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Carcass mass can influence rate of decomposition and release of ninhydrin-reactive nitrogen into gravesoil

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ABSTRACT

To investigate the use of ninhydrin-reactive nitrogen (NRN) in gravesoil to estimate early postmortem interval (PMI), we conducted an experiment to decompose swine (*Sus scrofa*) carcasses of contrasting mass (~1 kg, ~20 kg, ~40 kg, ~50 kg). Carcasses were placed on the soil surface during June 2007 to monitor mass loss and the concentration of gravesoil NRN over an interval of 15 days. Carcasses of a mass $\leq$ 20 kg decomposed more rapidly than larger carcasses. However, 1 kg carcasses were associated with a slower release of NRN into gravesoil but a greater concentration of NRN per kg carcass (NRN_C). We conclude that carcass mass can affect the rate of decomposition and release of NRN into gravesoil, which reflects an interaction between carcass volume and blow fly colonization. Furthermore, we conclude that neonatal carcasses require a different equation than larger carcasses when using gravesoil chemistry to estimate PMI.

1. Introduction

One aim of forensic taphonomy is to estimate postmortem interval (PMI) [1]. Achieving this goal lets investigators reconstruct the series of events associated with the death scene, accept or reject alibis, and include or exclude suspects and victims from an investigation. At present, the development of blow fly larvae (Diptera: Calliphoridae) is arguably the most effective method to estimate early (<30 days) PMI (e.g. [2]). However, the possibility that flies can be inhibited from colonizing a body during the perimortem period (e.g. [3]), prompts the need to develop new methods for the estimation of PMI. No single method to estimate PMI is effective at all scenes. Moreover, the development of separate, independent measures of PMI would provide a method for increasing the accuracy estimates by evaluating the overlap between independent estimators.

Carter et al. [4] recently developed a method for using ninhydrin-reactive nitrogen (NRN) to locate clandestine graves and reconstruct death scenes [5]. This method has the potential to act also as a basis for the estimation of early PMI. This application is based on the finding that cadaver decomposition is initially characterized by a lag phase, or Fresh stage of decomposition [6], where little decomposition occurs and an insignificant concentration of organic material is released into gravesoil [4,7,8]. Thus, some time is required before organic material, including NRN, is released into gravesoil. The time required for this influx of NRN is unknown. To develop the dynamics of NRN as a tool to estimate early PMI it is necessary to determine the time period required for the release of NRN into gravesoil.

Various biotic and environmental factors, such as temperature [9] and soil type [4], influence the rate of NRN movement into soil. Carcass mass is potentially an important factor in this regard. Because dead bodies have various masses, it is essential to determine the influence of cadaver mass on the release of NRN into gravesoil. Although cadaver mass is known to have an impact on the rate of decomposition [10] and release of cadaveric material into gravesoil [7], few studies have directly investigated the relationship between initial body mass and decomposition [11–15]. Consequently, the decomposition of a wide range of cadaver masses has yet to be investigated experimentally.

Here, we tested the hypothesis that cadaver mass has a significant effect on the rate of release on NRN into gravesoil so that larger carcasses will release a significant concentration of NRN into gravesoil more rapidly than smaller carcasses. To examine this question, swine (*Sus scrofa*) cadavers, three each of four different weight categories (1 kg, 20 kg, 40 kg, 50 kg) were placed on the soil surface to decompose for 15 days during the summer (June 2007). Swine were chosen as experimental subjects based on their availability in an appropriate range of masses and with common

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times of death. Moreover, swine are commonly used as human surrogates in decomposition studies, which allows for some application of results to human decomposition. The concentration of NRN in gravesoil and gravesoil pH were measured to assess the effect of cadaver mass on gravesoil chemistry and develop gravesoil NRN as a tool to estimate early PMI.

2. Materials and methods

2.1. Study site

The experimental site was at the University of Nebraska Agricultural Research and Development Center located approximately 48 km north of Lincoln, NE, USA at 41°10′24.47″N, 96°30′3.20″W. The site is a pasture that is intermittently grazed by cattle and horses. The soil at the site is a deep, silty clay loam of the Yutan series (Mollic Hapludalf) (Soil Survey Staff, 2003) with a texture of 15.1% sand, 53.6% silt and 31.3% clay. Soil physicochemical characteristics include total carbon (3.5% ± 0.1%), organic carbon (3.5% ± 0.1%), total nitrogen (0.32% ± 0.01%), ammonium (5.3 ± 0.6 μg g^{-1} soil), nitrate (6.1 ± 0.3 μg g^{-1} soil), Bray phosphorus (11.2 ± 0.4 μg g^{-1} soil), potassium (506 ± 11 μg g^{-1} soil), electrical conductivity (0.22 ± 0.01 mmhos cm^{-1}), and cation exchange capacity (17.9 ± 0.3 cmol kg^{-1}).

The climate at the experimental site is temperate midcontinental characterized by hot summers, cold winters, and moderately strong surface winds. Average annual precipitation is 695 mm with approximately 75% of the precipitation occurring between April and September. Mean annual temperature is 9.8 °C with mean minimum and maximum temperatures ranging from 0 °C (January) to 31 °C (July). The vegetation at the site is dominated by non-native grasses (predominately smooth brougham) and forbs (predominately white clover) with some native vegetation, including daisy fleabane, yellowwood sorrel nut sedge, and pasture rose. Coyotes (*Canis latrans*) and turkey vultures (*Cathartes aura*) are the primary scavengers in the area. The site was square-fenced with steel cattle fence to prevent the entry of the mammalian scavengers. Cattle fence was 6.3 m in length and 1.27 m in height with 15.24 cm × 10.16 cm mesh. This construction allowed the presence of avian scavengers, but this activity has not been observed in five years of study at this experimental site.

2.2. Experimental design

During June 2007 swine (*S. scrofa*) carcasses of contrasting masses (~1 kg, ~20 kg, ~40 kg, ~50 kg) related to age (neonate to adult) were acquired from the University of Nebraska Lincoln Swine Center. Carcasses were acquired within 20 min of death, which was caused by blunt force trauma to the skull with a bolt gun. Ethical approval for carcass use was acquired through the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Carcasses were transported to the experimental site and were oriented on their right side facing west within 60 min of death. Carcasses were placed on a weighing frame (2.5 cm^2 polypropylene mesh bound to a 85 cm × 40 cm PVC frame) placed directly on the soil surface (Fig. 1). Decomposition was also measured through the calculation of Total Body Score [16]. Recording thermocouples were located on and around the carcass to record temperature at intervals of 1 h. Soil samples were collected (0–5 cm depth) from adjacent to the cadaver at the time of placement and at intervals of 24 h for the initial seven days followed by sampling on day 9 and day 15. Control (no cadaver) samples were also collected. However, sampling was not conducted on day 3 and day 8 due to severe thunderstorms. This experiment was replicated three times resulting in a total of 12 carcasses. Soils were analyzed for NRN following the method by Carter et al. [4] with the calculation of micrograms NRN per gram soil (μg NRN g^{-1} soil) and NRN g^{-1} soil kg^{-1} carcass (NRN_C). NRN_C was calculated using carcass mass at death and using carcass mass at each time of sampling. Accumulated Degree Days (ADDs) were calculated after Arnold [17] using a base temperature of 0 °C [7]. Soil pH was analyzed using a 1:5 soil:water ratio [18].

2.3. Data analysis

Normality and homogeneity of variance were tested using the Kolmogorov–Smirnov and Levene's test, respectively. Parametric statistics were generated using a univariate analysis of variance with SPSS version 16.0.1 (Chicago, IL, USA).

3. Results

3.1. Temperature

Daily temperatures ranged from 13.7 °C to 32.9 °C (Table 1). A severe thunderstorm occurred on day 3 and day 8, consequently, sampling could not be conducted on those dates.

3.2. Carcass decomposition

Carcass decomposition followed a pattern typically [8] associated with death caused by blunt force trauma to the head (Fig. 1). More specifically, blow flies (Diptera: Calliphoridae) were present on the 20 kg carcass within seconds of placement on the soil

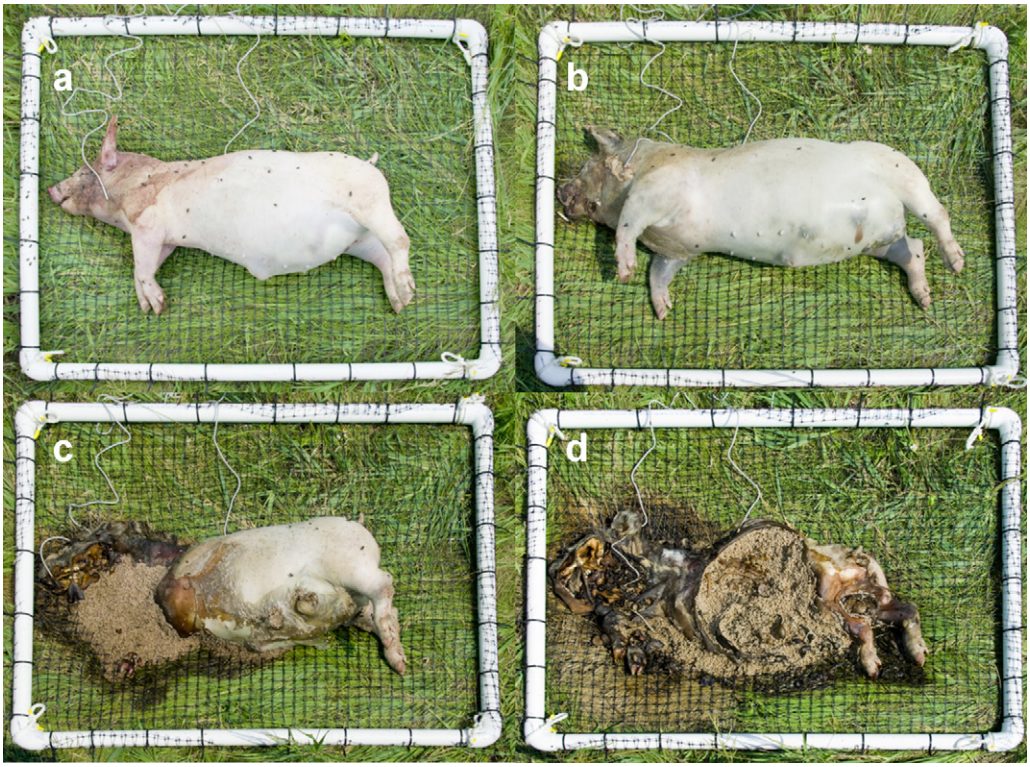

Fig. 1. 20 kg swine (*Sus scrofa*) carcass placed on the soil surface of a pasture near Mead, NE, USA following blunt force trauma to the skull during June 2007 after one day (a), two days (b), three days (c), and four days (d) postmortem.

Table 1
Temperature (°C) during the decomposition of swine carcasses (1 kg, 20 kg, 40 kg, 50 kg) on the soil surface near Mead, NE during June 2007. Accumulated Degree Days were calculated using a base temperature of 0 °C (see [7]).

Day	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Accumulated Degree Days (°C)
0	28.9	18.9	23.6	23.6
1	29.5	18.8	24.0	47.6
2	24.3	20.3	22.1	69.7
3	28.2	19.6	23.1	92.8
4	31.6	18.6	25.0	117.8
5	32.2	19.4	25.8	143.6
6	30.4	19.9	25.1	168.7
7	25.6	16.8	22.8	191.5
8	26.6	13.7	21.2	212.7
9	31.8	16.4	24.5	237.2
10	32.3	20.3	26.7	263.9
11	31.6	20.7	25.5	289.4
12	25.2	17.9	21.8	311.2
13	29.4	15.8	22.6	333.8
14	32.9	20.4	26.4	360.2
15	31.3	21.1	26.2	386.4

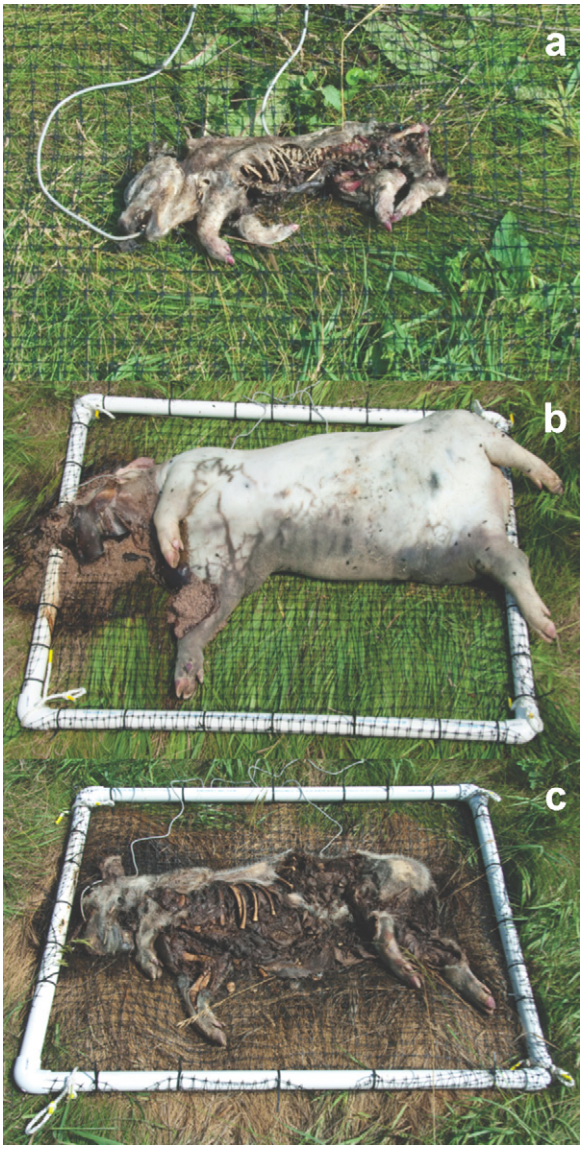

Fig. 2. Decomposition of swine (*Sus scrofa*) carcasses placed on the soil surface of a pasture near Mead, NE, USA following blunt force trauma to the skull during June 2007 including 1 kg (neonatal) carcass five days postmortem (a), 50 kg carcass four days postmortem, and (c) 40 kg carcass 9 days postmortem.

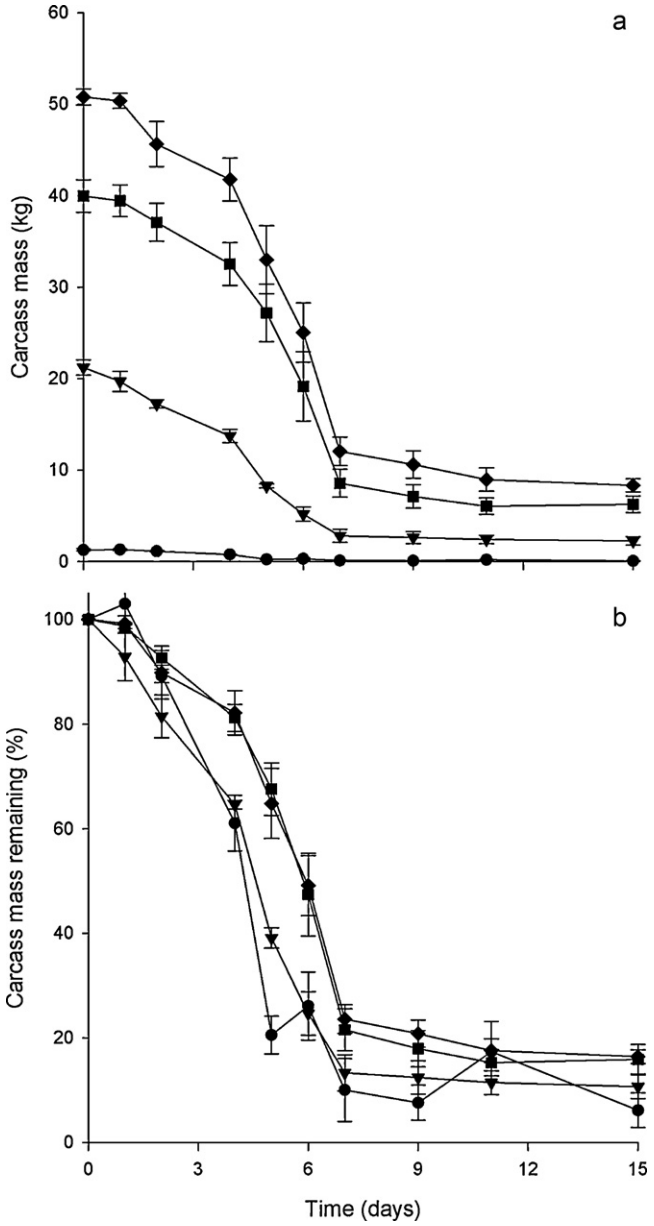

Fig. 3. Absolute (a) and percentage (b) mass loss of 1 kg (●), 20 kg (▼), 40 kg (■), or 50 kg (◆) swine (*Sus scrofa*) carcasses placed on the soil surface of a pasture near Mead, NE, USA following blunt force trauma to the skull during June 2007.

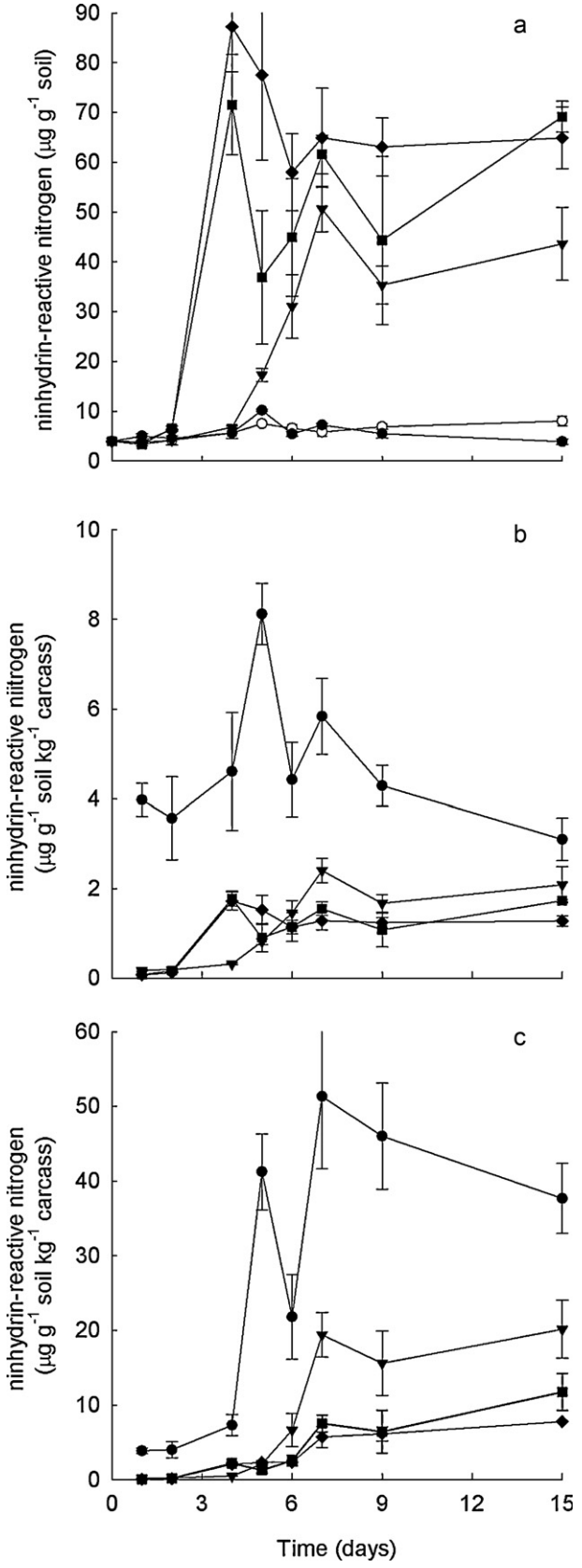

Fig. 4. Ninhydrin-reactive nitrogen per unit soil (a: μg g^{-1} soil) and per unit carcass (μg g^{-1} soil kg^{-1} carcass) using carcass mass at death (b) and carcass mass at time of sampling (c) in gravesoil associated with no carcass (○) or the decomposition of 1 kg (●), 20 kg (▼), 40 kg (■), or 50 kg (◆) swine (*Sus scrofa*) cadavers on the soil surface of a pasture near Mead, NE, USA following blunt force trauma to the skull during June 2007.

surface and eggs were present in the facial orifices by one day postmortem (Fig. 1a). Initial maggot masses were present in the facial region and initial discoloration (blackening) of the soil surface occurred initially 3 days (92.8 ADDs) postmortem (Fig. 2c). These phenomena corresponded with the Bloat stage of decomposition and a Total Body Score (TBS) of 20.7 ± 0.4. By four days postmortem (117.8 ADDs) the carcass has progressed into Active Decay (TBS = 25.0 ± 0.8) which coincided with the maggot mass inhabiting the anterior third of the carcass and discoloration of the soil surface extending to the posterior third of the carcass (Fig. 1d). By five days postmortem (143.6 ADDs) the entire carcass was inhabited by the maggot mass and discoloration of the soil was associated with full length of the carcass, primarily on the caudal side. This corresponded to a Total Body Score of 26.3 ± 0.7. By 9 days postmortem (237.2 ADDs) maggots had migrated from the 20 kg carcass, which designated the onset of Advanced Decay (TBS = 31.3 ± 0.9).

Although all carcasses followed a similar decomposition pattern, carcass decomposition was influenced by carcass mass. Neonatal carcasses were associated with little, if any, discoloration of the soil surface and had reached Advanced Decay (TBS = 27.0 ± 1.3) by 5 days postmortem (143.6 ADDs) (Fig. 2a). Also, the skulls of the 40 kg and 50 kg (Fig. 2b) only were being consumed by maggots at 4 days postmortem (117.8 ADDs). This was associated with a Total Body Score of 17.7 ± 1.9 (40 kg) and 21.0 ± 2.1 (50 kg), which was associated with more pronounced marbling and skin slippage in the larger cadavers, presumably because the majority of the carcass was being decomposed microbially and abiotically. However, the 20 kg, 40 kg (Fig. 3c), and 50 kg carcasses reached Advanced Decay by 9 days postmortem (237.2 ADDs). At this stage, the Total Body Score equaled 31.3 ± 0.9, 28.3 ± 0.2, and 28.0 ± 0.5, respectively for these cadavers.

3.3. Carcass mass loss

Carcass decomposition followed a sigmoidal curve where approximately 80% of carcass mass was lost by day 9 (237.2 ADDs) (Fig. 3a). On a percentage basis, the neonatal and 20 kg carcasses decomposed significantly ($P < 0.05$) faster during the initial six days of death (Fig. 3b). By 11 days postmortem (289.4 ADDs), however, initial carcass mass had no effect on the percentage of carcass mass remaining.

3.4. Ninhydrin-reactive nitrogen

The NRN concentration of gravesoil associated with 20 kg, 40 kg, and 50 kg carcasses was significantly ($P < 0.01$) greater than in neonate gravesoil and control soils by 4 days (117.8 ADDs) postmortem (Fig. 4a). These elevated concentrations persisted throughout the experiment (15 days/386.4 ADDs). In contrast, the concentration of gravesoil NRN associated with neonate carcasses required 5 days (143.6 ADDs) to increase to a level that was significantly ($P < 0.05$) greater than control soils.

The concentration of NRN per unit carcass (NRN_C) in neonate gravesoil was significantly ($P < 0.05$) greater than in all other gravesoils throughout the experiment (Fig. 4b). Another, albeit brief, significant difference in NRN_C was observed in gravesoil under the 20 kg carcasses, which was significantly ($P < 0.05$) less than that from all other gravesoils at 4 days postmortem (117.8 ADDs) (Fig. 4b). Calculations of NRN_C based on carcass mass at the time of sampling (Fig. 4c) were generally consistent with calculations based on carcass mass at the time of death. However, they did show that NRN_C associated with 20 kg carcasses was also greater than NRN_C associated with the 40 kg and 50 kg carcasses.

3.5. Gravesoil pH

The pH of control soils ranged from 6.3 to 6.6 during the experiment. No significant differences between gravesoils and control soil were observed through day 9 (237.2 ADDs). On day 15 (386.4 ADDs), however, a significantly ($P < 0.05$) greater pH was associated with 50 kg (7.4 ± 0.1), 40 kg (7.4 ± 0.2), and 20 kg (7.5 ± 0.2) carcasses relative to neonate gravesoil (6.7 ± 0.1), which was significantly ($P < 0.05$) greater than control soil (6.5 ± 0.1).

4. Discussion

The current results show a relationship between carcass mass, rate of decomposition, and NRN in gravesoil. Neonatal and 20 kg carcasses decomposed more rapidly than larger carcasses for the initial 6 days postmortem (168.7 ADDs), which is generally supported by the findings of Vass et al. [7] and Simmons et al. [10]. However, more rapid decomposition did not result in a more rapid release of NRN into gravesoil. Rather, the release of NRN from the neonate carcass was slower than from the larger carcasses. However, the concentration of NRN_C was significantly greater than larger carcasses. In addition, neonatal carcasses did not have a persistent effect on gravesoil pH. Gravesoil NRN during the initial 9 days of decomposition (up to 237.2 ADDs) likely was organic N (protein, peptide, amine, amino acids) primarily. The lack of difference in pH is probably associated with no significant change in ammonium formation within the initial 15 days of decomposition (up to 386.4 ADDs). Later, the change observed on day 15 associated with the neonatal carcass might be consistent with an increase in nitrate (see [19]). Based on these results, we conclude that carcass mass affects the rate of decomposition, the time required for NRN to be released into gravesoil, and the concentration of NRN in gravesoil. Furthermore, we conclude that neonatal carcass require a different equation than larger carcasses when using gravesoil chemistry to estimate PMI.

Carcass breakdown typically follows a sigmoidal pattern [8]. This pattern is initially characterized by a lag phase, which can represent the time required for the decomposer community to become established and consume a significant component of an organic resource (e.g. [20]). In the current study, the primary decomposer community consisted of blow flies (Diptera: Calliphoridae) and microbes (largely bacteria, given the absence of fungal growth on carcasses). Establishment of blow flies on a carcass occurs first through egg laying, and second through maggot growth and development. We expected and observed similar oviposition on all carcasses irrespective of size: blow flies laid eggs in the eyes, nose, mouth, and head wound. This is not to say that initially the same number of blow fly eggs were laid on all carcasses, rather the initial ovipositional substrates were completely used by blow flies in the first two days postmortem. Subsequently, as larvae developed they opened the body cavities of smaller (neonatal and 20 kg) carcasses much sooner than with larger carcasses. Although these differences undoubtedly reflect a continuum across carcasses masses, in this study we note that carcass masses $\leq$ 20 kg had demonstrably faster rates of decomposition (as indicated through mass loss) than larger (40 kg and 50 kg) carcasses. Such a relationship is expected based on differences in surface area to mass ratios across an order of magnitude difference in carcass masses. Yet, our observations differ from Hewadikaram and Goff [12], where a 15.1 kg swine (*S. scrofa*) carcass decomposed more rapidly than a 8.4 kg swine carcass. It would be helpful if the Hewadikaram and Goff [12] work were conducted again, but replicated, to determine of the observed differences in mass loss are significant.

We also observed that carcass mass influenced the rate of NRN release into gravesoil, which is likely due to the consumption of N by maggots. Maggots take up carcass N while feeding. In doing so, they affect the release of NRN into gravesoil. Because of the small surface area of neonatal carcasses, it is possible that blow fly larvae can take up most available N while they are active. This leaves NRN to enter the gravesoil when blow fly larvae begin to migrate for pupation. In contrast, the amount of available NRN associated with a 40 kg and 50 kg carcass is greater and cannot be consumed completely by the maggot biomass. This allows NRN to enter the gravesoil earlier than with smaller carcasses.

Insect activity can also explain the differences observed in NRN_C between carcass mass. Neonatal carcasses do not have the surface area to support an insect biomass similar to that observed in association with 20 kg, 40 kg, and 50 kg cadavers (e.g. [12]). Thus, the higher concentration of NRN_C associated with neonatal cadavers is probably because more NRN was released into gravesoil rather than being consumed by fly larvae. In other words, maggots were consuming NRN as it was becoming available, however, maggots were able to use relatively little of the neonatal carcass which left a greater proportion of NRN to be released from the carcass into the soil following maggot migration. The lag phase observed with NRN_C associated with 20 kg on day 3 (92.8 ADDs) and day 5 (143.6 ADDs) might be due to an optimal insect biomass that was able to consume NRN as it was being released from the body. After 5 days (143.6 ADDs), however, the rate of NRN release was greater than maggot feeding activity.

If we accept that maggot biomass regulates the release of cadaveric nitrogen into gravesoil, then the current results might indicate the presence of a phenomenon analogous to Michaelis–Menten kinetics (see [21]). In this scenario, a cadaver of approximately 40 kg represents the optimal cadaver mass for release of NRN into gravesoil. In other words, carcasses with an initial mass of 40 kg or greater will release similar amounts of NRN into gravesoil because a zero-order reaction has been met, i.e. insect activity is at its greatest and an increase in carcass mass will not increase the amount of NRN release into the soil (a zero-order reaction). In contrast, the smaller carcasses demonstrated that insect activity and release of NRN were dependent on carcass mass (a first-order reaction). A 20 kg cadaver might represent the maximum carcass mass at which first-order kinetics are met, as demonstrated by the nearly 100% uptake of available NRN by the maggot mass for the initial few days of decomposition. The meta-analysis conducted by Simmons et al. [10] supports the current work by providing additional evidence that insect biomass and carcass mass affect decomposition.

The current measurements of NRN g^{-1} soil and NRN_C have significant implications for the use of soil-based estimations of PMI. Vass et al. [7] proposed that accounting for cadaver mass compensated for differences in victims of different masses. Within a particular mass range (they presented a range of approximately 40–60 kg) this appears to be correct. However, the current results show that smaller carcasses, those representing neonates and juveniles, do not follow the same dynamics that are associated with adult masses. Consequently, estimates of PMI based on larger cadavers [7] should not be used to estimate the PMI of cadavers with an initial mass less than 40 kg.

We conclude that the release of NRN into gravesoil is influenced by carcass mass, which is related to insect activity. Carcasses with a mass $\leq$ 20 kg can decompose more rapidly than larger carcasses because they contain less material for insects to consume. A significant increase in gravesoil NRN associated with carcasses with a mass $\geq$ 20 kg occurred at 4 days postmortem (117.8 ADDs) while significant increase in gravesoil NRN associated with carcasses of 1 kg occurred at 5 days postmortem (143.6 ADDs). Therefore, an increase in gravesoil NRN associated with $\geq$20 kg carcasses represented a minimum PMI of approximately 118 ADDS. In addition, an increase in gravesoil NRN associated with

≤1 kg carcasses represented a minimum PMI of approximately 144 ADDs.

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