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IN SUBSURFACE WATERS**

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**North Dakota Water Resources Research Institute
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ABSTRACT

17ß-estradiol (E2) is a natural estrogenic hormone found in animal manure and urine. It has been reported to dissipate rapidly in soil laboratory studies but is frequently detected in the environment at concentrations that could potentially have adverse affects on wildlife. The objective of this study was to assess the persistence and detections of manure-borne E2 in soil on a farm-scale. Soil cores were taken from various locations at a swine (*Sus scrofa domesticus*) farm over 2006 and 2007. The producer applied liquid manure slurry to one of the selected sites each year. Cores were taken to the depth of the water table and separated into 15-cm increments. Porewater was extracted and analyzed for E2, using a combination of liquid chromatrography and tandem mass spectrometry (LC/MS-MS). Estradiol was detected at all sites but was highly variable. Estradiol was detected in 249 out of 589 extractions (42.3%), and concentrations ranged from 0 to 733 ng-E2 kg^{-1}-dry soil (or 0-7,712 ng L^{-1} porewater equivalents). Analysis of variance indicated a significant difference between sites and time ($p \leq 0.05$). Beginning in Spring 2006, concentrations trended upward, reaching a maximum in Spring 2007, and then declined. Manure slurry surface application did not appear to have a significant affect on the amount of E2 extracted. A similar range of concentrations was detected on all sites, and trends for both manure-treated and non-treated sites appeared related to climate, possibly to precipitation. Where E2 was detected, highest concentrations favored the upper profile, while the greatest frequency of detections was in the lower profile and near the water table. After removing the effects of space and time, analysis of covariance indicated significant effects from the following soil properties: percent organic matter ($p<0.007$), clay content ($p<0.03$), and sand content ($p<0.003$). However, relationships appeared bifurcated between the upper and lower portions of the soil profile, with E2 decreasing with soil coarseness in the upper 0.75 m and decreasing with coarseness in the lower 0.75-2 m. The presence of organic material appeared to affect the persistence of E2 in the upper 0.6 m, while the presence of the water table seemed influential at lower depths.

ACKNOWLEDGEMENTS

INTRODUCTION

17ß-estradiol (E2) is a natural estrogenic hormone found in animal manure and urine. While it has been reported to dissipate rapidly in soil laboratory studies (Casey et al., 2003; Colucci and Topp, 2003; Colucci et al., 2001; Das et al., 2004; Jacobsen et al., 2005; Lee et al., 2003), it is frequently detected in the environment at concentrations that could potentially have adverse

affects on wildlife (10 ng L^{-1}) (Thorpe et al., 2003). These effects have been well documented and are generally indicated by feminization of male wildlife species and abnormal reproductive development, such as the production of vitellogenin (an egg yolk precursor protein) and other physiological alterations (Irwin et al., 2001; Jacobsen et al., 2005; Purdom et al., 1994). The hormone is moderately hydrophobic with a water solubility of around 13 mg L^{-1} (Shore and Shemesh, 2003; Lai et al., 2000) and has been shown to sorb readily onto clays and organic matter (Das et al., 2004; Hanselman et al., 2003; Lee et al., 2003; Shore and Shemesh, 2003). Common pathways to the environment include effluent from sewage treatment plants and the application of animal manures and municipal sewage sludge as fertilizers (Lai et al., 2000). There has been increasing concern over the toxicological effects of E2 with the growth of concentrated animal feeding operations (CAFOs), where concentrated amounts of animal waste are often disposed of on limited agricultural fields.

BACKGROUND

In 2003 Thompson (2005) conducted a field experiment in Ransom county, North Dakota to measure the movement and persistence of 17ß-estradiol (E2) under different applications of swine manure in four different plots (lagoon slurry, raw manure, compost, and control). Thompson used passive capillary samplers (PCAPS), or fiberglass wicking pan lysimeters (Holder et al., 1991), placed at a 61-cm depth under each of four plots to collect water samples and measure dissolved E2. He found that E2 peaked twice: first in late June, with a mean maximum concentration of about 400 ng L^{-1} and a second time in late July with a mean maximum concentration of about 150 ng L^{-1}. There was considerable variability in E2 measurements and times between individual plots. One surprising outcome of Thompson's finding was that E2 was detected in an untreated plot at concentrations similar to those of the treated plots. This raised questions of what could be the possible sources or processes leading to the detections in the non-treated plot. This was particularly concerning because it was assumed that the main source of E2 was from the manure applications.

In 2006 and 2007, this study was initiated to further investigate the findings of Thompson on a more extensive farm-scale. Five locations in 2006 and six locations in 2007 were selected for seasonal stratified soil and porewater samples, from the same swine farm as Thompson's study. Sites sampled included two sites with recent swine manure slurry treatments and four sites with no known recent history of manure application. The soil core sites were chosen based on proximity to surface conditions seen as possible estrogen sources, i.e. next to an uncovered compost heap, near a holding pond for lagoon slurry, and in a field injected with lagoon material.

OBJECTIVES

This study was conducted to:
(1) Determine whether the previous 2003 lysimeter study's findings were unique or widespread
(2) Investigate the effects of manure slurry application on E2 in porewater
(3) Assess the persistence and detections of E2 on a farm-scale
(4) Investigate the potential sources and/or site characteristics contributing to detections of E2 in soil porewater (including soil properties and climatic effects)

MATERIALS AND METHODS

Site Description

Research was conducted at a swine (Sus scrofa domesticus) farm located near Milnor in Southeastern ND. The facility accommodates around 4,000 swine of varying type (i.e. gestation, farrowing, nursery, and finishing) at any given time. Based on their weights, the animals are relocated from a series of nurseries and confined pens, and later to hoop barns. At each nursery and pen various diet formulas are given to the pigs, based on their weight and nutritional needs. In the hoop barns the pigs are held until they achieve market weight. Animals are kept in nursery and confined pens with slotted flooring, which allows urine and feces to drop through and be held in a cistern below the barns. Periodically, plugs that hold the liquid manure in these sub-pen tanks are pulled, which allows the manure to be pumped or drained by gravity into a large earthen manure storage pit (MSP). The MSP is about 3 m deep with an area of 47 x 34 m and is constructed above natural land-level due to the sandy soils. Liquid manure from sub floor pits in the barns is generally emptied into this MSP on a monthly basis. When the MSP becomes full, the liquid slurry is then applied as fertilizer to nearby fields, either by injection beneath the upper 15-cm of soil, or by direct surface application. The finishing barns are un-insulated structures built on compacted earth that is covered with bedding of straw or wood shavings. The animals eat on one side of the barns and deposit their manure on the other side. Twice a year dry matter, consisting of the bedding/manure mixture from the barns, is scraped into an uncovered static manure pile (i.e. it is not rotated) atop bare soil east of the facility.

In previous work by Thompson (2005), four passive capillary samplers (PCAPS) were installed approximately 61 cm beneath the soil surface under four square 9.29 m^2 experimental plots (Holder et al., 1991); soil water leachate was collected from these lysimeters in 2003 and tested for hormones. Nine regularly monitored shallow water wells were also in place on this site. Water table elevations and concentrations of testosterone and 17ß-estradiol were measured in the wells. The wells were placed in the shallow zone of the Milnor Channel aquifer. According to a North Dakota State Water Commission Office memo, the Milnor Channel aquifer is a shallow unconfined aquifer, which receives recharge from local infiltration and runoff of precipitation and snowmelt, and underflow from undefined aquifers upland to the west. In some places the water table and root zone overlap, thereby constraining water table gradients by evapotranspiration (Shaver, 1999). Hydraulic conductivities range from 10.7 to 210.3 m day^{-1} with a mean value of 68.6 m day^{-1}. Groundwater at the study flows in a southeasterly direction.

Weather data obtained from the North Dakota Agricultural Weather Network (NDAWN) weather station for the town of Wyndmere, ND, about 25 km from the study area, indicate a mean annual precipitation of 33.2 mm for 2006 and 47.5 mm for 2007. The average monthly temperature was 13.9ºC for the combined months of April through November for both 2006 and 2007 (Enz et al., 1989).

Soil Description

Soils at the study site are predominantly loamy sands belonging to the Hecla (Sandy, mixed, frigid Oxyaquic Hapludoll), Garborg (Sandy, mixed, frigid Typic Endoaquoll), Wyndmere (Coarse-loamy, mixed, superactive, frigid Aeric Calciaquoll), and Ulen (Sandy, mixed, frigid Aeric Calciaquoll) Soil Series. These soils are of glaciofluvial and glaciolacustrine origins. Soil characterization is based on soil profile descriptions from each of the sites, soil survey information, and laboratory analysis. The overall textural breakdown of the samples, according to particle size analysis and USDA textural delineations, is approximately 47% loamy sands, 26% sands, 20% sandy loams, and less than 1% of the following: clay, loam, sandy clay, sandy clay loam, silt loam. Percent organic matter decreases linearly with depth in the top 0.6 m of soil, from 2.14% in the top 15-cm to 1.60%, 0.91%, and 0.65%, respectively, in the following 15-cm increments. Thompson (2005) estimated the following soil properties using the Rosetta program (Schaap et al. 1998) and actual cores collected from the area: Saturated water content (vol.%) range=0.33-0.48 cm^3 cm^{-3}, residual water content (vol.%) range=0.024-0.027 cm^3 cm^{-3}, saturated hydraulic conductivity (cm d^{-1}) range=22.40-140.85. Soils in this area are aquic and exhibit redoximorphic features (i.e. faint mottling) within 0.16 m of the soil surface, suggesting large fluctuations in the water table. These high water tables are more prevalent in the spring when the temperatures are still low.

Sampling protocol

Soil samples were collected from various locations on the farm (Fig. 1). Sites were chosen based on nearness to surface conditions likely to provide an estrogen source, e.g. next to the static manure pile, near the MSP, and in fields where MSP material was applied as fertilizer. One site, labeled the State Well site (W) had no known manure application history. A total of six sites were chosen and labeled according to their associated surface features (Table 1).

Duplicate core samples were collected from each site except for fields with manure application during 2006 and 2007, where four sets of cores were collected instead of two. This was done, because a primary interest was whether or not the manure fertilizer application could be affecting E2 concentrations reaching subsurface waters. Additional replicates allowed more degrees of freedom in the statistical analysis. Samples were collected in the spring, summer, and fall of 2006 and the spring and fall of 2007.

Table 1. Site names, abbreviated symbols, and brief descriptions.

Site	Abbrev.	Description
Compost	C	Directly next to the static manure pile
Lysimeter	LS	Next to the lysimeters
Lagoon	LG	Field adjacent to the lagoon holding pond
State Well	W	Hutterite field next to a state well with no known manure application history
2006 Injection	INJ	Field where manure slurry was applied in 2006
2007 Injection	NF	Field where manure slurry was applied in 2007

Fig. 1. Diagram of 2006 and 2007 sample sites. Red circles indicate where a core was collected. Lagoon slurry was applied to different fields in 2006 and 2007.

Field Procedure

Core samples were collected from the designated plots using a Giddings Probe and 1.2-meter long, 56-mm diameter polyethylene liners. The ends were secured with plastic liner caps, and the cores were enclosed in pipe insulation to keep them cool and avoid photodegradation during transport. Bore holes were then filled with sand to about 15 cm from the surface; the remaining area was filled with bentonite. Locations were recorded for replication with a global positioning system (GPS) receiver. Cores were then transported to the USDA-ARS Biosciences Research Laboratory in Fargo, ND where they were refrigerated (approx. 2.5ºC) for up to one month until they could be sectioned, and then frozen (five days to three months) until extraction.

In a parallel study, manure samples were collected from different areas of the farm, to obtain input E2 concentrations. These concentrations were useful for a secondary experiment, described later, which applied E2 to soil columns within a range found in manure.

Laboratory Procedure

Cores were segmented into 15.2 cm increments. Individual samples were weighed, bagged, and mixed to promote homogeneity. Subsamples from each soil segment were used to determine soil moisture contents by oven drying and organic matter contents, using the loss-on-ignition method (Ben-Dor and Banin, 1989). Particle size analysis was performed, using a modified hydrometer method as described by Gee and Bauder (1986). Bulk densities were also calculated using the core method (Blake and Hartge, 1986). Additional cores were collected from each site in November of 2007 for soil descriptions.

Extraction Procedure

Porewater in the soil cores was extracted using a 0.01M $CaCl_2$ solution. Calcium was added to promote clay flocculation and provide a clear filtrate. One hundred grams of moist soil and 200 mL extracting solution were placed in a 500-mL Erlenmeyer flask and stirred vigorously for 20 minutes with a mechanical shaker. The mixture was refrigerated (≈2.5ºC) for one hour to allow particulates to settle, in order to improve the filtering time. Mixtures were gravity filtered through #2 Whatman (Maidstone, England) filter paper into 250-mL polyethylene Nalgene® brand bottles. Extracts were submitted to the USDA-ARS Biosciences Research lab for sample preparation and analysis using a combination of high performance liquid chromatography and tandem mass spectrometry (HPLC/MS-MS).

Solid Phase Extraction

All aqueous soil extracts were purified by solid phase extraction (SPE) by a modification of the methods described in Waters (2002). Briefly, 100 mL of aqueous extract was spiked with 1000 pg of a standard ethanolic d4-estradiol solution (CDN Isotopes, Pointe-Claire, Quebec, Canada), then filtered through 0.45 µ disk filters (Whatman, Florham Park, NJ) attached to a 25 mL syringe. The entire filtrate was applied to a Water HLB Oasis® SPE cartridge by programming a RapidTrace Workstation (Caliper Life Sciences, Hopkinton, MA) to perform such a large volume. The SPE cartridge had been pre-equilibrated with 3 mL of diethyl ether, 3 mL methanol, and 3 mL NanoPure (NP) water. Following sample loading the cartridge was washed with 1 mL of 40% methanol in NP water, 1 mL of NP water, and 1 mL of 10% methanol/2% NH_4OH in NP water. The steroid hormones were then eluted from the SPE cartridge with 2 mL of methanol. The solvent was blown dry on a centrifugal rotary evaporator (SpeedVac, Savant Instruments, Farmingdale, NY) before being reconstituted in 100 µL with 1:1 NP water:acetonitrile and analyzed by LC/MS-MS.

Chemical Analysis

The liquid chromatograph of the LC-MS-MS was an Alliance 2695 Separation Module (Waters, Beverly, MA) equipped with a 50 µL sample loop. The chromatographic column was a Symmetry C18, 3.5 µm, 2.1x100 mm (Part Number: WAT058965) with a C18 packed guard column (2.1x10 mm). A linear gradient starting at 40:60 Millipore water:acetonitrile to 100% acetonitrile over 10 min was used at 0.2 mL min^{-1}. The MS portion of the LC-MS-MS system was a Waters Q-TOF Ultima API-US, Quadrupole-Time of Flight mass spectrometer (Waters). The system was equipped with an electrospray ionization source, and analyses were done in negative ion mode (ES-). The capillary and cone voltages were 2.33 and 55, respectively. The source and desolvation temperatures were 120 and 400ºC, respectively. The cone and desolvation gas flows were 20 and 500 L h^{-1}, respectively. The E2 limit of detection of 0.005 µg L^{-1} (based on signal-to-noise of 3:1) has been achieved under the conditions described above. The samples (10 µL injection volume) were fragmented in the collision cell at a collision energy of 15, such that parent ion, m/z 271, and two prominent daughter ions, m/z 145 and 183 could be summed and quantified to three significant figures. Coefficient of determination was >0.995 over a nine-point E2 standard curve ranging from 5 to 2000 pg on-column. Deuterated E2 and T (d4-E2 and d3-T) were added prior to SPE sample prep to allow method recoveries to be determined.

Statistical Analysis

Initial E2 measurements were expressed in soil gravimetric units (ng-E2 kg^{-1}) and were obtained by dividing by the dry soil weight. Given the method of extraction, however, it seemed useful and perhaps more appropriate to express E2 concentrations as porewater equivalents (ng-E2 L^{-1}). Because the soil was shaken in an approximate 2:1 solution to soil ratio, it would be expected that some of the sorbed E2 would enter solution as governed by the appropriate E2 desorption processes and also the kinetics of desorption in a more dilute solution. So, the measured fraction of E2 was likely representative of mainly the dissolved porewater fraction, slightly augmented by a portion of the sorbed fraction. This was best expressed in units of E2 per unit volume of water, which was calculated by dividing the soil gravimetric units (unit mass of E2/unit mass of soil) by the gravimetric water content (unit mass of water/unit mass of soil) in order to obtain E2 porewater concentrations in ng L^{-1}. The water concentration also lends itself as a better comparison for toxicological standards, for environmental impact assessment, and also for comparison with previous field porewater studies, particularly that of Thompson (2005), which previously analyzed subsurface porewater E2 concentrations from PCAPS lysimeters. In this report, analyses involving E2 concentrations and other variables were conducted using the calculated porewater E2 concentrations.

The SAS software package, JMP, was used for all statistical analysis (Sall et al., 2005). Log-transforms of some of the data were required in order to normalize them for statistical analysis (Fig 2).
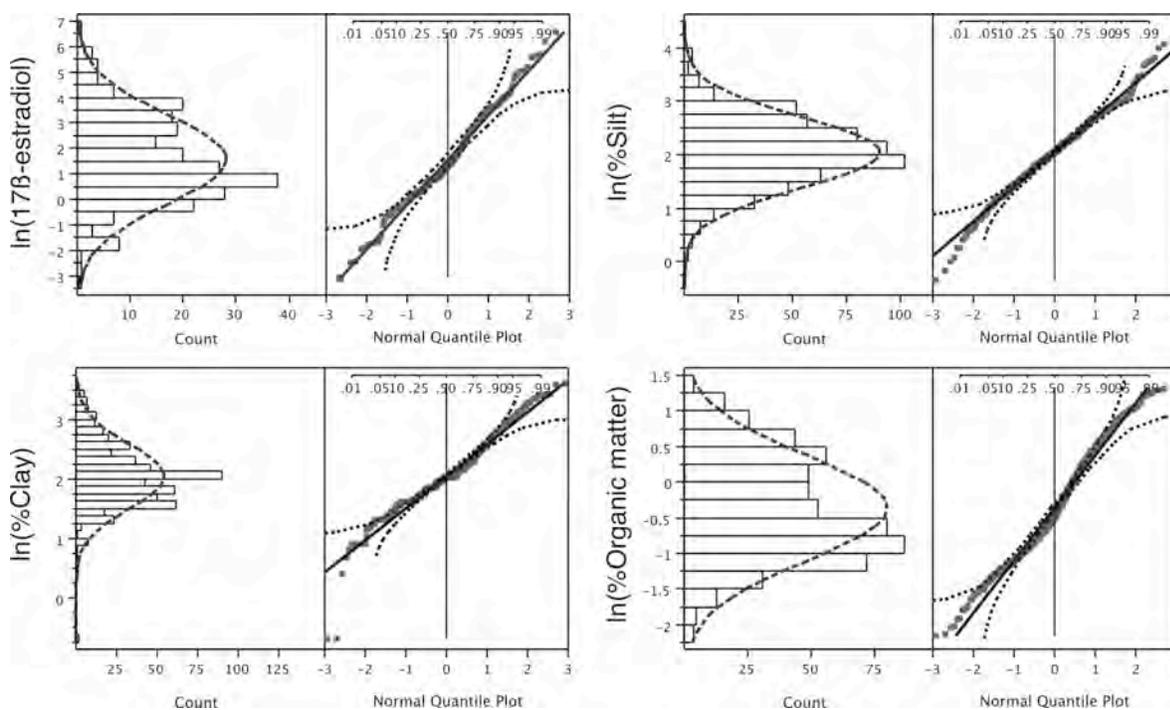

Figure. 2. Histograms and normal quantile plots for lognormally transformed E2, percent silt, percent clay, and percent organic matter. Distributions are fitted with normal curves.
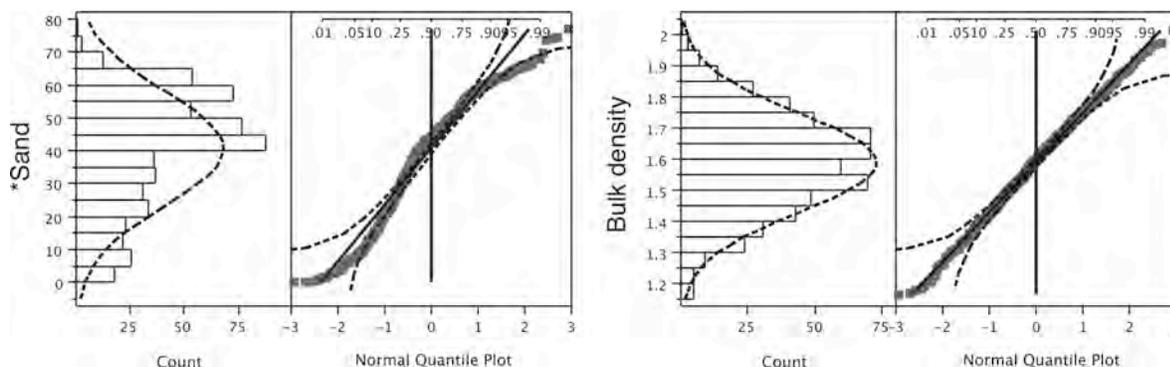
Figure. 3. Histograms and normal quantile plots for transformed sand and bulk density variables.

The log transformation fit poorly with sand. However, this variable could be approximately normalized using the following transformation: $Sand* = \dfrac{\% Sa^5}{10^8}$. The purpose of the denominator (10^8) was to normalize the sand variable within the range common to percent values. Some bulk density values, especially in the lower profiles, were unrealistically high or low, because of loss of sample integrity in the saturated zone. One calculation of 2.84 g cm^{-3} was above the likely particle density for a siliceous mineral soil (generally assumed to be approx. 2.65 to 2.70 g cm^{-3}), and therefore, clearly an outlier. All bulk density data outside the 95 percent prediction interval (1.23 to 1.93 g cm^{-3}) were excluded from analysis (Fig. 3).

Effects on E2 were evaluated over space and time, looking at the frequency of detections as well as the magnitude and distribution of detections. The frequency analysis was a qualitative analysis comparing the percentage of detections over sites, depths, and time. The log-transformed concentrations (lnE2) were a quantitative analysis for statistic comparisons with soil properties. The use of log-transformed E2 data excluded all non-zero measurements, because zero values could not be log-transformed. The question addressed was: "If there was a detection of E2, what effect did a given soil property have on the magnitude of the detection?" Or, in statistical terms, the appropriate null hypothesis would be: "If there is a detection, arbitrary property 'A' or 'B' has no effect."

The layout of the reconnaissance study was complex, involving six sites, thirteen depths, and five sample sets or times. Comparisons between these target categories implied the influences of many measured and non-measured soil properties and spatial and temporal effects, including climate, farming operations, and other factors. All attempts at simple correlations with soil physical variables were non-significant. This was partially due to the larger significant differences between sites, depths, and sample times. Therefore, any reasonable attempt to discern the effects of individual soil properties required an effort to separate the effects of the soil properties from other factors influencing the measurements. To filter the effects of time and space, analysis of covariance was used, employing site, set (sample time), and depth intervals as discrete variables, and various soil physical properties as continuous variables. Sample "set" refers to the time of sampling as a sequential number (1-5) without reference to season.

A Type III general linear model was used on the transformed variables. The effect was similar to blocking in that it provided measurements of the strength of the effect of the target properties on E2 concentrations after filtering the effects of other variables in the models. The objective was to determine whether a model for predicting lnE2 concentrations would be improved by addition of a specified property variable, after adjusting for the affects of site, sample set, and depth; and if so at what level of confidence.

Because the 2007 Injection site was not included until the second year of sampling (beginning spring of 2007), data from this site was excluded for comparisons with soil properties to better approximate a balanced design. Data from this site was necessary for analyses of the effects of manure application on E2, since this was the only field treated with manure in the second year.

Laboratory Dissipation Study

A separate bench study was conducted, in addition to the field study described above, to further investigate factors controlling E2 detections in the field. Between 2003-2007 manure from the manure storage pit (MSP), the hoop barn, and the static manure pile at the study site were collected and characterized for hormones using LC/MS-MS in order to obtain background concentrations of E2. As an auxiliary study, E2 was applied to soil columns at a concentration of 5,000 ng L^{-1}, based on the span of E2 concentrations measured in the collected manure samples (raw hoop barn manure=2,989-7,744 ng kg^{-1}, static manure pile mixture=1,016-5,820 ng kg^{-1}, and MSP=509-3,767 ng L^{-1}).

17ß-estradiol was purchased from Sigma (St. Louis, MO) and a 5,000 ng L^{-1} solution was prepared using 0.01 M $CaCl_2$ (1% EtOH) and stored at ~2.5°C. Soil, classified as a Wyndmere fine sandy loam (Coarse-loamy, mixed, superactive, frigid Aeric Calciaquoll) was collected to a depth of 15 cm from a cultivated field in the study location. The soil was air-dried and machine-ground (2-mm maximum particle size), and 105 g of soil were packed into each of eight acid-washed glass columns, measuring 3 cm in diameter and 11 cm in length. The soil was packed in one-cm increments to obtain an approximate bulk density of 1.30 g cm^{-3} (est. bulk density of top 15 cm based on previous core bulk densities). A stainless-steel screen was affixed to the bottom of each column with teflon tape so that the soil was held in but any excess moisture could flow through, even though the amount of solution added was determined so that none was expected to elute. Twenty mL of the 5,000 ng L^{-1} 17ß-estradiol solution was slowly dripped onto the surface of the soil using a burette, so that a total of 100 ng of E2 was applied to each column. Columns were covered with parafilm to prevent evaporation and set in front of a window, where they were exposed to sunlight. The surrounding room temperature was recorded throughout the day.

At designated times (1 hr, 2 hr, 4 hr, 8 hr, 16 hr, 32 hr, 48 hr, 64 hr), soil from the columns was removed and the pore water was extracted with 200 mL of 0.01 M $CaCl_2$ solution following the soil core sample extraction procedure described above. Samples were then prepared and analyzed according to the procedure described above in the Chemical analysis section of Methods. All treatments were in triplicate, and results are reported at the end of the following Results and Discussion section.

RESULTS AND DISCUSSION

Analysis of Composite Data

E2 was detected at all sites and all depths and on a total of 249 out of 589 extractions (42.3%). The mean E2 concentration was 10 ± 1.9 ng kg^{-1}–dry soil with a range of 0-733.9 kg^{-1} and median concentration of 0 ng kg^{-1}. Concentrations were converted to porewater equivalents by dividing E2 per unit-mass of soil by the gravimetric water content measured for each sample. This was done to obtain units of E2 in ng L^{-1}, which was more representative, given the method of extraction, and more appropriate for comparisons with toxicological standards, environmental impact assessment, and other studies. The mean E2 concentration in porewater was 65 ± 16.8 ng L^{-1} with a range of 0-7,712 ng L^{-1}. The maximum mean porewater concentrations by site were generally in the range of 100-300 ng-E2 L^{-1} on all but the State Well site, which was much larger at >700 ng-E2 L^{-1} (Table 2). This is comparable with concentrations from Peterson et al. (2000) who reported E2 in the range of 6-66 ng L^{-1} in a karst aquifer and Thompson et al. (2005) who collected E2 in lysimeter leachate in the range of 0-1,082 ng L^{-1} with a mean near 100 ng L^{-1}.

Table 2. Means of E2 porewater concentrations (ng L^{-1}) for each site and sample set

Site	(Set 1) Spring 06	(Set 2) Summer 06	(Set 3) Fall 06	(Set 4) Spring 07	(Set 5) Fall 07
06 Injection	0.9	1.4	18.73	202.55	16.35
Compost	83.18	0	1.87	330.67	11.99
Lagoon	0.04	1.06	10.88	205.43	12.39
Lysimeter	3.32	1.63	2.41	78.88	19.35
State Well	2.73	40.53	763.06	93.25	11.31
07 Injection	-	-	-	93.39	12.23
All Sites	12.38	10.3	126.09	157.72	14.06

Spatial and Temporal Variability

Site, depth interval, and sample set (time) exhibited significant relationships with porewater lnE2 (p<0.001). Significant differences between sample sets represented a time progression with increasing E2 in the Fall of 2006 that continued to increase in the Spring of 2007 and then decrease by the Fall of 2007 (Fig 4). However, the time progression of detections appeared to have no relationship to seasonal fluctuations.

This compared to trends in lysimeter E2 concentrations from the Thompson (2005) study, which served as the groundwork for this study and was conducted at one of the same sites. Concentrations from the Thompson (2005) study peaked in the summer rather than the spring. However, both studies indicated temporal trends visible across data sets rather than repetitive seasonal trends. This may suggest that peak E2 detections were more related to climatic events than seasonality.
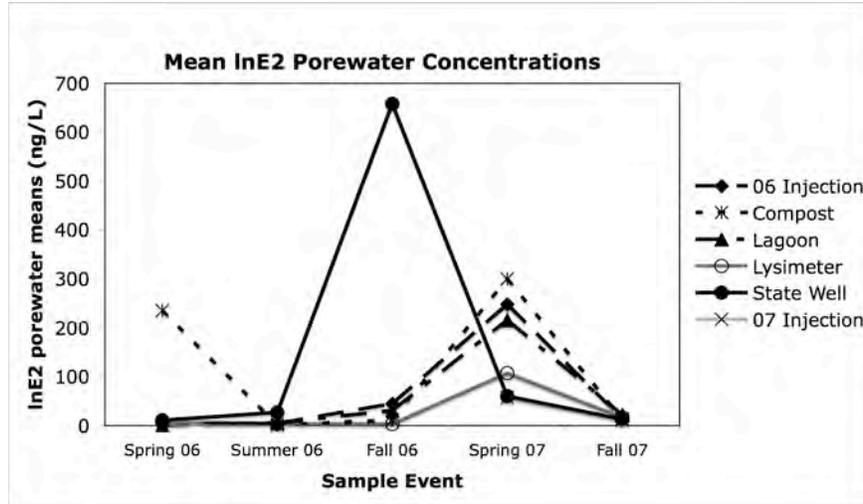
Fig. 4. Mean lnE2-water concentrations. Values are averaged by site and over time and re-adjusted to non-logarithmic E2 values.

Possible climatic effects on temporal variation

Mean E2 concentrations from both Thompson's 2003 lysimeter effluent samples and porewater samples from this study (2006 and 2007) corresponded approximately to precipitation (Figs. 5 and 6). Precipitation data were obtained from NDAWN for the city of Wyndmere (approx. 25 km from the study site). Differences in response to precipitation events may be related to the individual hydrology or cropping practices at different locations. However, our data is not sufficient to determine these potential effects.
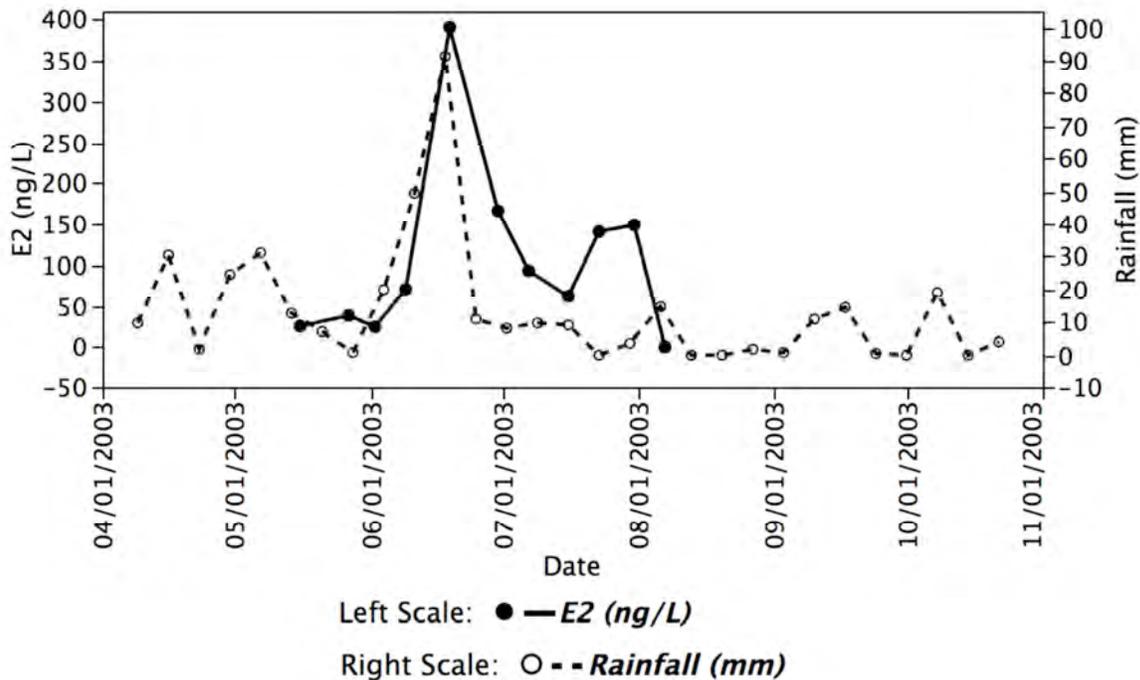
Figure 5. Thompson's E2 Concentrations (ng-E2 L^{-1}) plotted with local rainfall (mm).
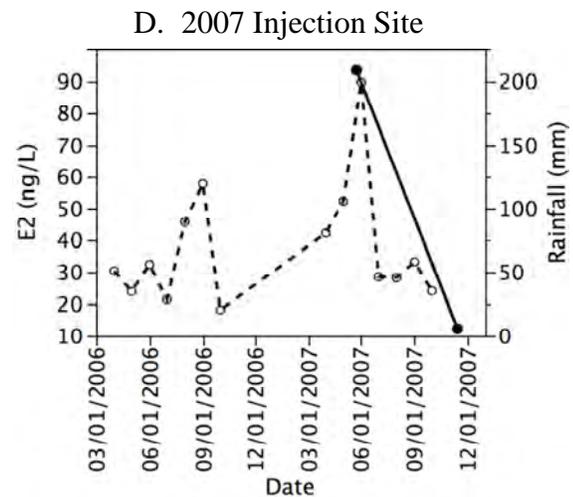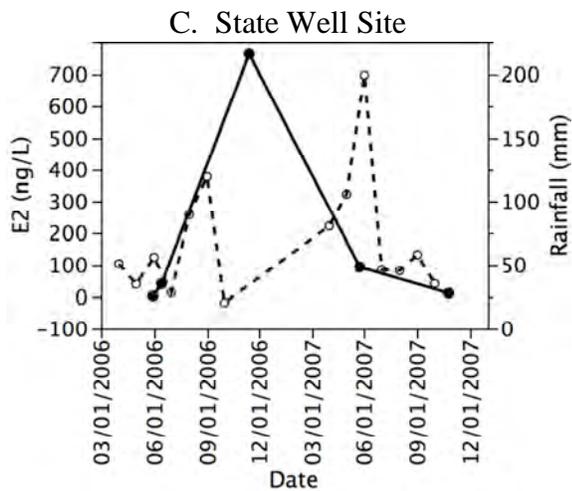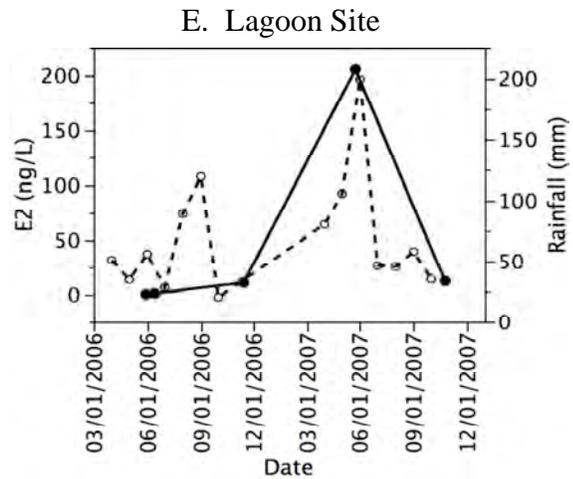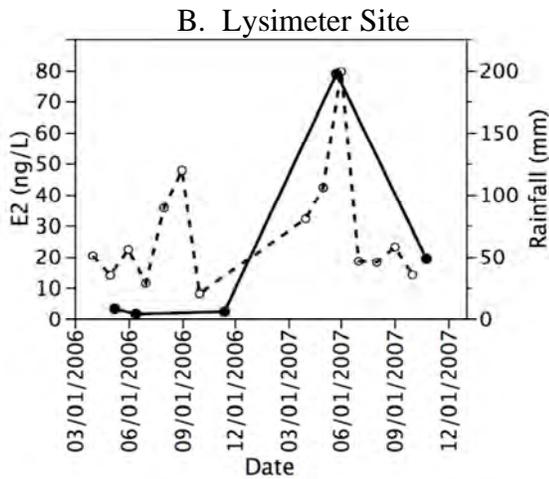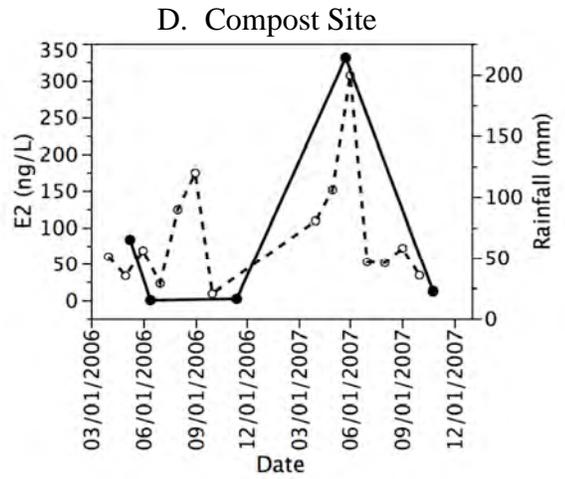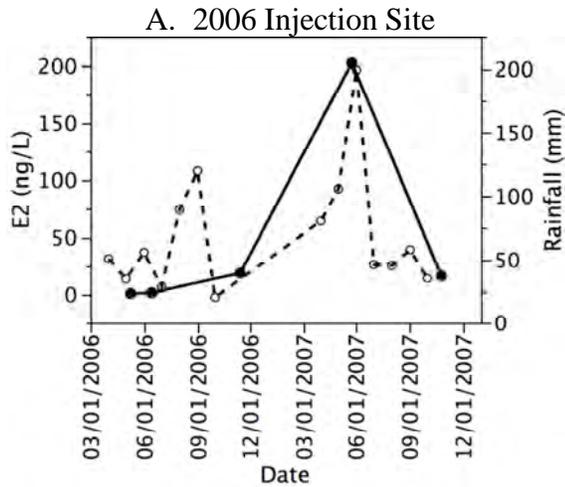
Figs. 6A-F. Porewater E2 concentrations plotted with monthly precipitation (mm).

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To better understand the effect that precipitation may have on E2 concentrations, it was necessary to examine the depth distribution of E2 concentrations, the relationship between local groundwater hydrology and E2 distributions in relation to precipitation events, and the relationship between soil physical properties and E2 detections.

Depth Effects

Analysis of variance showed the effects of depth interval on lnE2 (ng L^{-1}) to be significant (p<0.01) once the effects of site and set were removed. However, Tukey's HSD indicated that the upper profile (0-61 cm) was only statistically separable from the very bottom interval (183-198 cm). The removal of two outliers (on the State Well site) increased the significance of depth to p=0.0006.
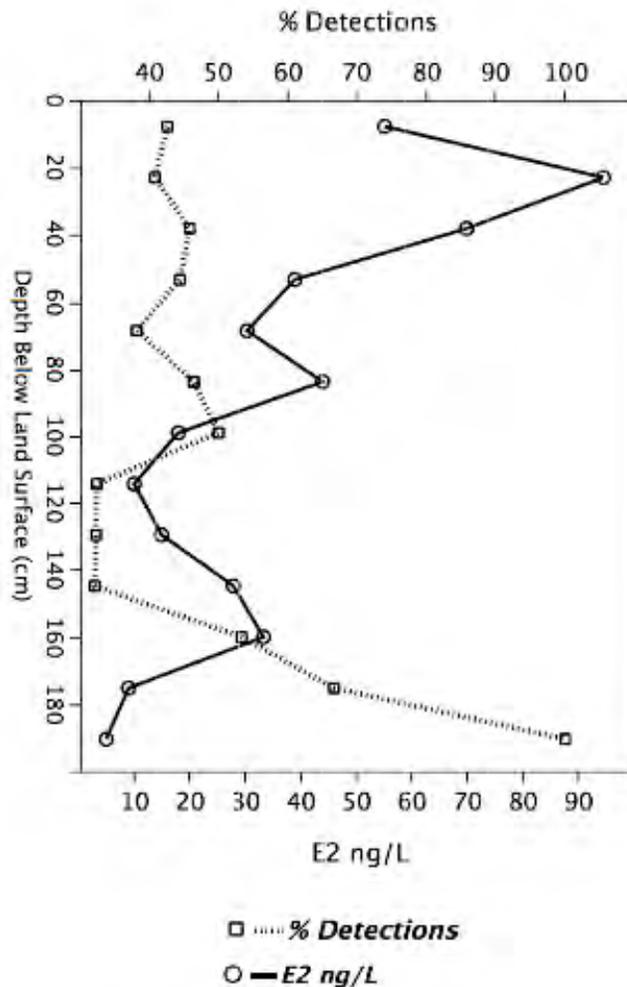
Fig. 7. Percentage of E2 detections and concentrations with depth. Values are averaged over time and across sites. Two outlier concentrations from the State Well site were excluded.

Overall, highest concentrations of E2 were found in the upper soil profile, while the higher proportional frequencies of detections were in the lower soil profile (Fig. 7). This may suggest that preferential source locations and flow paths carry E2 to the water table, where it is integrated or mixed through diffusion and/or convective movement in water-saturated pores. Kjaer et al. (2007) noted preferential flow as a governing influence in the transport of estrogens from manure-treated soil to tile drainage water. Other studies have also suggested rapid preferential transport of E2 in karst stystems (Peterson et al., 2000, 2005; Wicks et al., 2004). Kolodziej et al. (2004) also attributed sporadic detections of E2 in subsurface waters to preferential flow paths. The expected product of these integrations would be more detections, but also lower concentrations through dilution, because of the saturated state of the lower profile and also because of dispersion. Decreasing frequency of detections with vertical distance from the water table could also suggest upward movement of E2 with water and/or soil colloids under the capillary control of the water table.

Mean concentrations from the 2003 lysimeter effluent and porewater samples from this study (2006 and 2007) appeared related to the depth of the water table, with highest E2 concentrations corresponding approximately to shallowest water-table depths (Figs. 8 and 9). This may be caused directly by precipitation effects, indicated above (Figs. 5 and 6), which also cause high water tables, but in some cases it is possible that the rising water table itself may carry E2 upward into the soil profile or perhaps remobilize colloid-bound E2 resident in the soil profile. Casey et al. (2008) gave evidence of antecedent hormones in the soil, while Holbrook et al. (2004) suggested a strong association between E2 and colloidal organic carbon. Other studies have noted hydrological connections with E2 in karstic springs, either in correspondence with recharge (Peterson et al., 2000) or discharge events (Wicks et al., 2004).
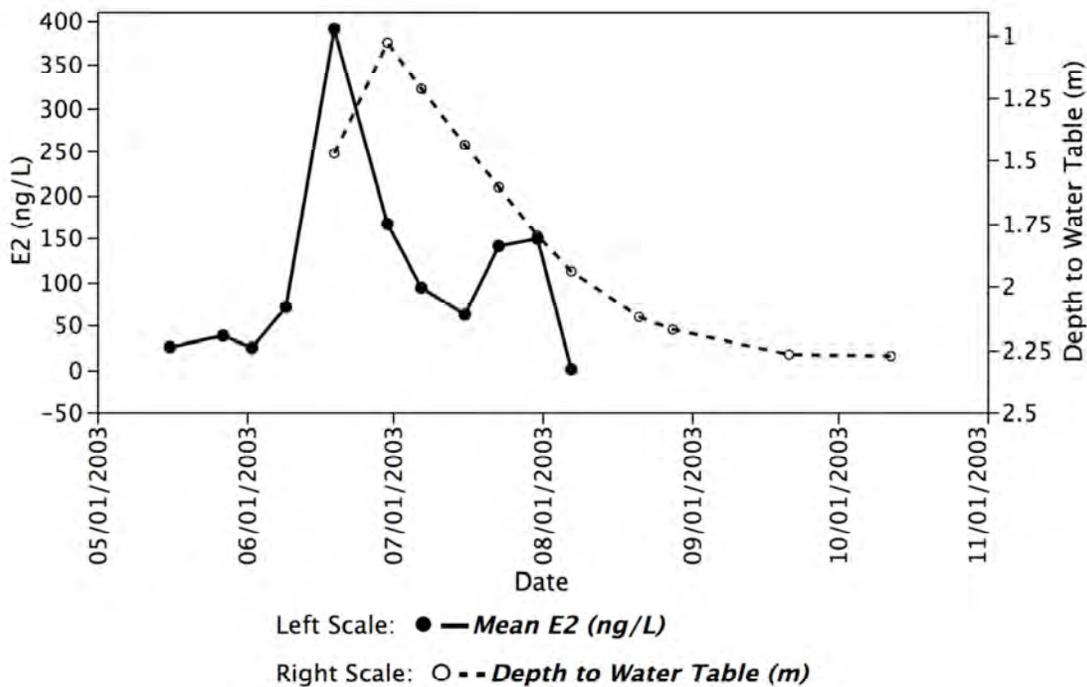
Fig. 8. Thompson's data: Porewater E2 versus Distance from Surface to Water Table
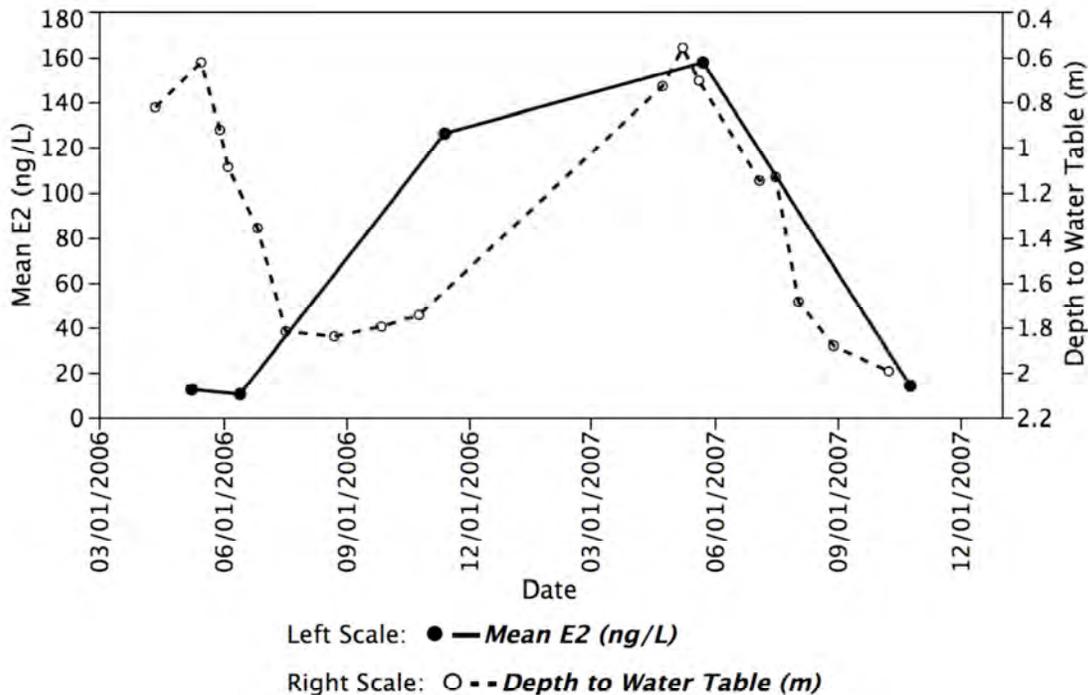
Fig. 9. 2006 and 2007 data: Porewater E2 versus Distance from Surface to Water Table

Effects of soil properties

None of the measured soil physical variables (particle size, organic matter content, moisture content, bulk density) exhibited a significant relationship with E2 without adjusting for the effects of site and sample set (time). A Type III linear covariance model was applied individually for each measured soil physical property, controlling for the effects of Site, Set, and Depth to improve discrimination of the target variable. One site (the 2007 Injection site) only contained data for one year and was excluded from soil property analysis in order to allow for a balanced data set.

After removing the effects of space and time, porewater lnE2 exhibited significant relationships with sand ($p < 0.003$), organic matter ($p < 0.007$), clay ($p < 0.03$), and gravimetric water content ($p < 0.04$). However, relationships were not simple and varied with positions in the soil profile. Relationships for lnE2 with silt ($p < 0.09$) or bulk density ($p < 0.16$) were non significant.

Examination of the interaction between sand and depth indicated that lnE2 was directly related to sand in the lower horizons (~75-200 cm), but inversely related to sand in the upper horizons (~0-75 cm) (Fig 11). The result for lnE2 and clay was similar but reversed. Inclusion of a silt and depth interaction was significant at $p < 0.04$ and indicated that lnE2-water increased with silt in the upper soil profile and decreased with silt in the lower soil profile. Overall, in the shallow soil profile, E2 increased with finer soil textures, but in the deeper soil profile, E2 decreased with finer soil textures. There was a relationship with lnE2 and organic matter with depth (generally, E2 increased with OM in the upper profile and decreased with OM in the lower profile), but it was less clear than for sand and clay.
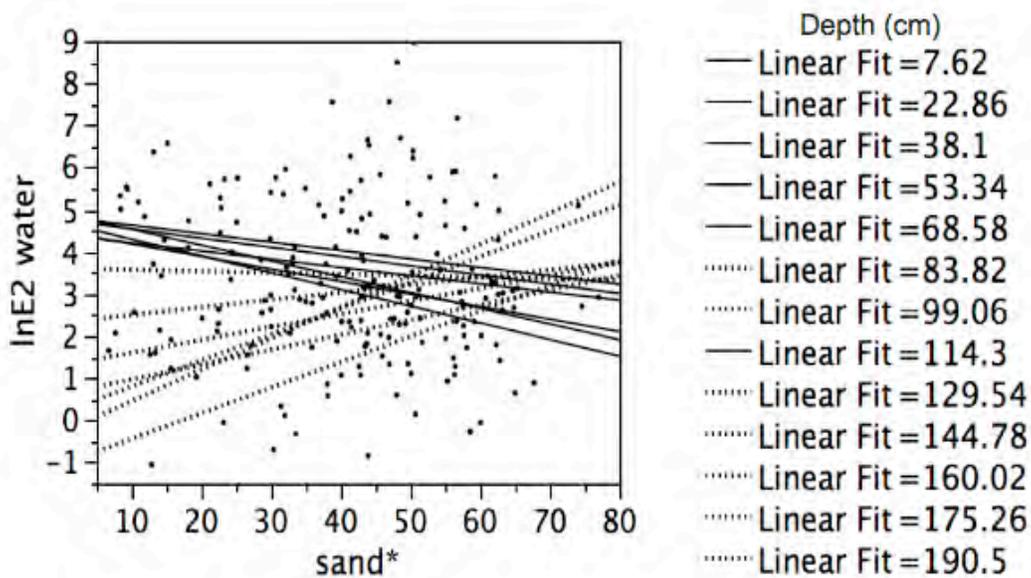
Fig. 10. Linear relationship between transformed sand and porewater lnE2 by depth (cm). Solid lines show depths at which lnE2 decreases with sand. Dotted lines show depths at which lnE2 increases with sand.

A logical presumption for a process governing the relationships between E2 and clay or organic matter would be sorption. However, the results of the depth interactions indicate that the relationships may be complex. Based on published work of Casey et al., (2003), Lai et al., (2000), Lee et al., (2003), and others, one might expect lower water-extractable E2 concentrations with greater clay and organic matter. However, analysis of covariance indicated that in the upper profile there were larger detections on finer soils and higher organic matter, where intuitively one would expect lower detections due to sorption.

Considering previous observations, concerning precipitation and water table relationships with E2, one possibility is that E2, sorbed onto organic matter, might be released through the breakdown of organic matter, induced by increased moisture following rainfall events. The result of this organic breakdown would be the loss of sorption sites and the potential release of detectable E2 and/or the possible release of E2 coupled with mobile organic acids or colloidal materials, which would allow transport to the lower profile.

Holbrook et al. (2004) suggest as much as 60% of aqueous E2 and wastewater could be associated with colloids and that colloids could contribute substantially to E2 movement through convective transport. Casey et al. (2008) concluded that colloids likely facilitated transport of E2 and that greater amounts of flowing water could mobilize hormone-bound colloids in lysimeters and result in more effluent detections. Kolodziej et al. (2004) noted field studies showing that pesticides, which are moderately hydrophobic and strongly adsorbing like E2, can be released from overland flow after rainfall events.

The possibilities of E2 immobilized by organic matter are supported by Fan et al. (2007), who found that "non-extractable hormones remaining in the soil after solvent extraction were mostly associated with humic substances, the naturally occurring organic matter, which represents more than 50% of the OM in soils." Their data indicated that 50-73% of E2 was bound by humic substances.

There is also increasing evidence that dissolved organic matter (DOM), defined as the fraction that passes a 0.45 μm filter, may play a significant role in transporting organic chemicals from terrestrial to aquatic ecosystems, since it is both reactive and mobile in water. The formation of organic complexes can affect the transport and persistence of chemicals, since "much of the terrestrially-borne DOM is microbially consumed, photo-degraded, or adsorbed in soils and sediments" (Bolan et al., 2004). Kolodziej et al. (2004) attributed the sporadic nature and lack of quantifiable levels of steroids in tile drains to strong adsorption and/or degradation during infiltration of dairy wastewater to ground water for distances of 10-100 m. Herman and Mills (2003) found that the migration of chemicals applied at the surface was mainly due to infiltration and subsurface flow rather than overland flow and noted that soil water typically has more dissolved organic carbon than surface water (0.05 g L^{-1} compared to 0.01 g L^{-1}), suggesting the role of sorption in chemical transport. Bolan et al. (2004) noted that a possible sink for dissolved organics could be their preferentially distribution between DOM fractions of different mobility. This preferential distribution onto dissolved organic matter could serve as a link between terrestrial and aqueous bionetworks and account for pockets and variability in E2 concentrations measured in the field.

Holthaus et al. (2002) have reported that E2 and EE2 were more attracted to fine bed sediments than those with high organic carbon, so it's possible that the effects of clay on E2 would have been stronger and more defined if the study were not conducted on such a sandy soil. On the other hand, in the present study, clay effects could be acting as a surrogate for organic matter, which was often closely associated with clay content. Further mineralization of organic acids and colloids during transport could contribute to the increase in low-level percent detections near the water table. Once transported and released, E2 would be less likely to be resorbed on coarser materials in the deep profile, which could account for the statistical inverse relationship of E2 with increasing soil fineness in the lower profile.

Herman and Mills (2003) suggest that the inconsistency between rapid biodegradation in laboratory studies and persistence of E2 in watersheds suggests that "either the rates of biodegradation are reduced compared with the laboratory experiments or that E2 probably interacts with the components of the natural environment through complexation, sorption, or abiotic transformation in the ageing process that leads to diminished bioavailability."

There are a number of factors that could affect the bioavailability of E2 in a field setting, including the formation of organic complexes (Bolan et al., 2004) and sorption onto soil particles (Casey at al., 2003, 2004; Fan et al., 2007). Das et al. (2004) indicated that E2 degradation occurs mainly during flow interruptions rather than during active transport. Fan et al. (2007) cited a review of studies conducted by Harms and Bosma (1997), which concluded that "organic contaminants, including non-aqueous phase liquids, solid compounds, and sorbed substrates have

to be dissolved or desorbed into the aqueous phase, to be available for microbes." Therefore, attached to colloids, E2 may have increased mobility yet remain biologically unavailable and thus more persistent. The question is whether E2 is bioavailable or whether it is bound to colloids.

If bioavailability were the inhibiting factor in the field, E2 detections would be expected to increase with optimal conditions for microbial breakdown, such as favorable moisture conditions created by infiltration in an aerated soil. Degradation of E2 through transformation to estrone was found to be mainly a biological process under both aerobic and anaerobic conditions (Fan et al., 2007; Jacobsen et al., 2005), so both mineralization and degradation into other chemicals appear to be biotically driven processes.

If sorption were a controlling factor, one would expect lower E2 in the upper layers, where clay and organic matter were highest. This conflicts with the results of this study. However, it is possible that E2 sorbed to DOM could have remained in the water-extraction and been forced off dissolved organic colloids during the solid phase extraction step of the chemical analysis. The result would be higher E2 in the upper profile, where there would be higher organic content and finer particles, but it would not explain the apparent hydrological connections. Colloids could carry E2 downward, but less would be expected with distance from the surface. In this case, the relationship with infiltrating water would be due to kinetic desorption.

Bolan et al. (2004) reported a study in which topsoil horizons exhibited weaker DOM sorption due to high organic carbon contents (which occupy sorption sites) and fewer sorbants (such as clay). This is interesting in relation to figure 7, which showed that E2 concentrations were lower in the top layer (%OM=2.13) and reached a maximum at a depth of approximately 15-30 cm (%OM=1.59), directly below the higher organic layer and depth of the applied manure, in soil with a higher mineral fraction.

It is also interesting that E2 concentrations decreased linearly from the top 15 cm until a depth of about 60 cm. Percent organic matter also decreased linearly until this depth before leveling out at about 0.52% (Fig. 12). Above this depth E2 decreased with sand and increased with clay and organic matter, indicating increased sorption. This increased E2 was measurable in the water extractable portion of the soil samples, whether as a result of desorption or organic decomposition with increased rainfall, or due to the stripping of E2 bound to dissolved organic matter during the SPE process of the chemical analysis. Below this depth E2 increased with sand and decreased with clay and organic matter, indicating less sorption and greater leaching. Bolan et al. (2004) cited a soil column study in which leaching of DOM leaching was nearly un-retarded. They also stated that DOM is easily oxidisable in an aquatic environment, so once in the saturated zone, E2 could be released and distributed through dilution. This could account for the higher frequency and lower concentrations near the water table, compared to the variable pockets of detections in the upper profile that appear to coincide with areas of increased moisture. Lysimeters installed in the Thompson (2005) study were placed at a depth of approximately 61 cm, directly below the area indicated by this study to be influenced by organic matter and where the relationship with sand began to change. It is likely that the sporadically high and spatially variable E2 concentrations measured in the lysimeter leachate were affected by the same processes governing the upper soil layers in this study.
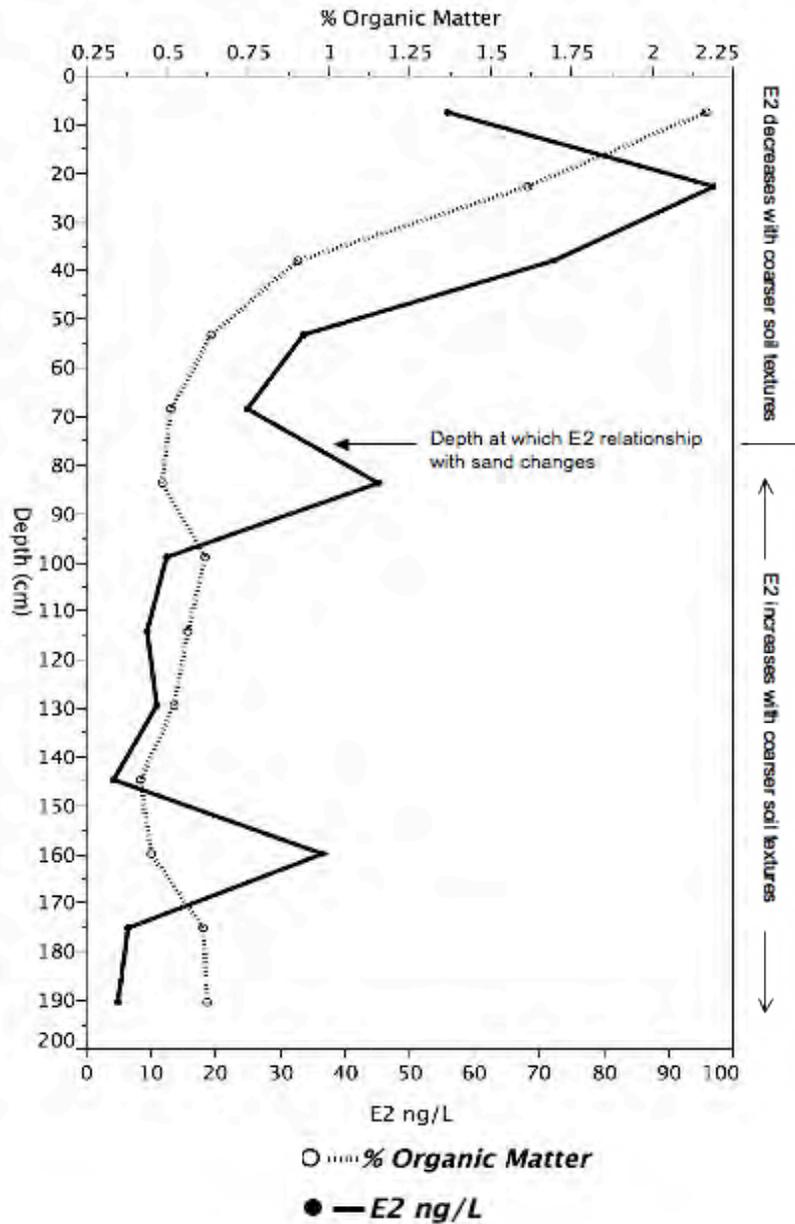
Figure 11. Relationships between percent organic matter and E2 concentrations with depth. Data from the 2007 Injection site and two outlier concentrations were excluded.

Results of this field study indicate that the fate of E2 in the natural soil environment is highly complex, relating to sorption, localized hydrology, and microbial degradation, and it appears bifurcated between the upper and lower portions of the soil profile. The presence of organic material appeared to strongly affect the persistence of E2 in the upper 0.6 m, while the presence of the water table seemed influential at lower depths. Elevated concentrations often appeared with slugs of infiltrating water. As others have pointed out, these processes governing the dissipation of E2 are interconnected and occur simultaneously, making them difficult to evaluate.

Results of Laboratory Dissipation Study

Field results indicated a likely strong role of sorption in maintaining and periodically releasing E2 during wet periods. A bench study was conducted to examine the effects of sorption and transformation within the field-detected range of E2 concentrations, using a topsoil sample, under controlled laboratory conditions. 17ß-estradiol was applied to soil columns at a concentration of 5,000 ng L^{-1}, based on the range of measured E2 concentrations in the field (mean=65 ng L^{-1}, max.=7,712 ng L^{-1}) and in manure samples from the farm (raw hoop barn manure=2,989-7,744 ng kg^{-1}, static manure pile mixture=1,016-5,820 ng kg^{-1}, and MSP=509-3,767 ng L^{-1}).

Under the set conditions, E2 was rapidly dissipated in the Wyndmere fine sandy loam soil maintained at a temperature of approx. 20 ºC. By 1 hour, LC/MS-MS of soil extracts could not detect E2 in any soil extracts beyond the assumed antecedent concentrations of approximately 18 ng L^{-1}. Therefore, concentrations of E2 in the soil columns decreased by approximately 98% of the applied amount within the first hour of incubation and appeared to change little in the columns over time (Fig. 13). Values ranged from 10.7 ng L^{-1} to 27.5 ng L^{-1} (Table 3). Tukey's HSD showed no significant difference between the concentrations detected in the controls, to which no E2 was applied, and in the supernatants of the following timed extractions (p=0.997).

Thus, it appears that the amount of E2 in the soil to which the hormone was applied reached antecedent concentrations (represented by the blank samples≈2% of the concentration applied to the columns) within one hour from the time of application (Fig. 13). This is consistent with Fan et al. (2007) who found in a soil column study that after 5 days, only about 2% of applied E2 was water-extractable. There was a question of what has caused the dissipation of water-extractable E2 to be so complete within an hour of application.

Since "dissipation" in this study was defined as a decrease in the concentration of water-extractable 17ß-estradiol, the applied E2 could have dissipated due to one or all of the following processes: Transformation into another chemical, mineralization, or sorption onto soil particles. Studies have shown that E2 should dissipate rapidly into estrone within hours (Hanselman et al., 2003; Shore and Shemesh, 2003; Soto et al., 2004). However, there was no observed increase of estrone with decreasing E2 concentration as evidence of the transformation from E2 to E1 taking place. However, since E1 and E2 have similar reported K_d values, E2 would dissipate just as fast because of sorption (Casey et al., 2005; Fan et al., 2008).

Table 3. Concentrations of E2 (ngL^{-1}) recovered per extraction time. Means and standard deviations are included in the table.

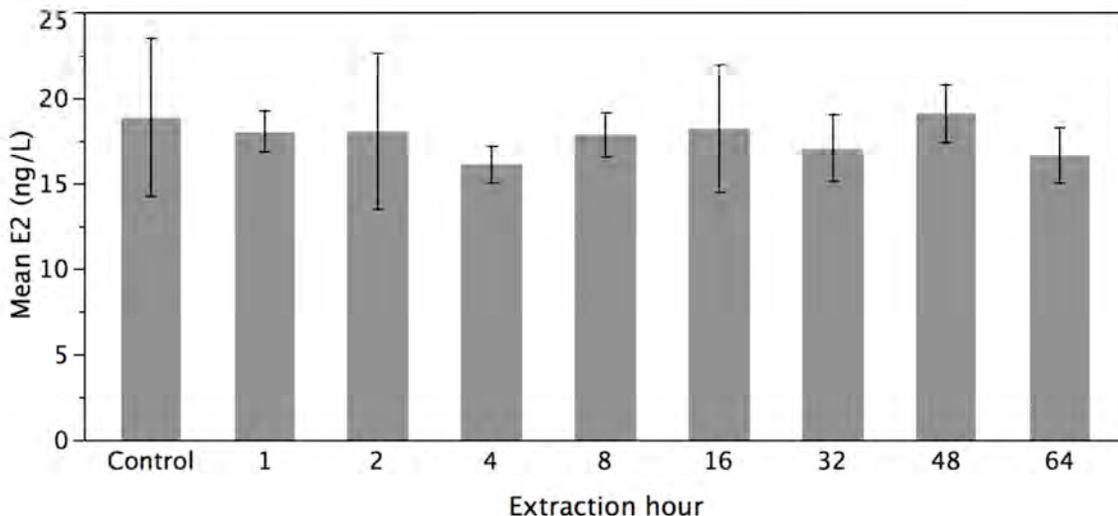|	Blank	1 hr	2 hr	4 hr	8 hr	16 hr	32 hr	48 hr	64 hr
Set 1	11.7	*	10.7	18.3	17.4	25.6	14.7	16.5	13.8
Set 2	17.4	16.8	26.3	14.7	15.9	15.3	20.9	22.3	19.4
Set 3	27.5	19.2	17.1	15.3	20.3	13.7	15.5	18.5	16.6
Mean	18.9	18.0	18.0	16.1	17.9	18.2	17.0	19.1	16.6
StDev	8.0	1.7	7.8	1.9	2.2	6.5	3.4	2.9	2.8

* Discarded reading

Fig. 12. Mean E2 values for controls and other sample times (ng L^{-1}). Standard error bars are included in the chart.

Fan et al. (2007) reported mineralization of only 6% of E2 in native soils under aerobic conditions within 5 d of incubation. Thus, mineralization was an unlikely cause of low recoveries. Several studies have reported rapid sorption of E2 and its metabolites on soil and organic matter (Lai et al, 20002; Hanselman et al., 2003; Shore and Shemesh, 2003; Lee et al., 2003). Das et al. (2004) observed large K_d values for both E2 and its metabolites and suggested that much of the hormone or metabolite mass is present in the sorbed phase. For this experiment, the solution was applied to the surface at a slow rate and was almost fully retained in the column. Given the speed of dissipation, it was possible that the virtually immediate disappearance of E2 from the columns can be explained by sorption alone.

For simplicity, a linear sorption isotherm was used to evaluate potential sorption within the column. Holthaus et al. (2002) reported K_d values ranging from 4 to 74 L kg^{-1} for E2 on bed sediments. Applying the lowest K_d value of 4 L kg^{-1} to 5000 ng L^{-1} (E2 applied), resulted in an estimated sorbed concentration of 20,000 ng-E2 kg^{-1} soil. There were 0.105 kg of soil in the column. Thus, 2,100 ng of E2 could potentially sorb. Since, there were only 100 ng of total E2 actually applied the column, to approximate field capacity in the column without flow through, all of the applied E2 would be capable of sorption within a fraction of the column.

Lee et al. (2003) and Van Emmerick et al. (2003) reported rapid sorption kinetics. However, the extraction method for this experiment consisted of shaking the soil from the columns for 20 minutes in a 200 ml solution of 0.01 CaCl$_2$. Because Lee et al. (2003) also reported rapid desorption, the dilution may have been expected to extract sorbed E2. If the field capacity of the sand were estimated at approximately 0.15 cm^3 cm^{-3}, then for a bulk density of 1.3 g cm^{-3} and 0.105 kg of soil, the estimated water in the column would be 0.012 L. By readjusting the water volume for the addition of the 200 ml extracting solution to 0.212 L, the new aqueous E2

concentration (100 ng/0.212 L) would be 472 ng L^{-1}. Even with this new diluted concentration, the 100 ng E2 added would still be less than the 189 ng estimated sorption capacity after adjusting the linear isotherm. And this would be assuming equilibrium, which would not likely be reached in 20 min. shaking time with the extracting solution. Thus, even during extraction the absence of E2 could be accounted for by sorption alone.

Since the bench study indicated that the concentrations measured in the soil porewater should be rapidly adsorbed in and of themselves, this indicated that much larger concentrations of E2 must be sorbed in the soil that was not measurable in a water extraction. This supports the previous hypothesis, which corresponds to periods of higher moisture, that measured E2 concentrations may be related to the breakdown of organic matter and temporary release of E2 from sorption sites. Given that shaking in 200 ml of water was the standard extraction for all samples, desorption into water alone, without further release mechanisms, was not adequate to explain the differences in detected concentrations.

Effects of Manure Application

First Field Season (2006)

The effects of surface manure application on the 2006 Injection site are shown on Fig. 14. The west side of the field was sampled on 5-9-06. The east side of the field was sampled on 5-30-06 due to wet conditions earlier. Manure was applied the weekend of 6-3-06 and 6-4-06, and the field was sampled again on 6-5-06. 17ß-estradiol was detected on 18% of the pre-application samples and on 26% of the post-application samples. Percent detections before and after the manure application were not significantly different at $p<0.05$ using Student's t.
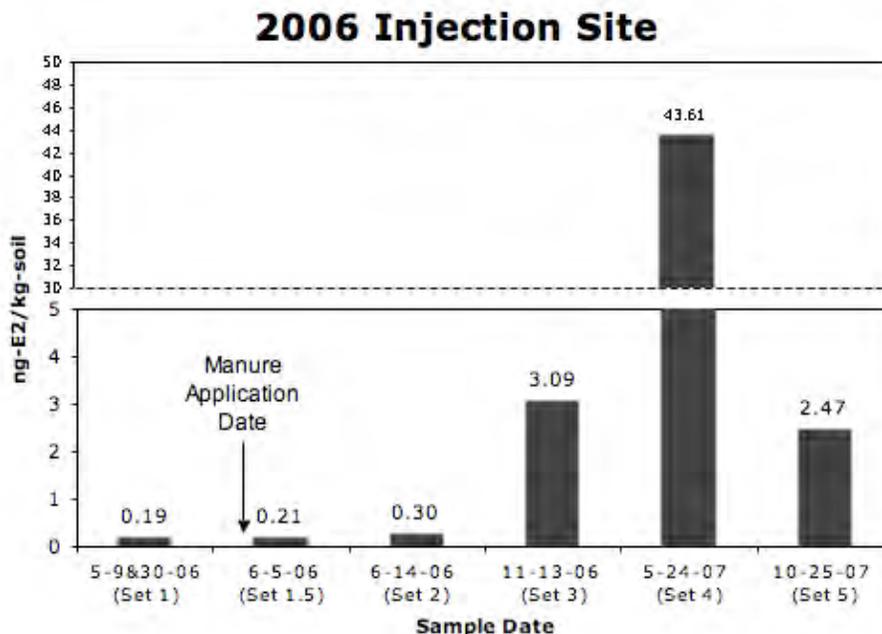

Fig. 13. Mean (non-transformed) E2 concentrations for each sample date. Values are averaged across replicate core locations. Mean E2 concentrations are listed by set above the bars.

Differences between mean concentrations for pre-application and post-application samples were non-significant ($p<0.05$). If E2 concentrations were affected by manure additions, effects were nullified within the one to two-day sampling interval following the application on 6-3-06 and 6-4-06. The lack of detections could be affected by numerous factors causing dissipation of E2. These include mineralization, transformations, and sorption, as well as transport from the system under field conditions. A considerable body of research supports the occurrence of rapid dissipation of E2 (Lee et al., 2003, Casey et al, 2003; Colucci et al., 2000, Colucci and Topp, 2002, Das et al., 2004, and Lorenzen et al., 2005). Colucci et al. (2000) reported that on a loam soil a 1 mg kg^{-1} supplement of E2 was 90.7% non extractable after three days, while on a sandy loam 70.3% was non extractable within 3 d. Fifty percent of E2 was dissipated in less than 0.5 d. Fan et al. (2007) reported that 70-73% off E2 was non-extractable from native soils after 5 d of incubation. Therefore, the 1-2 day field incubation period, following manure application, represents a relatively short period for dissipation. Jacobson et al. (2005) reported that on fields amended with swine manure, E2 was almost fully dissipated within a few days of application and that swine manure enhanced the rate of dissipation. In addition, the rapid dissipation rates described in the bench study of this report also confirm the lack of significance of manure on E2 detections.

In this experiment, only the water extractable fraction was measured, so that sorbed E2 following manure application, would not have been detected. Both bench experiments and the field data indicated that a substantial amount of E2 could have remained in the soil, likely sorbed to soil and organic materials and non-detectable in a water extraction. Kjaer et al. (2007) reported that on a field with applied swine manure, E2 was able to leach and move to tile drains up to three months after the manure application. Thus, the lack of increased detections and concentrations following manure application did not necessarily indicate that no E2 was retained in the field following the application. Evidence of later elevated detections indicates that E2 release into soil water may have been delayed by climatic and hydrologic conditions.

Second field season (2007)

Lagoon material was applied to the 2007 Injection site field over 5-16-07 and 5-17-07. Because of wet field conditions in the spring and early summer and later crop growth, which prevented sample access, no pre-injection or late-summer post-injection samples were collected. A first set of post-injection samples was taken on 5-24-07. A second set was taken on 11-14-07. A comparison of spring and fall samples indicates that the percentages of detections were 78% and 65%, respectively. The difference was not significant at $p<0.05$, using Student's t-test.

Spring and Fall E2 mean concentrations were significantly different at $p<0.05$. The amount of detected E2 was slightly elevated in the spring after manure was applied and decreased by the fall sampling period. However, E2 concentrations at the 2007 Injection site were similar to sites across the farm, and the mean was significantly lower than one (the State Well site) with no history of manure application.
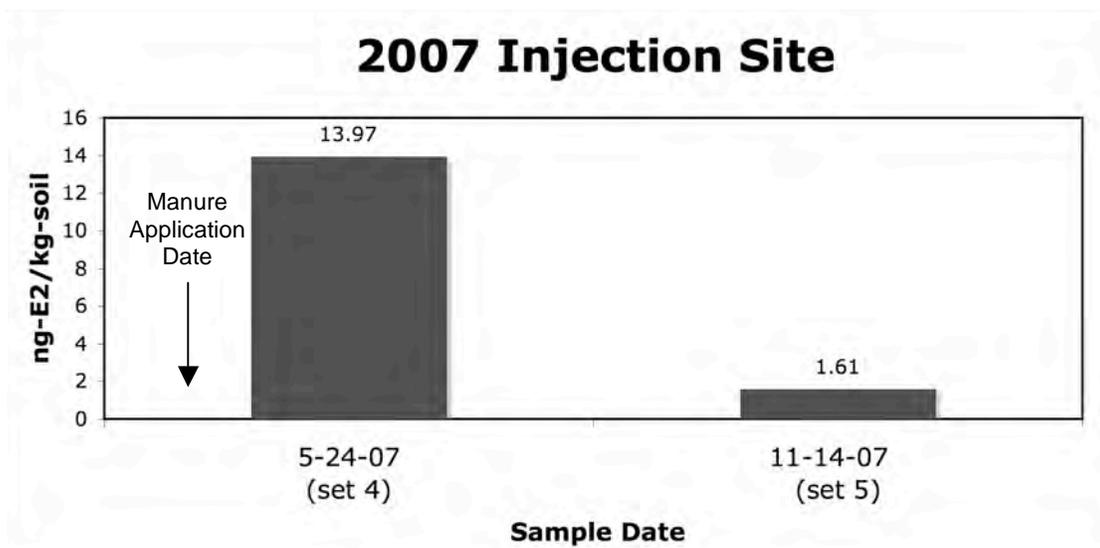
2007 Injection Site

Fig. 14. Mean (non log-transformed) E2 concentrations from the 2007 Injection site for each sample date. Values are averaged across replicate core locations.

Neither of the cases of manure application showed strong evidence of an effect on E2 concentrations. High Spring concentrations at both sites appeared related to temporal trends, likely related to climatic variables, across the farm rather than the manure treatments.

Toxicological Comparisons

Porewater E2 concentrations found on the study site were within the range of concentrations found to impact wildlife. Hanselman et al. (2003) cited 10-100 ng L^{-1} as the threshold of concern for aquatic organisms. Panter et al. (1998) reported that male fathead minnows in water with 30 ng L^{-1} E2 for 21d exhibited vitellogenin production and abnormal testicular growth. Irwin et al. (2001) found 1-7 ng L^{-1} for 28 d caused female turtles to have increased vitellogenin. Peterson et al. (2000) found that salmon in waters with 250-50,000 ng L^{-1} had 84-100% females, compared to about 50% in control ponds. Thorpe et al., (2003) reported the "predicted-no-effect-concentration" to be 1.0 ng L^{-1} and a lowest observable effect level (LOEL) affecting vitellogenin production in juvenile female rainbow trout to be 14 ng L^{-1}. The overall mean porewater concentration in this study (65±16.8 ng L^{-1}) was four times this published LOEL.

Consideration of E2 Sources

One of the purposes of this study was to investigate possible causes for elevated E2 on sites without manure applications indicated by Thompson (2005). The results of this study were similar to Thompson (2005), which indicated that concentrations in the field had little relationship to manure applications, and were related to other trends and factors, principally precipitation. The presence of E2 detections on non-manured sites, that were similar to application sites, seems to indicate that E2 may be widespread in some soil environments. This raises the question of what the sources may be. Without detailed historic documentation, there

was the possibility that manure could have been applied at some time, however, the dissipation rates of E2 indicated that it should not persist long in the environment.

Identifying potential ongoing sources other than manure applications may be a worthwhile research objective. For example, the natural fauna of a soil environment, including insects, arthropods, and amphibians, which are common in agricultural fields may provide sources of E2 that have not been previously considered. Experiments recreating a controlled environment for soil fauna and testing their effect on soil E2 may be productive. Another possibility may be the uptake and preservation of E2 in plant roots, although, there is no indication in the literature that this is probable. While the occurrence of vertebrate steroids in plants is rare, estrone has been found in some plants, such as in pomegranates and apple seeds (Shore and Shemesh, 2003; Choi et al., 2006). These are not common crop plants in North Dakota. However, the fact that hormones related to estradiol are found in some plants shows that they may be present in others. Finally, field reconnaissance studies in other environments, where livestock are not present and where predominant farming practices consist of cash cropping may be useful to test the limitations of the ubiquity of E2.

CONCLUSIONS

A whole farm field reconnaissance study to detect the soil concentrations of 17ß-estradiol was conducted on a swine farm in southeastern ND. Sites were selected for proximity to different manure sources (i.e. one near a manure pile, one near a manure lagoon, one near a compost heap, one with surface applied manure slurry, one with injected manure slurry and one with no known manure history). Two years (2006-2007) and five sample sets that are stratified with depth on six sites have indicated no detectable effects from the addition of manure slurry that was either surface applied or injected. Detections of E2 were widespread, regardless of treatment history and soil conditions. These results were similar to a lysimeter study conducted on the same farm by Thompson (2005). Concentrations were significantly different between sites, sample sets, and depth intervals. The largest overall mean concentration was detected on the site for which there was no known application history. 17ß-estradiol was detected at all depths on all sites. Highest concentrations of E2 were in the upper soil profile, while higher frequencies of detections were in the lower soil profile. Significant differences between sample sets represent a time progression with increasing E2 in fall 2006 that continued to increase in spring 2007 and then decrease by fall 2007. The time progression of detections appeared to have no relationship to seasonal fluctuations. The main factors affecting E2 concentrations appeared to be climate and hydrology. Increasing E2 concentrations appeared to correspond to increasing precipitation for all sites of this study and for the previous study conducted by Thompson (2005) on the same farm. Elevated concentrations in some soil profiles seemed to indicate that high E2 corresponded mainly with elevated moisture in the upper soil profile, but elevated concentrations also appeared to correspond to the depth of the water table on others, indicating that some E2 could be moving in the soil profile through preferential flow to the water table and then following the water table as it fluctuated. Concentrations exhibited significant relationships with transformed sand, clay, and organic matter. Relationships were not simple, however, and varied with positions in the soil profile. Concentrations increased with increasing coarseness in the lower soil profile and decreased with coarseness in the upper soil profile. Greater concentrations with finer soils in the upper profile may indicate the release of sorbed E2 from clay surfaces

during wetting or from the loss of sorption sites caused by the breakdown of organic matter under moist conditions following precipitation. Release from wetting alone would not be a likely cause, since all soil samples were extracted using a 200 ml aqueous solution. A consequence of sorption would be less leaching. The higher concentrations with increasing coarseness in the lower profile may indicate the effects of leaching, or may be a consequence of E2 left in solution because of the lack of sorption in the lower profile after transport. Organic matter effects were similar to clay, with decreasing E2 concentrations corresponding to decreasing organic matter. Because organic matter is frequently associated with clay particles, one may serve as a surrogate for the other, or both may be affecting concentrations. Depth interaction effects of organic matter were similar to clay. There was no significant relationship between bulk density and E2 concentrations. 17ß-estradiol was detected on 42.3% of all samples collected, with no detects in 57.7% of the samples. The range of individual pore water concentrations for all sites was 0-7,712 ng L^{-1} with a mean value for all of 65 ng L^{-1}. These compare with the published lowest observable toxicological effect level of 14 ng L^{-1} (Thorpe et al., 2003). These results indicate that E2 may be widespread in some soil environments. They also raise the possibility of other unidentified sources than livestock manure.

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