophenone		.5	Fixative for perfumes and soaps
beta-Sitosterol		2	Plant sterol
beta-Stigmastanol	Stigmastanol	2	Plant sterol
Bisphenol A		1	Manufacturing of polycarbonate resins, antioxidant, flame retardant
Bromacil		.5	Herbicide, noncrop usage
Tribromomethane	Bromoform	.5	Wastewater ozonation byproduct, military/explosives
Caffeine		.5	Beverages, diuretic
Camphor		.5	Flavor, odorant, ointments
Carbaryl		1	Insecticide, crop and garden use
Carbazole		.5	Manufacturing of dyes, explosives and lubricants
Chlorpyrifos		.5	Insecticide, termite and pest control
Cholesterol		2	Fecal indicator, plant sterol
Cotinine		1	Primary nicotine metabolite

Table 1–1. Wastewater-method compound names, U.S. Geological Survey National Water Quality Laboratory reporting limits, and possible compound uses or source.—Continued

[RL, Laboratory reporting limit; reporting limits in micrograms per liter; PAH, polycyclic aromatic hydrocarbon; possible use or sources from Zaugg and others, 2001]

Analyte	Synonym	RL	Possible Use or Sources
Diazinon		0.5	Insecticide, ants and flies
Dichlorvos		1	Insecticide, pet collars
d-Limonene		.5	Fungicide, antimicrobial, antiviral, fragrance
Fluoranthene		.5	Component of coal tar and asphalt
Hexahydrohexamethyl-cyclopentabenzopyran	HHCB	.5	Musk fragrance
Indole		.5	Pesticide inert ingredient, fragrance in coffee
Isoborneol		.5	Fragrance in perfume
Isophorone		.5	Solvent for lacquer, plastic, oil, silicone and resin and some pesticides
Isopropylbenzene	Cumene	.5	Manufacturing of phenol/acetone, fuels and paint thinner
Isoquinoline		.5	Flavors and fragrances
Menthol		.5	Cigarettes, cough drops, liniment, mouthwash
Metalaxyl		.5	Herbicide, fungicide, general use pesticide
Methyl salicylate		.5	Liniment, food, beverage, sun block
4-Nonylphenol, total	*para*-Nonylphenol	5	Nonionic detergent metabolite, pesticide adjuvant
Metolachlor		.5	Herbicide
N,*N*-Diethyl-*meta*-toluamide	DEET	.5	Insect repellant, urban use on mosquitoes
Naphthalene		.5	Fumigant, moth repellent, component of gasoline
Nonylphenol, diethoxy- (total)	NPEO2	5	Nonionic detergent, pesticide adjuvant
Nonylphenol, monoethoxy- (total)	NPEO1	5	Nonionic detergent, pesticide adjuvant
Octylphenol, diethoxy-	OPEO2	1	Nonionic detergent
Octylphenol, monoethoxy-	OPEO1	1	Nonionic detergent metabolite
para-Cresol		1	Wood preservative
Pentachlorophenol		2	Herbicide, fungicide, wood preservative, termite control
Phenanthrene		.5	Manufacturing of explosives, component of tar, diesel and crude oil
Phenol		.5	Disinfectant, used in the manufacturing of many products
Prometon		.5	Herbicide, noncrop, before blacktop
Pyrene		.5	Component of coal tar and asphalt
Tetrachloroethylene	PCE	.5	Solvent, degreaser, veterinary anthelmintic
Tri(2-butoxyethyl)phosphate		.5	Flame retardant
Tri(2-chloroethyl)phosphate		.5	Plasticizer, flame retardant
Tri(dichlorisopropyl)phosphate		.5	Flame retardant
Tributyl phosphate		.5	Antifoaming agent, flame retardant
Triclosan		1	Disinfectant, antimicrobial
Triethyl citrate	Ethyl citrate	.5	Cosmetics and pharmaceuticals
Triphenyl phosphate		.5	Plasticizer, resin wax, roofing paper

Table 1–2. Antibiotic analytes by liquid chromatography/mass spectrometry.

[CAS, Chemical Abstract Service; RL, laboratory reporting level in micrograms per liter]

Compound name	CAS number	RL
Pharmaceuticals		
Carbamazepine	61336-70-7	0.005
Ibuprofen	69-53-4	.005
Macrolides		
Anhydro-erythromycin	--	.008
Erythromycin	114-07-8	.008
Roxithromycin	80214-83-1	.005
Tylosin	1401-69-0	.005
Virginiamycin	11006-76-1	.005
Quinolones		
Ciprofloxacin	85721-33-1	.005
Enrofloxacin	--	.005
Lomefloxacin	98079-51-7	.005
Norfloxacin	70458-96-7	.005
Ofloxacin	83380-47-6	.005
Sarafloxacin	98105-99-8	.005
Sulfonamides		
Sulfachlorpyridazine	80-32-0	.005
Sulfadiazine	68-35-9	.050
Sulfadimethoxine	122-11-2	.005
Sulfamethazine	57-68-1	.005
Sulfamethoxazole	723-46-6	.005
Sulfathiazole	72-14-0	.020
Tetracyclines		
Chlorotetracycline	57-62-5	.010
Anhydrochlorotetracycline	4497-08-9	.010
Doxycycline	564-25-0	.010
Oxytetracycline	79-57-2	.010
Tetracycline	64-75-5	.010
Anhydro-tetracycline	13803-65-1	.010
Other		
Lincomycin	154-21-2	.005
Ormetoprim	6981-18-6	.005
Trimethoprim	738-70-5	.005