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ECOSPHERE

Delayed insect access alters carrion decomposition and necrophagous insect community assembly

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Abstract. Vertebrate carrion in terrestrial ecosystems is an unpredictable, ephemeral resource pulse that contributes to local biodiversity and nutrient transformation dynamics. Carrion ecology is infrequently studied compared to other decomposition systems, such as leaf litter detritus, despite its importance as a resource subsidy in most ecosystems. We hypothesized that delayed insect access to carrion (insects excluded for five days) would demonstrate marked shifts in necrophagous insect community structure, turnover rates and assembly with overall effects on carrion decomposition. Despite similarities between taxon arrival patterns, once insects were allowed to colonize carrion previously excluded from insects there was an increased necrophagous insect taxon richness and increased community turnover rates. Additionally, during the first five days of decomposition, insect exclusion carcasses remained in bloat stage while those naturally colonized were well advanced in active decomposition. This resulted in substantial differences in decomposition and highlighted the importance of insect community assembly in the decomposition process. Carrion decomposition has been a neglected field of study compared to other organic matter processes (e.g., leaf detritus), and these data suggest the ecology of carrion-arthropod interactions can contribute to a broader understanding of decomposition processes and ecosystem function.

Key words: carrion; community assembly; decomposition ecology; ephemeral resource; necrobiome; necrophagous insects.

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INTRODUCTION

Decomposition of ephemeral resources such as carcasses and leaf litter is important to consumer dynamics at both population and community levels (Strickland et al. 2009, Barton et al. 2012). Decomposition ecology research has been dominated by leaf litter studies (Dickinson and Pugh 1974, Melillo et al. 1982, Hattenschwiler et al. 2005) with fewer studies focusing on carrion

resources. When carrion has been studied, there have been important discoveries and reported broad impacts to ecosystem structure and function. Resource pulses such as carrion are defined as infrequent, short-lived resources that can have large impacts on ecosystem processes (Yang et al. 2008). For instance, carrion nutrient transfer between ecosystems can result from blow fly (Diptera: Calliphoridae) consumption and dispersal (Hocking and Reimchen 2006, Hocking et al. 2009, Parmenter and MacMahon 2009) or vertebrate scavenging (Wilson and Wolkovich 2011, Beasley et al. 2012). Necrophagous arthropod attraction, colonization, development, and migration can affect nutrient transformation and release and thus local biodiversity (Hocking and Reimchen 2006, Lisi and Schindler 2011, Tomberlin et al. 2011, Hawlena et al. 2012), which can ultimately impact landscape biodiversity depending on the density and frequency of carrion (Yang et al. 2008, Barton et al. 2013). While many organisms utilize these ephemeral resources the mechanisms governing attraction, consumption, utilization and nutrient transformation of carrion are only beginning to be investigated. Further, microbial community structure and function associated with carrion have began to be identified in terrestrial habitats (Pechal et al. 2013, Pechal et al. 2014) and microbial communities are known to mediate competition among higher trophic level consumers in aquatic ecosystems (Burkepile et al. 2006), while interkingdom communication with bacteria has been reported to regulate blow fly attraction and oviposition on carrion resources (Ma et al. 2012, Tomberlin et al. 2012). Additionally, there have been novel implications for the use of necrophagous insects (i.e., blow flies) as a means to assess mammalian diversity (Calvignac-Spencer et al. 2013).

Carrion resources represent ecological units within larger ecosystems that result in energy and nutrient surges to the immediate soil, arthropod and plant communities (Towne 2000). Decomposing carcasses such as whale falls (Smith and Baco 2003) or anadromous salmon, *Oncorhynchus* spp. (Salmoniformes: Salmonidae) (Hocking and Reimchen 2006), can be the primary resource subsidy for their associated ecosystems (Lisi and Schindler 2011). Carrion decomposition reintroduces essential nutrients such as nitrogen, potassium, calcium and magnesium into an ecosystem (Towne 2000, Carter et al. 2007, Parmenter and MacMahon 2009). In a recent study, the differences in carcass quality (i.e., grasshoppers nonstressed vs. stressed by predators) was reported to affect soil microbial communities and subsequent decomposition processes; the addition of stressed carcasses significantly reduced plant litter decomposition rates (Hawlena et al. 2012). It is clear from these studies that carrion plays an important, but understudied, role in ecosystem structure and function.

Despite the importance of carrion in all ecosystems, there have been few examinations of the mechanisms and driving factors that regulate carrion decomposition (DeVault et al. 2003, Carter et al. 2007, Yang et al. 2008). There has yet to be a study to experimentally test the hypothesis that delayed necrophagous insect access to carrion alters arthropod community assembly as a mechanism that mediates decomposition, even though arthropod communities are known to play a large role in carrion decomposition (Payne 1965, Srivastava et al. 2009). For example, the introduction of ungulate carrion into a terrestrial system has been reported to facilitate a localized succession of insect colonizers, such as blow flies (Carter et al. 2007). Blow fly larvae consume most carrion soft tissue with reported biomass loss of up to 90% in less than six days (Payne 1965), suggesting an important functional role of this community to the surrounding habitat. Delaying insect access to carrion by physical barriers (e.g., buried remains), abiotic conditions (e.g., temperatures below insect activity thresholds), or in forensically related instances when a human body is wrapped in a barrier to necrophagous insect access (e.g., plastic bags) could ultimate affect nutrient reintroduction into the surrounding environment. However, the role of necrophagous arthropods in the ecology of decomposition and ecosystem nutrient cycling remains limited (Parmenter and MacMahon 2009).

Early work bridged decomposition processes and rates to arthropod succession patterns (Schoenly and Reid 1987). Arthropod community composition poorly correlated with stage of decomposition, and was more representative of a continuous pattern of insect arrival to a carcass (Schoenly and Reid 1987). Stages of decomposi-

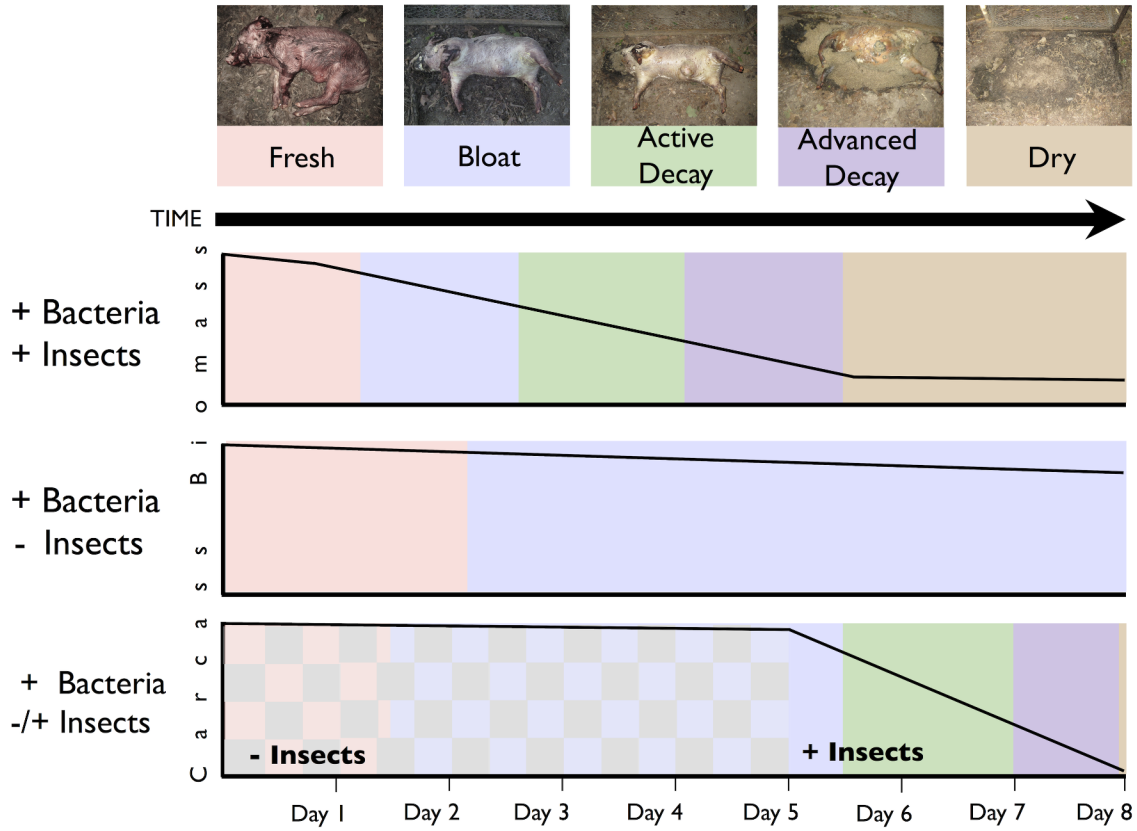

Fig. 1. Biodiversity of microbe and arthropod communities dictates carrion decomposition. Initial access of microbe and arthropod (+ microbe, + arthropod) results in a sigmoidal decomposition process with a decrease of biomass by arthropod consumers until dry stage. Delaying arthropod access (+ microbe, − arthropod) prolongs the decomposition process and decreases the rate of biomass loss over time. Allowing microbial communities to proliferate and condition the resource prior to arthropod access (+ microbe, −/+ arthropod) is predicted to accelerate decomposition rates after subsequent arthropod colonization. Duration of each decomposition stage (represented by the colored boxes in each row with examples of the carcass along the top arrow) is indicated by the width of the box and is a temperature dependent process.

tion have been commonly defined by the physical appearance and insect activity associated with the remains, and there is a variable number of classification schemes ranging from two to eight stages (Payne 1965, Greenberg 1991, Villet 2011). Despite the variation in the number of stages and classification, decomposition follows the same general process. Fresh carrion begins to bloat shortly after death, resulting from increased enteric bacterial activity; followed by putrification and loss of soft tissue from either vertebrate or arthropod consumption; and finally all that remains is the dried out carcass consisting of skeleton, hair and any unconsumed soft tissue. Delaying or excluding arthropods from colonizing carrion has been qualitatively described to alter the decomposition process (Payne 1965, Simmons et al. 2010); yet, the mechanisms and quantitative effects have not been thoroughly investigated.

In this study, we hypothesized that initially excluding necrophagous insects to carrion would delay decomposition, specifically during early stages of decomposition, through undisturbed proliferation and maturation of microbial communities (Fig. 1). We predicted that controlling the temporal access of arthropods to the resource would initially slow and then subsequently accelerate the decomposition process because of initial microbial conditioning compared to con-

trol carcasses allowed to be naturally colonized by necrophagous insects (Fig. 1). We tested the null hypothesis that delayed colonization would not affect the insect taxon arrival sequence, community composition or assembly on carrion. Alternatively, we predicted that delayed colonization would be associated with reduced necrophagous insect community composition, taxon richness, and diversity; alter the taxa associated with each stage of decomposition; and lower taxa turnover rates during community assembly. Therefore, these changes in biotic communities would mediate the decomposition process.

Materials and Methods

Site description and experimental design

Swine carrion decomposition was studied in a Midwestern temperate forest habitat surrounded by agricultural fields in Xenia, Ohio, USA (39°38′14.83″ N, 84°1′37.82″ W). Carcasses were sampled during two summer seasons, from 5 August 2010 until 14 August 2010 and 26 July 2011 until 2 August 2011. The dominant tree fauna consisted of oak (*Quercus* spp.) and maple (*Acer* spp.) with a 95% canopy cover that was similar over all carcasses. In 2010, six male carcasses ranging from 10.4 to 30.1 kg, euthanized by cranial blunt force at approximately 16:30 h, were purchased from a local farm on 5 August 2010. Carcasses were transported to the field using methods previously described (Benbow et al. 2013). Briefly, the carcasses were double-bagged, transported for approximately 1 h, and randomly placed a minimum of 20 m apart along three transects 2 h before the National Oceanic and Atmospheric Administration (NOAA) defined sunset, which occurred at approximately 19:00 h on 5 August 2010. In 2011, using the same methods, six carcasses (three females and three males) were purchased from the same local farm on 26 July 2011 after being euthanized at approximately 17:45 h. Carcasses (5.0–7.3 kg) were transported to the field using methods described in 2010 and randomly placed along three new transects at approximately 18:30 h on 26 July 2011.

All carcasses were oriented in the field with the anterior end pointed towards cardinal north and the dorsal side towards the east. Each carcass was labeled alphabetically with "A" through "F" representing the 2010 field season and "G" through "L" representing carcasses in 2011. During each field season, three random carcasses were immediately exposed to insect access (ACC). The remaining carcasses were enclosed in individual 1.8 m^3 Lumite screen (18 × 14 mesh size) portable field cages (BioQuip Products, Rancho Dominguez, California, USA) to exclude necrophagous insect access for five days. These carcasses were considered a delayed insect access (for five days) treatment, or heretofore referred to as insect exclusion carcasses (EXC). All carcasses were covered with anti-vertebrate scavenging cages (0.9 × 0.6 × 0.6 m) constructed of wooden frames enclosed with wire poultry netting. It should be noted that while some arthropods had access to the EXC remains the focus of this study was to assess the necrophagous insect community and not other crawling arthropods (e.g., spiders and mites) that may have accessed the EXC carcasses.

The decomposition progress was categorized according to stage as defined by Payne (1965): fresh, bloat, active decay, advanced decay and dry. NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Alpha, Ohio, USA) were placed within 0.6 m of each carcass, approximately 0.3 m above the ground to measure local (carcass-associated) ambient temperature in 15 min intervals. Temperature data were converted into accumulated degree hours (ADH), which accounts for temperature variation over decomposition time; this is important to insect life history, growth and development (Megyesi et al. 2005) and has been used to make estimates of the carrion decomposition time course (Michaud and Moreau 2011).

Insect sampling

Trapper max glue traps (16.5 × 11 cm) (Bell Laboratories, Madison, Wisconsin, USA) were used to quantify community composition, turnover and assembly of adult flying insects attracted to the carrion. A single glue trap was attached to the anti-vertebrate scavenging cage at the anterior and posterior region of the carcass, each approximately 0.15 m from the remains. Glue traps were replaced every 12 h. The exclusion cages were removed from EXC carcasses after five days of insect exclusion at

approximately 19:30 in 2010 and 18:30 in 2011. The same insect sampling protocol was followed in 2011; however, sampling of ACC carcasses concluded after four days due to the rapid decomposition resulting in the carcasses in dry decomposition with little biomass remaining. Insects were identified to the lowest taxonomic level possible while remaining on the glue trap using a stereomicroscope (Triplehorn and Johnson 2005, Whitworth 2006) with an emphasis on known necrophagous Diptera and Coleoptera (Byrd and Castner 2001), while incidental taxa were not included in the analyses. A post hoc power analysis was performed using G*Power 3 (version 3.1.9) to assess the robustness of the insect sampling regime (Faul et al. 2007).

Statistical analyses

We tested differences in ambient temperature and ADH between years by two-way analysis of variance using Prism 5 (GraphPad Software, La Jolla, California, USA). Insect relative abundance data were arcsine-square root transformed to accommodate assumptions of normality and homogeneity of variance for parametric analyses. Simpson's diversity, Shannon-Wiener diversity, richness, and evenness were calculated according to methods of Zak et al. (1994). Rank-abundance plots were used to visualize similarities in taxon abundance between the insect access and exclusion treatments (Longino and Colwell 1997). Furthermore, a Spearman rank correlation analysis for the total log abundances was performed to test for differences between treatments (ACC and EXC) within each year, as previously described for carrion related insect communities by Schoenly et al. (2007). Two-way repeated measures analysis of variance (RM-ANOVA) with multiple comparisons evaluated after Bonferroni corrections using Prism 5 to test the hypothesis that excluding insect access to carcasses would affect diversity, richness and evenness among decomposition days and their interactions. Taxa turnover rates among replicate ACC ($n = 3$) and EXC ($n = 3$) carcasses was calculated according to Whittaker's beta diversity (Whittaker 1960). Beta diversity measures taxa turnover rates, or changes in community assembly, along spatial and temporal gradients (Whittaker 1960, Scheiner 1992).

To test the hypothesis that insect community assembly differences would result from initially excluded insect access to the remains, nonmetric multidimensional scaling (NMDS) was used to evaluate insect community composition between treatments (ACC vs. EXC) and over decomposition time in the vegan 2.0-3 library in the R statistical package (R Development Core Team 2010) using Bray-Curtis dissimilarity matrix (McCune and Grace 2002). NMDS is a nonparametric ordination technique that does not require the assumption of linearity among community variables (McCune and Grace 2002). Outliers were identified and removed prior to ordination using Jackknife distances in JMP 9.0.0 (SAS Institute, Cary, North Carolina, USA) as recommended by McCune and Grace (2002). Multiresponse permutation procedure (MRPP) was used to statistically test for differences in insect community structure between or among covariates (i.e., treatment and day) within the ordination using methods described elsewhere (Biondini et al. 1985). Indicator species analysis (ISA) complemented MRPP by assigning significant indicator values to specific insect taxon that were indicative of community separation between treatments (ACC vs. EXC) and over decomposition in days (McCune and Grace 2002). The indicator value represents the taxon that best predicts decomposition day or treatment with 0 representing no indication and 100 being a perfect indication for each covariate grouping.

Results

Abiotic conditions

The local daily ambient temperature among carcasses was 23.2 ± 2.1°C (mean ± SE) during 2010 and 25.1 ± 1.0°C in 2011. Mean ADH associated with the carcasses in 2011 was significantly higher ($F_{1,7} = 432.9$, $P < 0.0001$) by 8–18% than 2010 (data not shown) throughout decomposition, except at initial placement in the field when ADH for all carcasses was zero. Because of these significant differences in temperature we analyzed insect community assembly response to delayed insect access to carrion in each year separately.

Insect arrival patterns

Twenty-one necrophagous insect taxa, repre-

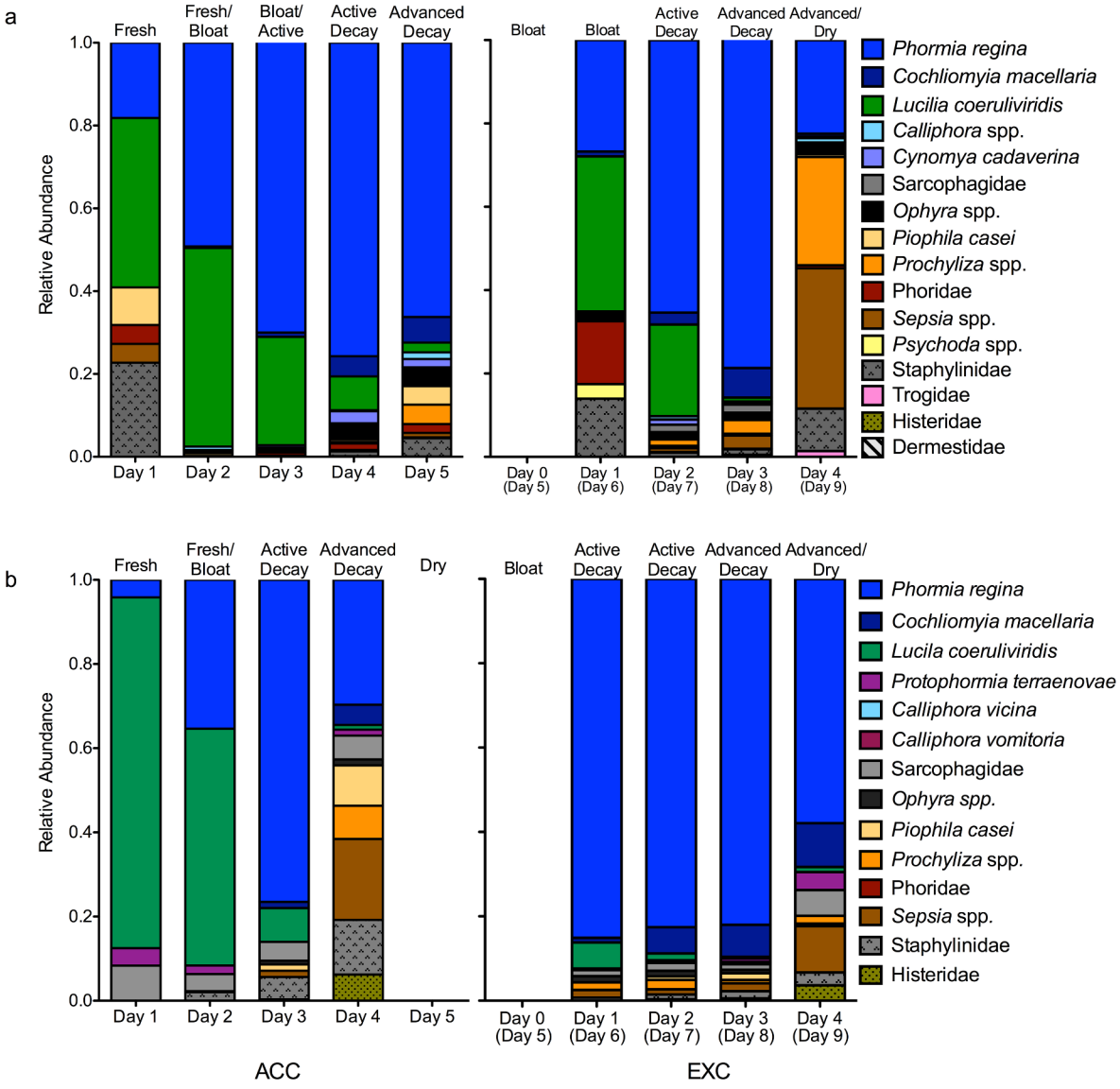

Fig. 2. Adult insect relative abundance during (a) 2010 and (b) 2011. Carcasses initially exposed to insects (ACC) were in either advanced decay (2010) or dry with no insect activity (2011) by the fifth day in the field. Each day represents immediate days of insect access and post-exclusions insect access to the carcass for ACC and insect exclusion carcasses (EXC) carcasses, respectively. Due to the insect exclusion for five days, days in parentheses along the x-axis of EXC columns represent the number of days the carcasses were in the field, and Day 0 (Day 5) is the fifth day of decomposition but prior to insect exclusion cages being removed.

senting two orders and eleven families, were collected over both years (Appendix: Table A1) resulting in 10,127 and 3,503 adult insects for 2010 and 2011, respectively.

Insect access carcasses.—In 2010, the blow flies *Lucilia coeruleiviridis* (Macquart) and *Phormia regina* (Meigen) (Diptera: Calliphoridae) were the dominant taxa (41–76%) for carcasses with natural necrophagous insect colonization (Figs. 2 and 3). Similarly in 2011, *L. coeruleiviridis* and *P. regina* were the dominant taxa (56–83%) throughout active decay. However, *P. regina*, *Sepsia* spp. (Diptera: Sepsidae) and staphylinid beetles (Coleoptera: Staphylinidae) were the dominant taxa

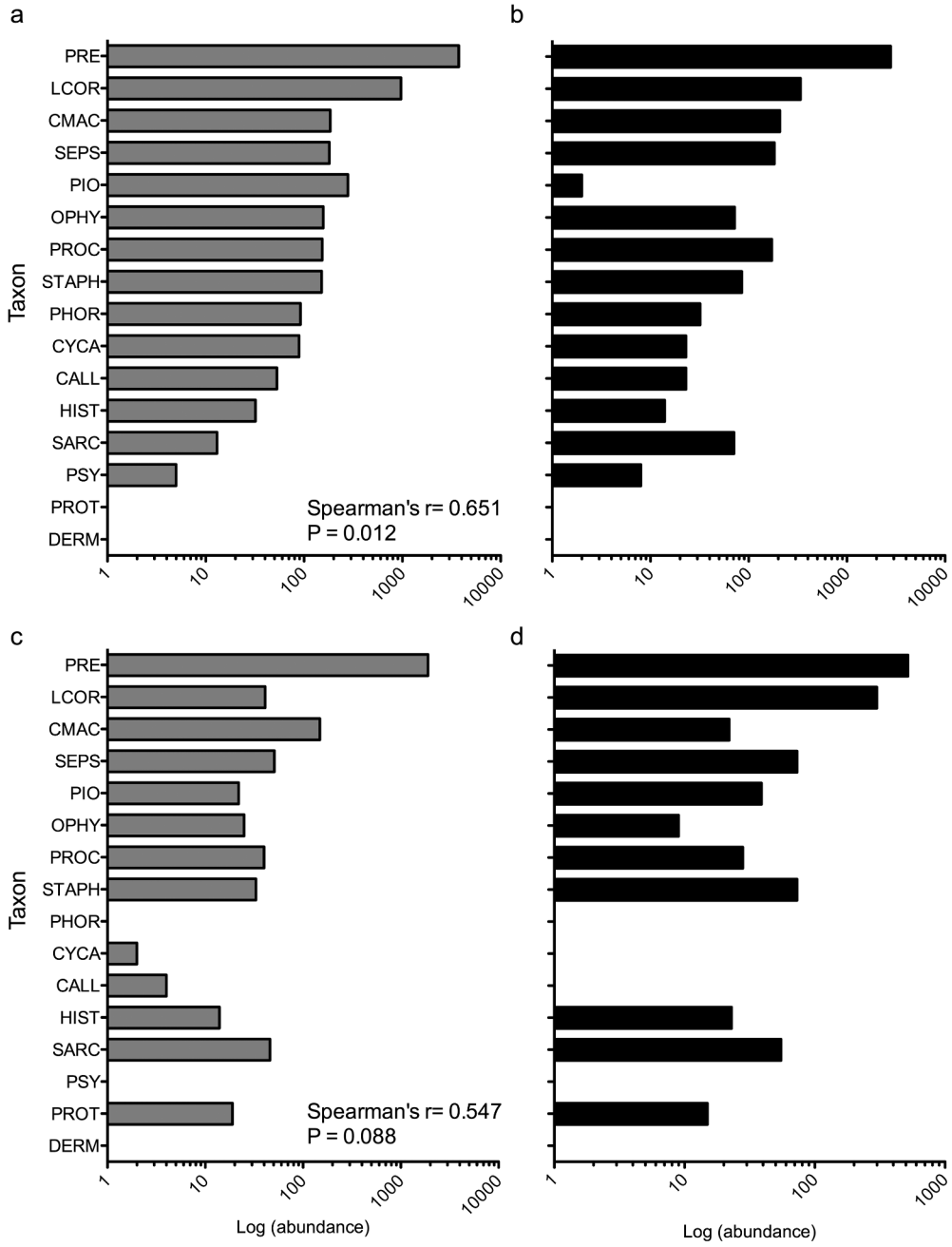

Fig. 3. Matched rank-abundance plots for the total insect taxa collected over the sampling periods during the decomposition of (a) access (ACC, gray) and (b) exclusion (EXC, black) remains in 2011 and throughout the decomposition of (c) access (ACC, gray) and (d) exclusion (EXC, black) remains in 2011. The plot is vertically oriented based on the most abundant taxon of the 2010 ACC carcasses at the top. A logarithmic scale is used for abundance. The taxa list on the far left of each figure corresponds to the taxa list in Appendix: Table A1. The Spearman rank correlation between treatments within each year is located in the bottom right of each ACC treatment figure.

(Figs. 2 and 3) in the latter stages of decomposition. There were no insect collections made on Day 5 in 2011 due to accelerated decomposition with no adult insects present at the carcasses during the sampling period.

Insect exclusion carcasses.—Insect exclusion cages were 99% efficient in 2010, but did not exclude ground dwelling arthropods. Post-exclusion insect access will be used henceforth to define the period of time when exclusion carcasses were exposed to insect colonization after removing the exclusion cages on day 5. *Lucilia coeruleiviridis* and *P. regina* were the dominant adult taxa (37–79%) throughout advanced decay. As advanced decomposition ended and the dry stage began, there were three dominate taxa on the carcasses: *Sepsia* spp. (34%), *P. regina* (26%), and *Prochyliza* sp. (Walker) (Diptera: Piophilidae) (26%). In 2011, insect exclusion cages were 95% effective but did not exclude ground dwelling arthropods. *Phormia regina* was the dominant taxon (58–85%) after insect exclusion (Figs. 2 and 3).

Insect community composition

2010.—There were significant effects of decomposition time, insect exclusion and their interaction on taxon richness (Appendix: Fig. A1, Table A2), confirming our hypothesis that delayed insect access would affect necrophagous insect communities over decomposition. Taxon richness was higher for insect exclusion carcasses during bloat and active decay decomposition. Evenness (Appendix: Fig. A1, Table A2) and Simpson's diversity (Appendix: Fig. A1, Table A2) was not different between the beginning and end of decomposition, and there were no significant differences in these metrics between insect access and insect exclusion carcasses, and no interaction was observed between these factors. Failure to detect significant effects may reflect lack of statistical power (power = 0.21, $\alpha = 0.05$, effect size = 1.22). However, Shannon-Wiener diversity was significantly higher for insect exclusion carcasses and generally higher during the later stages of decomposition (Appendix: Fig. A1, Table A2). Maximum taxa turnover rates occurred within five days for carcasses exposed to insects (Fig. 4), while highest turnover rates occurred on the first day post-exclusion insect access for those that were excluded from initial insect colonization (Fig. 4). Taxa turnover rates increased by 76.6% and 75.8% over decomposition for ACC and EXC carcasses, respectively (Fig. 4). Furthermore, there was a positive linear relationship between taxon richness and accumulated degree hours for carcasses initially exposed to insects; however, this relationship was unimodal for insect exclusion carcasses (Fig. 4), which confirmed our hypothesis that delayed insect access would reduce insect biodiversity throughout the decomposition process.

There were significant differences in community composition between insect access and insect exclusion carcasses (MRPP: $T = -9.38$, $P < 0.0001$) and among days of decomposition (MRPP: $T = -19.53$ $P < 0.0001$). A two-dimensional NMDS ordination explained 92.3% of the variation (stress = 0.144) in carcass necrophagous insect community composition (Appendix: Fig. A2). Thus demonstrating significant insect community change throughout decomposition associated with initial insect access and confirming our hypothesis that there would be significant differences in indicator taxa between carcass types (ACC vs. EXC).

Further, *Piophila casei* (L.) (Diptera: Piophilidae) was the lone indicator taxon of carcasses with immediate insect access. *Phormia regina,* flesh flies (Diptera: Sarcophagidae), *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae), *Ophyra* spp. (Diptera: Muscidae), *Prochyliza* sp., and *Psychoda* sp. (Diptera: Psychodidae) were significant indicator taxa (ranked in order from highest to lowest indicator values) when necrophagous insect colonization was delayed by five days (Appendix: Table A3). Accounting for only decomposition time and not the treatment effects (ACC vs. EXC), the significant indicator taxa for decomposition time were composed of: *Cynomya cadaverina* (Robineau-Desvoidy) (Diptera: Calliphoridae) for the third day of decomposition, which was characterized as the active decay stage; *Phormia regina*, *C. macellaria*, *Prochyliza* sp., and Sarcophagidae for the fourth decomposition day; while staphylinid beetles were significant indicators of the dry stage, which occurred on the fifth day of decomposition (Appendix: Table A3).

2011.—There were no significant effects of decomposition time, nearly significant effects of insect exclusion ($P = 0.058$) and no significant

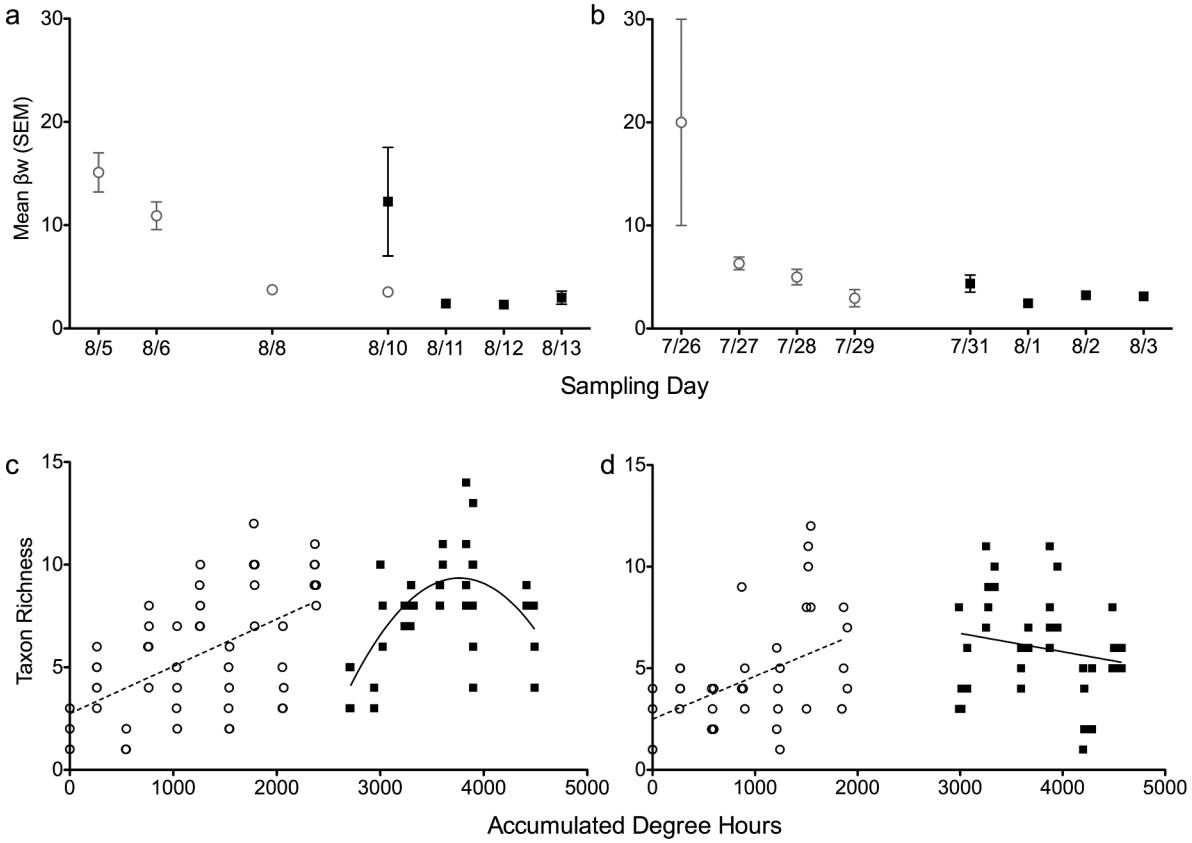

Fig. 4. Taxa turnover rates (βw) during 2010 (a) and 2011 (b) for insect access carcasses (ACC-open circles) and insect exclusion carcasses (EXC-filled squares). In 2010 (c), ACC carcasses increased ($y = 2.7 + 0.0004x$, $r^2 = 0.33$, $P = 0.8948$) in taxon richness over decomposition. EXC Carcasses showed a unimodal distribution ($y = -57.74 + 0.035x - 4.73e{-}006x^2$, $r^2 = 0.42$, $P < 0.0001$) with maximum richness occurring within two days (3893 ADH) of insect exposure. In 2011 (d), taxon richness increased linearly over decomposition ($y = 2.5 + 0.0021x$, $r^2 = 0.24$, $P = 0.6575$) for ACC carcasses; however, decreased for EXC carcasses over decomposition ($y = 9.4 - 0.009x$, $r^2 = 0.04$, $P = 0.1673$).

interaction on taxon richness (Appendix: Table A2). Overall taxon richness was higher during the first two days of decomposition for carcasses immediately exposed to insect colonization (Appendix: Fig. A1). Evenness increased over decomposition and was nearly significantly different ($P = 0.057$) between carcasses initially allowed insect access compared to carcasses that where initially excluded from necrophagous insects. However, evenness was significantly higher for carcasses with initial insect access (Appendix: Fig. A1). Simpson's diversity (Appendix: Fig. A1, Table A2) increased significantly over decomposition with significant differences between insect access and insect exclusion carcasses but there was not a significant interaction. Shannon-Wiener diversity significantly increased over decomposition (Appendix: Fig. A1, Table A2) but there was no insect access effect. Failure to detect significant effects may reflect lack of statistical power (power = 0.64, $\alpha = 0.05$, effect size = 2.52). Maximum taxa turnover rates occurred within five days for access carcasses (Fig. 4), while they remained similar across sampling days when insects were initially excluded from carcasses (Fig. 4). Taxa turnover rates for carcasses initially exposed to insects increased by 85.5% (Fig. 4) over decomposition; however, carcasses initially excluded from insects maintained a mean taxa turnover rate of 23.5 ±

20.4% (Fig. 4). There were positive and negative linear relationships between taxon richness and decomposition time for access and exclusion carcasses, respectively (Fig. 4) that confirmed our hypothesis that delayed insect colonization would alter necrophagous insect assembly during carrion decomposition.

A two-dimensional NMDS ordination explained 87.3% of the variation (stress = 0.166) in necrophagous insect communities (Appendix: Fig. A3). There were significant differences between insect access and insect exclusion necrophagous insect communities (MRPP: $T = -18.55$, $P < 0.0001$) and among days (MRPP: $T = -18.69$, $P < 0.0001$). These data demonstrate significant insect community change throughout decomposition when initial access is delayed.

Lucilia coeruleiviridis was the only significant indicator of carcasses with initial insect access (Appendix: Table A4). *Phormia regina, C. macellaria, Ophyra* spp., *Prochyliza* sp., and *Calliphora vicina* were significant indicator taxa (ranked in order from highest to lowest indicator values) for insect exclusion carcasses (Appendix: Table A4). Similar to 2010, after accounting for only decomposition time and not treatment effects (ACC vs. EXC), *L. coeruleiviridis* was a significant indicator taxon for the second day of decomposition while *P. regina* was an indicator of the third day; while the following five taxa were significant indicators (ranked in order from highest to lowest indicator values) for the fourth day of decomposition: *Sepsia* spp., histerid beetles, *Prochyliza* sp., *Piophila casei*, and staphylinid beetles (Appendix: Table A4).

Effects on decomposition

There were marked differences between the decomposition of carcasses allowed insect access compared to those with delayed insect access (Fig. 5; Appendix: Fig. A4). For example, in 2010 the insect access carcasses were approaching the dry stage with calliphorid larval masses covering two carcasses (carcasses "B" and "E"), while for carcass "F" there had already been a larval dispersal event (Fig. 5) by the end of day 5. Meanwhile, insect exclusion carcasses (carcasses "A", "C", and "D") were in the bloat stage of decomposition (Fig. 5) on the fifth day. In 2010, insect access carcasses decomposed to the dry stage between the sixth and seventh day of decomposition; however, in 2011 these carcasses were in the dry stage within five days. During both 2010 and 2011, insect exclusion carcasses were in dry stage by the ninth day of decomposition, which corresponded with the fourth day of post-exclusion insect activity. This suggests that once insects were allowed to colonize the resource it had an accelerated decomposition process, thus confirming our initial hypothesis.

Discussion

This study demonstrates that insect arrival patterns and community assembly are important to the process of decomposition, and delayed insect access to carrion affects subsequent insect community structure and successional trajectories, and retards the carrion decomposition process during insect exclusion. These arrival data are important for understanding the ecological interactions of organisms utilizing carrion (e.g., members of the necrobiome) and to help identify future research directions of insect behavioral cascades on community level dynamics and ecosystem processes (Tomberlin et al. 2011, Benbow et al. 2013). Our results confirm reported arrival patterns of blow flies to carrion, followed by secondary colonizers such as beetles and lesser flies (e.g., *Prochyliza* spp. and *Piophila casei*) (Reed 1958, Payne 1965). There was negligible overlap of insect taxa utilizing both carcass types (ACC and EXC) since the insect community structure of access carcasses on the fifth day was clearly different from the insect community structure of the first day of post-exclusion carcasses (Fig. 2). However, a moderate (Spearman's $r = 0.651$) and significant ($P = 0.012$) correlation was found between the access and exclusion carcasses in 2010; while the carcasses in 2011 demonstrated a moderate (Spearman's $r = 0.547$) but non-significant ($P = 0.088$) correlation between access and exclusion remains. A significant correlation indicates a similarity between the access and exclusion communities (Fig. 3), but does not take into account the arrival times of when each taxon is utilizing the carcass. Further, the low power of our statistical analysis may have made it difficult to statistically detect differences between the insect access and exclusion treatments. Vertebrate decomposition studies typically have practical limitations that

Fig. 5. Images of 2010 carcasses on the fifth day of decomposition (10 August at 19:00). Insect exclusion cages were removed from the carcasses on this day. (a) Carcasses with insect access (B, E and F) were in active or advanced decay while (b) insect exclusion carcasses (A, C, and D) were still in the bloat stage.

impede proper replication in the experimental design (see Michaud et al. [2012]), but the inherent lack of statistical power resulting from a small number replicates does not imply a lack of biological significance.

Progression through the decomposition stages was qualitatively different between carcasses naturally colonized by insects and those excluded from insect access. Insect exclusion carcasses remained in bloat (or beginning stages of active decomposition), as hypothesized (Fig. 1), for approximately 2–3 times longer than carcasses with immediate insect access. Quantitative measurements of biomass loss through recorded weights, e.g., Payne (1965), were not documented throughout decomposition as these carcasses

were used as part of previous studies documenting bacterial community structure (Pechal et al. 2014) and function (Pechal et al. 2013) from the external surfaces of carcass over decomposition. Weighing the carcasses at each sampling interval may have disrupted proliferating bacterial communities through artificially introducing near-by environmental bacterial communities and thus affecting the results. We hypothesized that decomposition is initially governed by microbial community proliferation and subsequent insect arrival patterns is based on the microbial communities, as has been documented in arthropod attraction to microbe-laden carcasses in a marine habitat (Burkepile et al. 2006).

Altering carcass quality has been reported to govern microbial communities in the soil beneath the decomposing organism, which in-turn influences decomposition rates of organic matter (Hawlena et al. 2012). We demonstrated that delayed insect access altered subsequent insect community assembly and was associated with a slower decomposition process. Changes in decomposition is known to modify the rate of nutrient transformation and availability in the local habitat, and overall effect the ecosystem function of removing decomposing organic matter from a terrestrial habitat (Parmenter and MacMahon 2009, Hawlena et al. 2012, Woodward et al. 2012). Reducing the quantity or rate of nutrient reintroduction into the environment could ultimately impact other members of the community that respond to nutrient pulses (Post and Kwon 2000).

Immediately after death, a succession of taxa occurs on carrion that will often reach a climax community (Horn 1974, Yang et al. 2008). However, in this study insect arrival patterns and community composition (i.e., abundance, richness and evenness) were variable between insect access and insect exclusion carcasses between years within the same habitat, thus confirming our hypothesis of differences between insect communities attracted to insect access and delayed insect access carcasses. The variation between years could be attributed to differences in resource size (Braack 1987) or priority effects of initial colonizers altering subsequent community structure (Chase 2010, Fukami and Nakajima 2011). However, while differences in taxon richness occurred between years, it has been reported that carcass size does not influence insect arrival patterns (Hewadikaram and Goff 1991). Once insect exclusion carcasses were exposed for insect access after five days, they attracted a different insect community. Coexistence of blow flies on carrion resources is common (Hanski 1987), and can be explained by aggregations of individual taxa consuming a resource patch (e.g., head or anal region) while not consuming other areas of the carcass, thus, providing opportunities for additional taxa to utilize the same resource (Hartley and Shorrocks 2002). However, our results demonstrated the impact of delayed colonization on insect arrival patterns that resulted in a shift in taxon richness and taxa turnover once access was restored. The unimodal and negative linear relationship between taxon richness in 2010 and 2011, respectively, for insect exclusion carcasses supported our hypothesis that delayed access would reduce the number of insect taxa attracted to the carcasses.

Carrion arthropod community structure change over time has often been described as a competition between micro- and macro-organisms for an ephemeral resource (Norris 1965, Schoenly and Reid 1987, Burkepile et al. 2006). Leaf litter decomposition studies have reported substantial variation in the influence of microbial and insect communities and substrate type on decomposition rates. For example, microbial and insect communities have been shown to facilitate nutrient cycling and decomposition rates in leaf litter systems (Hieber and Gessner 2002, Srivastava et al. 2009). Alternatively, another study demonstrated that leaf litter composition drives decomposition rates with little or no influence of microbial and insect communities (Kominoski et al. 2011). The influence of microbes in succession patterns of carrion decomposition has been greatly overlooked, and interactions between microbes and necrophagous insects have yet to be thoroughly analyzed for carrion systems in the natural environment (Ma et al. 2012).

A commonly overlooked component in carrion decomposition ecology is the role and mechanisms of microbial communities in relation to carrion-associated insects (Tomberlin et al. 2011). Microbial communities associated with the carcasses could be altering the quality of the resource and, thus, mediating insect community

assembly, as previously demonstrated with fungi governing phytophagous insect composition in a plant-based system (Tack et al. 2012). Indeed, when insects were denied access and colonization of carrion the indigenous microbial communities continue to change as a response to changing nutrient and chemical conditions after death (Vass 2001), although this has rarely been studied (Tomberlin et al. 2011). This microbial community succession is thought to provide distinct volatile signatures that can influence Calliphoridae behavior, and it has been hypothesized that these profiles can alter the sequence and subsequent community assembly of the necrophagous insect community (Ma et al. 2012). There is an on-going effort to identify and expand the current knowledge of carrion microbial communities and their interactions with higher-level consumers (Pechal et al. 2013, Pechal et al. 2014). A better understanding of mechanisms controlling community assembly patterns that influence carrion biodiversity is key since decomposition is a consumer driven process and changes in the organisms utilizing the remains could ultimate dictate decomposition rates (Hawlena et al. 2012).

In summary, we demonstrated delayed insect access altered insect community structure, assembly, and the overall process of carrion decomposition. Altering the insect community composition and arrival patterns by delaying insect access decreased decomposition. Better understanding the mechanisms dictating rate and progress of carrion decomposition is important since carrion is a vital resource subsidy in many ecosystems. This is especially true in systems with annual mass die offs (e.g., salmon runs), large unpredictable pulses such as locust outbreaks, or large-scale population die offs such as livestock population declines from the 2011 drought in the southern part of the US (Sivakumar et al. 2011). Thus, decomposition of carrion could have major impacts on nutrient cycling, food web dynamics, and biodiversity of an ecosystem at multiple spatial and temporal scales.

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SUPPLEMENTAL MATERIAL

APPENDIX

Table A1. The presence and absence of adult necrophagous insects collected throughout decomposition from the insect access (ACC) and delayed insect access (EXC) carcasses. The ACC taxa represent specimens collected after the insect exclusion cages were removed (post-exclusion insect access). The abbreviation for each taxon follows the name in parentheses.

Order	Family	Species	2010	2011
Diptera	Calliphoridae	*Phormia regina* (PRE)	ACC, EXC	ACC, EXC
		Cochliomyia macellaria (CMAC)	ACC, EXC	ACC, EXC
		Lucilia coeruleiviridis (LCOR)	ACC, EXC	ACC, EXC
		Protophormia terraenovae (PROT)		ACC, EXC
		Calliphora vicina (CALL)	ACC, EXC	EXC
		Calliphora vomitoria (CALL)		EXC
		Cynomya cadaverina (CYCA)	ACC, EXC	
	Sarcophagidae	Unknown spp. (SARC)	ACC, EXC	ACC, EXC
	Piophilidae	*Piophila casei* (PIO)	ACC, EXC	ACC, EXC
		Prochyliza sp. (PIO)	ACC, EXC	ACC, EXC
	Sepsidae	*Sepsia* spp. (SEPS)	ACC, EXC	ACC, EXC
	Psychodidae	*Psychoda* spp. (PSY)	EXC	
Coleoptera	Staphylinidae (STAPH)	*Platydracus maculosus*	ACC, EXC	ACC, EXC
		Creophilus maxillosus	EXC	ACC, EXC
		Philonthus caeruleipennis	ACC, EXC	
		Unknown sp.	ACC, EXC	ACC, EXC
	Histeridae	Unknown sp. (HIST)	ACC, EXC	ACC, EXC
	Dermestidae	Unknown sp. (DERM)	EXC	

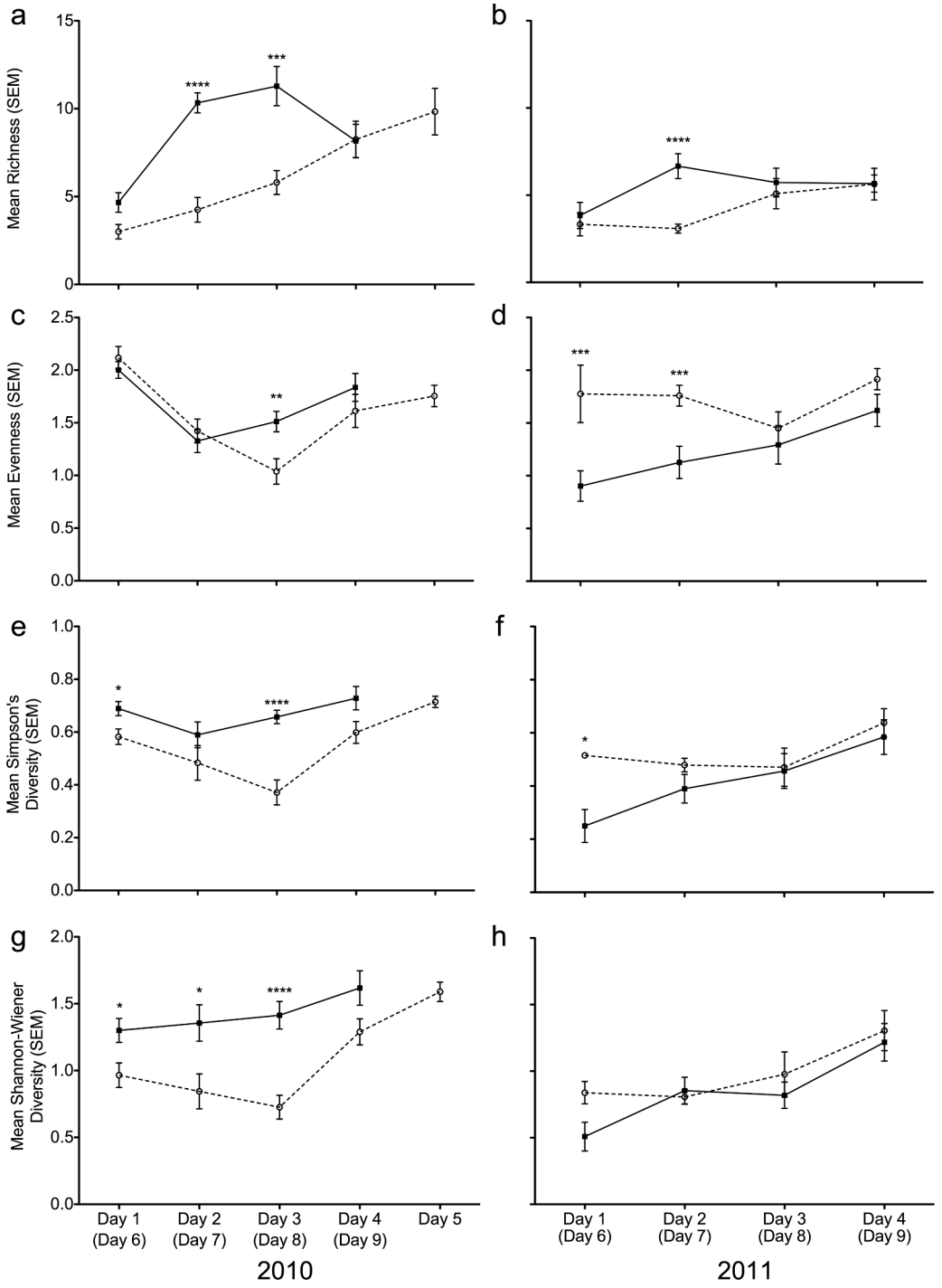

Fig. A1. Necrophagous insect community taxon richness, evenness, Simpson's diversity and Shannon-Wiener diversity in 2010 (a–d) and 2011 (e–h) over decomposition for carcasses allowed initial insect access (open circles) and those with delayed access for five days (filled squares). Each sampling day (Day 1–5) represents insect access to the carcass. However, for the delayed access treatment, the days in parentheses represent the number of days the carcasses were in the field (Day 6–9). * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, **** $P < 0.0001$.

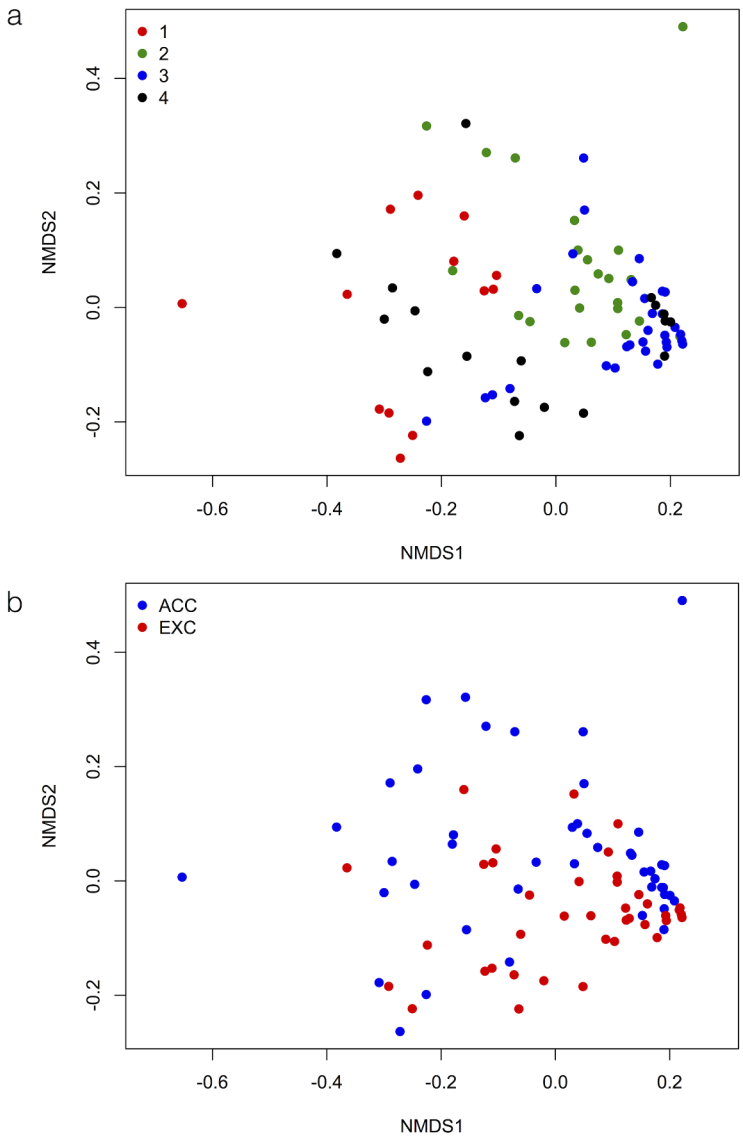

Fig. A2. Nonmetric multidimensional scaling analysis ordination of 2010 carcass necrophagous insect communities with significantly different communities among days (MRPP: $T = -9.38$; $P < 0.0001$) (a) and insect access (ACC) and delayed access (EXC) treatments (MRPP: $T = -19.53$; $P < 0.0001$) (b). This ordination explained 89.4% of the variation (stress = 0.144).

Table A2. RM-ANOVA results testing necrophagous insect community metrics (richness, evenness, Simpson's diversity and Shannon-Wiener diversity) between carcasses with delayed insect access and those given immediate access (Treatment) over decomposition day (Day) including the interaction.

Ecological metric	Factor	*F*	df	*P*
2010				
Richness	Day	8.830	3	<0.0001
	Treatment	20.53	1	<0.0001
	Day × Treatment	4.216	3	0.0085
Evenness	Day	12.37	3	<0.0001
	Treatment	1.435	1	0.2350
	Day × Treatment	1.918	3	0.1347
Simpson's diversity	Day	4.012	3	0.0108
	Treatment	18.50	1	<0.0001
	Day × Treatment	1.410	3	0.4740
Shannon-Wiener diversity	Day	3.809	3	0.0138
	Treatment	25.97	1	<0.0001
	Day × Treatment	0.874	3	0.4599
2011				
Richness	Day	2.254	3	0.0906
	Treatment	3.730	1	0.0579
	Day × Treatment	1.731	3	0.1694
Evenness	Day	2.639	3	0.0570
	Treatment	16.43	1	0.0001
	Day × Treatment	1.800	3	0.1561
Simpson's diversity	Day	4.318	3	0.0078
	Treatment	5.017	1	0.0286
	Day × Treatment	1.384	3	0.2558
Shannon-Wiener diversity	Day	6.131	3	0.0010
	Treatment	1.727	1	0.1935
	Day × Treatment	0.6096	3	0.6112

Table A3. Indicator taxa for 2010 necrophagous insect communities. Insect taxon is given along with the indicator value and p value for the respective group. All pair-wise comparisons were significantly different using $\alpha = 0.0071$ and $\alpha = 0.0063$ after Bonferroni correction for multiple pair-wise comparisons of treatment (EXC and ACC) and day, respectively, and are indicated by an asterisk (*).

Indicator group	Insect taxon	Indicator value	Mean	SD	*P*
ACC	*Piophila casei*	25.6	13.0	3.11	0.0040*
EXC	*Phormia regina*	63.9	33.8	3.48	0.0002*
EXC	Sarcophagidae	46.1	16.3	3.24	0.0002*
EXC	*Cochliomyia macellaria*	39.8	20.7	3.39	0.0004*
EXC	*Ophyra* spp.	39.6	29.2	3.55	0.0120
EXC	*Prochyliza* sp.	36.1	25.4	3.62	0.0120
EXC	*Psychoda* spp.	13.3	6.1	2.22	0.0096
Day 3	*Cynomya cadaverina*	18.3	9.2	4.99	0.0498
Day 4	*Phormia regina*	32.3	19.1	4.38	0.0176
Day 4	*Cochliomyia macellaria*	29.6	14.0	4.95	0.0156
Day 4	Sarcophagidae	23.6	12.0	4.85	0.0324
Day 5	*Sepsia* spp.	55.5	15.9	5.24	0.0004*
Day 5	*Prochyliza* sp.	45.2	16.2	5.13	0.0020*
Day 5	Staphylinidae	30.4	18.1	4.81	0.0294

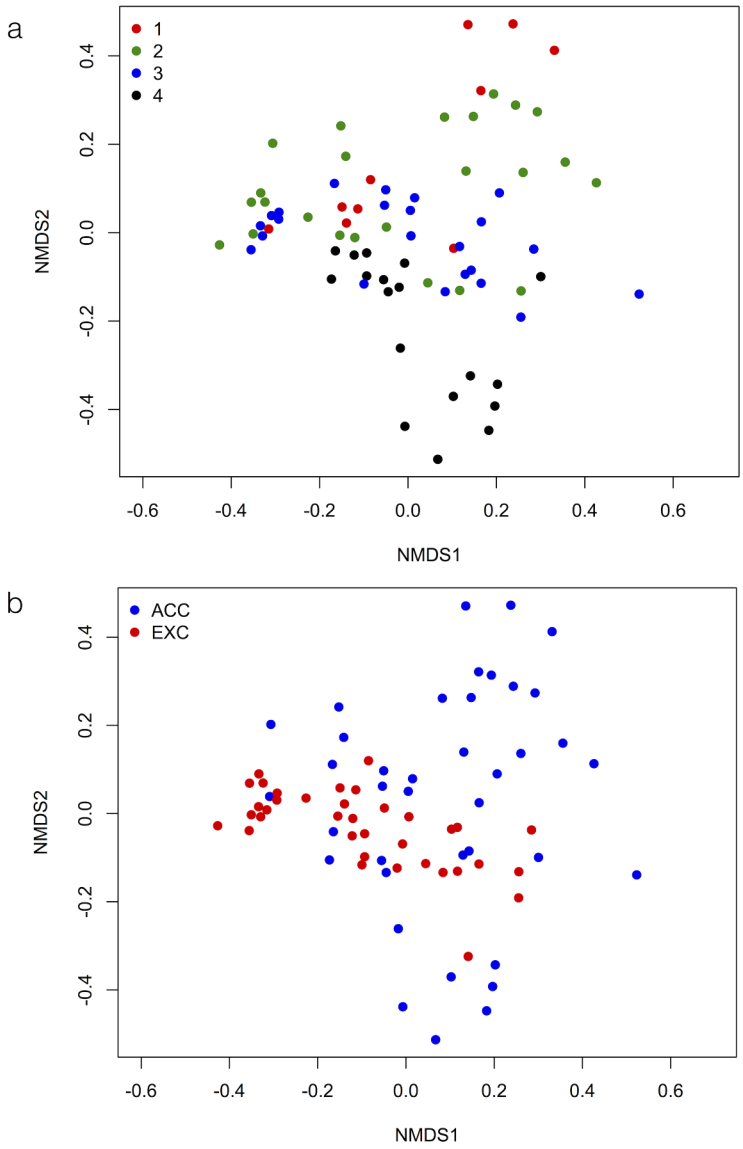

Fig. A3. Nonmetric multidimensional scaling analysis ordination of 2011 carcass necrophagous insect communities with significantly different communities among days (MRPP: $T = -18.55$; $P < 0.0001$) (a) and insect access (ACC) and delayed access (EXC) treatments (MRPP: $T = -18.69$; $P < 0.0001$) (b). This ordination explained 92.3% of the variation (stress = 0.166).

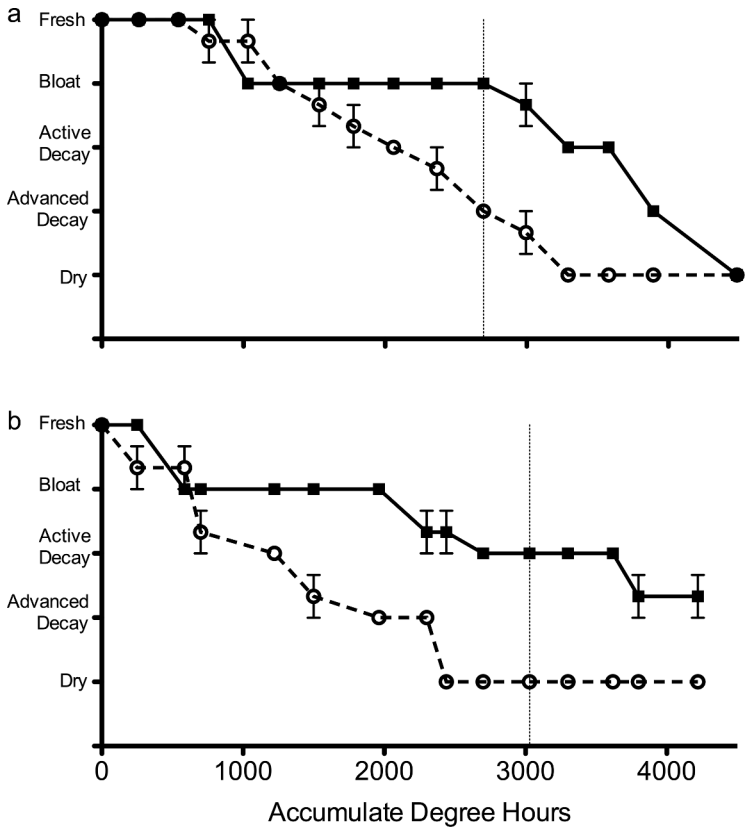

Fig. A4. Decomposition process from fresh to dry stages (Payne 1965) for carcasses with initial insect access (open circles) and those with delayed access for five days (filled squares) during 2010 (a) and 2011 (b). The vertical dashed line at 2,694 accumulated degree hours (ADH) and 3,028 ADH indicates when exclusion cages were removed from the delayed insect access carcasses for 2010 and 2011, respectively. Each datum represents the mean categorical stage of decomposition and standard error mean (SEM) for three carcasses with integers representing the decomposition stages from fresh (5) to dry (1).

Table A4. Indicator taxa for 2011 necrophagous insect communities. Insect taxon is given along with the indicator value and p value for the respective group. All pair-wise comparisons were significantly different using $\alpha = 0.0071$ and $\alpha = 0.005$ after Bonferroni correction for multiple pair-wise comparisons of treatment (EXC and ACC) and day, respectively, and are indicated by an asterisk (*).

Indicator group	Insect taxon	Indicator value	Mean	SD	*P*
ACC	*Lucilia coeruleiviridis*	56.3	35.0	4.40	0.0004*
EXC	*Phormia regina*	68.5	43.8	3.25	0.0002*
EXC	*Cochliomyia macellaria*	52.6	24.1	4.01	0.0002*
EXC	*Ophyra* spp.	25.5	16.8	3.84	0.0292
EXC	*Calliphora vicina*	11.1	5.2	2.23	0.0366
Day 2	*Lucilia coeruliviridis*	35.5	22.7	4.32	0.0098
Day 3	*Phormia regina*	33.6	25.3	2.72	0.0070
Day 4	*Sepsia* spp.	65.9	18.2	4.39	0.0002*
Day 4	Histeridae	51.8	13.2	4.69	0.0002*
Day 4	*Prochyliza* sp.	33.8	14.2	4.29	0.0028*
Day 4	*Piophila casei*	29.3	13.6	4.36	0.0068
Day 4	Staphylinidae	27.5	19.0	4.17	0.0420