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Research article

Effects of tillage and poultry manure application rates on *Salmonella* and fecal indicator bacteria concentrations in tiles draining Des Moines Lobe soils

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ABSTRACT

Application of poultry manure (PM) to cropland as fertilizer is a common practice in artificially drained regions of the Upper Midwest United States. Tile-waters have the potential to contribute pathogenic bacteria to downstream waters. This 3-year study (2010–2012) was designed to evaluate the impacts of manure management and tillage practices on bacteria losses to drainage tiles under a wide range of field conditions. PM was applied annually in spring, prior to planting corn, at application rates ranging from 5 to 40 kg/ha to achieve target rates of 112 and 224 kg/ha nitrogen (PM1 and PM2). Control plots received no manure (PM0). Each treatment was replicated on three chisel-plowed (CP) plots and one no-till (NT) plot. Tile-water grab samples were collected weekly when tiles were flowing beginning 30 days before manure application to 100 days post application, and additional grab samples were obtained to target the full spectrum of flow conditions. Manure and tile-water samples were analyzed for the pathogen, *Salmonella* spp. (SALM), and fecal indicator bacteria (FIB), *Escherichia coli* (EC), and enterococci (ENT). All three bacterial genera were detected more frequently, and at significantly higher concentrations, in tile-waters draining NT plots compared to CP plots. Transport of bacteria to NT tiles was most likely facilitated by macropores, which were significantly more numerous above tiles in NT plots in 2012 as determined by smoke-testing. While post-manure samples contained higher concentrations of bacteria than pre-manure samples, significant differences were not seen between low (PM1) and high (PM2) rates of PM application. The highest concentrations were observed under the NT PM2 plot in 2010 (6.6×10^3 cfu/100 mL EC, 6.6×10^5 cfu/100 mL ENT, and 2.8×10^3 cfu/100 mL SALM). Individual and 30-day geometric mean ENT concentrations correlated more strongly to SALM than EC; however, SALM were present in samples with little or no FIB.

1. Introduction

Poultry manure (PM) is an excellent source of phosphorous, nitrogen, potassium, and other nutrients essential for plant growth, and is therefore is applied to cropland in the United States (US), Canada, the European Union, and China, in lieu of, or in addition to, commercial fertilizer (Oenema et al., 2007; Sistani et al., 2010). In the US state of Iowa, alone, over 15 billion eggs are produced annually (UDSA-NASS, 2014), resulting in the generation of over 5.6 million Mg of fresh layer manure (Naber and Bermudez, 1990). Along with beneficial nutrients, PM commonly contains pathogenic bacteria, including *Salmonella* (SALM) (Kraft et al., 1969; Rodriguez et al., 2006; Berghaus et al., 2013). Once released to the environment, pathogens can be transported to recreational, irrigation, or drinking waters and pose a risk to human health (Rogers and Haines, 2005; Craun et al., 2010; Dale et al., 2010; USEPA, 2013), or compromise the bio-security of poultry facilities (CDC, 2010; Castiglioni Tessari et al., 2012).

In addition to pathogens, PM commonly contains non-pathogenic bacteria, including *Escherichia coli* (EC) and

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enterococci (ENT) (Terzich et al., 2000; Rogers et al., 2011). Studies of human exposures have confirmed the increased risk of negative health outcomes from swimming in waters containing these and other fecal indicator bacteria (FIB) (Pruss, 1998). Alternative indicators, such as wastewater chemicals and genes unique to pathogenic bacteria, have been tested (Haack et al., 2009); however, despite the potential for regrowth and poor correlations to pathogens in some environmental settings (Field and Samadpour, 2007; Payment and Locas, 2011), EC and ENT have remained the preferred indicators for assessing the risk to human health (USEPA, 2012).

Watershed-scale models commonly used to evaluate microbial fate and transport often underestimate microbial contributions via drainage tiles, or assume that all leached bacteria die-off (Gassman et al., 2007). To improve these models, source-specific data sets that document the variability of bacterial concentrations in tiles under common agricultural practices are needed, along with research relating pathogens to the more readily-modeled FIB (Benham et al., 2006).

Most PM generated in Iowa confinements is stockpiled, and solid manure is broadcast on cropland as fertilizer. Transport of bacteria from applied PM can occur via runoff or through the subsurface. In regions with poor natural drainage, including formerly glaciated landscapes, tile-drainage systems are commonly installed to remove excess water and facilitate plant growth. These systems move water quickly to surface waters, decreasing the soil's natural capacity for filtration. Bacterial transport in runoff from application of various types of manures, including poultry, has been widely studied (Soupir et al., 2006; Jenkins et al., 2008; Brooks et al., 2009; Harmel, 2009; Guzman et al., 2010; Sistani et al., 2010; Delgado et al., 2011). Laboratory-scale studies have shown that SALM and other pathogens can be transported through over 1 m of soil, and transport is controlled by soil types, hydrodynamic forces, physical filtration, and interactions between bacterial surface-charges and air, water, and soil interfaces (Haznedaroglu et al., 2009; Bech et al., 2010; Chen, 2012). Studies show increased bacterial transport under saturated conditions; however, transport has also been shown to occur under unsaturated conditions when preferential flow paths, such as vermicular macropores, are present (Beven and Germann, 1982; Abu-Ashour et al., 1998; McMurry et al., 1998; Bottinelli et al., 2013).

Timing of manure application relative to precipitation has been shown to play an important role in determining transport to drainage tiles (Samarajeewa et al., 2012). Recent studies of PM-amended soils have reported survival of FIB and pathogens for weeks and months after application (Rogers et al., 2011; Cook et al., 2014), highlighting the potential for continued release of bacteria to subsurface waters.

Despite the documented potential for bacteria transport to tile-waters, no previous studies have addressed the impacts of PM on microbial tile-water quality. Here, we present analyses of both SALM and FIB concentrations in drainage tiles waters under realistic field conditions, following PM application. The objectives of this study were to evaluate the effects of time relative to manure application, PM application rates, and tillage practices (CP and NT) on bacteria concentrations in tile waters. This 3-year study was conducted under a wide range of moisture conditions, and flow regimes, allowing for characterization of bacteria losses to tiles under exceptionally wet and dry conditions up to 100 days after manure application. Our findings can be used to improve predictive models and risk assessments, and to inform producers and watershed managers interested in minimizing the microbial impacts of PM application.

2. Materials and methods

2.1. Study site

Field experiments were conducted from 2010 through 2012 at Iowa State University's Agronomy and Agricultural Engineering Research Farm, west of Ames, Iowa, United States. The site is located in the Des Moines Lobe landform region, a landscape formed by the last glacial maximum that occurred in the state during the late Pleistocene Epoch, between 18,000 and 15,000 years ago. The research plots are located on soils with a Canisteo-Clarion–Nicollet association, which are loamy soils formed in glacial till under prairie vegetation, characterized as moderately permeable, with drainage classifications ranging from well-drained to poorly drained. Soil texture typically ranges from 30 to 45% sand, 35–42% silt, and 20–30% clay content (NRCS, 2014). Topsoil (0–30 cm) measurements for all plots (2010–2012) range from 2.0 to 4.4% organic matter content. Plot slopes range from 0 to 5 percent. Drainage tiles are installed along the midline of each plot at a depth of approximately 1.2 m. At the edge of each plot, tiles outlet into sumps, which are protected from the elements and accessible for sampling.

A long-term study of the effects of PM on water quality began at this site in 1998, with nine chisel-plowed (CP) plots under a corn-soybean rotation. For 12 years, PM was applied in the spring to the portion of each plot that was to be planted in corn as (Nguyen et al., 2013). In 2010, all CP plots were converted to a continuous corn rotation, and 3 established NT plots were included as part of the PM study. PM and urea ammonium nitrate (UAN) were applied each spring, prior to planting, at application rates based on nitrogen (N) goals as described in Table 1. Control plots have not received manure since the initiation of the long-term study in 1998. The PM2 goal achieves the maximum recommended rate of N application for continuous corn production in Iowa (Sawyer et al., 2006).

Fig. 1 shows the layout of the study plots and the location of the study site on the Des Moines Lobe. Plot areas range from 0.08 to 0.51 ha (ha). Tiles are spaced 36.3 m (m) apart. The experimental treatments on CP plots were arranged in a randomized design with 3 plots receiving PM1 treatment, 3 plots receiving PM2 treatment, and 3 plots receiving no manure as controls (PM0). Two of the control plots were fertilized with urea ammonium nitrate (PM0 – UAN), and one that received no fertilizer (PM0 – NONE). Individual NT plots were fertilized with PM1, PM2, and PM0 – UAN treatments. The NT PM1 and PM2 plots are split, so that separate tiles capture the upper and lower portions of the plots, allowing for replication of tile-water samples. No manure was applied to either type of PM0 plot; thus, we will not distinguish between PM0 treatments for the remainder of this report. The six possible combinations of tillage and treatment are as follows: CP PM0, CP PM1, CP PM2, NT PM0, NT PM1, and NT PM2.

2.2. Precipitation and tile flow measurements

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