Published November 10, 2016

Journal of Environmental Quality

TECHNICAL REPORTS

WASTE MANAGEMENT

Estrogen Transport in Surface Runoff from Agricultural Fields Treated with Two Application Methods of Dairy Manure

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Abstract

This study compares two methods of dairy manure application—surface broadcast and shallow disk injection—on the fate and transport of natural estrogens in surface runoff from 12 field plots in central Pennsylvania. Ten natural surface runoff events were sampled over a 9-mo period after fall manure application. Results show that the range of estrogen concentrations observed in surface runoff from the broadcast plots was several orders of magnitude higher (>5000 ng L^{-1}) than the concentrations in runoff from the shallow disk injection plots (<10 ng L^{-1}). Additionally, the transport dynamics differed, with the majority of the estrogen loads from the surface broadcast plots occurring during the first rainfall event after application, whereas the majority of the loads from the shallow disk injection plots occurred more than 6 mo later during a hail storm event. Total estrogen loads were, on average, two orders of magnitude lower for shallow disk injection compared with surface broadcast. Independent of the method of manure application, 17α-estradiol and estrone were preserved in the field for as long as 9 mo after application. Overall, injection of manure shows promise in reducing the potential for off-site losses of hormones from manure-amended soils.

Core Ideas

- Estrogens in surface runoff after dairy manure application were monitored for 9 months.
- Estrogen detection from broadcast plots was higher (67%) than from injection plots (41%).
- 17α-E2 and E1 were detected in runoff for up to 9 months for both applications types.
- Estrogen loads were on average 400 times less for injection than surface broadcast plots.

LIVESTOCK PRODUCTION is increasingly tied to water quality concerns, in part due to the management of manure on farms. In a National Water Quality Inventory, the USEPA assessed approximately 33% of US waters. Of the assessed waters, runoff from agricultural lands was reported as a primary source of impairment for approximately 40% of streams, 45% of lakes, and 50% of estuaries (USEPA, 2000). In the United States, farm animals produce over 300 million t of manure each year, with the manure often applied to agricultural fields as a nutrient source (USDA, 2008). Reuse is known to contribute to water quality concerns, including transport of estrogens to the aquatic environment. On an annual basis, land application of manure is estimated to introduce more than 200 times the mass of estrogens introduced from biosolids applications (Lange et al., 2002; USEPA, 1999). Various studies have reported estrogens in surface runoff and tile drainage after land application of manure (Dutta et al., 2010, 2012; Finlay-Moore et al., 2000; Gadd et al., 2010; Gall et al., 2015; Kjær et al., 2007; Shappell et al., 2016; Snow et al., 2015), with some concentrations exceeding the lowest observable effect levels (LOELs) for fish and other aquatic organisms (Leet et al., 2011; Shull and Pulket, 2015).

Soil physical properties of the mid-Atlantic and northeastern United States, typically shallow and stony, have limited the adoption of newer methods of manure application and the use of deeper injection tools due to severe soil disturbance, horsepower requirements, and the increase in frequency of maintenance (Johnson et al., 2011). More recently, however, shallow disk injection of manure has been found to be less susceptible to failure compared with deep injection systems (Dou et al., 2001) because shallow disk injection requires little contact between the injection system and the soil matrix, with disks only penetrating the soil a few centimeters.

Strong emphasis has been placed on manure application method, timing, form, and rate as key determinants of the impact of manure on off-site concerns, especially the fate and transport of nutrients. Various field studies have been conducted to quantify phosphorus (P) transport in surface runoff under natural and simulated rainfall (Daverede et al., 2004; Kleinman and Sharpley, 2003; Volf et al., 2007; Withers et al., 2001). Daverede

J. Environ. Qual. 45:2007–2015 (2016)
doi:10.2134/jeq2016.05.0173

Supplemental material is available online for this article.
Received 10 May 2016.
Accepted 9 Sept. 2016.
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Abbreviations: LOEL, lowest observable effect level; MDL, method detection limit.

et al. (2004) reported load reductions for dissolved and total P of >80% when swine manure was injected compared with surface applied. Volf et al. (2007) found that total P and dissolved reactive P concentrations in runoff from simulated rainfall on plots that received incorporated solid beef cattle manure (one double disk pass) were not significantly different compared with concentrations from nonincorporation for sites that were saturated with P. Kleinman and Sharpley (2003) found that differences in P losses from fields applied with different manure types (swine, poultry, and dairy) and application rates were greatest shortly after applications occurred but diminished over time. Withers et al. (2001) also found that differences in release of P from sewage sludge and cattle manure were greatest in the first runoff event after application.

Despite the prevalence of studies relating manure application method, timing, form, and rate on P losses, few studies have quantified the impact of these factors on hormone losses. Stumpe and Marschner (2010) examined the effects of organic waste (manure and biosolids) application history (short-term, on the order of weeks; long-term, on the order of decades) on estrogen behavior and found that mineralization was generally higher in the short-term applications, whereas sorption was generally enhanced by long-term applications due to increased organic carbon levels in the soil. Dutta et al. (2010) compared the effects of poultry litter application (raw vs. pelletized) on estrogen transport in surface runoff for 10 runoff events and found that the masses exported from the pelletized litter were up to ~7 times lower than the masses exported from the plots that had received raw litter. In this study, the litter applications contained different levels of estrogens, with the raw poultry litter containing approximately 1.5 times the concentrations of estrogens of the pelletized litter. DeLaune and Moore (2013) reported an exponential decrease of 17β-estradiol concentrations in runoff with time from fields receiving various application rates of poultry litter. However, no studies have been conducted to compare dairy manure application methods on estrogen transport in surface runoff, even though estrogens excreted annually by pregnant cattle are estimated to be more than 15 times higher than those excreted by chickens (Lange et al., 2002).

The goal of this study was to compare the effects of two application methods of dairy manure—surface broadcast and shallow disk injection—on the transport of estrogens in surface runoff during natural rainfall events after fall application of dairy manure for an extended period of time (9 mo). The specific objectives were (i) to compare the concentrations of 17α-estradiol (17α-E2), 17β-estradiol (17β-E2), estrone (E1), and estriol (E3) in the surface runoff; (ii) to quantify the loads of estrogens transported in the surface runoff; (iii) to compare the effects of application method on the estrogen transport dynamics, such as the timing of the majority of their export; and (iv) to gain insight into the role of application method on the potential for winter preservation of estrogens and the implications for transport during spring snowmelt and rainfall events. The findings of this research have important implications for best management practices that can help protect aquatic ecosystems from manure-borne hormone exposures.

Materials and Methods

Study Site

This research was conducted at the Russell E. Larson Agricultural Research Center in Rock Springs, PA, near the Pennsylvania State University's University Park Campus (Fig. 1). The site contains 12 field plots (27 m wide × 15 m long), with the dominant soils being well-drained Hagerstown silt loam (fine, mixed, semiactive mesic Typic Hapludalf) and Opequon silty clay loam (clayey, mixed, active, mesic Lithic Hapludalf). Slopes range from 7 to 15%.

Details of the plots can be found in Duncan et al. (2016). Briefly, the plots were designed to be hydrologically isolated using berms and drains to intercept flows from the exterior of each plot. Within each plot, surface runoff is routed to PVC drains via earthen berms and directed into huts (near Plots 4 and 8; Fig. 1) where the flow is measured with tipping buckets and samples are collected (see below for additional details). Flow data were recorded with a datalogger at a 5-min temporal resolution. Six of the 12 plots were amended with dairy manure slurry via surface broadcasting (Plots 2, 4, 5, 6, 9, and 11), and the remaining six plots received manure via shallow disk injection (Fig. 1). More details are provided in the supplemental material. The history of manure applications at the site included a total of three dairy manure applications prior to the one that occurred on 2 Oct. 2014, which coincided with the beginning of our study period. Manure was applied at rates determined to meet crop nitrogen (N) requirements, and rates of application were approximately 55 Mg ha^{-1} (wet weight, 6.8% solids content), providing nutrient application rates (dry weight) of 172 kg N ha^{-1}, 2.95 kg P ha^{-1}, and 14.5 kg K ha^{-1}. Corn was harvested 1 wk before manure application.

Sampling and Analysis

At the time of manure application (2 Oct. 2014), dairy manure slurry samples were collected in 1-L amber glass bottles, with one sample collected from each of the two tankers that delivered the manure to the site for application. The two manure samples were

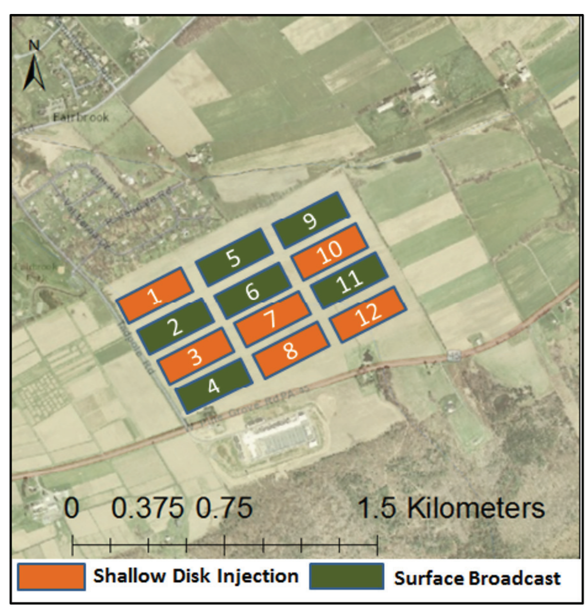

Fig. 1. Aerial image of the study site, located at 40.72° N, 77.92° W in Centre County, PA.

composited into a single sample, which was analyzed for the natural estrogens 17α-estradiol (17α-E2), 17β-estradiol (17β-E2), estrone (E1), and estriol (E3). Sample processing and analysis information is provided in the supplemental material.

Surface runoff was sampled by attaching a 3.8-L trace-cleaned amber glass bottle to the tipping buckets to capture a representative fraction of the total runoff for each event, generating a flow-weighted sample. Continuous monitoring and sampling occurred throughout the 9-mo study period, with a flow-weighted sample collected for each runoff event after the fall 2014 manure application. The duration of the study period was intended to begin immediately after manure application and to go into the following summer when evapotranspiration rates are highest and the frequency of surface runoff decreases. A total of 10 rainfall and/or snowmelt events generated surface runoff during the study period of October 2014 through June 2015. This study period duration is similar to or longer than most estrogen field studies, which are generally on the order of months (Dutta et al., 2010, 2012; Gall et al., 2014, 2015, 2011; Kjær et al., 2007; Shappell et al., 2010, 2016).

For water samples in which more than 1 L was collected in the 3.8-L amber glass bottle, the larger bottles were shaken, and a representative 1-L subsample was collected in trace-cleaned amber glass bottles. All samples were collected within 24 h of each runoff event and shipped overnight on ice to ALS Environmental, ALS Group, USA, Corp. in Kelso, WA, for analysis of 17α-E2, 17β-E2, E1, and E3. Sample processing and analysis details are provided in the supplemental material.

For quality assurance/quality control, ALS Environmental spiked surrogates before extraction and analysis into all blanks, standards, and samples. The labeled compound spiking solution is 5 μg mL^{-1} stock standard of each of the stable isotope-labeled standards estradiol-d5, estrone-d4, and estriol-d2. Samples were spiked with 10 μL before extraction, which equated to 50 ng mL^{-1} of estradiol-d5, estrone-d4, and estriol-d2 in the extract final volume. Each 1 mL of sample extract undergoing analysis was spiked with the internal standard solution to provide a concentration of 50 ng mL^{-1} of each internal standard. Percent recoveries were reported for each surrogate with an average (± SD) of 70 ± 32.9% for estradiol-d5, 79 ± 35.2% for estrone-d4, and 27 ± 23.4% for estriol-d2. The lower control criterion (control limits 66–134) was exceeded for the estriol-d2 surrogate in the field samples due to matrix interferences. The presence of nontarget components affected the resolution of the surrogate, and accurate quantitation was not optimal. Assuming the native analyte performed similar to the labeled analog, the effect on the reported result is minimal. More details are provided in the supplemental material.

Data Analysis

Estrogen loads were calculated by multiplying the volume of surface runoff and the flow-weighted concentration. Concentrations below the method detection limit (MDL) were assumed to have no contribution to the total load, providing a conservative load calculation. Loads were also calculated using MDL/√(2) and are reported in the supplemental material for completeness. For samples with concentrations between the MDL and the method reporting limit, the concentrations estimated by ALS Environmental were used to calculate the load.

All statistical analyses were conducted using Minitab 17 software. The data were tested for normality and were determined to be non-normal. Data were analyzed using the Mann–Whitney nonparametric version of the independent *t* test. The Mann–Whitney U test was run to determine the difference in exported loads between the surface broadcast and shallow disk injection treatments. The tests were conducted using a significance level of $p < 0.05$.

Results and Discussion

Dairy Manure Estrogen Concentrations

The estrogen concentrations applied in the dairy manure, reported on a dry weight basis, were 91 μg kg^{-1} for 17α-E2 (MDL, 29 μg kg^{-1}), 130 μg kg^{-1} for E1 (MDL, 27 μg kg^{-1}), 150 μg kg^{-1} for E3 (MDL, 9.7 μg kg^{-1}), and <MDL of 28 μg kg^{-1} for 17β-E2. The 17α-E2 content is comparable to concentrations reported in the literature for other dairy manures: 88 μg kg^{-1} in Gall et al. (2014) and 155 μg kg^{-1} in Vethaak et al. (2002). However, the E1 concentrations in the manure were higher compared with these studies (31 and 50 μg kg^{-1}, respectively), whereas the 17β-E2 concentrations were lower (7.9 and 48 μg kg^{-1}). Differences in storage methods and holding times may contribute to these differences because E1 is a metabolite of both 17α-E2 and 17β-E2 under aerobic conditions and reversion back to the parent compounds can occur under anoxic conditions (Czajka and Londry, 2006; Mashtare et al., 2013a, 2013b). Composting can also affect estrogen concentrations, with composting reported to remove steroid hormones in beef cattle manure at rates ranging from 79 to 87% (Bartelt-Hunt et al., 2013).

Estrogen Detection and Concentration Summary

A total of 10 surface runoff events occurred over the 9-mo study period (October 2014 through June 2015) (Table 1). From these runoff events, 90 samples were collected because not every runoff event triggered runoff from all plots (details are provided in the supplemental material). The samples were roughly evenly distributed between the two manure application methods: 46 samples were collected from the surface broadcast plots and 44 from the shallow disk injection plots. A total of 67% of the samples from the surface broadcast plots had estrogen concentrations >MDL (Table 2). For the shallow disk injection plots, a total of 41% of the samples collected had estrogen concentrations >MDL.

Table 1. Dates of sample collection and description of each runoff event.

Date of sample collection	Runoff event number	Precipitation	Average intensity
		mm	mm h^{-1}
4 Oct. 2014	1	12.4	1.3
16 Oct. 2014	2	39.9	4.1
10 Mar. 2015	3	snowmelt	snowmelt
11 Mar. 2015	4	snowmelt	snowmelt
13 Mar. 2015	5	snowmelt	snowmelt
15 Mar. 2015	6	snowmelt	snowmelt
11 Apr. 2015	7	7.1	3.0
22 Apr. 2015	8	18.0	7.7
1 June 2015	9	6.9	2.1
24 June 2015	10	19.1	19.1

Table 2. Data summary, including frequencies of detection, maximum and median observed concentrations, and total number of samples (*n*) collected for 10 surface runoff events. None of the runoff plots generated runoff from all 10 observed events, and therefore the maximum *n* at each plot was 9. Details are provided in the Supplementary Material. Median concentrations are calculated using the sample concentrations reported above the method detection limit.

Plot no.	17α-Estradiol				17β-Estradiol				Estrone				Estriol			
	Median, max.	% *n* > MDL†	% *n* > MRL‡	*n*	Median, max.	% *n* > MDL	% *n* > MRL	*n*	Median, max.	% *n* > MDL	% *n* > MRL	*n*	Median, max.	% *n* > MDL	% *n* > MRL	*n*
	ng L^{-1}				ng L^{-1}				ng L^{-1}				ng L^{-1}			
									Surface broadcast							
2	375, 750	22	11	9	NA,§ 1500	11	11	9	1.65, 1500	67	11	9	NA, NA	0	0	9
4	191, 380	22	11	9	NA, NA	0	0	9	0.87, 1100	56	22	9	NA, NA	0	0	9
5	NA, 0.90	14	0	7	NA, 3.3	14	14	7	8.05, 720	57	29	7	NA, NA	0	0	7
6	NA, 0.99	20	0	5	NA, 18.0	20	20	5	4.2, 8.75	60	20	5	NA, NA	0	0	5
9	1.0, 310	43	14	7	NA, 1.3	14	0	7	6.90, 1900	43	29	7	NA, 470	14	14	7
11	1200, 2400	22	11	9	NA, NA	0	0	9	3.55, 4000	89	22	9	NA, 3.2	11	0	9
									Shallow disk injection							
1	NA, NA	0	0	9	0.71, 1.2	11	0	9	3.80, 10.09	67	11	9	NA, NA	0	0	9
3	NA, NA	0	0	8	NA, NA	0	0	8	0.70, 0.77	25	0	8	NA, NA	0	0	8
7	0.49, NA	13	0	8	NA, NA	0	0	8	2.50, 2.55	38	0	8	NA, NA	0	0	8
8	0.87, NA	14	0	7	NA, NA	0	0	7	1.50, 1.80	43	0	7	NA, NA	0	0	7
10	0.65, NA	25	0	4	NA, NA	0	0	4	0.95, 0.97	50	0	4	4.8, NA	25	25	4
12	0.75, NA	13	0	8	NA, NA	0	0	8	0.70, 0.94	25	0	8	NA, NA	0	0	8

† Method detection limit.

‡ Method reporting limit.

§ NA, maximum concentration not applicable because all concentrations were <MDL. Bold type indicates that only one sample concentration was reported above the MDL, and therefore calculating a median is not applicable.

Regardless of application method, E1 was the most frequently detected estrogen, with 63% of the surface broadcast plot samples having concentrations >MDL, ranging from 0.63 to 4000 ng L^{-1}; and 41% of the shallow disk injection samples were also reported >MDL, and concentrations ranged from 0.45 to 10 ng L^{-1}. Other estrogens were detected in fewer than 25% of the samples collected from the surface broadcast plots, with 17α-E2 detected in 24% of samples and concentrations ranging from 0.65 to 2400 ng L^{-1}, 17β-E2 detected in 9% of the samples and concentrations ranging from 1.3 to 250 ng L^{-1}, and E3 detected in 4% of samples and concentrations ranging from 3.2 to 470 ng L^{-1}. The shallow disk injection plots yielded similar frequencies of detection for these estrogens but at much lower concentrations in the surface runoff, with 17α-E2 detected in 9% of samples and concentrations ranging from 0.49 to 0.87 ng L^{-1}; 17β-E2 was detected in one of the samples with a concentration of 1.2 ng L^{-1}, and E3 was detected in 1 sample at 4.8 ng L^{-1}.

This difference in estrogen detection frequencies in the surface runoff is of interest because the concentrations of 17α-E2, E1, and E3 applied were all on the same order of magnitude (90–150 μg kg^{-1}). Our data suggest that the higher frequency of detection of E1 relative to E2 isomers and E3 is the result of transformation of 17α-E2 to E1 and degradation of E3 to nonestrogenic metabolites, which is consistent with known transformation pathways (Mashtare et al., 2013b) and other field studies that identified E1 as the most frequently detected estrogen (Gall et al., 2011; Kjær et al., 2007).

Estrogen Discharge Dynamics

Rainfall Events Immediately after Application

Over the 9-mo study period, differences in estrogen loads and timing of transport in surface runoff between the surface broadcast and shallow disk injection treatments were most significant during the first rainfall event after application. Estrogen concentrations in samples collected from the plots that received the surface broadcast had the highest concentrations during the first event after application (4 Oct. 2014), with E1 and 17α-E2 concentrations ranging from 720 to 4000 ng L^{-1} and from 310 to 2400 ng L^{-1} (Fig. 2). The event was relatively small, with a 24-h return period of less than 1 yr, but it occurred less than 48 h after the manure application. Five out of the six surface broadcast plots generated surface runoff, whereas none was generated from the shallow disk injection plots.

Other studies have reported a significant decrease in surface runoff with disk application (Johnson et al., 2011; Liu et al., 2016; Verbree et al., 2010) and after disk tillage (Biswas et al., 2013) due to the creation of microtopographical ridges that prevent the development or reduce the connectivity of runoff flow paths (Kibet et al., 2011; Pote et al., 2011). Liu et al. (2016) also consistently reported lower runoff during the first rainfall event after application for subsurface poultry litter wastes application compared with surface application. The surface runoff samples collected after the first rainfall event, which occurred less than 48 h after the manure application on 2 Oct. 2014, contained high levels of particulates, and therefore less than optimal sample volumes were extracted through the solid phase extraction cartridges. The detection limits were therefore elevated due to the high level of particulates in the samples, and the reporting limits were adjusted to reflect the volumes extracted (see details in the supplemental material). However, despite these analytical issues, the concentrations were high enough to be above the reporting limits for this first event.

The second rainfall event occurred on 16 Oct. 2014, 2 wk after the manure application, and was about three times larger in depth and intensity than the first rainfall event (Table 1). This rainfall event generated runoff from four (Plots 1, 3, 7, and 12) of the six shallow disk injection plots, and only Plot 1 had estrogen

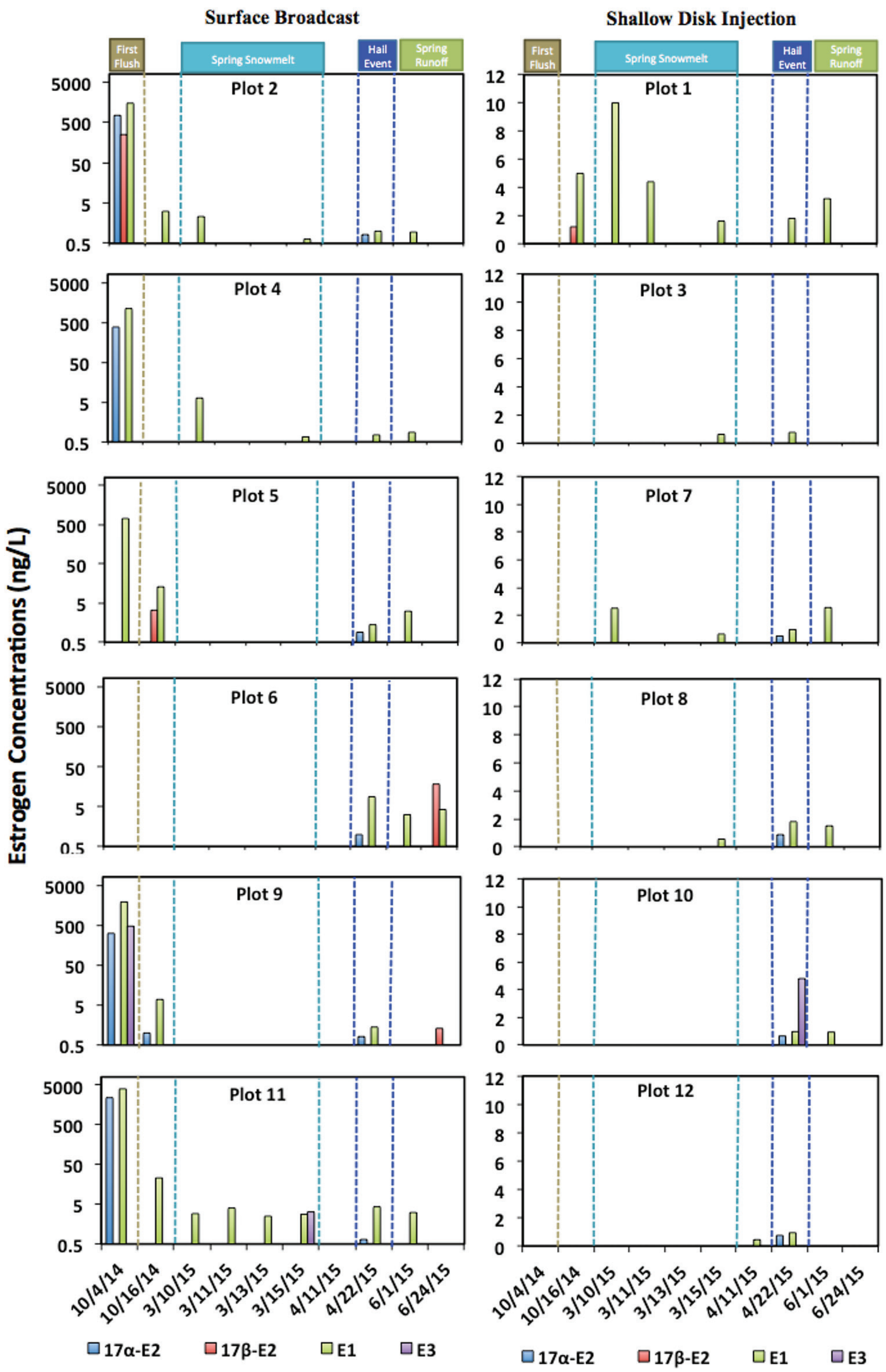

Fig. 2. Concentrations of each estrogen: 17α-estradiol (17α-E2), 17β-estradiol (17β-E2), estrone (E1), and estriol (E3) for each runoff event at each plot for surface broadcast (left) and shallow disk injection (right) application methods. Different events are denoted with colored lines and specified at the top of each graph (first flush, spring snowmelt, hail event, and spring runoff). Dairy manure was applied to all plots, surface broadcast and shallow disk injection, on 2 Oct. 2014. Note the *y* axis scale differences for surface broadcast versus shallow disk injection plots.

concentrations >MDL, with 17β-E2 and E1 concentrations of 1.2 and 5 ng L^{-1}, respectively (Fig. 2). Estrogen concentrations in the surface runoff from the surface broadcast plots were one to two orders of magnitude lower than those observed during the first rainfall event. This is consistent with previous studies that have shown lower concentrations of estrogens in subsequent events (Dutta et al., 2010; Gall et al., 2011). This finding may have two potential explanations: (i) depletion of the source at the surface due to translocation into the soil where sorption and/or degradation occur or (ii) preservation of the estrogens in the manure solids, with a slow release over time that occurs at longer time scales than the inter-rainfall event time.

Spring Snowmelt Events

Runoff samples due to snowmelt events were collected in March 2015, 5 mo after manure application. The E1 concentrations in the snowmelt runoff samples collected from the surface broadcast plots ranged from <MDL to 6.3 ng L^{-1}, and all other estrogen concentrations were <MDL. Snowmelt runoff samples from the shallow disk injection plots had E1 concentrations on the same order of magnitude, with concentrations ranging from <MDL to 10.0 ng L^{-1} and all other estrogen concentrations were <MDL. However, the loads exported by the snowmelt events were low relative to fall and spring transport. The snowmelt loads contributed to a maximum of 18% of the total loads observed

over the study period for the surface broadcast plots and 9% for the shallow disk injection plots.

Transport of estrogens during these snowmelt events suggests their preservation in the soil profile (likely within the first few centimeters near the soil surface). Similar spring snowmelt dynamics were observed by Gall et al. (2011) in tile-drained fields in the midwestern United States but could not be linked to a specific application method or manure type given the array of estrogen sources and application methods used in that study. Here, we see evidence of estrogen persistence over the winter months for both surface and subsurface injection methods, suggesting that both methods can lead to similar estrogen transport potential (i.e., concentrations on the same order of magnitude) during the early spring. However, for both application types, the observed concentrations of E1 were below the LOEL (14 ng L^{-1}) for vitellogenin induction in zebrafish (Holbech et al., 2006).

Transport during a Hail Event

On 20 Apr. 2015, a storm with an average rainfall intensity of 7.7 mm h^{-1}, introducing hail over 25.4 mm in diameter, passed through the study site. This event was the first to produce runoff from all 12 plots simultaneously and with at least one estrogen concentration >MDL in all of the runoff samples. Estrogen concentrations in the runoff generated from the broadcast plots ranged from 0.75 to 8.7 ng L^{-1} for E1 and from <MDL to 0.99 ng L^{-1} for 17α-E2. For the shallow disk injection plots, E1 concentrations ranged from 0.77 to 1.8 ng L^{-1} and from <MDL to 0.87 ng L^{-1} for 17α-E2. This was the first event to generate samples with detectable levels of 17α-E2 for the shallow disk injection plots with a median concentration of 0.70 ng L^{-1}, whereas 17α-E2 was detected in runoff from the surface broadcast plots during the first event (median concentration, 565 ng L^{-1}) and again during this event (median concentration, 0.82 ng L^{-1}). This suggests that preservation over the winter and early spring months occurred within the soil profile for both E1 and 17α-E2, perhaps due to lower microbial activity and therefore less transformation in the coldest months of the year. Preservation of the parent compound might be expected for shallow disk injection, where there is more physical protection of the manure compared with surface application. However, these results suggest that 17α-E2 also persists in the surface broadcast plots.

Summer Rainfall Events

Surface runoff samples were collected from all 12 plots during a rainfall event (6.9 mm) (Table 1) on 1 June 2015. Five of the six surface broadcast plots had detectable levels of E1 ranging from 0.87 to 3.1 ng L^{-1}, whereas the other estrogens had concentrations <MDL. Four of the shallow disk injection plots had similar levels of E1 (0.93–3.2 ng L^{-1}), whereas the other estrogens had concentrations <MDL. For samples collected during the final rainfall event of the study period (24 June 2015; 19.1 mm), only two samples contained concentrations >MDL. These results suggest that, although estrogens can be preserved in the soil profile and continue to be transported through the spring months and into the summer, independent of manure application method (surface broadcast vs. shallow disk injection), concentrations in the runoff were below the LOEL.

Estrogen Loads and Delivery Ratios

Cumulative Export Dynamics

The masses of total estrogens transported in surface runoff from each plot during each surface runoff event and the percent contribution of that event to the total load discharged over the study period are given in Table 3. The median loads exported in each runoff event from the surface broadcast treatment (7.9 ng) and shallow disk injection (0 ng) were statistically significantly

Table 3. Total estrogen loads exported from each plot during surface runoff events and percent contribution of each event to the total loads exported over the 9-mo study period. Loads were considered to be zero both when no runoff was observed and when the concentrations in the runoff were below the method detection limit.

Plot no.	Runoff events									
	4 Oct. 2014	16 Oct. 2014	10 Mar. 2015	11 Mar. 2015	13 Mar. 2015	15 Mar. 2015	11 Apr. 2015	22 Apr. 2015	1 June 2015	24 June 2015
	μg ha^{-1}									
	Surface broadcast									
2	3,764 (98.8%)	2.35 (0.06%)	33.3 (0.88%)	<MDL† (0%)	<MDL (0%)	3.44 (0.09%)	NR‡ (0%)	6.05 (0.16%)	2.18 (0.06%)	<MDL (0%)
4	24.1 (67.4%)	1.66 (4.56%)	5.84 (0.36%)	<MDL (0%)	<MDL (0%)	0.53 (0.03%)	NR (0%)	2.64 (0.16)	0.98 (0.06%)	<MDL (0%)
5	967 (91.4%)	85.6 (8.35%)	<MDL (0%)	<MDL (0%)	NR (0%)	NR (0%)	NR (0%)	2.36 (0.23%)	0.50 (0.05%)	<MDL (0%)
6	NR (0%)	NR (0%)	NR (0%)	<MDL (0%)	<MDL (0%)	NR (0%)	NR (0%)	51.3 (38.6%)	0.38 (0.28)	81.0 (61.1%)
9	8,002 (98.8%)	50.8 (0.63%)	NR (0%)	<MDL (0%)	<MDL (0%)	NR (0%)	NR (0%)	29.5 (0.36%)	0 (0%)	17.8 (0.22)
11	38,509 (99.7%)	35.4 (0.09%)	10.6 (0.03%)	21.6 (0.06%)	4.08 (0.01%)	13.9 (0.04%)	NR (0%)	35.0 (0.09%)	1.19 (0.003%)	<MDL (0%)
Avg. ± SD	8,536 ± 14,998	29.3 ± 34.7	8.28 ± 13.0	3.60 ± 8.81	0.68 ± 1.67	2.98 ± 5.52	0 ± 0	21.1 ± 20.5	0.87 ± 0.77	16.5 ± 32.4
	Shallow disk injection									
1	NR (0%)	1.65 (22.2%)	0.18 (2.39%)	ND§	ND	ND	ND	3.03 (40.9%)	2.55 (34.5%)	<MDL (0%)
3	NR (0%)	<MDL (0%)	<MDL (0%)	<MDL (0%)	<MDL (0%)	1.07 (21.3%)	NR (0%)	3.94 (78.7%)	<MDL (0%)	<MDL (0%)
7	NR (0%)	<MDL (0%)	<MDL (0%)	1.09 (8.5%)	<MDL (0%)	NR (0%)	<MDL (0%)	7.36 (57.2%)	4.42 (34.3%)	<MDL (0%)
8	NR (0%)	NR (0%)	<MDL (0%)	<MDL (0%)	<MDL (0%)	0.35 (4.53%)	NR (0%)	5.57 (71.4%)	1.88 (24.1%)	<MDL (0%)
10	NR (0%)	NR (0%)	NR (0%)	<MDL (0%)	NR (0%)	NR (0%)	NR (0%)	68.8 (96.8%)	2.28 (3.21%)	<MDL (0%)
12	NR (0%)	<MDL (0%)	<MDL (0%)	<MDL (0%)	<MDL (0%)	NR (0%)	0.007 (0.14%)	4.86 (99.9%)	<MDL (0%)	<MDL (0%)
Avg. ± SD	0 ± 0	0.27 ± 0.67	0.03 ± 0.07	0.22 ± 0.49	0 ± 0	0.28 ± 0.46	0.001 ± 0.003	15.6 ± 26.1	2.23 ± 1.58	0 ± 0

† Method limit of detection.

‡ No runoff.

§ No data due to problem with recorder.

different, with $p < 0.05$ (0.008). Loads were considered to be zero both when runoff from a specific plot was zero (but with runoff occurring on some of the plots) and when concentrations in the runoff were <MDL (Table 3).

Differences in the hydrologic responses of the various plots are explored in more detail in Duncan et al. (2016) and contribute to the high SDs. Briefly, variability in the hydrology was observed across the 12 field plots, with Plot 6 reporting lower-than-expected recoveries of overland flow of <1% of annual precipitation in 2012 and 2013 and <4% of annual precipitation in 2014, suggesting runoff losses that likely bypassed the monitoring infrastructure. The timing of estrogen loads transported in surface runoff from the two different application methods exhibited pronounced differences (Fig. 3). For five of the six surface broadcast plots, more than 90% of the total estrogens loads (reported average load ± SD, 10,903 ± 16,380 μg ha^{-1}) exported from the plots occurred during the first rainfall event, with this relatively small event (24 h, <1 yr return period) as the "hot moment" or "hot event" most significant to overall estrogen transport.

In contrast, the "hot event" for the plots receiving shallow disk injection was the hail event, which occurred more than 6 mo after the manure application date and in most cases exported >70% of the total estrogen loads transported via surface runoff from those plots (Fig. 3). These exported loads during the hot event (hail event) for the shallow disk injection were two orders of magnitude lower (335 ± 777 μg ha^{-1}) than the exported loads (10,903 ± 16,380 μg ha^{-1}) transported during the hot event from the surface broadcast plots. The exported loads from the surface broadcast plots during the hail event (21.8 ± 21.1 μg ha^{-1}) were one order of magnitude lower than the exported loads transported during that same event from the shallow disk injection.

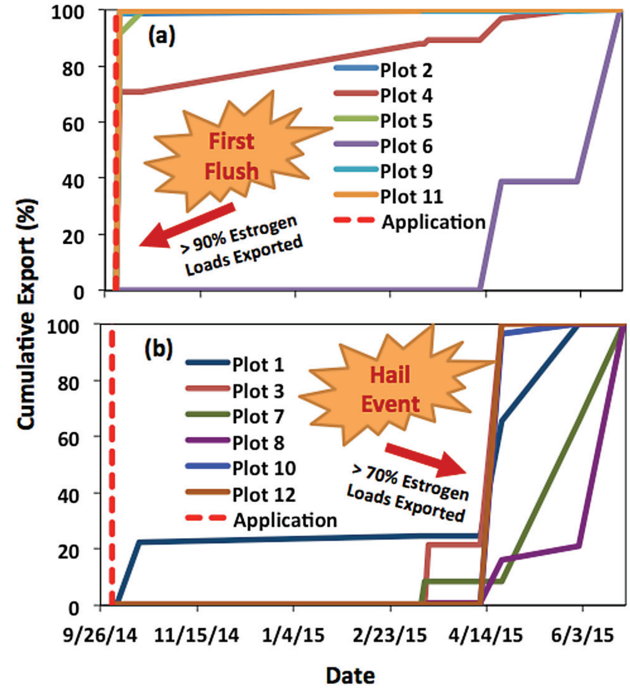

Fig. 3. Cumulative export (percent) of total estrogens discharged over the 9-mo study period for the plots that received dairy manure slurry by (a) surface broadcast and (b) shallow disk injection. The first flush event was the most important for hormone loads in (a); a hail event was the most important event for hormone loads in (b).

Delivery Ratios

Estrogen delivery ratios for each plot were calculated as the total estrogen load exported during the study period divided by the total estrogen mass applied (Table 4). The delivery ratios are given in Table 5. The average delivery ratio from the surface broadcast plots was 0.04%, which was two orders of magnitude higher than the average delivery ratio from the shallow disk injection plots (0.0001%). The lower delivery ratios for the sites that received manure slurry via shallow disk injection suggest that the potential for estrogens to be transported via surface runoff is significantly lower (one to two orders of magnitude) compared with the same type of manure applied via surface broadcasting. For each application method, the highest loads observed were for Plots 10 and 11, which had the highest slopes (14.6 and 13.7%, respectively) (Supplemental Table S1).

The results of the current study are low relative to other delivery ratios reported by Gall et al. (2014) for manure-amended soils (0.23–3.1%). However, those soils had received multiple applications in both solid and liquid (lagoon effluent) form and had an approximately 20-yr history of land application prior to the other study period. In this current study, the soils had less than a 3-yr manure history and received a total of four slurry applications during that period. The HERD (Hormone Export and Recovery Dynamics) model developed by Gall et al. (2016) showed that the land application history at a site matters in predicting the delivery ratio.

Conclusions

The total estrogen loads transported from the shallow disk injection application were on average two orders of magnitude lower than the total estrogen loads transported from the surface broadcast plots. The significant reduction in total estrogen load transport from the shallow disk injection application relative to the surface broadcast application is an important finding that adds to previous findings (Maguire et al., 2011) of improved N use efficiency through less NH_3 volatilization, reduced odors, and decreased nutrient losses in surface runoff. The observed estrogen concentrations in surface runoff from the surface broadcast plots that occurred less than 48 h after the manure application on 2 Oct. 2014 were several orders of magnitude higher than concentrations in the surface runoff that occurred throughout the study period (October 2014 through June 2015) from the plots that received shallow disk injection application.

Table 4. Average dairy manure masses per hectare applied to surface broadcast and to shallow disk injection plots and average mass per hectare of 17α-estradiol, estrone, and estriol applied to surface broadcast and to shallow disk injection plots. Each plot was of 27 m × 15 m.

Manure application method	Dairy manure slurry†	17α-Estradiol	Estrone	Estriol	Total estrogens
	kg ha^{-1}	mg ha^{-1}			
Surface broadcast	56,379	5131	7330	8457	20,918
Shallow disk injection	59,343	5401	7716	8902	22,019

† 17β-Estradiol was not detected in the manure sample.

Table 5. Total estrogen masses exported and estrogen delivery ratios for each plot, and average delivery ratios for each application method.

Plot number	Exported mass	Delivery ratio
	μg ha^{-1}	%
	Surface broadcast	
2	3,793	2.06×10^{-2}
4	35.7	1.69×10^{-3}
5	1,025	4.43×10^{-3}
6	133	6.42×10^{-4}
9	8,100	3.63×10^{-2}
11	38,631	1.94×10^{-1}
Avg.	8,619	4.27×10^{-2}
	Shallow disk injection	
1	7.65	3.94×10^{-5}
3	5.18	2.14×10^{-5}
7	13.3	5.29×10^{-5}
8	38.2	3.71×10^{-4}
10	73.4	3.18×10^{-4}
12	4.97	2.18×10^{-5}
Avg.	23.8	1.37×10^{-4}

These findings have important implications for downstream receiving water bodies. Although shallow disk injection is not commonly used in the eastern United States, this study shows that this method of manure application has the potential to reduce the loads of estrogens exported in surface runoff after dairy manure application and the potential threat that endocrine disrupting compounds may pose to aquatic organisms. In addition, the "hot events" associated with estrogen transport appeared to depend on the method of manure application, with timing of the first rain event after application being the most important factor in estrogen transport from surface broadcast application, whereas intensity of an event rather than timing appeared to be the most important factor in estrogen transport for the shallow disk injection method.

The frequency of detection of E1, 17α-E2, and 17β-E2 and E3 in surface runoff from the surface broadcast plots was higher than the frequency of their detection from the shallow disk injection plots. Winter preservation of 17α-E2 and E1 was observed for 9 mo after application, and the observed preservation was independent of the method of manure application.

Acknowledgments

This research was supported, in part, by the Penn State Institutes of Energy and the Environment. Construction of the field plots and field management activities were funded, in part, by a grant from the Northeast Sustainable Agricultural Research and Education (NESARE) program. The authors thank Dr. Heather Karsten for providing with manure application information; Gordon Folmar, Nicolas Florentino Assalim dos Santos (supported by the Brazil Scientific Mobility Grant), Marc Gluberman (supported by the College of Agricultural Sciences Undergraduate Research Program), Alexandera Everhart (supported by the College of Agricultural Sciences Undergraduate Research Program), Daniel Schultz (supported by the College of Agricultural Sciences Undergraduate Research Program and Erickson Discovery Grant), and Dr. Malcom Taylor for their help in the field and laboratory; and three anonymous reviewers, whose comments and suggestions greatly improved the manuscript.

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