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Science of the Total Environment 358 (2006) 164–177

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Fecal bacteria and sex hormones in soil and runoff from cropped watersheds amended with poultry litter

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Received 10 December 2004; accepted 1 April 2005
Available online 31 May 2005

Abstract

The application of poultry litter to agricultural fields can provide plant nutrients for crops and forage production, but fecal bacteria and the sex hormones estradiol and testosterone are components of litter that can be detrimental to the environment. Our objective was to determine if applications of poultry litter to small watersheds would contribute to the load of fecal bacteria and sex hormones to soil and runoff. We, therefore, investigated the fate and transport of fecal bacteria, estradiol and testosterone from surface applied poultry litter to four small cropped watersheds. Poultry litter was applied to meet the nitrogen requirements of pearl millet (*Pennisetum glaucum* L.) in 2000 and grain sorghum [*Sorgham bicolor* (L.) Moench] in 2001. Neither *Salmonella* nor *Campylobacter* were detected in the litter but the fecal indicator bacteria were. The average load of total coliforms, *Escherichia coli*, and fecal enterococci applied with the litter was 12.2, 11.9, and 12.7 $\log_{10}$ cells ha^{-1}, respectively. The average load of estradiol and testosterone was 3.1 and 0.09 mg ha^{-1}, respectively. Runoff events first occurred 7 months after the first litter application in 2000, and 3 weeks after the second application in 2001. Only for the 25 July 2001 runoff event 3 weeks after the second litter application were the concentrations of total coliforms, *E. coli*, and fecal enterococci in runoff greater than background concentrations which were on average 5.2, 2.9, and 1.1 $\log_{10}$ MPN 100 ml^{-1}, respectively. Average background levels of total coliforms, fecal enterococci, and *E. coli* in surface soil were 8.2, 7.9, and 3.5 $\log_{10}$ cells kg^{-1} soil. At the rate of litter application the concentrations of estradiol and testosterone in the litter did not appear to impact the background levels in the soil and runoff. Because concentrations of sex hormones in litter from other broiler operations are known to be greater than in the litter we applied, further study on the connection between concentrations of sex hormones in poultry litter and operational practices is recommended.
Published by Elsevier B.V.

Keywords: Poultry litter; Fecal indicator bacteria; Estradiol; Testosterone; Soil; Runoff

1. Introduction

In 2002, over 8.7 billion chickens were produced in the U.S. (Georgia Agricultural Statistics Service,

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0048-9697/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.scitotenv.2005.04.015

2004). A byproduct of this multi-billion dollar industry is poultry litter, a mixture of feces, bedding material, and feathers. Considering that one chicken produces approximately 1.5 kg of litter (Perkins et al., 1964), 13 million metric tons of litter was produced in 2002. Most poultry litter is applied as a fertilizer to agricultural lands as it contains the plant nutrients N, P, and K (Moore et al., 1995). Since a significant component of poultry litter is fecal material from the birds, it can contain pathogenic bacteria such as *Salmonella*, *Campylobacter* (Kelley et al., 1994; Jeffrey et al., 1998), and fecal indicator bacteria: total coliforms, *Escherichia coli* and fecal enterococci. In addition poultry litter contains appreciable concentrations of the sex hormones 17β-estradiol and testosterone. As the application of poultry litter increases across the country, the risk of contaminating surface waters with fecal bacteria and sex hormones could increase.

A few researchers have reported on the presence of fecal bacteria from poultry litter in runoff from field soils (Giddens and Barnett, 1980; Coyne and Blevins, 1995; Edwards and Daniels, 1994). Giddens and Barnett (1980) performed rain simulation experiments on fallow soil and coastal bermuda grass on which various rates of fresh poultry litter (consisting of manure and wood shavings) were applied, and total coliforms in runoff were enumerated. As expected, their results indicated that concentrations of total coliforms in runoff increased with increasing tonnage of applied litter. In another study of runoff from field plots amended with broiler litter, Edwards and Daniels (1994) measured fecal coliforms and observed elevated concentrations. Elevated concentrations of fecal coliforms were reported in runoff in similar studies (Coyne and Blevins, 1995). Because *E. coli* and fecal Enterococci are the preferred fecal indicator bacteria (EPA, http://www.epa.gov/OWOW/tmdl/pathogens_all.pdf), it can be inferred that runoff from agricultural fields to which boiler litter had been applied could have unacceptable concentrations of these fecal indicator bacteria, and could threaten to impair recreational and drinking waters.

Concentrations of estrogens have been observed to range from 14 to 65 μg kg^{-1} dry weight of litter, and concentrations of testosterone have been observed to be as high as 133 μg kg^{-1} dry weight of litter (Shore et al., 1993). These naturally occurring sex hormones have been detected in surface waters across the U.S. and Europe (Koplin et al., 2002; Adler et al., 2001; Kuch and Ballschmiter, 2001), and have raised concern by the general public because of their potential adverse ecological and public health effects. Tyler and Routledge (1998) demonstrated the adverse effects of estrogens from sewage treatment plants on wild fish. They reported that estradiol concentrations between 10 and 100 ng l^{-1} can affect the development of trout. Several researchers have reported that environmental estrogens have been linked to decreased sperm counts, testicular, prostate and breast cancer, and male reproductive disorders (Harrison et al., 1997; Epstein, 1997; Toppari et al., 1996; Sharpe and Skakkebaek, 1993). The presence of these hormones in surface waters can adversely affect both aquatic life and human health.

A few studies have reported on the transport and fate of estradiol and testosterone in soil and runoff from fields on which poultry litter was applied. Shore et al. (1995) reported estrogen (presumably estradiol and estrone) concentrations ranging from 4 to 23 ng l^{-1}, and testosterone concentrations ranging from 1 to 34 ng l^{-1} of pond water receiving runoff from fields to which poultry litter had been applied. Nichols et al. (1997) demonstrated that runoff concentrations of estradiol increased with increased application of poultry litter. The highest runoff concentration was 1280 ng l^{-1} and corresponded to a litter application of 7.05 Mg ha^{-1}. In another study, Nichols et al. (1998) looked at the effects of grass buffer strips on runoff concentrations of estradiol from litter applications, and demonstrated significant decreases in estradiol concentrations with increased widths of the buffer strips. Finlay-Moore et al. (2000) measured the edge-of-field losses of estradiol and testosterone in runoff from fescue pastures to which broiler litter was applied. Concentrations ranged from 20 to 2330 ng l^{-1} and 10 to 1830 ng l^{-1} for estradiol and testosterone, respectively. They also measured concentrations of estradiol and testosterone in field soil after litter application; the highest concentrations of estradiol and testosterone reached 675 ng kg^{-1} and 165 ng kg^{-1}, respectively.

None of the studies mentioned above investigated the effects of poultry litter application on the concentrations of *Salmonella*, *Campylobacter*, *E. coli*, fecal enterococci, estradiol, and testosterone in runoff from cropped watersheds. The practice of conservation tillage or no-till agriculture has been widely adopted in the

many parts of the United States because it reduces soil erosion and increases water retention (Langdale et al., 1979; Reicosky et al., 1977). Under conservation tillage surface applications of poultry litter are not incorporated into the soil by mechanical means. It is a management practice that may enhance the transport of fecal bacteria and sex hormones associated with the litter in runoff from rain events. Our objective, therefore, was to conduct a study to determine if applications of poultry litter to four small cropped watersheds would contribute to the load of fecal bacteria and sex hormones to soil and runoff from rain events.

2. Material and methods

2.1. Experimental sites and their instrumentation

The research was conducted on four small cropped watersheds, designated P1, P2, P3, and P4, in Watkinsville, GA; the dominant soil in all four watersheds was a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludult) (Smith et al., 1978). The hectarage of each watershed was 2.70, 1.29, 1.26, and 1.40 for P1, P2, P3, and P4, respectively. Before this study, all four watersheds were under continued no-till management for at least 10 years. Summer crops were pearl millet (*Pennisetum glaucum* L.) in 2000 and grain sorghum [*Sorghum bicolor* (L.) Moench] in 2001. Crops were fertilized with broiler litter in July 2000 and 2001 (Table 1). The goal was to apply equal amounts of total N at each watershed. Poultry litter was applied immediately after planting and before seed germination. The standing stubble from the previous crop was 10 to 15 cm tall. For the hormone study, another instrumented watershed, designated W1, was used as a comparison to the watersheds that received poultry litter. It was a pastured watershed 7.8 ha in area, and all runoff was measured and subsamples collected. This watershed received no poultry litter, and was grazed by various classes of cattle. The following is the grazing schedule for watershed W1 for years 2000 and 2001: in 2000, 109 cow/calf pairs for 9 days in March, 38 cow/calf pairs for 15 days in May, 37 cow/calf pairs for 5 days in June, 85 cow/calf pairs for 7 days in July, 109 cows for 3 days in September, 109 cows for 5 days in October, and 40 cows for 8 days, and 70 cows for 8 days in December; in 2001, 61 cow/calf pairs for 7 days in March, 38 cow/calf pairs for 26 days in April, 59 cow/calf pairs for 5 days in June, 39 cow/calf pairs for 27 days in July, and 84 cows for 26 days in November.

Rainfall and runoff were measured with an automated system consisting of a Texas Electronics Inc. TR525M tipping bucket rain gauge, a flume fabricated to a 0.762 m (2.5 ft) H-flume of USDA specification (Brakensiek et al., 1979), a 17.24 kPa (2.5 psi) flow depth sensing Druck Incorporated (New Fairfield, CT) transducer located in the stilling well of the flume, and a Campbell Scientific Inc. (Logan, UT) CR10X data logger. Flumes are located at the lowest (outlet) part of each watershed. The rain gauge and transducer are integrated with the data logger such that 5-min cumulative rainfall and runoff amounts are saved in the data logger memory for downloading to a computer through a phone line or manually on to a storage module. Each data logger was programmed to convert the transducer readings into runoff rates using the standard flume

Table 1
Rates of broiler litter (dry weight basis), total N, total P, total K, total coliforms (TC), *E. coli* (EC), fecal enterococci (FE), estradiol and testosterone applied to the four P-watersheds

Date	Watershed	Litter (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	TC ($\log_{10}$ MPN ha^{-1})	EC ($\log_{10}$ MPN ha^{-1})	FE ($\log_{10}$ MPN ha^{-1})	Estradiol (mg ha^{-1})	Testosterone (mg ha^{-1})
5 July 2000	P1	2210	72.9	30.1	47.7	12.6	12.4	13.7	2.9	0.06
	P2	2530	83.5	34.4	54.6	12.7	12.5	13.7	3.3	0.07
	P3	2650	87.4	36.0	57.4	12.7	12.5	13.7	3.4	0.07
	P4	2170	71.6	29.5	46.9	12.6	12.4	13.7	2.8	0.06
2 July 2001	P1	2080	66.8	27.9	50.5	11.7	11.4	11.7	2.8	0.09
	P2	2420	77.7	32.4	58.8	11.8	11.5	11.8	3.2	0.10
	P3	2830	90.8	32.4	58.8	11.8	11.6	11.8	3.8	0.13
	P4	2270	72.9	30.4	55.2	11.7	11.5	11.7	3.0	0.10

calibration curve. When the water depth in the stilling well is at or below the flume base, it means there is no runoff. A refrigerated American Sigma (Denver, CO) 900 Max sampler housed in a shelter just below each flume was used to collect and store runoff samples until removed for processing and analysis. Samples were collected in 24 polyethylene bottles each of 1-l size arranged in a circle. The bottles were thoroughly cleansed with acidified and deionized water then sterilized before being placed in position. The sampler features a positive displacement and programmable peristaltic pump head that allows for time or flow-weighted sampling, as well as purging of the intake line before and after event sampling. Once the data logger senses runoff, it sends a signal to the sampler to activate itself per programmed sampling settings after a fixed time interval of every 10 min. The sampler keeps samples at 4 °C until collected, usually within 24 to 36 h after a runoff event. The runoff collected in each 1-l bottle was made up of two 500 ml samples taken every 10 min during a runoff event. This produced a total of three 1-l bottle samples every hour. Depending on the duration of the runoff event (usually from 1 to 5 or more hours) we obtained from 3 to 15 or more 1-l samples. Immediately after a runoff event, 10 ml subsamples were removed from each of these bottles, composited, and analyzed to obtain an average concentration for that event. Mass was determined using this average concentration and the total runoff volume for the event. This was performed for each watershed. Constraints on resources did not allow for analysis of individual samples. Only for the 25 July 2001 event was a more detailed analysis undertaken by determining the concentration of subsamples from composited bottles representing hourly runoff, determining the hourly runoff volume, and developing a temporal distribution of concentration and mass through a runoff event from which a mean concentration was estimated for the whole event.

2.2. Poultry litter

The source of litter was a local broiler producer. Immediately after the chickens were removed from the broiler house, the litter was loaded onto the truck that was to spread the litter over the watersheds. At each watershed the truck was weighed before and after spreading the litter. At each watershed and just before spreading the litter, at least five random grab samples of litter were taken from the litter in the spreader and placed in sterile whirl-pack containers for microbiological and hormone analysis. Grab samples were also taken for moisture and nutrient analysis which were performed by the Soil Testing Laboratory at the University of Georgia. The Litter samples were placed on ice while in transit to the laboratory for analysis. Litter samples were analyzed within 24 h of collection for total coliforms *E. coli*, and fecal enterococci with IDEXX Colilert and Enterolert kits, respectively. The samples were also analyzed for *Campylobacter* and *Salmonella* as described below. Subsamples to be analyzed for hormones were immediately put in storage at −80 °C.

2.3. Soil sampling

Soil sampling was done with a flame sterilized steel auger (2 cm diameter). A composite of five soil cores to a depth of 5 cm were taken at random at each of four sub-areas of each watershed. Each set of composite soil samples was placed in sterile zip-lock bags and stored on ice at 4 °C until processed for analysis. The five soil cores per sub-area were thoroughly mixed. Subsamples of fresh soil were set aside for the MPN determination of total coliforms, *E. coli*, and fecal enterococci. To express bacterial data on a dry weight basis, soil gravimetric water content determinations were made following standard protocol. The remainder of the soil was air-dried, passed through a 2-mm sieve and stored at −80 °C for hormone analysis. Soil samples were taken before and after litter application. Soil moisture was also routinely measured by the time domain reflectometry (TDR) technique with a Type A ESI MoisturePoint probe that was interfaced with an MP-917 cable tester (ESI Environmental Sensors, Inc, Victoria, BC, Canada). Four of these Type A moisture point probes were installed near the center of the four sub-areas of the four watersheds.

2.4. Analysis of manure bacteria

Initial determinations of *E. coli* in soil were made as follows. Ten grams of fresh soil were suspended in 90 ml (first 10^{-1} dilution) of EC broth (Difco), shaken on a reciprocal shaker for 20 to 30 min. After the larger soil particles settled, 1 ml of the suspension was

pipetted to 9 ml of EC broth. Each tube of EC broth contained a durham tube. Five replicate tubes per dilution, 10^{-2} to 10^{-6} were prepared. The inoculated tubes of EC broth were incubated at 44.5 °C for 24 h. Positive tubes displayed growth and gas production. A loop (10 μl) of each positive tube was used to inoculate a tube of 5 to 10 ml of EC MUG broth containing a durham tube. Inoculated tubes were incubated at 44.5 °C in a water bath for 24 h. Positive tubes fluoresced blue and displayed gas production. At the time of the first litter application, total coliforms, *E. coli* and fecal enterococci in litter, soil, and runoff samples were each assayed for with IDEXX Colilert and Enterolert commercial kits as described below.

Analysis for *Campylobacter* and *Salmonella* were undertaken as follows. The litter samples were decaked and homogenized inside the Whirl-Pak bags by hand. Ten grams of the litter was placed in 50 ml of sterile Bolton broth with antibiotics and 50 ml of buffered peptone water (BPW), both in duplicate. The Bolton slurry was incubated microaerobically at 42 °C for 24 h for the enrichment of *Campylobacter* and the BPW slurry was incubated aerobically at 37 °C for 24 h for pre-enrichment of *Salmonella*. After incubation the slurry of *Campylobacter* was streaked out on Campy Line Agar (CLA), and the plates were incubated microaerobically at 42 °C for 48 h. Following the pre-enrichment for *Salmonella*, 1 ml of the slurry was transferred to 9 ml of Tetrathionate enrichment broth (TT broth) and incubated aerobically for 24 h at 37 °C. After the TT broth incubation, 1 ml of the TT broth was transferred to 9 ml of Rappaport Vassiliadis enrichment broth (RV broth) and incubated aerobically for 24 h at 37 °C. After the incubation in RV broth, it was streaked on Brilliant Green Sulfa (BGS) agar and also on Modified Lysine Iron Agar (MLIA) plates.

Total coliforms, *E. coli*, and fecal enterococci in runoff, soil, and litter samples were measured with commercial Colilert and Enterolert kits (IDEXX, Atlanta, GA). These kits represent a defined substrate technology (Edberg and Edberg, 1988; Edberg et al., 1988, 1990). Both Colilert and Enterolert are semi-automated most probable number (MPN) methodologies. For both litter and soil, 10 g fresh weight were suspended in sterile 90 ml of phosphate buffered saline (PBS) (Clesceri et al., 1998) and shaken 100× by hand. The suspensions were allowed to settle for 5 to 10 min after which additional 10-fold dilutions were prepared. Ten milliliters of appropriate dilutions were added to 90 ml of Colilert and Enterolert substrate. Ten milliliters of runoff or 10 ml of a serial dilution of runoff was added to 90 ml of substrate. The inoculated substrate was then poured into a Quanti-Tray, sealed, and incubated for 24 h at 35.5 °C for Colilert and 24 h at 41 °C for Enterolert. At the end of the incubation the plates were read according to the manufacturer and a most probable number per 100 ml runoff and per g soil and litter was derived.

2.5. Analysis of estradiol and testosterone

Estradiol and testosterone concentrations in unfiltered runoff, soil and litter were measured with a commercial competitive enzyme-linked immunosorbent assay (Caymen Chemical Company, Ann Arbor, MI). Soil, litter, and runoff samples were stored at either −20 or −80 °C before their analysis. The assay procedure for runoff, soil and litter samples has been described in detail by Finlay-Moore et al. (2000). Each sample was analyzed in duplicate.

2.6. Data analysis

Soil and runoff samples analyzed by Colilert and Enterolert methodology that resulted in no cells detected were considered to have a concentration of at most 0.5 cells g^{-1} soil or 0.5 cells 100 ml^{-1} runoff. For performing statistical analysis on the MPN determinations for total coliforms, *E. coli*, and fecal enterococci from soil samples, the original data were transformed into natural log numbers before performing analysis with procedures proc means and proc mixed of SAS (version 8.2). After analysis the data were transformed into $\log_{10}$. To compare soil communities of total coliforms and fecal enterococci and populations of *E. coli* the four watersheds were treated as replicate sites. Hormone data from runoff events were analyzed with procedure proc mixed of SAS (version 8.2).

3. Results and discussion

3.1. Rain events, runoff, and soil moisture

The period in which this research was conducted coincided with an extensive drought that lasted from

mid-May 1998 to mid-November 2001. Before the first application of poultry litter on 5 July 2000 runoff was limited to one rain event on 1 February 1999 and one on 10 January 2000. Although precipitation occurred during June through December 2000 (Fig. 1) and was reflected in the rise and fall of soil moisture at the watersheds (Fig. 2), runoff events were not recorded until a series of storms beginning 23 February 2001 (Table 2) followed by four successive storms in March 2001. The amount of runoff was limited during the first three of these five rain events as the soil profile was generally dry. But 86 mm of precipitation split equally in a 2-day period brought soils to saturation and a large runoff event on 15 March 2001. In addition, the 15 March 2001 storm lasted 7 h with an average intensity of 4.7 mm h^{-1} for the first half and 8 mm h^{-1} for the second half. Runoff started 5 h into the storm and lasted for 6 h, and peaked at 13.2 l s^{-1}. Despite another 45 mm storm 5 days later, the resulting runoff was much reduced compared to the previous event. This 20 March 2001 storm lasted 10 h with an average intensity of 3 mm h^{-1} for the first 8 h and then a sustained 8 mm h^{-1} for 1.5 h before terminating. Runoff from this last storm started 9 h after initial precipitation and in response to the 8 mm h^{-1} intensity. It lasted 3 h and peaked at only 0.9 l s^{-1}. These watersheds have been managed under no-tillage since the mid-1970s and show high infiltration rates compared to periods when they were managed under conventional tillage of disking and harrowing (Endale et al., 2000).

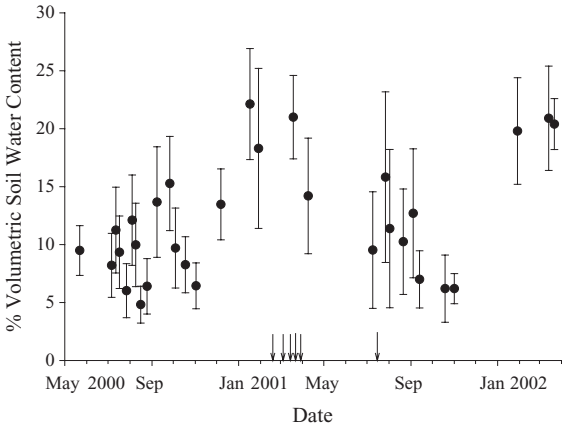

Fig. 2. Mean % volumetric soil water content (± S.D.) for depth segment 0 to 15 cm for watersheds P1, P3, and P4 read periodically from May 2000 to June 2002. Arrows along date axis indicate runoff events.

3.2. Fecal indicator bacteria

Analysis of the poultry litter for *Campylobacter* and *Salmonella* yielded negative results. These manure pathogens were not detected in the two loads of litter applied to the watersheds during this study.

E. coli were not detected in the surface 5 cm of soil collected on 31 May 2000, 5 days before the first application of poultry litter (Table 3). Soil samples analyzed 20 days after litter application yielded substantial concentrations of total coliforms, and fecal enterococci, but populations of *E. coli* were 10^5 times less than total coliforms and fecal

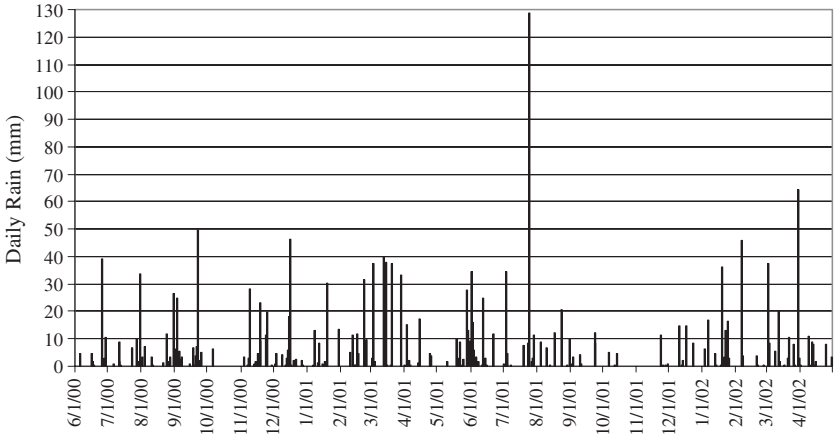

Fig. 1. Daily precipitation (mm) for Watkinsville, GA from June 2000 to April 2002.

Table 2
Mean (± S.D.) rainfall for the four P-watersheds and corresponding total runoff at each of the four watersheds after poultry litter application

Date	Rainfall (mm)	P1 (l ha^{-1})	P2 (l ha^{-1})	P3 (l ha^{-1})	P4 (l ha^{-1})
23 February 2001	31.5 ± 2.3	NR[a]	NR	6987	350
5 March 2001	43.6 ± 1.2	350	350	16,940	350
13 March 2001	41.0 ± 1.4	350	490	12,530	350
15 March 2001	45.4 ± 1.4	33,390	8750	77,420	16,660
20 March 2001	44.8 ± 1.3	1050	4060	32,830	5950
25 July 2001	166.7 ± 0.0	522,023	552,565	434,611	375,115

[a] NR = no runoff.

enterococci. Soil samples analyzed 9 and 11 months after the first litter application and after the runoff events in March 2001 indicated that *E. coli* was marginally detectable. In contrast, a decline in concentrations of total coliforms and fecal enterococci was slight but significant at $P > 0.01$ (Fig. 3). The soil samples of 7 August 2001 were obtained 3 weeks after the second litter application and after the significant storm event of 25 July 2001. Again a minimal concentration of *E. coli* was detected; whereas, concentrations of total coliforms and fecal enterococci were maintained at a comparable level as the previous two sampling times. Runoff events appeared to have little or no effect on the soil communities of fecal bacteria. By 29 November 2001, more than 4 months after the second poultry litter application, the concentrations of total coliforms, *E. coli* and fecal enterococci were not signif-

Table 3
Mean MPN (± S.E.) surface soil concentrations of total coliforms (TC), *E. coli* (EC), and fecal enterococci (FE) before and after litter application to the four P-watersheds

Date	Watershed	TC ($\log_{10}$ MPN kg soil^{-1})	EC ($\log_{10}$ MPN kg soil^{-1})	FE ($\log_{10}$ MPN kg soil^{-1})
31 May 2000	P1	ND[a]	nd[b]	ND
	P2	ND	nd	ND
	P3	ND	nd	ND
	P4	ND	nd	ND
25 July 2000	P1	ND	ND	ND
	P2	ND	ND	ND
	P3	9.3 ± 3.1	4.9 ± 5.7	8.9 ± 3.5
	P4	9.4 ± 0.0	3.7 ± 4.9	8.9 ± 3.3
11 April 2001	P1	8.1 ± 3.4	≤2.7	7.8 ± 3.1
	P2	7.9 ± 3.2	≤2.7	8.1 ± 3.2
	P3	8.2 ± 3.3	3.9 ± 5.5	7.6 ± 3.1
	P4	8.3 ± 3.1	≤2.7	7.6 ± 3.3
25 June 2001	P1	8.2 ± 3.3	≤2.7	8.5 ± 3.2
	P2	8.4 ± 0.0	≤2.7	8.3 ± 3.1
	P3	7.6 ± 3.2	≤2.7	7.6 ± 3.1
	P4	>8.4	4.2 ± 4.9	7.6 ± 3.5
7 August 2001	P1	>8.4	≤2.7	8.2 ± 3.1
	P2	>8.4	4.8 ± 4.5	8.3 ± 3.4
	P3	>8.4	3.5 ± 4.7	7.6 ± 3.3
	P4	>8.4	≤2.7	8.1 ± 3.2
29 November 2001	P1	>8.4	3.3 ± 4.3	7.6 ± 3.3
	P2	9.4 ± 3.0	4.5 ± 5.6	8.2 ± 3.3
	P3	7.4 ± 3.4	3.5 ± 4.1	7.9 ± 3.1
	P4	7.7 ± 3.8	≤2.7	7.5 ± 3.2

Litter was applied on 5 July 2000, and 2 July 2001.
[a] ND = no data.
[b] nd = not detectable.

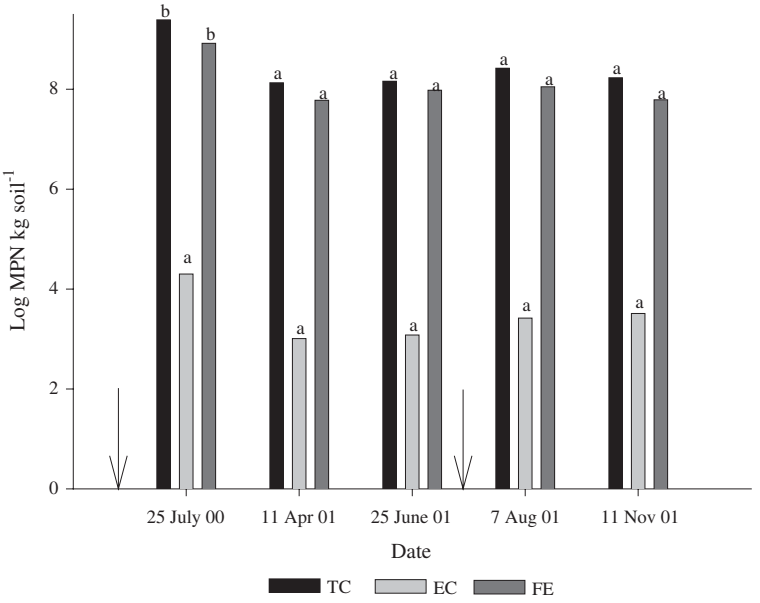

Fig. 3. Mean log MPN cells g soil^{-1} of total coliforms (TC), *E. coli* (EC), and fecal enterococci (FE) in soil before and after litter application. Means are based on the MPN determinations for the four watersheds. Different letters above the bars indicate least square differences at $P=0.01$ between communities of TC, and FE, and populations of EC. Litter was applied on 7/05/2000, and 7/02/2001. Arrows along date axis indicate litter applications.

icantly different from the previous sampling times. The general pattern of the soil communities of total coliforms, *E. coli* and fecal enterococci before and after litter application, and before and after runoff events appeared to fluctuate, but fluctuations after 25 July 2000 were statistically insignificant (Fig. 3). In contrast to the apparent normal distribution of total coliforms and fecal enterococci, the distribution of *E. coli* across the watersheds, based on standard deviations, appeared to be either a Poisson distribution or a clustered distribution (Table 3).

As mentioned, the first runoff event did not occur until 7 months after the first litter application in July 2000. Based on the observations that the fluctuations of the soil communities of total coliforms and fecal enterococci were on the whole not significant, and that they maintained concentrations between 7 and 8 $\log_{10}$ MPN kg soil^{-1}, we inferred that the soil concentrations of these two soil communities were at this same level at the time of the first runoff event. The level of the soil *E. coli* population at the time of the first runoff event was most likely minimal as was observed for the sampling times from 11 April 2001 and after. The soil concentrations of total coliforms, *E. coli*, and fecal enterococci after 25 July 2000 appeared to represent a background level for the four watersheds. The concentrations of the fecal indicator bacteria in the runoff for the events from 23 February 2001 to 20 March 2001 most likely, therefore, represented background levels. Overall, total coliforms fluctuated little between runoff events that occurred in February and March of 2001 (Fig. 4A) although significant differences were observed when the MPN data were normalized (Fig. 4B). Over the runoff events of February and March the fecal enterococci appeared to fluctuate more than the total coliforms and at levels 10^2 to 10^3 times less than total coliforms. The concentrations of *E. coli* were, on the whole, minimal (Fig. 4). The elevated concentrations of these bacteria observed for the major runoff event on 25 July 2001, soon after the second litter application, may be attributed to the added load from the litter as both the non-normalized and normalized data appeared to indicate (Fig. 4). Because of the relatively constant background concentrations of total coliforms and fecal enterococci, their concentrations in runoff may not be indicative of fecal bacteria from litter applications; whereas, elevated concentrations of *E.*

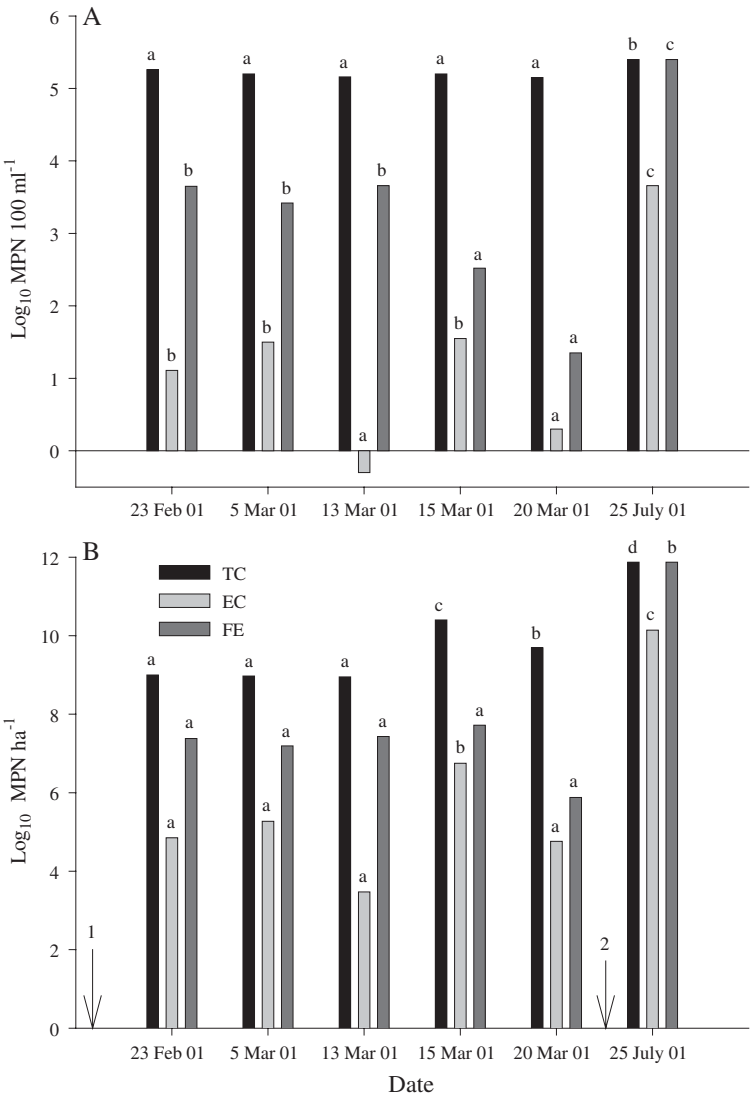

Fig. 4. (A) Mean log MPN 100 ml runoff^{-1} and (B) mean log MPN ha^{-1} of total coliforms (TC), *E. coli* (EC), and fecal enterococci (FE) in runoff from storms before and after poultry litter applications. Different letters above the bars indicate least square differences at $P=0.05$ between communities of TC, and FE, and populations of EC. Arrow 1 indicates litter application on 5 July 2000, and arrow 2 indicates litter application on 2 July 2001.

coli may be a more reliable indicator of manure applications to agricultural fields.

3.3. Estradiol and testosterone in litter, soil, and runoff

The concentrations of estradiol and testosterone in litter applied to the watersheds (Table 1) were substantially less than the concentrations of these hormones in poultry litter that Finlay-Moore et al. (2000), Nichols et al. (1997, 1998), and Shore et al. (1993) reported. With an average poultry litter application of 2395 kg ha^{-1} we applied an average of 3.1 mg estradiol ha^{-1} and 0.08 mg testosterone ha^{-1}. In contrast, Finlay-Moore et al. (2000) applied between 50 and 180 mg estradiol ha^{-1} and between

50 and 280 mg testosterone ha^{-1} which corresponded to 2550 and 4750 kg litter ha^{-1}, respectively; and Nichols et al. (1998) applied 234 mg estradiol ha^{-1} which corresponded to 1760 kg ha^{-1}. This difference suggests a possible wide spectrum of estradiol and testosterone concentrations between different litters and different operational practices. The bedding material of the litter we used was wood shavings, and only one flock of birds was grown on it before cleanout. In contrast, the litter that Finlay-Moore et al. (2000) applied to their experimental fields had four to five flocks of birds before cleanout.

The average concentration of testosterone in the upper 5 cm of soil ranged from 1.1 to 72.4 ng kg^{-1}, and the average concentration of estradiol ranged from 65.3 to 636 ng kg^{-1} (Fig. 5). The concentration of testosterone in the surface 5 cm of soil for all sampling times was less than or in the range of concentrations that Finlay-Moore et al. (2000) had reported. Finlay-Moore et al. (2000) observed that 14 days after their first litter application soil concentrations of estradiol were not different from background levels. Their observations were congruent with the data that Colucci et al. (2001) reported that indicated that estradiol dissipates in soil in a matter of a few days by being abiotically transformed into estrone which is then biodegraded. The concentrations we observed for 25 July 2000, 20 days after the first litter application, and 25 June 2001, a week before the second litter application, may also represent background levels of soil estradiol. The background levels of soil estradiol that Finlay-Moore et al. (2000) observed varied overtime and before and after litter applications. The variation that they observed, however, was not as extreme as the variation that we observed. These variations in concentrations of soil estradiol may indicate two things: (1) significant spatial variability of soil concentrations of estradiol is a factor that needs further study to understand better the potential that agricultural soils appear to be a source of estradiol; and (2) inputs from wildlife and avian activity may be a significant factor in accounting for this variability.

Although the concentrations of estradiol and testosterone in runoff varied between watersheds and runoff events (Table 4), a statistical analysis of the data indicated that the concentrations in runoff from the cropped watersheds were not significantly different from the grazed watershed W1 to which we compared the watersheds receiving poultry litter. Although Finlay-Moore et al. (2000) observed in their study that the presence of cattle did not contribute to

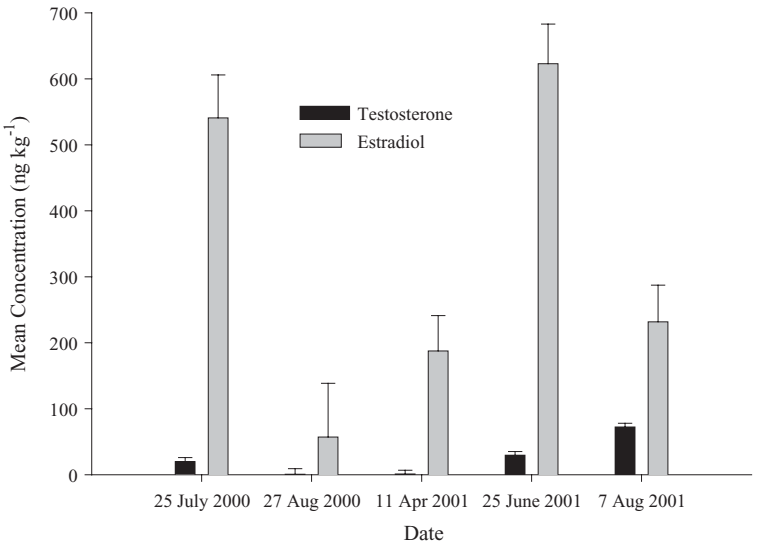

Fig. 5. Mean concentrations (with standard error bars) of estradiol and testosterone in soil. Means are based on the determinations for the four watersheds.

Table 4
Mean (±S.D.) concentrations of estradiol and testosterone in runoff from the four P-watersheds during rainfall events and compared to W1 which received no litter but on which cattle had grazed

Date	Watershed	Estradiol (ng l^{-1})	Testosterone (ng l^{-1})
23 February 2001	P3	20.4 ± 0.02	12.3 ± 0.01
	P4	14.3 ± 0.01	8.2 ± 0.01
	W1	7.9 ± 0.01	10.2 ± 0.01
5 March 2001	P1	ND[a]	ND
	P2	21.2 ± 0.02	6.1 ± 0.01
	P3	22.1 ± 0.02	15.4 ± 0.02
	P4	105.0 ± 0.11	21.5 ± 0.02
	W1	ND	ND
13 March 2001	P1	38.6 ± 0.04	23.6 ± 0.04
	P2	25.4 ± 0.03	16.4 ± 0.02
	P3	18.2 ± 0.02	8.5 ± 0.01
	P4	20.9 ± 0.02	16.4 ± 0.02
	W1	38.7 ± 0.04	38.6 ± 0.04
15 March 2001	P1	25.9 ± 0.03	11.1 ± 0.01
	P2	9.0 ± 0.01	14.8 ± 0.01
	P3	20.9 ± 0.02	8.8 ± 0.01
	P4	7.4 ± 0.01	10.4 ± 0.01
	W1	14.5 ± 0.01	13.3 ± 0.01
20 March 2001	P1	13.7 ± 0.01	3.8 ± 0.004
	P2	23.8 ± 0.02	4.4 ± 0.004
	P3	40.4 ± 0.04	19.3 ± 0.02
	P4	16.4 ± 0.02	4.7 ± 0.005
	W1	23.3 ± 0.02	4.3 ± 0.004
25 July 2001	P1	133.6	4.4
	P2	38.7	3.3
	P3	196.3	7.4
	P4	116.9	5.3
	W1	147.2	6.0

Litter was applied on 5 July 2000 and 2 July 2001. Standard deviations for the individual means on which the total mean was based for the 25 July 2001 runoff event are indicated in Fig. 6.
[a] ND=no data.

the load of estradiol or testosterone, we cannot assume that the presence of cattle (61 cow/calf pairs grazed on W1 for the first week of March 2001, 39 cow/calf pairs grazed on W1 from July 5 to 31, 2001) did not contribute to the load of hormones in runoff from W1. The estradiol concentrations in the runoff for most of the watersheds, nevertheless, may have a deleterious effect on aquatic life based on the observations of Tyler and Routledge (1998).

The runoff from the major storm of 25 July 2001 was analyzed more closely than the other runoff events; concentrations of estradiol and testosterone were plotted as a function of cumulative runoff volume (Fig. 6). Concentrations of testosterone were significantly less than concentrations of estradiol and reflect the differences between these two hormones in the litter and surface soil. During the high volume of runoff from the storm on 25 July 2001, the average quantity of estradiol and testosterone contributed to the streams draining the watersheds were 69.7, 21.4, 85.3, and 43.9 mg estradiol ha^{-1}, and 2.30, 1.82, 3.22, and 1.99 mg testosterone ha^{-1} for watersheds P1, P2, P3, and P4, respectively. These quantities were greater than the load of estradiol and testosterone that the poultry litter contributed to the watersheds, and suggests that these soils may be a reservoir of these hormones.

In field studies, we, as well as Finlay-Moore et al. (2000), have shown that both estradiol and testosterone appeared to persist in soil. Either these hormones are protected by soil components such as organic matter, or, perhaps, a continuous source of input exists

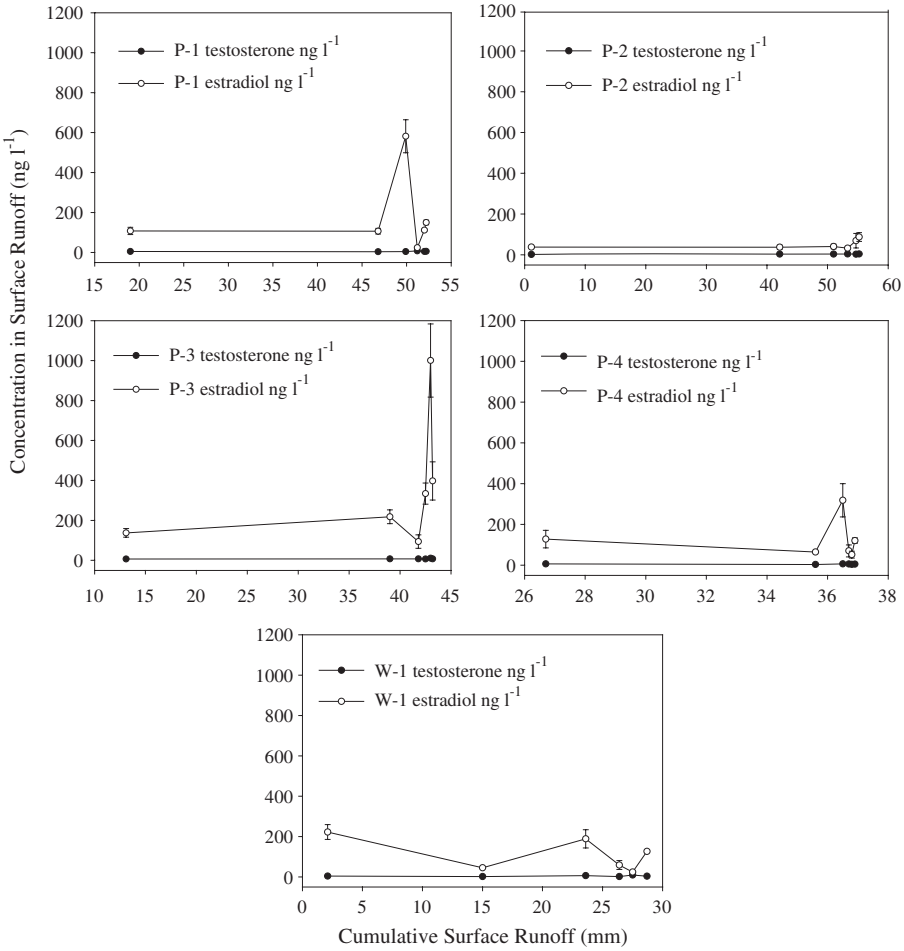

Fig. 6. Mean concentrations (± S.D.) of estradiol and testosterone in runoff samples collected at the four P-watersheds and the grazed watershed W1 during the rain event of 25 July 2001, 23 days following litter application. S.D. bars that are not visible are smaller than the symbols in the graph.

such as from wildlife or avian activity. Both of these hypotheses would need testing. Whether or not a field has received poultry litter as a fertilizer, agricultural soils nevertheless appeared to be a source of estradiol and testosterone that can contribute to runoff and surface waters.

4. Conclusions

Under the conditions of drought and conservation tillage, the rates at which we applied poultry litter to the four cropped watersheds appeared to have little or no significant effect on (a) soil community of fecal indicator bacteria, (b) concentrations of estradiol and testosterone in surface soil, and (c) quantities of estradiol and testosterone coming off the watersheds with runoff. The elevated concentrations of fecal bacteria observed in the runoff of 25 July 2001 indicated that litter can impact runoff when runoff occurs a few days after litter application. Because concentrations of fecal pathogens, indicator bacteria, estradiol and testosterone may vary widely between litters based on operational practices, litter with greater concentrations of fecal bacteria, estradiol and testosterone may impact runoff and surface waters. A survey of poultry litter for these constituents under different operational practices may be warranted to identify those practices

that reduce or increase the presence of fecal bacteria, estradiol and testosterone.

Acknowledgements

The authors wish to express their appreciation to project technical assistants S. Humayoun, T. Olexa, J. Tripp, S. Norris, R. Woodroof, and S. Knapp for their efforts in this study. We also wish to express our appreciation to E. Line for analyzing the litter for *Campylobacter* and *Salmonella*. This study was supported in part by the U.S. Poultry and Egg Association. The mention of trade or manufacturer names is made for information only and does not imply an endorsement, recommendation, or exclusion by USDA-Agriculture Research Service.

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