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ABSTRACT Necrophagous invertebrates have been documented to be a predominant driver of vertebrate carrion decomposition; however, very little is understood about the assembly of these communities both within and among seasons. The objective of this study was to evaluate the seasonal differences in insect taxa composition, richness, and diversity on carrion over decomposition with the intention that such data will be useful for refining error estimates in forensic entomology. *Sus scrofa* (L.) carcasses (n = 3–6, depending on season) were placed in a forested habitat near Xenia, OH, during spring, summer, autumn, and winter. Taxon richness varied substantially among seasons but was generally lower (1–2 taxa) during early decomposition and increased (3–8 taxa) through intermediate stages of decomposition. Autumn and winter showed the highest richness during late decomposition. Overall, taxon richness was higher during active decay for all seasons. While invertebrate community composition was generally consistent among seasons, the relative abundance of five taxa significantly differed across seasons, demonstrating different source communities for colonization depending on the time of year. There were significantly distinct necrophagous insect communities for each stage of decomposition, and between summer and autumn and summer and winter, but the communities were similar between autumn and winter. Calliphoridae represented significant indicator taxa for summer and autumn but replaced by Coleoptera during winter. Here we demonstrated substantial variability in necrophagous communities and assembly on carrion over decomposition and among seasons. Recognizing this variation has important consequences for forensic entomology and future efforts to provide error rates for estimates of the postmortem interval using arthropod succession data as evidence during criminal investigations.

KEY WORDS Calliphoridae, Coleoptera, decomposition ecology, necrobiome, succession

The decomposition process is critical to nutrient cycling and energy flow in most ecosystems (Carter et al. 2007, Barton et al. 2012). Thus, understanding this process has broad application to ecological and environmental science but also in medicolegal entomology when arthropods are collected and interpreted as evidence (Byrd and Castner 2010). Necrophagous invertebrates are responsible for the majority of vertebrate carcass decomposition (Payne 1965, Carvalho and Linhares 2001, Goff 2009), defined here for the first time as part of the 'necrobiome.' Here we define the necrobiome as the community of species (e.g., prokaryotic and eukaryotic) associated with decomposing remains of heterotrophic biomass, including animal carrion and human corpses. The carrion invertebrate species composition of this community can vary among habitats, regions, days, and seasons (Matuszewski et al. 2010). Many species consume the carrion organic material directly (e.g., Diptera: Calliphoridae) (Campobasso et al. 2001), while others use the resource as habitat or as a location to find other prey insects attracted to the carrion as food sources (e.g., Coleoptera: Silphidae) (Gibbs and Stanton 2001). The dipteran and coleopteran taxa comprise the majority of insects involved in terrestrial carrion decomposition (Byrd and Castner 2010). During decomposition, there is a qualitative, recognizable pattern of necrophagous invertebrate (mostly insects) succession and community assembly associated with the different stages of decomposition (Arnaldos et al. 2005, Kreitlow 2010); however, most studies of this process have been qualitative and with little or no statistical replication (Michaud et al. 2012).

In terrestrial habitats there are five recognized stages of decomposition: fresh, bloat, active decay, advanced decay, dry, and skeletal stages (Payne 1965), and summarized by Byrd and Castner (2010). The fresh stage begins at death and continues until the carcass begins to bloat. Bloating results from increased microbial metabolic activity that produce gaseous byproducts that cause carrion to inflate, which also attracts or repels certain necrophagous insects to the carcass (LeBlanc 2008, Matuszewski et al. 2010). Active decay follows the bloat stage and is obvious when the body begins to rapidly decompose because of

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insect activity (Centeno et al. 2002). Advanced decay is associated with reduced entomological activity as the resource is consumed (Goff 2009). When all that remains are bones with dry skin and hair the carcass is considered to be in the dry stage and in the skeletal stage when only bones are left (Payne 1965). For the purposes of this article we use the stages defined here by Payne (1965), but combine the dry and skeletal stages.

Arthropod activity and community composition commonly shift between each of these stages of decomposition either because of alteration of the resource directly or through competitive and/or predatory interactions with other invertebrates, which results in a pattern of succession (Payne 1965). While this arthropod community assembly pattern provides a model for ecological research (VanLaerhoven 2010), it also has been applied to the field of forensics when entomological evidence is collected at a crime scene (Schoenly et al. 1992). Forensic entomologists are able to use this pattern of arthropod community assembly to estimate the period of insect activity (PIA), which is often synchronous with the minimum postmortem interval (PMI_{min}) (Amendt et al. 2007, Merritt and Benbow 2009, Wells and Lamotte 2010, Tomberlin et al. 2011b). However, there are instances where the PIA is not synchronous with the PMI_{min}, potentially introducing error in entomologically based estimates. In 2009, the United States National Research Council (NRC) called for a serious need to provide quantitative error rates in inferences derived from most forensic evidence and legal case work (National Research Council [U.S.] 2009; Tomberlin et al. 2011a,b). Therefore, it is becoming increasingly important for forensic entomologist to understand the natural variability in necrophagous arthropod community assembly to allow for better statistical predictions and generalization when using entomological evidence to estimate the PMI_{min} (Tomberlin et al. 2011b, Michaud et al. 2012).

We conducted a series of decomposition experiments to better understand variability in necrophagous insect community composition and assembly among seasons in a temperate mid-western United States forested habitat. We hypothesized that necrophagous insect taxa composition as overall community structure, richness, and diversity would vary through decomposition, and that these community characteristics would significantly vary across seasons.

Materials and Methods

Study Site and Design. This study was conducted within Morris Bean Reserve (39° 45′53.67″ N, 83° 54′41.12″ W) of Greene County, OH, a mid-west temperate forest of 12.2 ha with ≈90% canopy cover surrounded by agricultural fields described in more detail by Lewis and Benbow (2011). There was a small tributary stream running adjacent to the reserve that emptied into the Little Beavercreek River. The predominant trees were honey locust (*Cleditsia triacanthos* L.) and several maple species (*Acer* spp. L.), while the most common subcanopy cover was Amur honeysuckle (*Lonicera maackii*). Five 1 m^2 plots, along each of six, 50 m transects were established running north–south for a total of 30 plots that were 10–80 m from each other.

Sus scrofa (swine) (L.) carcasses weighing from 14 to 18 kg were used as models of vertebrate carrion decomposition (Schoenly et al. 2007) during four seasonal trials: spring (15 April–2 June 2009), summer (22 July–31 August 2009), autumn (11 November 2009–14 April 2010), and winter (8 February–23 April 2010). Six replicate carcasses were used in spring and summer trials whereas three were used during autumn and winter trials; the latter trials only included three replicates because of cost constraints and expected longer decomposition time during the colder months. The sizes of the carcasses were based on availability. Research has shown that swine carcasses are suitable models for understanding the process of human remains decomposition making this research relevant to the forensic sciences (Catts and Goff 1992; Carvalho et al. 2000; Schoenly et al. 2006, 2007), especially because human remains are difficult and expensive to acquire.

Carcasses were purchased from a local farm immediately after (i.e., within minutes) euthanization by cranial blunt force trauma. Each carcass was immediately double bagged in 1 mm thick garbage bags that were tightly sealed with tape to prevent access by any invertebrates. The carcasses remained in the bags from 2 to 3 h during transport to the study site where one carcass was placed at a randomly chosen plot along each of three or six transects (depending on season). Each plot was new, without any previous carcass decomposition from a preceding trial. Carcasses were exposed at 2 h before sunset defined by the National Oceanic and Atmosphere Administration (NOAA) on 15 April 2009 (1,814), 22 July 2009 (1,858), 11 November 2009 (1,623), and 8 February 2010 (1,704), for the spring, summer, autumn, and winter trials, respectively. Each carcass was placed under a wood exclosure cage (0.6 × 0.95 × 0.6 m) with 2.5 cm mesh chicken wire to prevent disturbance by vertebrate scavengers (e.g., raccoons, coyotes, and vultures).

Temperature was recorded every 15 min through the duration of each season experiment using NexSens DS1921G micro-T data loggers (Fondriest Environmental Inc., Beavercreek, OH) that were attached to each exclosure cage. These were used to calculate accumulated degree hours (ADH) that are units for accumulated heat over time that is related to invertebrate growth rates (Byrd and Butler 1996, Amendt et al. 2007), and can be used to thermally standardize the progression of decomposition. Rainfall was monitored using data from a local weather station (Station code: KSGH, Springfield, OH) ≈8 km away from the study location. Soil conditions were relatively homogenous throughout the study site and among carcass replicates as described by Lewis and Benbow (2011). We classified each stage of decomposition by ranges of ADH to examine community structure differences

Table 1. Accumulated degree-hour (ADH) ranges for each stage of decomposition (defined by Payne [1969]) during each season

Stage	Summer	Autumn	Winter
Fresh	0–315	0–566	0–1,385
Bloat	316–1,605	567–4,448	1,386–3,083
Active	1,606–2,517	4,449–14,517	3,084–9,823
Advanced	2,518–3,510	—	—
Dry	3,511–17,906	—	—

— indicates that the carcasses for those seasons never reached that stage of decomposition.

during each stage of decomposition in relation to ADH (Michaud and Moreau 2009). To avoid confusion, hereafter we use the stage terminology set forth by Payne (1965), standardized and described with the field-measured ADH ranges that are given in Table 1 for each stage of decomposition in each season.

Invertebrate Community Assessment. Sampling frequency for carrion invertebrate communities was as follows: daily from fresh through active decay (if there were insects present depending on season), every second day through post decay, and every fourth day through the dry stage and until activity of necrophagous insects was no longer prevalent. Sampling frequency was based on the relative length of each stage in each season so that we were able to represent the primary insect activity associated with each stage, but without daily sampling over several months of decomposition (e.g., winter season). Aerial sweeps using a 36 cm wide net were used to collect adult flies over the carcass. To represent the adult fly community per replicate carcass we standardized our methods by collecting flies from two out of three passes. We used hand collections with forceps to sample ground and carcass dwelling invertebrates from three different areas of each plot: the first area (A1) included the ground and vegetation surrounding the carcass for a 5 m radius; the second area (A2) included collections of invertebrates taken from the carcass not in contact with the soil (i.e., on the body); and the third area (A3) was underneath the carcass, which was done by lifting the carcass by the legs; this was possible for most carcasses. When the carcass could not be lifted by the legs (e.g., advanced decay stage) would gently move one side of the torso away for access to the underneath habitat.

We used standardized hand collections to collect representative taxa in each of these areas and to minimally disturb the carcass invertebrate community during decomposition, while also maintaining consistent sampling effects among replicates and seasons. To standardize sampling from the A1 and A2 areas, specimens were collected for 5 min or for a total of 10 specimens, whichever occurred first, to minimize the sampling affect on the community. For A3, sampling occurred for 30 s or until 10 specimens were collected. These standardized collections, as well as the pit-fall traps were established after the completion of the spring trial. Pitfall traps were used to collect mobile invertebrates that were drawn to the carcass when sampling was not conducted (e.g., at night). For this collection technique, we used three pitfall traps per carcass (0.95 liter plastic cups with cup lip flush to ground), filled approximately halfway with soapy water and randomly placed with regard to direction 1 m away from each carcass. Traps were replaced every sample date. For the spring trial, collections were made in all three areas, but without a standardized time or number of specimens. However, we collected representative species from all taxa present so that we could compare taxon richness among seasons. Because of the sampling discrepancy in the spring trial, we could not compare overall community diversity, composition or assembly and so the spring trial was omitted from those analyses. Blow fly larvae were collected in a tiered approach to minimize the effects of sampling on larval interactions and activity during decomposition. When total larval abundance was estimated to be low (≈100–500 larvae), we only collected ≈10–50 larvae or ≈10% of the population. For larger masses (>1,000 larvae), a total of ≈100 larvae were collected from each larval mass on, or surrounding, the carcass.

Blow fly larvae were placed into vials and transported to the laboratory for rearing to adults, or parboiled (≈100°C) for 30 s and then preserved in 70% ethanol (Amendt et al. 2007). The adult rearing process involved placing larvae or eggs onto 85–145 g beef liver in a 0.95 liter glass jar or a mosquito breeder (BioQuip, Rancho Dominguez, CA) filled with 2–5 cm of wood chip substrate that was used as a pupation medium. Beef liver was provided ad libidum until larvae reached the postfeeding, dispersal stage. Larvae were then transferred to a new container filled with 10–15 cm of fresh wood chip substrate (Byrd et al. 2010). Emerged adult blow flies were then euthanized using ethyl acetate and preserved in 70% ethanol. Dipteran and coleopteran specimens were identified to lowest taxonomic level using Hall (1977), Cutter and Dahlem (2004), and Whitworth (2006); Arnett (2000), Arnett and Thomas (2001), and Arnett et al. (2002) were used to identify Coleoptera and incidental taxa.

Data Analysis. For all analyses, the identified organisms counted from each sampling method (i.e., aerial sweep nets, pitfall traps, and hand collection) were pooled together to represent the community for each carcass on each sampling date. We calculated taxa composition and relative abundance, taxon richness, and Shannon diversity index for each season. To ease data interpretation and visualization, Coleoptera were grouped at family level. One-way analysis of variance (ANOVA) with Bonferroni posttests was used to test for seasonal differences in the relative abundance of the five most abundant taxa that made up >1.5% of the total population from all seasons. Three rare taxa, *Lucilia illustris* Meigen (Diptera: Calliphoridae), *L. sericata* Meigen (Diptera: Calliphoridae), and *Pollenia* sp. F. (Diptera: Calliphoridae), were omitted from further analyses. Additionally, after comparing taxon richness among seasons, the

Table 2. Mean (SD) necrophagous community taxa composition and variability (coefficient of variation [CV]) within and among seasons

Taxa	Spring			Summer			Autumn			Winter		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Diptera												
Phormia regina	11.9	2.8	0.2	4.3	2.7	0.6	0.6	1	1.7			
Cochlionayia macellaria	2.8	0.9	0.3									
Lucilia coeruleiviridis				8.8	5.4	0.6	3.3	2.2	0.7	0.1	0.2	1.7
Lucilia illustris	0.2	0.3	1.5							0.5	0.9	1.7
Lucilia sericata										0.2	0.4	1.7
Pollenia pediculate										0.5	0.4	0.9
Muscidae	1.6	1	0.6	7.5	5.1	0.7	3.2	1.3	0.4	3.1	2.2	0.7
Sarcophagidae	0.4	0.5	1.3									
Coleoptera												
Staphylinidae	20.9	5.7	0.3	61.9	10.1	0.2	21.4	5.9	0.3	14.3	6.7	0.5
Silphidae	51.9	4.4	0.1	5.9	2.4	0.4	60.5	9.4	0.2	70.1	8.9	0.1
Carabidae				2.8	2.7	1	3.2	3.2	1	6.1	3.6	0.6
Nitidulidae	5.5	2.3	0.4	4.7	1.9	0.4	4.9	4	0.8	1.4	1.2	0.9
Histeridae	1.5	0.6	0.4	2.2	0.6	0.3	1.4	1.3	0.9	0.9	0.6	0.7
Cleridae	2.3	2.1	0.9	0.9	0.7	0.8	0.5	0.4	0.9	0.3	0.5	1.7
Scarabidae	0.7	0.7	1	0.5	0.5	0.9	0.3	0.5	1.7	0.3	0.5	1.7
Trogidae	0.4	0.5	1.2	0.5	0.3	0.6	0.8	1.3	1.7	2.3	2.6	1.1

If no values are given the taxon was not present. Asterisks denote that there are significant differences ($P < 0.05$) for those taxa between seasons (one-way ANOVA with Bonferroni posttest, elaborated in Table 3).

spring insect community data were omitted from additional analyses as described above.

Overall invertebrate community structure was described using nonmetric multidimensional scaling (NMDS), followed by multi-response permutation procedures (MRPP), and indicator species (or in this case, taxon) analysis (ISA) using PC-Ord (MjM Software Design, Gleneden Beach, OR) on arcsine square root transformed data following recommendations by McCune and Grace (2002). Indicator values represent the taxon best predicting season or ADH, with 0 representing no indication and 100 being a perfect indication (McCune and Grace 2002). An overall ordination was performed using all of the seasons (except spring because of the different sampling techniques) pooled together, and then separate ordinations for each season. The ordinations were developed using only taxa that made up >1.5% of the total population for each individual season to avoid the effects of such rare taxa on ordination stability space (McCune and Grace 2002). For each of the ordinations, the axis that explained the most variation and the strongest orthogonality (lowest stress) were used for representing the data in multidimensional space (McCune and Grace 2002).

Results

Taxon Richness and Diversity. In total, 6,383 insects were collected and identified that were predominantly represented by Diptera and Coleoptera (Table 2). Taxon richness for spring and summer had similar patterns through decomposition (Fig. 1), with a unimodal trend of increased taxon richness during 5,000–10,000 ADH (spring) and 1,000–5,000 ADH (summer) followed by a decline in taxon richness for the remainder of decomposition. During the fresh stage (see ADH ranges in Table 1 that correspond with each stage), taxon richness was low (1–3 taxa) and uniform among replicates (≈±1 SD), while during the bloat and active decay stages taxon richness was higher (3–12 taxa) with more variation (≈±4 SD), and the advanced decay and dry stage communities were both low in richness and variation (≈±2.5 SD) (Fig. 1). Additionally, summer demonstrated the overall highest diversity (Fig. 1), which corresponded with increased taxon richness. Autumn and winter trials followed similar trends of both spring and summer, except that they never entered advanced decay, because of mummification, meaning they showed a constant increase in the taxon richness. Interestingly, autumn carcasses were occupied by the least amount of insect diversity throughout decomposition with higher diversity as decomposition progressed (Fig. 1). However, winter was the most consistent season for diversity, ranging from 0.5 to 1.0 throughout decomposition (Fig. 1).

Community Composition. We collected a total of 16 taxa, eight Diptera and eight Coleoptera families (Table 2). Several genera of Silphidae showed seasonal preference during this study. For instance, *Oiceptoma inaequalis* (F.) and *Oiceptoma novaboracense* (Forster) (Coleoptera: Silphidae) were present during the spring trial in high abundance, sometimes estimated to have hundreds of adults and larvae on and around a carcass at one time; however, during the summer trial, *O. inaequalis* was not present while <10 individuals of *O. novaboracense* were collected during the entire trial. *Necrodes* spp., *Nicrophorus* spp., and *Necrophila americana* (L.) (Coleoptera: Silphidae) were present during the summer trial, but were never collected during the spring trial. For the majority of taxa, relative abundance was consistent across seasons (Table 2; Fig. 2). The relative abundance of several taxa was significantly different among seasons: *Phormia regina* (Meigen) (Diptera: Calliphoridae), *L. coeruleiviridis* (Macquart) (Diptera: Calliphoridae), Muscidae, Staphylinidae, and Silphidae, but differ-

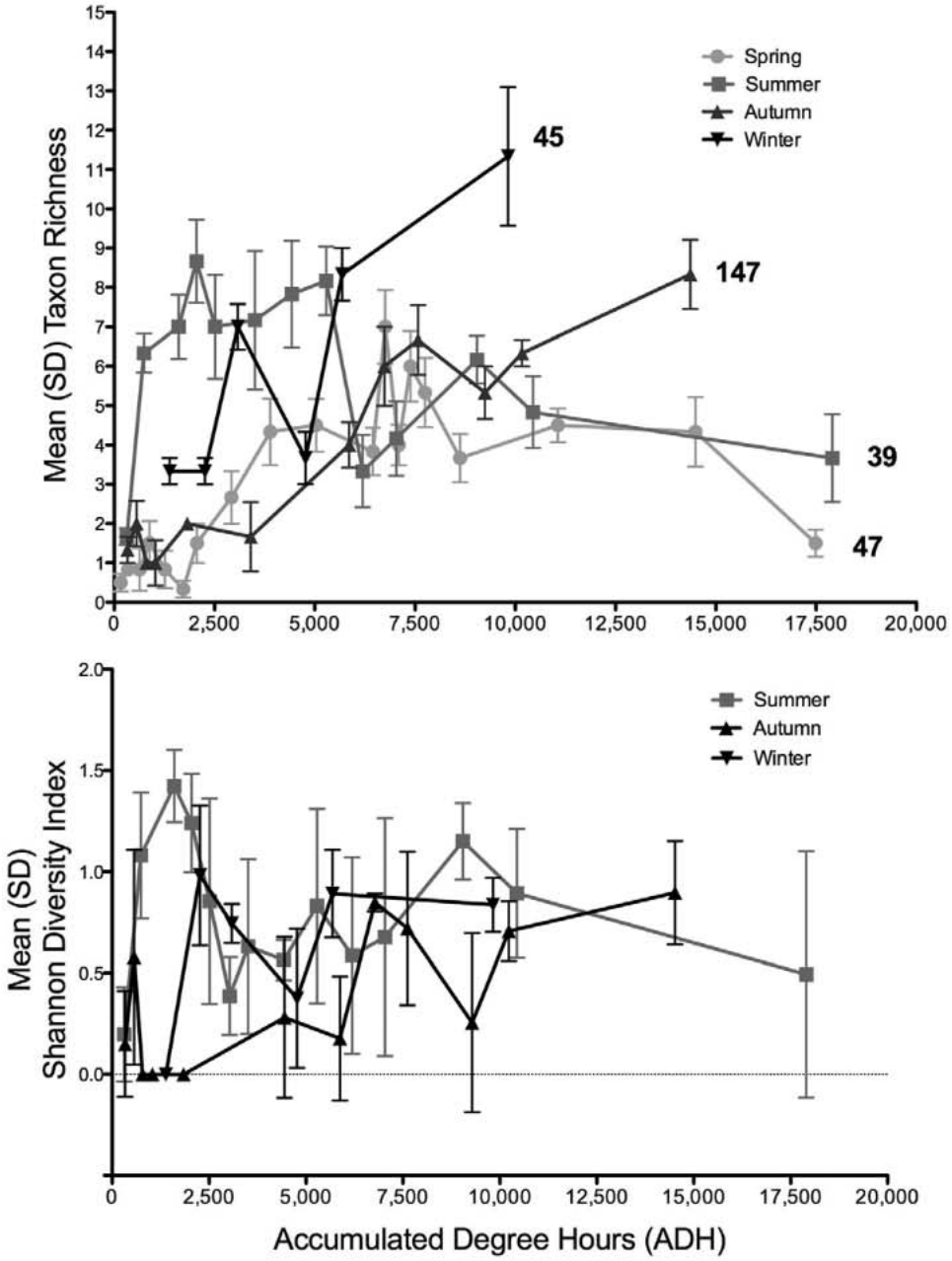

Fig. 1. (top panel) Mean (SD) necrophagous community taxon richness across decomposition expressed as ADH for each season. The duration of each seasonal trial, expressed as number of days, is given adjacent to the highest ADH datum. (bottom panel) Mean (SD) Shannon Diversity Index for each sampling date during decomposition expressed as ADH.

ences were season dependent (Table 3). Staphylinidae accounted for <22% of the populations for spring, autumn, and winter, while in summer accounted for >61% across replicates. The five most dominant taxa for each season were used to evaluate taxa composition changes during decomposition for each season (Fig. 2). The winter community taxa were very different compared with the other seasons. Blow fly species were not among the dominant taxa in the winter, as succession through the entire process of decomposition was dominated by Coleoptera.

Community Structure. Necrophagous insect community structure was significantly different between summer and autumn (MRPP: $P = 0.001$), and summer and winter (MRPP: $P < 0.001$), while communities were similar between autumn and winter (MRPP: $P = 0.073$) (Fig. 3). Further, there were significant necrophagous insect community assembly differences

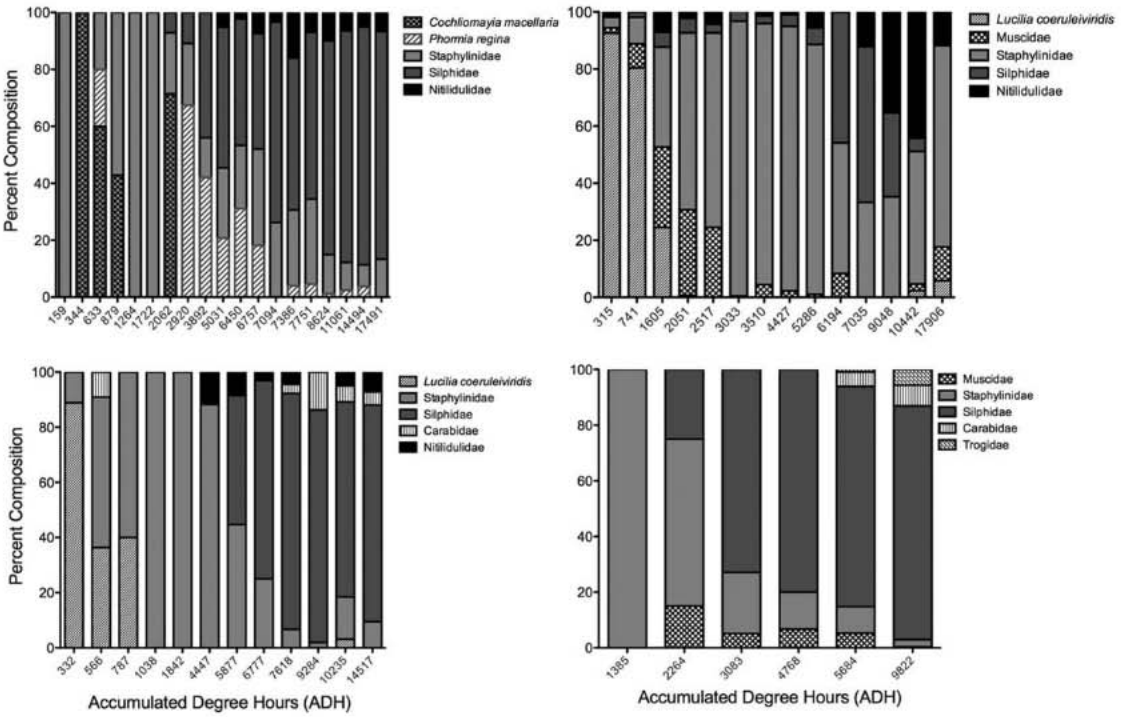

Fig. 2. Adult insect mean relative percent composition of the five dominant taxa over decomposition (expressed as ADH) in each season: (A) spring, (B) summer, (C) autumn, and (D) winter.

within each season (MRPP: $P < 0.001$) (Fig. 4), with significantly different community structure and indicator taxa for each stage of decomposition (Table 4). During summer and autumn *L. coeruleiviridis* was the strongest indicator of the fresh stage, while for winter it was Staphylinidae. Bloat stage was most strongly represented by *P. regina* during the summer, but by Staphylinidae during the autumn and Muscidae in winter. Staphylinidae and Histeridae represented the advanced decay stage communities in the summer, but these taxa were replaced by Silphidae, Muscidae, and Nitidulidae in the autumn and by Silphidae and Carabidae in the winter.

Discussion

In this study, necrophagous community assembly followed patterns similar to those reported in previous research (Payne 1965, Archer 2003, Tabor et al. 2004, Watson and Carlton 2005); however, we also supported this quantitatively using multivariate statistical techniques from replicate carcasses ($n = 3$–6) in four seasons. Calliphoridae larvae were responsible for the majority of organic tissue decomposition, which has also been reported elsewhere (Carter et al. 2007), with Coleoptera either aiding in the consumption of the carcass or preying on blow fly larvae. During advanced decay and dry stages, the only insects present at the carcass were a few remaining Calliphoridae larvae and Coleoptera consuming the dry remains, similar to a previous study (Arnaldos et al. 2004). The spring and summer communities followed similar trends as those described in other research (Joy et al. 2002, 2006; Watson and Carlton 2003; Tabor et al. 2004; Matuszewski et al. 2008). The autumn and winter carcasses exhibited a different pattern of decomposition and insect community assembly. While the carcasses in autumn did have blow fly oviposition, the eggs did not hatch. Winter carcasses never experienced oviposition, which drastically reduced the rate of decompo-

Table 3. Seasonal differences in dominant insect taxa using One-Way ANOVA and Bonferroni Post-tests

Taxa	One-way ANOVA statistics			Bonferroni's multiple comparison test of significance					
	F-value	R^2	*P* value	Sp vs Su	Sp vs Au	Sp vs Wi	Su vs Au	Su vs Wi	Au vs Wi
Phormia regina	8.20	0.64	0.0021	<0.001	<0.001	<0.001	ns	ns	ns
Lucilia coeruleiviridis	24.95	0.84	<0.0001	<0.01	ns	ns	ns	<0.05	ns
Muscidae	3.47	0.43	0.0451	<0.05	ns	ns	ns	ns	ns
Staphylinidae	40.86	0.90	<0.0001	<0.001	ns	ns	<0.001	<0.001	ns
Silphidae	120.20	0.96	<0.0001	<0.001	ns	<0.01	<0.001	<0.001	ns

Sp = spring, Su = summer, Au = Autumn, Wi = winter, and ns = no significant difference between those seasons.

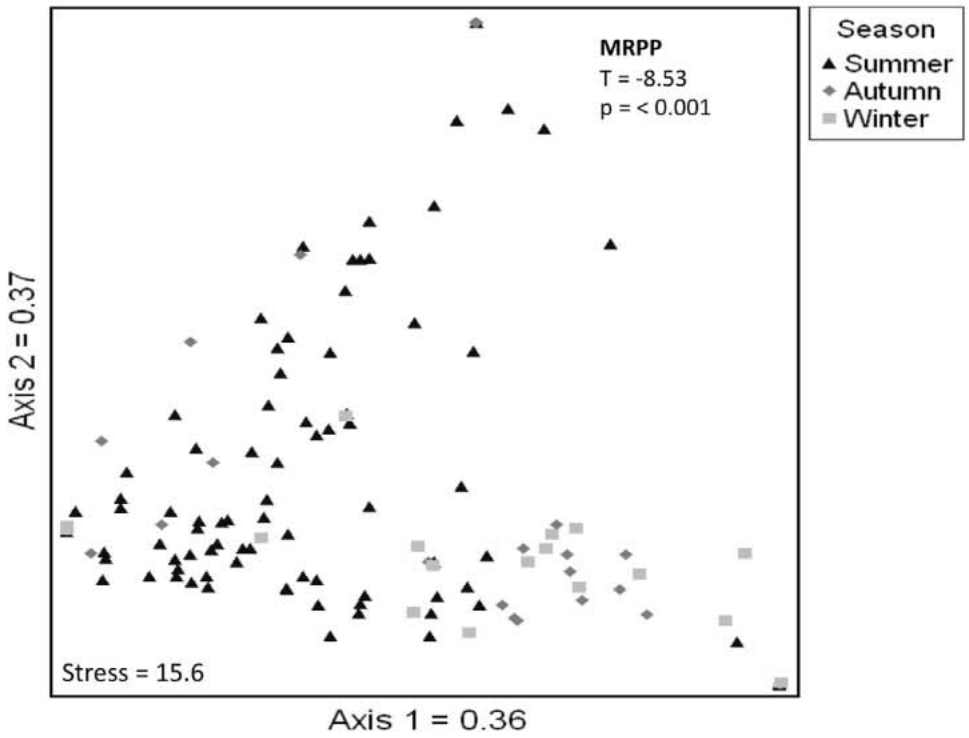

Fig. 3. Nonmetric multidimensional scaling ordination of the necrophagous insect community structure across seasons, with significant differences in communities between summer and both autumn and winter ($P < 0.0001$). The MRPP statistics (see Materials and Methods), stress and proportion of variation explained by each axis are provided.

sition (Gomes et al. 2006). The carcasses in the autumn trial only developed to the end of active decay stage because of mummification (M.E.B., unpublished data), while winter carcasses appeared to proceed through bloat and into the active decay stage, which has been noted by Watson and Carlton (2005) to occur during cooler seasons. While Calliphoridae activity was minimal for both autumn and winter, the Coleoptera communities followed a similar pattern to the spring and summer trials. It is hypothesized that the onset of cold weather in autumn and winter trials, along with the high degree of beetle activity, could have delayed blow fly oviposition on the carcasses (Mahat et al. 2009), perhaps because of microhabitat differences and activity of beetles compared with blow flies.

Most successional decomposition research has been conducted in either spring and summer during the warmer weather months (Tabor et al. 2004, Schoenly et al. 2007), or in geographical regions with milder winter temperature (Richards and Goff 1997, Carvalho and Linhares 2001, Archer 2003, Bharti and Singh 2003, Watson and Carlton 2005, Martinez et al. 2007). This is one of the first studies to use replicate carcasses (i.e., $n > 2$) to understand insect succession in different seasons of the temperate, mid-western region of the United States, and use multivariate statistical approaches to test for entire community structure differences both through decomposition and among seasons.

The spring and summer carcasses exhibited expected patterns of community assembly. There was low taxon richness at the beginning of decomposition; then as carrion entered bloat and active decay stages the taxon richness and variation greatly increased (Payne 1965, LaMotte and Wells 2000). This variation indicates the importance of using replicate carcasses in decomposition research and highlights areas of research interest related to community assembly rules in nature. With the bloating stage, the volatile byproducts produced by the microbial communities could act as signaling agents to attract or repel certain insects during the decomposition process (Tomberlin et al. 2011b, 2012). Taxon richness declined as the carcasses progressed to the advanced decay and dry stages. During the autumn and winter, carcasses went through the same pattern of decomposition as in spring and summer through the bloat stage, but progressed much more slowly, probably because of low dipteran activity compared with the other seasons. This left the carcass available to attract new taxa several months into the decomposition process.

Certain taxa demonstrated relative abundance changes among seasons or were more prevalent in specific seasons. *P. regina* and *L. coeruleiviridis* and the families Muscidae, Staphylinidae, and Silphidae had significant differences across seasons. There was also notable differences in the appearance of specific taxa in different seasons, such as *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae) only collected in spring

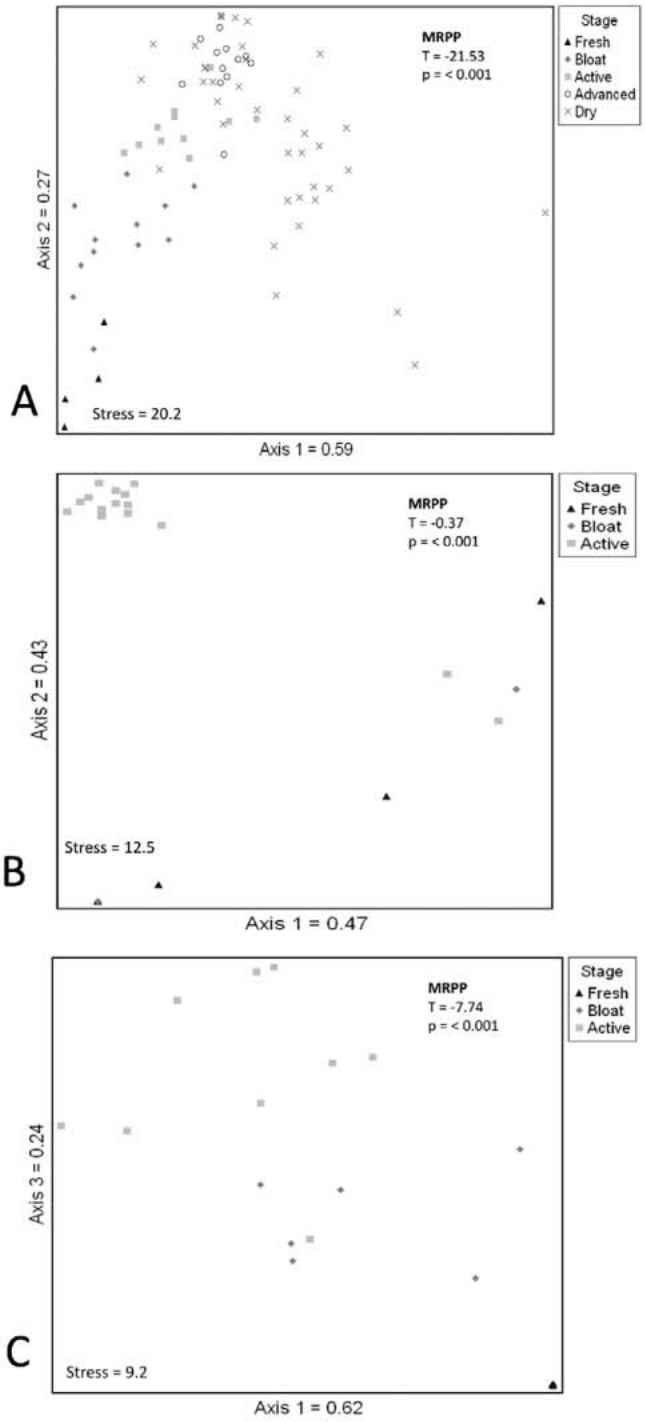

Fig. 4. Nonmetric multidimensional scaling ordinations of the insect community structure across the different stages of decomposition for each season: (A) summer, (B) autumn, and (C) winter. The MRPP statistics, stress and proportion of variation explained by each axis are provided.

that was similar to that reported by Centeno et al. (2002), Tabor et al. (2004), Arnaldos et al. (2004), Gill (2005), and Sharanowski et al. (2008). Through decomposition, Staphylinidae and Silphidae were the only consistent taxa captured in all seasons and Silphidae were dominant for every season except summer where Staphylinidae composed of >60% of the community. Based on indicator taxa, blow flies were

Table 4. Indicator insect taxa for each stage of decomposition in each season. Insect taxon is given along with the indicator value and value for each group

Season	Stage of decomposition	Insect taxon	Indicator value	*P* value
Summer	Fresh	*Lucilia coeruleiviridis*	50.7	0.001
	Bloat	*Phormia regina*	47.8	<0.001
	Active decay	Muscidae	30.8	0.025
	Advanced decay	Staphylinidae	33.3	<0.001
		Histeridae	25.9	0.043
	Dry	Cleridae	25.0	0.049
Autumn	Fresh	*Lucilia coeruleiviridis*	69.3	0.001
	Bloat	Staphylinidae	55.3	0.007
	Active decay	Silphidae	82.4	<0.001
		Muscidae	41.2	0.034
		Nitidulidae	41.2	0.034
Winter	Fresh	Staphylinidae	64.9	0.004
	Bloat	Muscidae	59.9	0.038
	Active decay	Silphidae	62.0	0.001
		Carabidae	55.6	0.039

For pair-wise comparisons a Bonferroni corrected = 0.006 should be used for statistical significant interpretation of taxon indicator value within each stage of decomposition.

the significant representative taxa of fresh and bloat stages during decomposition in both summer and autumn trials, with Coleoptera representing the later decomposition stages. These trends of blow flies arriving first followed by beetles have been thoroughly documented in carrion decomposition literature (Payne 1965, Byrd and Castner 2010). However, there were not high numbers of Diptera during the winter, which corresponds to known lower threshold limits of development for blow fly species (Davies and Ratcliffe 1994).

We found significant differences in insect community structure among seasons similar to qualitative findings in other studies (Centeno et al. 2002, Arnaldos et al. 2004, Tabor et al. 2004, Gill 2005, Sharanowski et al. 2008). Further, there were significant community structure changes among the stages of decomposition, indicating different community assembly characteristics among seasons. This has been described previously (Payne 1965, Schoenly et al. 2007). However, to our knowledge, while there has been excellent work to quantitatively describe and test insect succession on carrion and for forensic application (Schoenly 1992; Schoenly et al. 1992, 1996; LaMotte and Wells 2000), there have been no studies that have used an NMDS statistical approach to evaluate carrion insect community succession. Fresh stage was indicated by Diptera colonization, except for the winter trial that had minimal dipteran activity. The bloat and active decay stages for all seasons were best represented by taxa such as Silphidae, Staphylinidae, Nitidulidae, and Carabidae, which can be attracted to the carrion, as well as the invertebrates colonizing the carcasses (Watson and Carlton 2003, Arnaldos et al. 2004, Tabor et al. 2004). In the summer, the advanced stage was represented by Histeridae and the dry stage by Cleridae, which is consistent with previous literature (Byrd and Castner 2010).

In conclusion, this study contributes to a growing research base intended to enhance the science of forensics as noted recently in responses to the National Research Council (U.S.) (2009) by (Tomberlin et al. 2011a,b). This work contributes to a growing but limited number of studies on cold weather carrion decomposition (Gill 2005, Sharanowski et al. 2008, Matuszewski et al. 2010). Further, the use of replicate carcasses allowed for stronger statistical evaluation of community assembly variation within and among seasons in the study habitat (Tomberlin et al. 2012). The use of multiple seasons in this research demonstrated that taxa composition and community assembly vary across seasons (Archer 2003, Tabor et al. 2004, Matuszewski et al. 2010). However, we were limited by not having seasonal replication, and so strongly recommend additional studies that replicate by season or year to provide a foundation for broader statistical inference (Michaud et al. 2012). Our data can only be used to make inferences inherent to the variability of this one study site during the period of study. However, our data clearly show that season is important for understanding and describing insect community assembly in temperate regions of the United States. This variation should be considered when using entomological evidence in forensic investigations.

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