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Occurrence and pathways of manure-borne 17β-estradiol in vadose zone water

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ABSTRACT

The hormone 17β-estradiol (E2) can cause endocrine disruption in sensitive species at part per trillion concentrations. The persistence and transport pathways of manure-borne E2 in agricultural soils were determined by comparing its occurrence with the transfer of water and the transport of non-sorbing fluorobenzoic acid (FBA) tracers. This comparison was done using capillary wick lysimeters installed 0.61 m beneath three corn (*Zea mays* L.) plots that receive swine (*Sus scrofa domesticus*) manure from various sources. An additional control plot was included that received no manure. Soil water transfer was modeled to compare actual versus predicted percolation. On average, lysimeters collected 61% of the expected percolation and 8% of the FBA. There were frequent E2 detections, where there were an average of 8 detections for the 11 sample events. The average detection was 21 ng L^{-1} and its range was 1–245 ng L^{-1}. 17β-Estradiol was detected before manure was applied and also in the control plot lysimeters. Furthermore, the average mass recovery of E2 in all the lysimeters was >50%, which was greater than the FBA tracer recovery. Results indicated that tracer was transported with precipitated water infiltrating into the soil surface and percolating down through the soil profile. There was substantial evidence for antecedent E2, which was persistent and mobile. The persistence and mobility of the E2 may result from its associations with colloids, such as dissolved organic matter. Furthermore, this antecedent E2 appeared to overwhelm any observable effect of manure management on E2 fate and transport.

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1. Introduction

A pathway of environmental exposure to reproductive hormones is the agricultural use of animal manures as fertilizer. Runoff from livestock feedlots and manure fertilized fields can contribute several steroidal hormones in surface and subsurface waters (Finlay-Moore et al., 2000; Peterson et al., 2000; Kolpin et al., 2002). Thorough investigations are needed to clarify the role or lack of role of livestock production with respect to environmental exposure to hormones. Additionally, the fate and transport potential of hormones with respect to common manure storage and disposal practices needs to be evaluated.

As an exogenous hormone, 17β-estradiol (E2) is a potent endocrine disrupting compound at very low concentrations. It will bind to and activate hormone responsive organs (Melnick, 1999), disrupting normal sex differentiation and gametogenesis. Many laboratory studies indicate E2 will degrade rapidly (Lee et al., 2003; Das et al., 2004; Casey et al., 2005; Fan et al., 2007a) and its mobility in soil–water systems is minimal (Casey et al., 2003; Das et al., 2004; Fan et al., 2008). Nonetheless, field studies indicate moderate persistence and mobility of E2 in surface (Finlay-Moore et al., 2000; Kolpin et al., 2002) and subsurface (Peterson et al., 2000; Herman and Mills, 2003; Kjaer et al., 2007; Arnon et al., 2008) waters that are affected by animal agricultural and associated practices.

Kjaer et al. (2007) measured E2 and its metabolite, estrone, in subsurface tile drainage in a field that received swine manure. They suggested that cool anaerobic soil conditions and preferential flow were responsible for the estrogen persistence and greater than expected transport, respectively. Herman and Mills (2003) frequently detected E2 concentrations in lysimeters beneath fields that had received poultry manure. These lysimeters were installed at depths of 0.15–0.91 m and detection ranges were from 23 to 200 ng L^{-1}. Also, Arnon et al. (2008) recently measured substantial redistribution of estrogens through 40 m of vadose zone.

One possible explanation for hormone persistence and greater than expected mobility is the association of hormones with colloidal materials. Dissolved or colloidal fractions of soil and/or manure can potentially lead to greater mobility of hormones. Holbrook et al. (2004) indicated that up to 60% of aqueous E2 concentrations may be associated with organic colloids in wastewater. Studies on

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trace metal mobility in soil have found that organic colloids from wastewater biosolid can facilitate metal transport (Ashworth and Alloway, 2004). This facilitate transport can potentially be a process active in the fate and transport of hormones in the environment. Holthaus et al. (2002) found that E2 sorption was greater for fine sediments, potentially causing greater mobility in rivers. Herman and Mills (2003) contrasted surface and subsurface E2 concentrations and found that river E2 concentrations were mainly due to subsurface flow rather than overland flow, which may suggest the role of colloid facilitate movement of E2.

Greater than expected persistence of E2 may occur in the presence of fine mineral and organic fractions. At lower temperatures and higher dissolved organic carbon, Herman and Mills (2003) observed significantly higher persistence of E2 and attributed it to lower degradation rates of E2 in a more abundant energy source of DOC. Furthermore, Fan et al. (2007b, 2008) accurately modeled the fate of E2 and testosterone in soil by using an aqueous phase degradation. They based their aqueous phase degradation on a study by Harms and Bosma (1997) that concluded that organic contaminants have to be dissolved or desorbed from the solid phase to be available for microbes. Therefore colloid-bound E2 would not be available to microbial consumption.

The goal of this study was to assess manure application as a potential source of E2 and to determine the fate and transport of manure-borne E2 in soil.

2. Materials and methods

2.1. Study site, instrumentation, and soil characterization

Four study plots were established in southeastern North Dakota and instrumented with lysimeters and wells in the spring of 2003 (Fig. 1). The study soil was a Hecla–Hamar Series loamy fine sand (sandy, mixed, frigid oxyaquic hapludolls), which was developed from glaciofluvial and eolian deposits (Edland, 2002). This series consists of very deep, moderately well-drained soils formed in sandy sediments on lake plains and glacial outwash plains. The average concentrations of sand, silt, and clay in the 0.61 m profile were 78.9%, 11.3%, and 9.8%, respectively. The surface organic matter content was 2.23%.

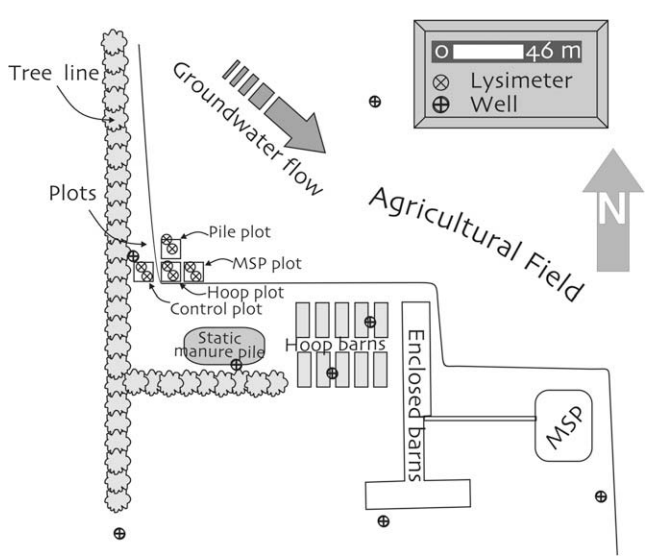

Fig. 1. Schematic of the animal operation indicating the location of the instruments and plots. The manure storage pit (MSP) receives liquid manure from sub-floor pits beneath the enclosed barn.

Animal production at the site was swine at varying developmental stages (sow, nursery, and finishing). Based on the stage of animal development, swine are located in a series of nurseries and pens within an enclosed barn, and later transferred to hoop barns (Fig. 1). Slatted floors are used with the sows and nursery animals in an enclosed barn whereas the finishing animals are housed in open-ended hoop barns. On a monthly basis the sub-floor pits beneath the slatted floors are drained and the liquid manure is pumped into a large earthen manure storage pit (MSP). The finishing hoop barns are un-insulated structures built on compacted earth that is covered with bedding of straw or wood shavings. Twice a year the bedding/manure mixture from the floors of the hoop barns is scraped into an uncovered static manure pile (i.e. not rotated) atop bare soil (Fig. 1).

Surrounding the animal operation facility is a cropped field. Four square 9.29 m^2 experiment plots were delineated on the very edge of this field, just inside a fence separating the field from the animal operation (Fig. 1). The plots were relegated to this area with permission from the livestock manager. The control plot was located outside the fenced agricultural field in a grass area that had no record of manure application. Manure had not been applied to the test plot area inside the agricultural field for at least three years. Additionally, this corner of the agricultural field is typically not cultivated nor receives manure, because farm implementation cannot conveniently pass over.

2.2. Lysimeters

Two passive capillary pan (PCAP) lysimeters were installed under each of the four plots at approximately 0.61 m depth. These pans are similar to those used for soil water sampling by Knutson and Selker (1994) and Zhu et al. (2002). The pans utilize the capillary suction of fiberglass wicks to produce a hanging water column to draw soil water samples across a range of pressures. Pressure potential arises from the length, flow rate, and hydraulic conductivity of the wick. Two PCAP pan lysimeters were installed per test plot. Each pan was a 30 by 33 cm by 15 cm tall stainless steel pan equipped with a stainless steel elbow and a 15.8-mm outside diameter compression fitting. Polypropylene tubing (15.8-mm outside diameter) was attached routed to a 28.7 cm tall, 15 cm outside diameter, 4-L polypropylene container positioned about 0.9 m below the pan, which was used as the sample-collection chamber. The sample-collection chamber was housed within a protective, 20 cm outside diameter PVC cylinder. The glass-fiber wicks used in this study were 20-strand and 2 cm in diameter ropes (Pepperell Braiding Co., East Pepperell, MA) and prepared according to Knutson et al. (1993) then coiled along the bottom of the pans and extended through the polypropylene tubing and into the top of the collection container. The materials of the PCAP in contact with soil water were all stainless steel, glass, or polypropylene; and were chosen for their resistance to microbial degradation and non-reactivity (non-adsorbing) to constituents (steroids and tracers) anticipated to be present in the soil solution.

When the PCAP lysimeters were installed the soil was excavated in 0.15 m and set aside separately. The soil was backfilled again in 0.15 m increments maintaining placement order. Each 0.15 m layer was carefully leveled, smoothed, and compacted to achieve the original soil bulk density.

The eight PCAP lysimeters are designated by the "L" prefix followed by their designation number. The experimental plots were designated by the manure source applied, which were hoop barn manure (Hoop plot), static pile manure (Pile plot), liquid MSP manure (MSP plot), and no manure (Control plot). Corn was also planted to these plots. In addition, eight wells were installed around the farmstead at locations close to potential E2 sources and near the plot study (Fig. 1). The well screening was made of

polypropylene, which does not sorb E2, and installed at depths between 2 and 3 m.

Six 8 cm long cylindrical (5.5 cm inside diameter) soil cores were taken to determine soil hydrological properties. Two cores were taken from the surface of the MSP plot and two from the surface of the Hoop plot. Two other cores were taken at depths of 0.30 m and 0.60 m outside the plot boundaries. Tempe pressure cells (Soilmoisture Equipment Corp., Goleta, CA) were used to determine volumetric water content (θ) as a function of soil water potential (ψ) between 0 kPa and −100 kPa (i.e. $\theta(\psi)$). The saturated water content (θ_s) was determined to be the porosity of the soil core. Pressure cells (Soilmoisture Equipment Corp., Goleta, CA) were used to determine this water retention function for the ψ ranges of −500 kPa to −1500 kPa. The van Genuchten water retention function parameters (residual water content (θ_r), α, and n) were obtained using the parameter optimization model RETC (van Genuchten et al., 1991). Also, the neural network program Rosetta (Schaap et al., 1998) was used to estimate the saturated hydraulic conductivity (K_s) from the following soil data: % sand, % silt, % clay, bulk density, θ (−330 kPa), θ (−1500 kPa), θ_r, θ_s, α, and n.

2.3. Manure application

Manure mixtures consisting of the hoop barn manure, static manure pile, and liquid MSP manure were obtained on-site from the farming operation (Fig. 1) and applied on 23 May 2003. Before application, manure sources were assessed for water-extractable E2 content. For a yield goal of 9416 kg ha^{-1}, the N recommendation for corn was 212 kg ha^{-1} (Rehm et al., 1995). This amounted to 0.021 kg m^{-2} or 200 g of N per 9.3 m^2 test plot. Manure management guidelines (Schmitt, 1999) recommended an application rate of 2.4 kg m^{-2} for the hoop barn manure and static pile manure and 12.2 L m^{-2} for MSP liquid manure. Using these application rates 2534, 72, and 205 ng of E2 was applied to the MSP (L01 and L02), Hoop (L03 and L04), and Pile (L05 and L06) plots, respectively. No manures were applied to the Control plot. The liquid MSP manure application also supplied an additional 10 mm of water to the MSP plot, which was recorded as a 1 d irrigation event for the water balance computation.

2.4. Tracers

Conservative, non-sorbing fluorobenzoic acids (FBAs) were used as tracers on the experiment plots to follow the downward movement of water and to serve as reference solutes for mass transport. The transport of these tracers would be similar to that of an anion such as bromide or chloride (Jaynes, 1994). These FBAs are resistant to chemical and microbial transformations and possess many of the properties required of non-reactive soil and groundwater tracers (Bowman, 1984). Moreover, there are multiple FBA tracers that have identical transport properties, but can be distinguished from one another using liquid chromatography (Jaynes, 1994). Three FBA derivates (Oakwood Products Inc., West Columbia, SC); *m*-trifluoromethylbenzoic acid (TFMBA), pentafluorobenzoic acid (PFBA), and difluorobenzic acid (DFBA); were applied to the experimental plots on the same day as the manure application, 23 May 2003. The three different tracers were applied to determine whether lateral transport between plots occurred. Tracers application amounts were equivalent to the electrolyte concentrations of the applied fertilizers, and were comparable to those amounts applied by Bowman and Gibbens (1992) in their vadose zone tracer test. The TFMBA was applied at a concentration of 0.06 M, PFBA at 0.27 M, and DFBA at 0.28 M. Tracer mixtures were evenly applied to the test plots in 2 L batches using a galvanized steel watering can. The application of the FBA tracer solutions was easily taken up by the soil and no runoff was noted.

Leachate from the PCAPS collection cylinder was collected using a peristaltic pump. The pump and tubing was rinsed with deionized water prior to each sampling, and approximately 50 mL of leachate was flushed through the pump tubing before sampling. Well sampling protocol was similar regarding the purging and flushing; however, polypropylene bailers were used instead of the peristaltic pump. Water samples were collected in 250-mL polypropylene bottles for E2 analysis and 10-mL glass scintillation vials for tracer analysis. Collection containers were made of materials non-sorbing to E2 or FBAs. Water sampling was more frequent during the late spring (April–June) because of low actual evapotranspiration (ET_c) and higher precipitation. Sampling frequency was weekly during the late spring and after heavy rain events. At the end of the season frequency decreased to bi-monthly.

2.5. Sample analysis

Manure and compost extracts were prepared according to the method discussed by Nichols et al. (1998) by adding 5 g of material to 100 mL of water. The source manure samples were extracted for 2 h and then centrifuged for 15 min in 50 mL centrifuge tubes at 2000 rpm (1124*g*). The aqueous layer was decanted and used for all assays. For the MSP liquid manure, a 5 mL aliquot was considered an aqueous extract. The extracts were individually prepared via a solid phase extraction method (Waters Corp., 2002) with Oasis HLB solid phase extraction (SPE) cartridges (Waters, Milford, MA), and E2 was eluted in 2 mL of methanol. Samples were dried and then reconstituted in 1 mL ethanol. A di-deuterium-labeled E2 internal standard was added to the final extract from the HLB SPE cartridge (10 pg μL^{-1}). Samples were separated into 10 μL injection volumes and analyzed in terms of picograms per injection. Samples were analyzed by negative-ion electrospray mass spectrometry. A Waters Q-TOF Ultima API-US, Quadrupole-Time of Flight mass spectrometer (Waters, Beverly, MA), equipped with an electrospray ionization source was used to characterize isolated metabolites. Analyses were performed in negative-ion mode for E2 samples. The capillary voltage was 2.33, cone voltage was 55, source and desolvation temperatures were 120 and 400 °C, respectively, and cone and desolvation gas flows (L h^{-1}) were 0 and 500, respectively. The LC system was an Alliance 2695 Separation Model (Waters) equipped with a 25 μL syringe and 100 μL sample loop. The column used was a Symmetry C18, 3.5 μm, 2.1 × 100 mm with a 2.1 × 10 mm guard column containing the same packing material (Waters). The initial mobile phase for E2 extracts consisted of 60% 95:5 Millipore water:acetonitrile (solvent A) and 40% acetonitrile (solvent B). Linear gradients to 100% B were used from time 0 to 10 min, an initial and final hold for 5 and 1 min, respectively, and a return to starting conditions over 5 min. The flow rate of the mobile phase was 0.2 mL min^{-1}. For lysimeter leachate analysis, a 100 mL aliquot was considered an aqueous field extract and analyzed identically to the compost, raw manure, and lagoon samples previously described.

A high performance liquid chromatographic (HPLC) analysis method was used to simultaneously quantify TFMBA, PFBA and DFBA (Lee and Casey, 2005), which was a variation of the procedure described by Bowman (1984). The instrumentation consisted of a Waters 600E Solvent Delivery System, Waters 717+ autosampler, Waters 746 data module integrator, and a Jasco FP-920 205 nm wavelength detector. The separation column was Alltech (Deerfield, IL) with Allsphere SAX 5 μm packing (250 × 4.6 mm). An injection volume of 10 μL was used for all chromatographic analyses. The mobile phase was 30 mM KH_2PO_4 buffer, pH adjusted to 2.85 with H_3PO_4, with 20% acetonitrile (v/v) as an organic modifier. The flow rate was 1.0 mL min^{-1}. The detection wavelength was 205 nm. Analyte concentrations were determined by comparisons of peak heights to those of external standards.

2.6. Water transfer and modeling

To evaluate the soil water flux measurements from the PCAPs, HYDRUS-2D (Šimůnek et al., 1996) was used to compute a predicted seasonal water balance for each lysimeter. Two hydraulic layers of soil were simulated and correspond to 0–0.30 m and 0.30–0.60 m depth. A third hydraulic layer was included to represent the influence of the wick that has associated hydraulic properties reported by Knutson and Selker (1994). Daily precipitation and ET data were used as the upper boundary conditions at the soil surface for the predicted simulations from April 10 to August 31. The Penman–Monteith method was used to calculate the potential evapotranspiration (ET_o; mm d^{-1}) (Allen et al., 1998). Values of ET_c (mm d^{-1}) were then obtained by multiplying ET_o with a crop coefficient (K_c). Empirically determined K_c values for corn versus cumulative growing degree days (GDD) were obtained from Southeastern North Dakota (Steele et al., 1996). There was significant spread in K_c versus GDD data, therefore a fifth-order polynomial was fit to this data and lower and upper 95% confidence intervals (CI) were used to obtain a range of K_c values for any given GDD. These best-fit and lower and upper 95% CI K_c values provided a range of ET_c estimates for calculating the predicted water flux. The idealness of the PCAP was then finally evaluated using leachate collection efficiency (LCE) calculations (Zhu et al., 2002). Lysimeter LCEs were determined by dividing the actual measured drainage (AD) by the predicted drainage (PD) during the same time period (LCE = AD/ED × 100).

2.7. Dissolved organic carbon analysis

Dissolved organic carbon (DOC) was determined for well samples using Standard Methods (APHA et al., 1998) with a total organic carbon analyzer (Phoenix 8000, Emerson Process Management: Tekmar-Dohrmann Division, Mason, OH, USA). The DOC analysis was done only on samples after the E2 analysis was completed, and if sufficient volume was available. Before analysis, all samples were first passed through a glass microfiber, 0.45 μm pore-size membrane filter (Whatman).

2.8. Statistical analyses

Lysimeter data was statistically analyzed by analysis of variances (ANOVA) and standard least-squares models. Components of the statistical models included treatment (i.e. plot), lysimeter, precipitation, drainage, depth to water table, and time. When the ANOVA revealed a significant difference in least-squares means,

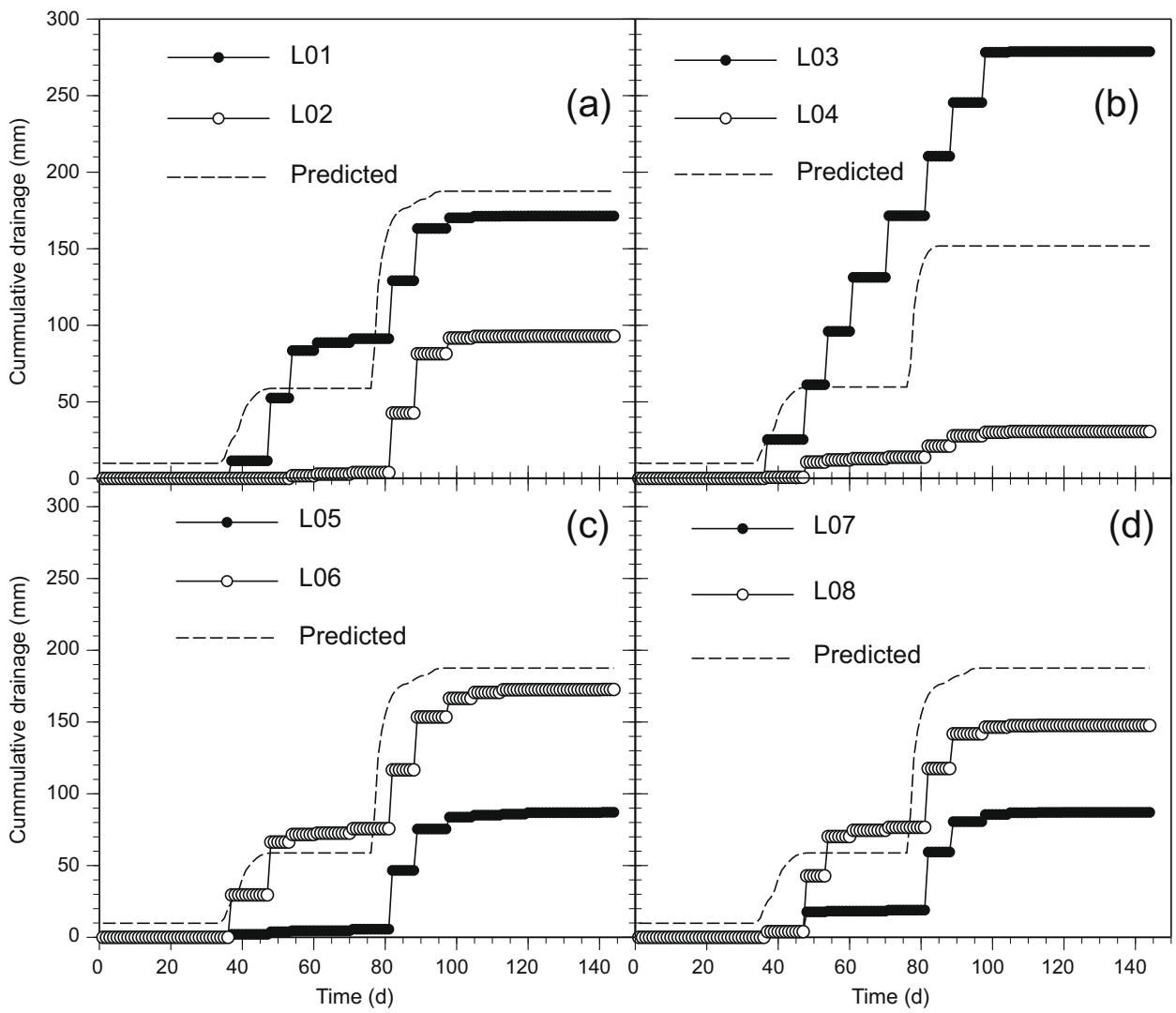

Fig. 2. Time series of measured cumulative drainage and predicted cumulative drainage in the lysimeters for the manure storage pit (MSP) plot, the Hoop plot, the Pile plot, and the Control plot. The time zero demarks the day of lysimeter installation.

Table 1
Drainage amounts and leachate collection efficiencies (LCE) per lysimeter for three different actual evapotranspiration (ET_c) scenarios. The three ET_c scenarios were calculated using crop coefficients (K_c) obtained from "best-fit" and "upper" and "lower" 95% confidence interval fit of K_c versus cumulative growing degree day curves.

Lysimeter ID	Cumulative expected drainage "best-fit" (mm)	Best-fit LCE (%)	Cumulative expected drainage "upper limit" (mm)	Upper limit LCE (%)	Cumulative expected drainage "lower limit" (mm)	Lower limit LCE (%)
L01	187	92	143	120	274	63
L02	187	50	143	65	274	34
L03	151	185	99	282	273	102
L04	151	20	99	31	273	11
L05	188	46	145	60	274	32
L06	188	92	145	119	274	63
L07	188	46	145	60	274	32
L08	188	79	145	102	274	54
s^2[a]		27		34		19
$\bar{x}$[a]		61		80		41
CV[a]		45		43		47

[a] Lysimeter L03 leaked, and was excluded from the calculation of standard deviation (s^2), average ($\bar{x}$), and coefficient of variation (CV).

then a Tukey–Kramer honestly significant difference (HSD) test for multiple comparisons was performed at $\alpha = 0.05$. Principal Components Analysis (PCA) was also performed, where the first and second principal components (PC1 and PC2, respectively) were used for further correlation analysis. The Spearman's ρ correlation analysis was used in case of non-parametric data distributions. Additionally, bivariate simple linear regression was used to determine any statistical relationship between E2 and DOC in well samples. Significance was accepted at probabilities (p) of 0.05 or less for all analysis. All statistical analyses were conducted using JMP IN statistical software (ver. 5.1.2, SAS Institute, Cary, NC).

3. Results and discussion

3.1. Water transfer

Except for L03, the drainage collected from all lysimeters ranged from 8% to 46% (average = 30%) of total precipitation, which agreed with other studies that used PCAP lysimeters (Brandi-Dohrn et al., 1996; Zhu et al., 2002). There was evidence for flooding in lysimeter L03 because the drainage pattern was independent of the expected pattern and the drainage patterns of the other lysimeters (Fig. 2). The least-squares statistical model indicated that the effects of lysimeter ($p < 0.0001$) and time ($p < 0.0001$) were significant in explaining cumulative drainage, and L03 was significantly greater than all other lysimeters. This significant difference was attributed to water leaking into the collection vessel, which was at a depth (between 1.55 and 1.83 m) that was below the water table for a portion of the study.

The amount of water collected per lysimeter per sampling event correlated well with the precipitation pattern, where correlation coefficients ranged from 96% to 99% (average = 97%). Also, least-squares statistical analysis indicated that precipitation ($p < 0.0001$) and depth to water table were significant, but not lysimeter ($p = 0.2013$), in explaining drainage per sampling event when leaky L03 was excluded. Although there was no significant difference, variability in drainage collected between lysimeters per event was high, with coefficient of variations (CV) ranging from 35% to 265% (avg. = 108%). This variability suggested that certain precipitation events may have caused different lysimeters to respond differently, which may result from factors such as disturbance when the lysimeters were installed (Brandi-Dohrn et al., 1996), water table variability, textural differences, or variation between PCAPs.

The LCEs reported in Table 1 correspond to ET_c scenarios for three K_c values (Steele et al., 1996) that represent the best-fit, the upper 95% CI, and the lower 95% CI of the crop coefficient curves. These LCE values, excluding the leaking L03, were similar to values reported by Brandi-Dohrn et al. (1996) for PCAPs collecting soil water over a similar duration of time, 155 d. Variability in LCE between adjacent lysimeters (Table 1) may be attributable to several factors. Ambient soil ψ values that exceeded the maximum ψ value of the wicks (−5.3 kPa) would have created diversion of the drainage water especially during high ET crop growth stages. The wicks will not draw water when it is held at ψ values less than −5.3 kPa of water. Additionally, local variation in the water table and capillary fringe may have influenced water sampled by the lysimeter, where groundwater would be sampled by the lysimeter

Table 2
Tracer recoveries from the lysimeters for *m*-trifluoromethylbenzoic acid (TFMBA), pentafluorobenzoic acid (PFBA), and difluorobenzoic acid (DFBA).

Treatment	Lysimeter	Tracer applied	Mass of tracer applied per lysimeter (g)	Mass of tracer recovered (g)	Percent tracer recovery (%)	First arrival of tracer (d)	Transport rate (cm d^{-1})
MSP	L01	TFMBA	23.30	2.31	9.91	4	15.3
MSP	L02	TFMBA	23.30	4.78	20.52	10	6.1
Hoop	L03	DFBA	0.90	0.0028	0.31	4	15.3
Hoop	L04	DFBA	0.90	0.0003	0.03	4	15.3
Pile	L05	PFBA	1.21	0.024	1.98	4	15.3
Pile	L06	PFBA	1.21	0.0012	0.10	4	15.3
Control	L07	PFBA	1.21	0.1385	11.45	38	1.6
Control	L08	PFBA	1.21	0.147	12.15	10	6.1
s^2[a]				1.8439	7.65	11.73	5.7
$\bar{x}$[a]				1.0573	8.02	9.75	11.3
CV[a]				174%	95.43	120%	50%
r[b]				−0.15 (−0.12–−0.17)	0.13 (0.11–0.14)		

[a] Lysimeter L03 leaked, and was excluded from the calculation of standard deviation (s^2), average ($\bar{x}$), and coefficient of variation (CV).
[b] Correlation coefficient (r) calculated for the best-fit lysimeter collection efficiencies (LCE; Table 1) and percent tracer recovery. Values inside parenthesis represent the same calculation for the lower and upper LCEs. Lysimeter L03 LCE was inaccurate due to leakage and was excluded from the r calculation.

and thus over estimating the amount of water due to percolation from the surface. The high LCE of the leaky PCAP, L03, demonstrated how water from below could exaggerate the LCE value.

3.1.1. Tracer transport

The range of tracer mass recoveries (Table 2) was low compared to the Brandi-Dohrn et al. (1996) study, which reported Br^- recoveries from PCAPS that ranged from 5.3% to 76.6%, with a mean of 29%. The low mass recoveries in the Brandi-Dohrn et al. (1996) study were attributed to lateral water movement processes. Chromatographic results of this study indicated that TFMBA had contaminated all the input solutions that were applied to the plots. Thus lateral water movement processes causing cross migration of TFMBA from the Hoop plot to the other lysimeters could not be ruled out. However, no other FBA tracer was detected in the lysimeter leachate that was applied on adjacent plots, thus indicating no lateral transport of FBA between plots and minimal lateral water transfer. Furthermore, the low recoveries of this study compared to others, could be partially attributed to the lower duration of leachate collection. Lysimeters were sampled for 144 d in this study compared to 1 yr by Brandi-Dohrn et al. (1996).

Fluorobenzoic acid tracer mass recovery was not correlated with LCE (r values in Table 2). Based on this weak correlation, FBA recovery did not appear to be a useful way to estimate lysimeter efficiency. For lysimeter L03, the lower mass recovery (Table 2) together with the greatest LCE (Table 1) was a further indication that this lysimeter leaked. If this lysimeter, L03, had a high LCE, then more tracer mass recovery would be expected. Additionally, on 30 June, the distance to water table was at its minimum and corresponded to an increase in FBA concentrations for all lysimeters except L03, which decreased (Fig. 3). The increase in FBA tracer, except in L03, was attributed to the advective transport from large amounts of precipitated water. The corresponding decrease in FBA tracer concentration in L03 on 30 June was attributed to

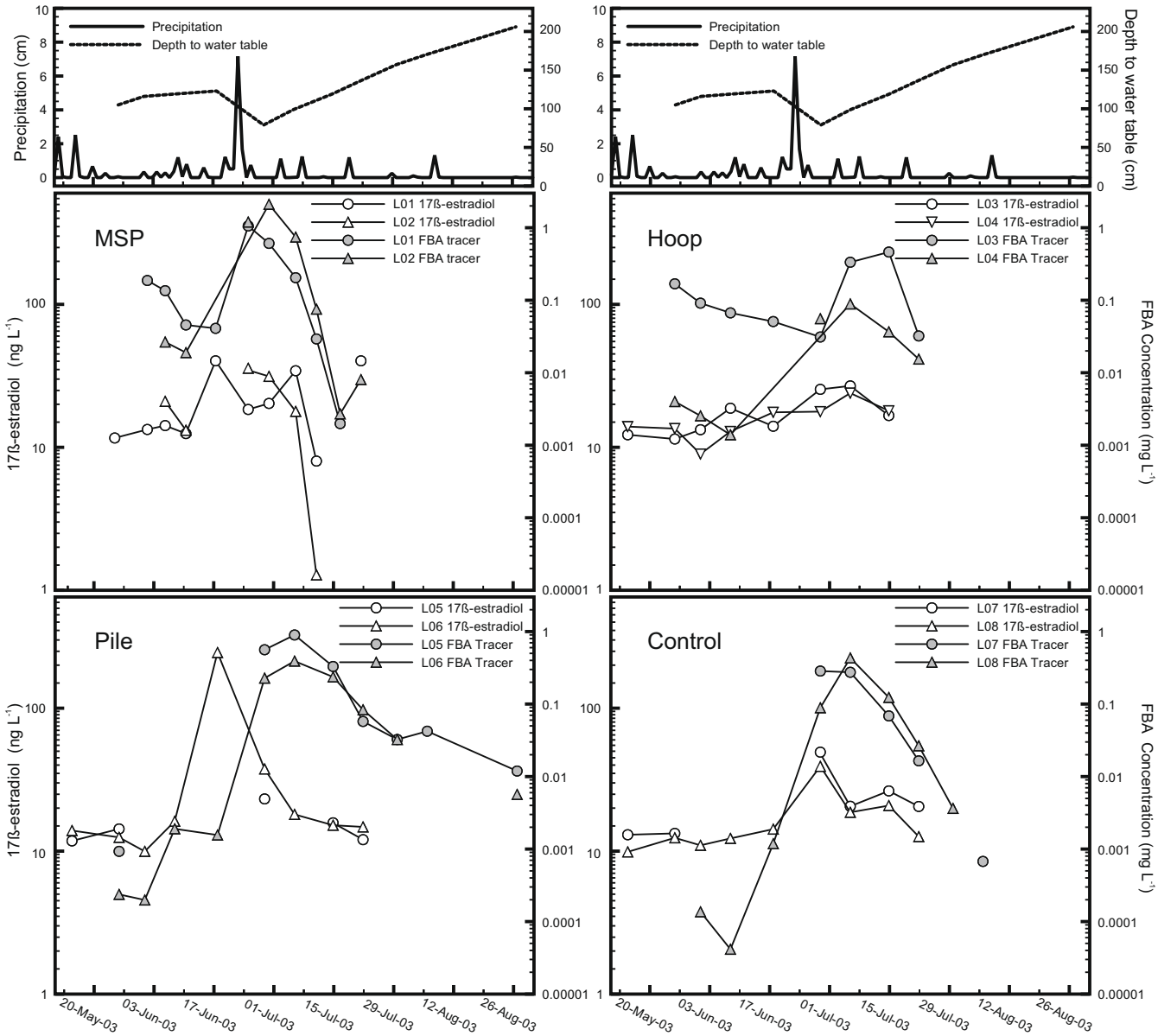

Fig. 3. The top two figures show the time series of precipitation and depth to the water table. The other figures are time series of 17β-estradiol and flurobenzoic acid (FBA) tracer concentrations measured in the two lysimeters installed beneath each of the treatment plots (MSP, Hoop, Pile, and Control).

dilution from subsurface water leaking into the collection container, which was under the water table at this time (Fig. 3). When L03 was removed from statistical analysis, the least-squares model determined precipitation ($p = 0.0084$), drainage ($p = 0.0039$), and depth to water table ($p = 0.0015$) were significant in explaining FBA detection, but not lysimeter, indicating no significant difference between lysimeters. Similar to the drainage, variability in tracer concentrations measured between lysimeters at each sampling event was high, where the CVs ranged from 65% to 256% (avg. = 163%). Again disturbances when the lysimeters were installed, water table variability, textural differences, or differences between PCAPs may have caused this variation.

3.1.2. 17β-Estradiol detections

Compared to the tracer mass recoveries, the E2 recoveries were high, based on E2 applied with the manure (Table 3). The average E2 mass recovery was nearly three times greater than the mass of E2 applied. The sum total of E2 in both lysimeters under the MSP, Hoop, Pile, and Control plots was 402, 521, 513, and 568 ng, respectively, which was a small variation considering the low part per trillion detection level. Least-square analysis showed that depth to water table was significant ($p < 0.0001$) but not precipitation ($p = 0.356$) and drainage ($p = 0.144$) in explaining E2 detections in the lysimeters. Also, there were no significant differences between lysimeters for E2 detections. The variation of E2 concentration detection across lysimeter per each sample event ranged from 7% to 141% (avg. = 53%). This variation in E2 concentration per sample event was lower compared to drainage collected ($35\% \leqslant CV \leqslant 265\%$; avg. = 108%) and tracer detections ($65\% \leqslant CV \leqslant 256\%$; avg. = 163%), which indicated a relative uniform input of E2 into all the lysimeters. Furthermore, the percent E2 mass recoveries were not related to lysimeter LCE, which was indicated by low correlations with the LCE (Table 3). However, the mass of E2 collected was well correlated to the lysimeter LCE (Table 3), which indicated that the more water collected corresponded to more E2 detected. Unlike the E2, the mass of FBA tracer collected was not correlated to lysimeter LCE (r values in Table 2), which suggests that there was an additional source of E2 not from surface applied manure.

17β-Estradiol concentrations were measured in all lysimeters except L03 on 16 May, before manure was applied, and in the Control plot where no manure was applied (Fig. 3). 17β-Estradiol was also consistently detected in all lysimeters at a depth of 0.61 m throughout the duration of the experiment, and recoveries were above 100% in many of the lysimeters (Table 3). Furthermore, there were three E2 detections of 64, 41, and 34 ng L^{-1} in the well closest to the plots on 27 May, 19 June, and 30 June, respectively, when the depth to water table was between 1.65 and 2.11 m. For all eight wells installed on the farmstead (Fig. 1), there were 27 E2 detections ranging from 16 to 100 ng L^{-1}, with an average of 48 ng L^{-1}. These lysimeter results were dissimilar to many laboratory studies that have shown E2 to degrade rapidly (Colucci et al., 2001; Casey et al., 2005; Fan et al., 2007a) and its transport is minimal (Lee et al., 2003; Das et al., 2004; Fan et al., 2008). However, other studies have shown that E2 can have moderate mobility and persistence in the field soil where animal manure management has occurred (Herman and Mills, 2003; Kjaer et al., 2007; Arnon et al., 2008). For example, Herman and Mills (2003) frequently detected E2 concentrations (range = 23–200 ng L^{-1}) similar to values of this study, in lysimeters at depths between 0.15 and 0.91 m.

The dominant processes that controlled FBA transport to the lysimeters seemed to be distinct from processes controlling E2 detections. The FBA detections were variable and significantly related to precipitation and drainage. However, the E2 detections were less variable and only significantly related to the depth to water table. These results indicate a process where precipitated water infiltrated and advectively transported the FBA tracer from the surface down, whereas E2 detections appeared to be determined by another source either resident at the depth of the lysimeter and/or perhaps from below. Use of principal component analysis on E2 and FBA concentrations, depth to water table, precipitation, and lysimeter drainage suggested other possible processes that may control E2 concentrations. Eigen values were greater than one for PC1 and PC2, and 69% of the data variation was explained in the first two PCs. The E2 concentrations were inversely correlated to the depth to water table (−0.64) and directly correlated to drainage (0.55). This suggests that the detections of E2 were related to the water status, as indicated by the relationship with water table and drainage, and likely points to another source of E2 other than the applied manure.

One potential source of the E2 was antecedent from natural historic wildlife or domestic animal manure inputs. This antecedent E2 could persist as a result of minimal microbial activity and/or colloid bound E2 (Holbrook et al., 2004; Fan et al., 2008). Also, dissolved or colloidal fractions of soil and manure can potentially lead to greater mobility of hormones (Bradford et al., 2003). It was hypothesized that the E2 detections in the lysimeters were related to the water table depth because of mobile and persistent antecedent E2. A relationship was found between aqueous E2 concentrations and DOC in the well water. There was insufficient sample for DOC analysis of the 2003 samples, during which the lysimeter study was conducted, therefore the DOC and E2 relationship was developed for 61 well samples taken in 2004. The

Table 3
Summary of 17β-estradiol recovery.

Treatment	Lysimeter	Mass of 17β-estradiol applied to lysimeters (ng)	Total 17β-estradiol recovered (ng)	Percent 17β-estradiol recovered (%)
MSP	L01	2534	282	11
MSP	L02	2534	120	5
Hoop	L03	72	471	654
Hoop	L04	72	50	69
Pile	L05	205	111	54
Pile	L06	205	402	196
Control	L07	0	272	–[a]
Control	L08	0	296	–
s^2[b]			145	77
$\bar{x}$[b]			193	67
CV[b]			75%	115
r[c]			0.86 (0.85–0.86)	0.31 (0.30–0.32)

[a] The designation of "–" indicates that division of zero error in the percent mass recovery calculation (percent mass recovered = (mass recovered)/(mass applied) × 100.
[b] Lysimeter L03 leaked, and was excluded from the calculation of standard deviation (s^2), average ($\bar{x}$), and coefficient of variation (CV).
[c] Correlation coefficient (r) was determined between the best-fit lysimeter collection efficiency (LCE; Table 1) and the mass or % 17β-estradiol recovered. Values inside parenthesis represent the same calculations but for the "lower" and "upper" LCEs (Table 1). Lysimeter L03 LCE was inaccurate due to flooding and was not used to calculate r.

distributions of DOC and E2 were lognormal. A bivariate correlation test indicated a correlation of 0.41 and the effect of the regression was significant ($p = 0.0003$), indicating a significant relation between log-transformed values of DOC and E2. This relationship suggests that E2 has the potential to be bound to DOC, which can facilitate its mobility and perhaps its persistence. Water either percolating down from the surface or moving up from the water table would mobilize antecedent E2 that was colloidally bound. Subsequently, the mobilized colloid-bound E2 could be rapidly transported and sampled in the lysimeters effluent. This hypothesis may explain the correlation of E2 detections with lysimeter drainage and water table. Additionally, the lower oxygen levels associated with the more saturated conditions would decrease E2 degradation thus increasing its persistence (Fan et al., 2007a; Kjaer et al., 2007).

4. Conclusions

Lysimeter plot studies were conducted to identify the fate and transport of manure-borne E2, from different manure types. This type of information is important from a management perspective in order to quantify the potential contributions of E2 from animal feeding operations. Results of this study indicated widespread detections of antecedent levels of E2, which diminished the significance of the surface manure management effects on E2 concentrations. 17β-Estradiol was shown to be mobile and persistent throughout the duration of the experiment, which was in contrast with other laboratory studies but consistent with some field observations. A relationship was observed between E2 and DOC, which could potentially explain its greater than expected mobility and persistence. Still, the extent of E2 spatial distribution is unknown and needs to be contrasted against pristine areas that have not received animal manures or are away from animal operations. Although the persistence and mobility of E2 appeared to be related to DOC, the exact cause is still not known and further controlled experiments are required.

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