Effects of Composting Swine Manure on Nutrients and Estrogens

Nathan E. Derby,[1] Heldur Hakk,[2] Francis X.M. Casey,[1] and Tom M. DeSutter[1]

Abstract: Application of raw manure to fields can contribute ammonia, pathogens, and volatile organic compounds at concentrations that may give rise to adverse odors and environmental concerns. In addition, raw manures contain reproductive hormones that could impact the endocrine systems of sensitive organisms. Composting manure, in which aerobic microorganisms destroy pathogens and convert organic compounds into more stable forms, may reduce its potentially harmful effects. Two piles of swine (*Sus scrofa domestica*) manure with corn stalk bedding were monitored for temperature, inorganic N, total N, P, K, pH, electrical conductivity, and the hormones 17β-estradiol (E2) and estrone (E1). A compost pile was mixed periodically, resulting in internal temperature increases to the thermophilic range (40°C–60°C) after each mixing. A static manure pile was not mixed, and the temperature stayed close to ambient throughout. After 92 days, the compost resembled a humus-like material with very little odor, whereas the manure pile had maintained much of its original physical characteristics. After 92 days, the compost had a pH closer to neutral, a lower electrical conductivity, and slightly lower total N content than the static manure pile. Both piles had greatly reduced ammonium concentrations. 17β-Estradiol concentrations did not decline, but rather fluctuated with time, and were much lower than E1 concentrations, which were initially high but decreased during the study. Static manure storage appeared to be just as effective in reducing total estrogenicity (estrogenicity = E2 + 0.1 × E1) compared with aerobic composting, in that estrogenicity was reduced by 74% in the static manure pile and 79% in the composted pile.

Key words: Swine manure, compost, estrogen.

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Worldwide, the estimated pig population is 941 million (FAO, 2009), which produces an estimated 1.88 billion Mg of manure annually, assuming an average annual manure production of 2 Mg per animal (Larson, 1991). Utilization or disposal of this manure may lead to detrimental environmental effects if not managed properly. Although application of swine manure to agricultural fields has benefits, such as increasing soil organic matter, reducing erosion, and supplying essential plant nutrients (Choudhary et al., 1996), best management practices must be used to avoid adverse environmental effects. Of the manure management options, including piling (static manure storage), composting, waste stabilization ponds (lagoon), and direct land application, aerobic composting of manures can effectively reduce odor, destroy pathogens and weed seeds, stabilize nutrient content, and reduce weight and volume before application to agricultural fields. The composting process has been widely studied. In general, increased aeration of the manure pile by turning or addition of a bulking agent such as straw promotes decomposition by microbial activity, which generates a large amount of heat. Temperatures of 45°C to 65°C destroy pathogens and speed decomposition of organic matter (Stentiford, 1996; MacGregor et al., 1981). Actively composting material normally exhibits heating, thermophilic, and cooling phases, which decrease NH_4^+-N, increase NO_3^--N, decrease pH, and slightly decrease total N content of the finished compost (Chiumenti et al., 2007; Jeong and Kim, 2001; Tiquia and Tam, 1998; Zhu, 2006). Composting has also been shown to result in varying degrees of degradation of different antibiotics in turkey manure (Dolliver et al., 2008).

In addition to nutrients, endogenous and/or veterinary organic chemicals, including estrogenic hormones, can also be present in manure. Although estrogens are present in all animal manures, the manure from swine farrowing operations has the highest measured concentrations of estrogenic compounds (Fine et al., 2003; Lorenzen et al., 2004; Raman et al., 2004). The estrogenic hormone, E2, can induce reproductive disorders in aquatic species at levels less than 14 ng L^{-1} (Thorpe, et al., 2003). Estrone, the metabolite of E2, is also considered an endocrine-disrupting compound, but is 2 to 100 times less potent than E2 depending on the assay (Allison and Omeljaniuk, 2000; Salste et al., 2007; Thorpe et al., 2003; Borgert et al., 2003). It is generally accepted that E2 is rapidly and strongly sorbed by soils (Casey et al., 2003) or rapidly transformed to E1 in the environment by a variety of microorganisms (Colucci et al., 2001; Hanselman et al., 2003). Findings of Thompson et al. (2009) and Kolpin et al. (2002) indicated E2 persistence and mobility in the environment.

The fate of estrogens present in swine wastes is not completely understood, and the management of manures may increase or decrease the levels introduced into the environment once the manures are land applied. Estrogens tend to be more persistent under anaerobic conditions, such as those found in anaerobic lagoons. However, increased aeration has been shown to decrease the persistence of estrogens in the environment (Fan et al., 2007). Although Hakk et al. (2005) reported decreasing E2 concentrations in composted poultry manure and Zheng et al. (2008) reported conversion of E2 to E1 in piled dairy manure, little if any work has been done investigating the changes in hormone concentrations within swine manure storage piles and during composting. It is expected that if the degradation of hormones is predominantly a biological process and composting encourages aerobic biodegradation, then composting will reduce the estrogenic activity in the manure. Therefore, the objective of this exploratory study was to determine the effects of composting of a manure/urine/bedding mixture from a swine farrowing operation on nutrients and the naturally occurring hormones E2 and E1 compared with static manure storage.

MATERIALS AND METHODS

The project was conducted in southeastern North Dakota in 2008. Swine manure solids were scraped from farrowing barns and placed in a large pile during normal barn cleaning operations. The solid manure material contained feces, urine,

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and large amounts of bedding, consisting primarily of corn stalks. This operation was similar to pig-on-litter composting mentioned by Tiquia and Tam (1998), where some degree of *in situ* composting was occurring before removal of the bedding and manure to an outside storage pile and the spent litter still contained an active microbial population, indicating that the composting process was not complete.

For this project, some of the material was used to create a smaller pile, which measured approximately 1.5 m high and 2 m wide at the base on a concrete pad. Five days later, on July 29 (Day 0), more material from the large pile was used to make another small pile on the concrete pad. These piles were outside, were not covered, and were subject to natural temperature fluctuations and rainfall. No attempt was made to control the moisture content of the piles. Each pile was equipped with four probes constructed of 1.9-cm-diameter high-density polyethylene rod with a thermocouple at the end to measure the interior temperature of the piles. The probes were inserted horizontally about 0.3 m from the top of the pile (TOP), slightly above the center (MID-UPPER), slightly below the center (MID-LOWER), and at the bottom of the pile (BOT) so that they were evenly spaced vertically. Laterally, the thermocouples were near the center of the piles. The thermocouples were connected to a data logger (CR10, Campbell Scientific, Logan, UT), and ambient air temperature and the temperature at each depth in the two piles were logged each hour and averaged daily (24 h). Rainfall was measured manually on-site as well as logged each hour at a nearby automated weather monitoring station (North Dakota Agricultural Weather Network, 2010). Cumulative rainfall data measured manually and each hour are included on Fig. 1.

One of the piles was not turned (NT) for the duration of the study. The NT pile is the pile that was placed on Day 0. The other pile was turned (T) with a skid-steer loader tractor approximately every 14 days starting 1 month after the piles were initiated until October 29 (Day 92). The total number of turnings/samplings was six, and these occurred at 0, 36, 50, 65, 78, and 92 days. Turning of the pile consisted of moving the material in multiple lifts with the loader tractor to an adjacent location, then moving it back again. This effectively mixed and aerated the material. Before turning the T pile, the temperature probes were removed from both piles so that subsamples (~150 g) of the manure could be taken from each depth. Samples were taken with a 1.9-cm-diameter stainless steel Oakfield soil-sampling probe that was cleaned between samples. The sampling probe was inserted part way into the hole left by the temperature probe, then angled slightly to collect material from the same depth as the temperature probe was located. Manure samples were collected into polyethylene bags and placed in a cooler until they could be frozen within 2 h of collection. After sampling and pile reconstruction, the temperature probes were reinserted into the T and NT piles. Because of the intensive solid phase extraction (SPE) clean-up procedures and cost-prohibitive nature of the cleanup and analysis for hormones, replicate samples were not taken and the samples from the four depths were composited.

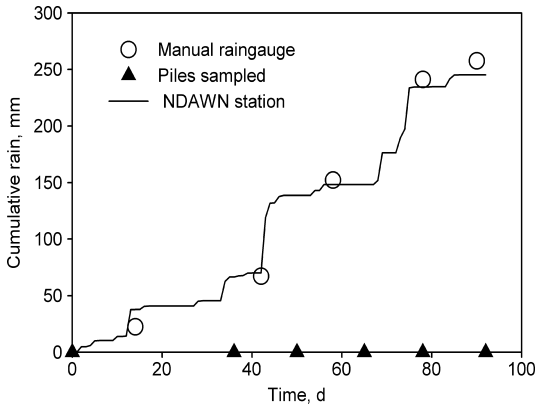

FIG. 1. Cumulative rainfall measured at a nearby automated weather station and manually at site in relation to manure sampling days. The Fingal, North Dakota, North Dakota Agricultural Weather Network station is located 21 km to the north-northwest of the research site.

Composite manure samples from the four depths of each pile were prepared for each sampling date and analyzed for NH_4^+-N, ($NO_3^- + NO_2^-$)-N, total Kjeldahl N, total P, total K, pH, electrical conductivity (EC), and the hormones E2 and E1. For inorganic N analysis, the wet manure was extracted with 2 *M* KCl and filtered according to the method described by Keeney and Nelson (1982) for inorganic N in soil. The extract was further filtered through a 0.2-μm syringe filter to remove particulates and diluted 1:10. Colorimetric determination of ($NO_3^- + NO_2^-$)-N *via* cadmium reduction and NH_4^+-N *via* a salicylate/sodium nitroferricyanide method was done on a flow injection analysis system (FIAlab 2500; FIAlab Instruments, Inc., Bellevue, WA). Total Kjeldahl N was determined by the salicylic acid–thiosulfate modification of the Kjeldahl method (Bremner and Mulvaney, 1982), which included digestion, followed by distillation and titration. Phosphorus (P) and potassium (K) were determined by atomic absorption after nitric acid and hydrogen peroxide digestion (Wolf et al., 2003). The pH and EC were measured with electrodes on 1:2 and 1:5 manure:water slurries, respectively.

Levels of E2 and E1 were measured *via* liquid chromatography and tandem mass spectrometry (LC/MS/MS) after an extensive extraction/clean-up process. The sample clean-up approach used for this very difficult matrix was a combination of two sample preparation methods used by Hanselman et al. (2006) and Ingrand et al. (2003). Approximately 10 g of compost was stirred in 100 mL of water for 1 h, centrifuged at 13,000*g* for 20 min, and decanted. To the residue, 30 mL of methanol was added and sonicated for 30 min, centrifuged at 4,000*g* for 15 min, and decanted. The methanol portion was repeated, and the three extracts combined were then filtered through glass fiber filter (GF/D, Whatman, Inc., Maidstone, England), then filtered through a 0.45-μm filter (Whatman). Fifty-milliliter portions were removed and extracted against 20 mL of hexane (2×). Combined hexane layers were then spiked with 43.4 μL of a 460 pg/μL internal standard spike of both d4-E2 and d3-T (20 ng). Sample was evaporated under a stream of N gas, reconstituted with 99:1 water:methanol (10 mL), applied to a C18 Bakerbond SPE cartridge, and equilibrated with 5 mL of hexane, 5 mL of ethyl acetate, 5 mL of methanol, and 5 mL of water. The SPE cartridge was dried under vacuum for 1 h, then eluted with 6 mL of ethyl acetate:methanol (5:1). Eluant was evaporated under N then resolvated with 2 mL ethyl acetate. The sample was then extracted with 1 mL of 5% NaCl (aq) by vortexing for 1 min and the supernatant was dried over Na_2SO_4 mini-column, washed with more ethyl acetate, evaporated under N, and reconstituted with 0.2 mL hexane:methylene chloride (1:1). The sample was then applied to a Florisil mini-column conditioned with 10 mL hexane:methylene chloride, and the steroid hormones were eluted with 7 mL of methylene chloride:acetone (95:5). Last, the sample was evaporated to dryness with N, solvated with 100 μL of water:acetonitrile (1:1) and submitted for negative ion LC/MS/MS analysis.

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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 estrogens in negative-ion mode. The capillary voltage was 2.33, cone voltage was 55, source and desolvation temperatures were 120°C and 400°C, respectively. Cone and desolvation gas flows (L h^{-1}) were 0 and 500, respectively. The LC system used was an Alliance 2695 Separation Model (Waters). 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 consisted of 60% 95:5 water:acetonitrile (solvent A) and 40% acetonitrile (solvent B). A linear gradient to 100% B was used from time 0 to 10 min followed by a hold for 5 min at a flow rate of 0.2 mL min^{-1}. Unlabeled estrogen standards were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical analysis (paired *t* test and analysis of variance) was performed using JMP software (SAS Institute Inc., 2009).

RESULTS

Physical Appearance

After each turning of the T pile, the material became successively more friable and the decomposition of the organic matter was more evident, with almost no large masses of bedding or manure present at the completion of the study. In contrast, the NT pile remained mostly unchanged, with large masses of bedding and manure still present at 92 days.

Temperature Profiles

Temperature profiles varied considerably for the T versus NT piles as well as by depth in the T pile (Fig. 2). Missing data are a result of a data logger malfunction between Day 14 and Day 23. Paired *t* test analysis (SAS Institute Inc., 2009) showed that, at $\alpha = 0.05$, the mean temperatures at every depth in both piles were significantly greater than ambient air temperature. In addition, T pile temperatures were greater than NT pile temperatures at corresponding depths, and within the T pile the temperature at MID-UPPER > TOP > MID-LOWER > BOTTOM. During the period after initiation of the piles, the internal temperatures of both piles was above ambient air temperature, with the NT pile being initially higher than the T pile. From 0 to 36 days, the temperatures in both piles continued to approach ambient. The drop in temperature of the T pile on the turning days was a result of introduction of air and cooler material from the outer surface of the pile, as well as temporary removal of the temperature probe. On Day 36, the T pile was turned for the first time, resulting in heating of the pile at all depths during a period of 110 h, reaching a maximum temperature of 68°C at the TOP level of the pile. After the heating spike, the temperature at the TOP level and, to a lesser degree, the MID-UPPER level, decreased. The temperature at the MID-LOWER and BOTTOM levels of the T pile increased for about 7 days, then maintained a nearly constant level of 50°C and 47°C, respectively, until the pile was turned again. In sharp contrast to the T pile, the NT pile temperatures did not vary by depth and were close to ambient, indicating limited thermophilic (45°C–80°C) microbial activity or decomposition. However, mesophilic (15°C–40°C) biological activity could still have been occurring. Between 50 and 65 days, after the second turning of pile T, all depths again showed an increasing temperature (heating) phase followed by a thermophilic phase of nearly constant temperature above 50°C for all depths. Again, the TOP and UPPER-MID levels reached the highest temperature (72°C) before decreasing very rapidly during a 12-h period to 42°C for the TOP. There was a second spike and a decrease in temperature for the TOP level before the temperature rose again and maintained relatively constant at 60°C until being turned again. After the initial heating phase for the UPPER-MID, LOWER-MID, and BOTTOM levels between Day 50 and Day 65, temperatures were relatively constant. Similar to the period of 36 to 50 days, the temperatures at all depths of the NT pile were near ambient. After the third turning of pile T on Day 65, the heating phase was less vigorous for all depths, with almost no heating at the BOTTOM level. The fourth turning of the T pile on Day 78 resulted in only slight heating at the MID positions of the pile and almost no heating at the TOP and BOTTOM levels, indicating that nearly no thermophilic microbial activity could be sustained and that the compost in the T pile was mature.

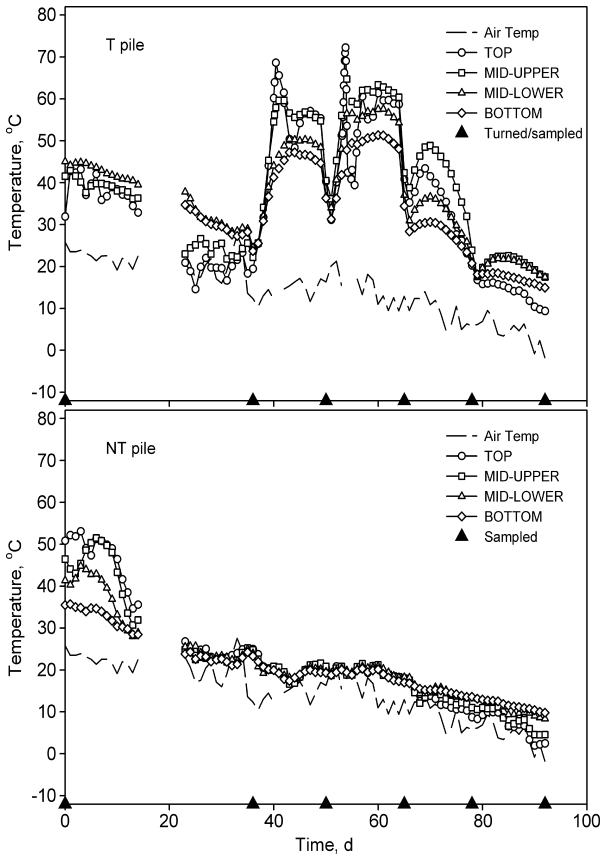

FIG. 2. Changes in temperature of air and at TOP, MID-UPPER, MID-LOWER, and BOTTOM positions of the T and NT piles. Values are 24-h averages. More frequent data are included at the T pile TOP maxima after the 36- and 50-day turnings, to show detail.

Nutrients, pH, EC, Moisture

Analyses of the 4-depth composite samples for each pile are shown in Fig. 3. Analysis of variance was performed using JMP (SAS Institute Inc., 2009), and average values for the five sampling dates including and after Day 36 were calculated to indicate changes caused by turning. Total N decreased from an initial value of 21.7 g kg^{-1} in both piles and fluctuated more for the NT pile. By the end of the study, the T pile had a lower total N concentration (13 g kg^{-1}) than the NT pile (15.9 g kg^{-1}), but the average N concentrations were not significantly different (15.8 g kg^{-1} for both piles). The initial concentration of NH_4^+-N and (NO_3^- + NO_2^-)-N of the material was 3.4 and 0.25 g kg^{-1}, respectively. In both piles, the NH_4^+-N concentration decreased dramatically after piling, to less than 1 g kg^{-1} on Day 36. There

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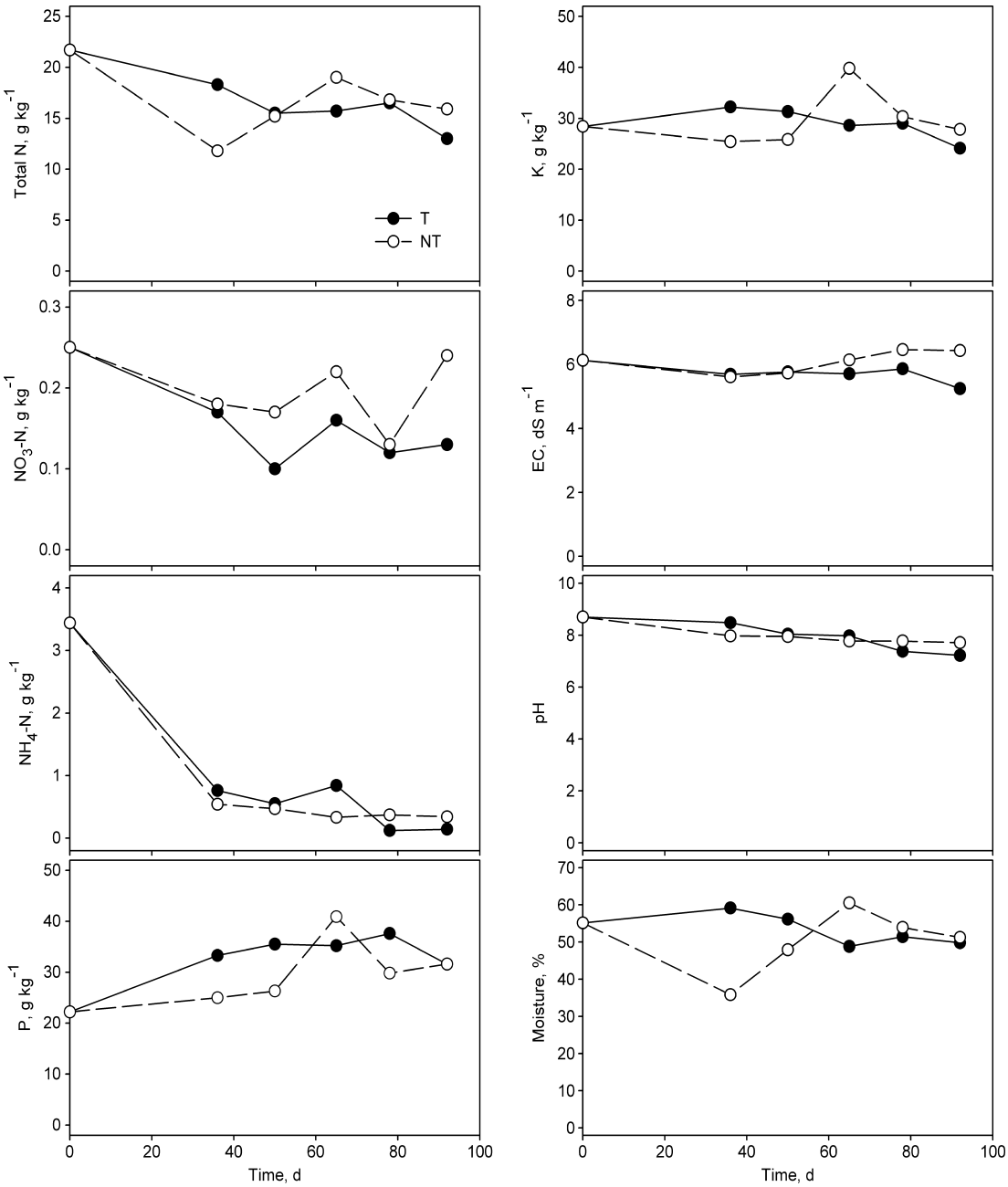

FIG. 3. Changes in total Kjeldahl N, NH_4^+-N, (NO_3^- + NO_2^-)-N, total P, total K, pH, EC, and moisture content for the T and NT manure piles. Nutrient values are reported on a dry weight basis.

was relatively little change in the NH_4^+-N of the NT pile after Day 36, whereas the T pile NH_4^+-N concentrations fluctuated. Average NH_4^+-N concentrations after Day 36 were 0.48 g kg^{-1} for T and 0.41 g kg^{-1} for NT, which were not significantly different at $P < 0.05$. The (NO_3^- + NO_2^-)-N in the T pile generally decreased during the study. The final (NO_3^- + NO_2^-)-N concentration was nearly the same as the initial concentration for the NT pile (0.24 g kg^{-1}) but declined in the T pile (0.13 g kg^{-1}), with post–36-day averages of 0.19 and 0.14 g kg^{-1} (significant at $P <$ 0.1) for NT and T, respectively. Phosphorous increased from the initial value of 22.2 g kg^{-1} to average 34.6 g kg^{-1} in the T pile and 30.7 g kg^{-1} in the NT pile (not significantly different at $P <$ 0.05) after Day 36. Average K concentrations after Day 36 showed little change from the initial value of 28.4 g $kg^{-1}$and were not significantly different (29.8 and 29.0 g kg^{-1} for NT and T, respectively). The pH of both piles decreased steadily throughout the study from the initial value of 8.7 down to 7.8 for the NT pile and 7.2 for the T pile at Day 92, but the averages were not different at $P < 0.05$ (7.8 for both piles) after Day 36. The EC of piles T and NT were almost identical through Day 50, after which, the T pile EC continued to be constant until Day 78, when it decreased slightly to 5.24 dS m^{-1}. During the same period, the EC of the NT pile increased to 6.46 dS m^{-1}. Average EC values for the study period, not including the initial sample, were significantly different at $P < 0.1$ and were 6.07 dS m^{-1} for NT and 5.65 dS m^{-1} for T. Moisture content was fairly constant at

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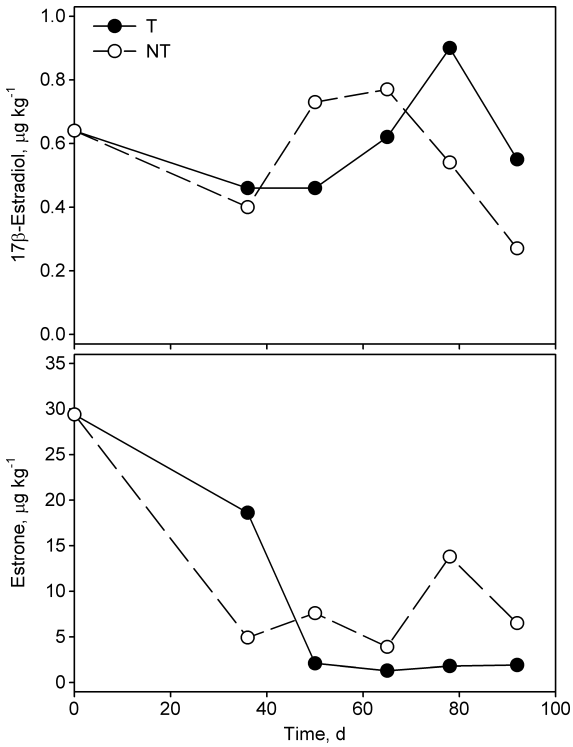

FIG. 4. Changes in dry weight basis 17β-estradiol and estrone concentrations in manure (NT) and composted (T) piles with time.

approximately 53% for the T pile. The NT pile moisture content fluctuated between 36% and 60% but averaged 49.9% for Day 36 or later, which was not significantly different than the T average. Although both piles were uncovered and exposed to the same precipitation, the turning process resulted in a more uniform moisture profile, with moisture contents near optimum (50%–60%) for the composting process (Tiquia et al., 1996).

Estrogens

17β-Estradiol and E1 concentrations in the T and NT piles are shown in Fig. 4. Estrone was initially relatively high (29.4 μg kg^{-1}) then decreased by Day 36. After Day 36, the T pile E1 decreased to a low stabilized level of less than 2 μg kg^{-1}. The E1 in the NT pile also decreased by Day 36 from the initially high concentration but did not stabilize at the same low level as in the T pile. The final concentration of E1 in the T and NT piles was 1.9 and 6.5 μg kg^{-1}, representing decreases of 94% and 78% from initial conditions, respectively. Average E1 concentrations after Day 36 were 7.3 and 5.2 μg kg^{-1} for the NT and T piles, respectively (not significant at $P < 0.05$). Overall, E2 concentrations were much lower than E1, both initially (0.64 μg kg^{-1}) and throughout the 92-day study. The E2 concentrations after Day 36 averaged 0.60 μg kg^{-1} for T and 0.54 μg kg^{-1} for NT, which were not significantly different at $P < 0.05$. The E2 concentrations in both piles decreased at similar rates after the turning at Day 78, with final concentrations of 0.27 μg kg^{-1} for the NT pile and 0.55 μg kg^{-1} for the T pile.

DISCUSSION

Temperature Profiles

The temperature of both piles was above ambient air temperature at the beginning of the study (0 day–13 days) because of increased microbial activity facilitated by aeration during pile placement (Fig. 2). Subsequently, the NT pile was also decomposing aerobically at a rate similar to the T pile at the start of the project. However, this condition did not exist later in the study as indicated by the near-ambient temperature of the NT pile after approximately 13 days, suggesting that either anaerobic conditions or aerobic conditions without thermophilic decomposition existed within the pile. In addition, the NT pile was initiated on the day the temperature readings were started (Day 0), whereas the T pile had been in place for about 5 days before commencement of temperature logging. Hence, the higher temperatures seen early in the NT pile were a result of said aeration at pile initiation. Aeration was probably partially maintained early in the NT pile because of the increased free air space from the high percentage of coarse bedding material present in the initial mixture.

The contrasting temperature profiles of the two piles after Day 36 clearly indicate that the process of aerobic composting was occurring in the T pile. The temperature at all depths rose very rapidly after the first and second turnings and, to a lesser degree, after the third turning of the T pile. This is a clear indication of increased thermophilic microbial activity caused by increased oxygen content of the material. After the first and second turnings of the T pile, the sharp drop in temperature (after a sharp rise in heating) at the TOP may be an indication that the microbial community responsible for decomposition and, hence, temperature increase was debilitated to some degree (MacGregor et al., 1981; Zhu, 2006). Previous research indicates that temperatures of 55°C to 65°C are necessary to destroy pathogens, and 45°C to 55°C must be maintained for maximum biodegradation (Stentiford, 1996), but that temperatures higher than 70°C are detrimental to thermophiles. In general, the temperature of the T pile was optimal for thermophilic decomposition through Day 65. After the third and fourth turnings on Day 65 and Day 78, respectively, the temperature did not increase as much as after previous turnings. This was an indication that the thermophilic microbial activity was reduced compared with the earlier periods, that the organic matter was more stabilized, and that the composting process was complete.

Nutrients

The (NO_3^- + NO_2^-)-N concentrations in this study generally decreased during the 92 days (Fig. 3). This is contrary to findings of increasing (NO_3^- + NO_2^-)-N concentrations in compost piles by other researchers (Tiquia and Tam, 1998; Zhu, 2006). On the other hand, NH_4^+-N concentrations decreased, as was observed in other studies. However, the inorganic N concentrations observed for this study were much lower than those reported by Tiquia and Tam (1998), by as much as an order of magnitude for the (NO_3^- + NO_2^-)-N. This is likely a function of the large proportion of high C content bedding material (corn stalks) present in the T and NT piles. The relatively low and stable (NO_3^- + NO_2^-)-N concentrations (compared with ammonium) also indicated that nitrification was not occurring to a great extent, and that ammonia volatilization, not nitrification, was the cause of the measured NH_4^+-N reductions early in the study. The conversion of the urea excreted in urine and feces to either ammonium or ammonia *via* the enzyme urease occurs rapidly. At higher pH levels, as was the case in the initial pile material (pH = 8.7), more is converted to the ammonia gas form (Gay and Knowlton, 2009). Later fluctuations of NH_4^+-N in the T pile may be an indication of increased gaseous ammonia emissions as a result of higher bacterial activity resulting from increased oxygen content (Chiumenti et al., 2007). Total Kjeldahl N, which included inorganic N, generally decreased during the course of the study for the T pile. This also may be a result of the increased

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breakdown of organic matter and gaseous nitrogenous emissions. Because the piles received natural rainfall, there may have been some movement of nitrate within the pile because of leaching. But because the concentration of the 4-depth composites did not change appreciably during the course of the study, no nitrate was leached out of the piles and N losses were mainly caused by ammonia volatilization.

The concentrations of both P and K were not very different between the T and NT piles at the end of the study, indicating that composting did not appreciably alter the P and K status of the material. From an agronomic perspective, the composting process had a positive effect on the pH and EC of the manure material, as a more neutral pH and lower EC were a result of composting. As stated in the previous discussion, ammonia production and volatilization are associated with higher pH levels. During the course of the composting process, lower pH values would help to lower N losses by limiting conversion of urea and other N sources to ammonia.

Estrogens

The estrogen concentration time series indicate that the predominant estrogen form in both piles was E1 (Fig. 4). Hanselman et al. (2003) reports that 96% of estrogens (E2 and E1) in swine are excreted in the urine (mostly in conjugated forms of glucuronide or sulfate) and that E2 and E1 were found in similar concentrations (160 and 217 μg kg^{-1}, respectively) in swine wastes derived from finishing hoop structures. At the onset of the current study, the E1 concentrations were nearly two orders of magnitude higher than E2, indicating that significant conversion of E2 to E1 or estrogen deconjugation, in the presence of fecal microorganisms (Jacobsen et al., 2005), had likely occurred between elimination from the animal to the time the first samples were taken. Therefore, the initial measure of estrogen levels of both piles likely represents E2 and E1 concentrations that have already undergone considerable transformations (e.g., conversion of E2 to E1, deconjugation) compared with parent estrogen forms originally released from the pigs.

In a review of methods comparing estrogenic activity, Borgert et al. (2003) reported that, depending on the assay used, the potency of E1 has been found to be between 0.14 and 0.6 times the potency of E2. In addition, Allison and Omeljaniuk (2000) found that the hormone receptor binding strength for E2 is 100 times stronger than E1 in the hypothalamus of a rainbow trout. Hence, for the current study, the concentration of E2 was added to 0.1× the E1 concentration as a measure of total estrogenicity (TE) in the samples (Fig. 5). Total estrogenicity decreased from the initial concentration of 3.6 μg kg^{-1} more rapidly in the NT pile after the piles were constructed. However, the TE concentration stabilized at a slightly lower level in the T pile after 50 days. The average TE concentrations for the last four sampling dates were 1.37 μg kg^{-1} for the NT pile and 0.81 μg kg^{-1} for the T pile, whereas the TE concentrations measured at the end of the study were nearly the same for the T and NT piles (0.73 and 0.92 μg kg^{-1}, respectively).

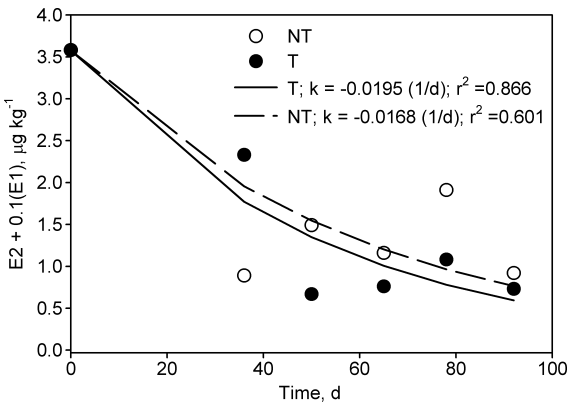

FIG. 5. Changes in dry weight basis of total estrogenicity (17β-estradiol plus 0.1 × estrone) concentrations in static manure (NT) and mixed compost (T) piles with time and nonlinear regressions with first-order-rate degradation constants.

The TE data in each of the piles was fit with a first-order degradation equation to obtain a degradation rate constant (k, d^{-1}) (Fig. 5). A standard degradation equation (i.e., $C = C_0e^{-kt}$; where C is concentration [M L^{-3}], C_0 is initial concentration [M L^{-3}], k is the first-order degradation coefficient [T^{-1}], and t is time [T]) was fit to the measured data using the following objective function (*E[k]*):

$$E(k) = \sum_{i=1}^{N} \{[C(t_i) - \overline{C}(t_i, k)]\}^2 \quad (1)$$

The k is sequentially optimized so that the difference between the observed C and calculated concentrations (C) at individual measurement time steps (t_i) is minimized. The Solver tool in Microsoft Excel, which uses a Newton method of minimization, was used to seek a k value that provided the smallest *E(k)*. Subsequently, the half-life of total estrogenic activity was calculated for each material as:

$$D_{T50} = 0.693/k$$

where D_{T50} is the half-life. The half-life of total estrogenicity in the T pile material was determined to be 36 days compared with 41 days for the material from the NT pile. This indicated that there was little difference in reduction of estrogenic activity by composting compared with static manure storage.

It should be noted that the estrogen concentrations reported here are water and methanol extractable from the solid manure and should not necessarily be directly compared with estrogen concentrations reported in surface or groundwater. It is not known from this study what the resulting aqueous concentrations might be, which could result from application of this material to an agricultural field. However, if application of the material were based on N fertility requirements, the following calculations could be made. The nutrient analysis indicated that application of 2 Mg ha^{-1} of the composted material would supply 13.1 kg N ha^{-1}. If 18 Mg ha^{-1} of compost (TE = 0.365 μg kg^{-1} wet basis, 50% moisture) was applied to supply a crop with 118 kg N ha^{-1}, the resulting total estrogenicity (0.1 × E1 plus E2) addition to the field would be 6.6 mg ha^{-1}. By comparison, the TE introduction to a field from the application of the same amount of material from the static NT pile (TE = 0.46 μg kg^{-1} wet basis) would be 8.3 mg ha^{-1}. If the material was applied directly from the barn (TE = 1.79 μg kg^{-1} wet basis) at the same rate, 32.2 mg ha^{-1} of TE would be introduced into the field. Hence, there would be a very large reduction in the amount of estrogenicity introduced to the environment if swine waste was either stored in piles or composted for at least 50 days before application to fields.

CONCLUSIONS

Two piles of manure/urine/corn stalk bedding, one mixed regularly and one static throughout the study, were constructed, and changes in temperature, nutrients, and hormone concentrations were monitored during a 92-day period. Temperature rise

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in the T pile after turning indicated high aerobic, thermophilic, microbial activity and decomposition of organic matter. Total N, P, and K concentrations were not altered significantly by piling or composting, although NH_4^+-N concentrations were reduced by more than 90% in each pile. Initial estrone concentrations were reduced by 94% as a result of composting compared with a reduction of 78% without turning. Total estrogenicity, based on the assumption that the potency of E2 = 1 and of E1 = 0.1, decreased by 74% in static manure and 79% in compost during the course of the study. It is impossible to determine from this study if the lower estrogenicity is resultant from degradation or increased sorption over time. It is clear, however, that storage of the manure in either static or compost piles reduced the amount of total estrogenicity that would potentially be applied to a field, and hence reduced the potential for surface and groundwater contamination from these potent endocrine-disrupting chemicals. Additional work needs to be done to validate these exploratory findings and to further investigate the fate of estrogen species in swine manure management systems.

ABBREVIATIONS

E2: 17β-estradiol;
E1: estrone;
TE: total estrogenicity;
T: turned compost pile;
NT: not turned manure pile.

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