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Research article

Special Collection: Filth Fly–Microbe Interactions

The Dynamic Maggot Mass Microbiome

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Abstract

Necrophagous insect studies have shown that decomposing vertebrate remains are an important ephemeral resource within an ecosystem. However, the microbes (e.g., bacteria and archaea) that were a part of the once living organism and the exogenous taxa that colonize this postmortem resource remain largely underexplored. Also, it is not well understood how these two kingdoms interact to recycle decaying biomass, an important mechanistic question for ecosystem function ecology. To better understand microbial community dynamics throughout decomposition, we used swine carcasses ($N = 6$) as models for mammalian postmortem decomposition to characterize epinecrotic microbial communities from: the abdominal skin of replicate carcasses; the internal microbiome of individual necrophagous dipteran larvae (maggots); and the microbiome of dipteran larval masses that had colonized the carcasses. Sampling occurred every 12 h for the duration of the decomposition process. We characterized these microbial communities over time using high-throughput 16S amplicon sequencing. The relative abundance of microbial taxa changed over decomposition as well as across sampling locations, suggesting significant interactions between the environment, microbes, and insect larvae. Maggot masses were represented by multiple blow fly species in each mass: *Phormia regina* (Meigen), *Lucilia coeruleiviridis* (Macquart), and *Cochliomyia macellaria* **(F.)**. Relative abundance of these species within the mass also changed as decomposition progressed, suggesting the presence of certain Calliphoridae species within a mass may be associated with temporal shifts of the microbial communities. These results provide new insight into the community ecology of carrion decomposition by providing new data on interactions of microbes and dipteran larvae over time.

Key words: Calliphoridae, decomposition, necrobiome, epinecrotic, community

Death is the beginning of new life within an ecosystem. After an animal dies, a wide range of organisms (i.e., vertebrate scavengers, insects, and microbes) will begin to decompose the carcass, utilize it as a resource, and reincorporate its constituents (e.g., nitrogen) into the ecosystem. All of these organisms coming together create a complex system of interkingdom interactions that are collectively described as the necrobiome (Benbow et al. 2013). Although carrion is an ephemeral resource, members of the necrobiome become integrated into food webs and ecosystems with potentially far reaching and lasting effects on ecosystem structure and function (Benbow et al. 2015b; Barton et al. 2013a,b; Parmenter and MacMahon 2009). Despite the significant impacts of carrion decomposition on an ecosystem, it remains a largely understudied area of ecology.

One aspect of carrion ecology that has received considerable attention is the succession of postmortem arthropod colonizers, specifically the blow flies (Diptera: Calliphoridae) (Payne 1965, Byrd and Castner 2001, Kreitlow 2009, Benbow et al. 2013). Arriving shortly after death, blow flies search for suitable habitat or substrates in which to reproduce, feed, and provide a food resource for their offspring (Byrd and Castner 2001). Their high fecundity and growth rates result in an efficient conversion of the carrion resource into insect biomass (Byrd and Castner 2001, Chaloner et al. 2002). This conversion efficiency is also evidenced in the growing interest in using saprophagous dipteran larvae to manage food and other organic waste (Pastor et al. 2015, Nguyen et al. 2015). Without blow flies and other scavengers, the turnover rate of carrion back into the ecosystem would be greatly reduced (Payne 1965, Pechal et al. 2014a). Nevertheless, even this well-studied area of carrion ecology has neglected an important component, the interaction of blow flies with the microbes (e.g., bacteria and archaea) of the decaying organic matter (Pechal et al. 2013, Pechal et al. 2014b). Indeed, studies of these interactions are beginning to demonstrate that microbes can play a role in the attraction behavior of carrion flies to resources (Zheng et al. 2013, Ma et al. 2012, Jordan et al. 2015), suggesting

additional interactions are likely important to the ecology of carrion within ecosystems.

Microbial communities are not only present throughout carrion decomposition but also have a predictable structural (community composition) and functional change over time (Pechal et al. 2013, 2014b; Metcalf et al. 2013, 2016), which reflects changes within entomological communities (Pechal et al. 2014b). Microbial communities are present within and on blow fly larvae as well as on the surface of the carrion itself (Pechal and Benbow 2016), but how these different communities interact over the course of decomposition is relatively unknown. In order to more fully understand these interkingdom interactions of carrion ecology, we identified the calliphorid larval community of decomposing carrion, as well as characterized the associated microbial communities of the carcass surface, (i.e., the epinecrotic community) the internal larval microbiome, and the larval (maggot) mass microbiome throughout swine carcass decomposition. We hypothesized that the relative abundance of certain microbial taxa in each sampling area would shift over time, and that taxa would become more or less abundant among the interacting communities of the skin, larvae, and larval masses. We predicted that the internal larval microbiome would be, in part, determined from their external environment and food source (i.e., the carcass). Therefore, we expected the internal and external microbiomes of the larvae to have increasingly similar microbial community composition as decomposition progressed. We also hypothesized there would be a detectable sequence of insect species colonizers that would change as the carcasses decomposed, consistent with previous studies on postmortem insect succession.

Materials and Methods

Study Site

The study was conducted from 19–27 August 2014 at Purdue University's Forensic Entomology Research Compound in West Lafayette, IN (40° 25′36.0″ N, 86° 56′57.0″ W). Six (three male and three female) swine carcasses (*Sus scrofa* L.), averaging ~19.0 kg and 81.0 cm in length, were purchased from a local farm, and therefore exempt from university IACUC review. Carcasses were immediately placed in plastic bags during travel to the site (~4 h), as previously described (Benbow et al. 2013; Pechal et al. 2013, 2014a,b). The carcasses were then placed on two East–West running transects ~50 m apart in a grassy field surrounded by deciduous forest. All carcasses were oriented on their left side with heads directed north and placed on top of stainless steel grates (~1.0 m^2), with ~5.0-cm^2 openings, located on the ground to allow for lifting the individual carcasses during sample collection. Cages (~1.0 m^3) constructed of chicken mesh wire and treated lumber were secured over the carcasses and the grates to prevent vertebrate scavenging. Microbial and insect communities were sampled every 12 h at ~0700 h and 1900 h for 8 d, which was the amount of time for the carcasses to fully decompose to only bones and skin, and dipteran larvae to develop and begin the postfeeding dispersal from the resources for pupation.

In order to accurately calculate accumulated degree hours (ADH) that are important for comparing rates of larval development of different populations, one HOBO temperature logger (Onset Computer Corporation, Bourne, MA) was attached to the east side of each antivertebrate scavenging cage and one was placed directly on the ground in the middle of the field; temperature (°C) and light intensity (lux) were recorded every 15 min throughout the duration of the study. An infrared thermometer (One World Technologies, Inc., Anderson, SC) was used to measure surface temperatures for the carcasses, blow fly larval masses, and the ground ~1 m away from each carcass at each 12-h sampling time period; the temperatures of three different locations for each substrate (e.g., the carcass or maggot mass) were recorded and then averaged. Temperature data were converted into ADH using thermal summation models with a base temperature of 0 °C (Pechal et al. 2014b, VanLaerhoven 2008). Data from the Purdue University Airport (40° 24′48.5″ N, 86° 56′26.1″ W) weather station, located 1.93 km northwest of the study site, were used to assess rainfall over the course of this study.

Microbe and Insect Sampling

The epinecrotic microbial community of the skin (i.e., external microbiome) for each carcass was aseptically collected every 12 h by swabbing a single transect (posterior to anterior orientation) along the abdominal skin with a 15 cm sterile DNA-free cotton-tipped swab (Puritan Medical Products, Guilford, ME), as described in Pechal et al. (2014b). The transect consisted of three combined areas (~2.54 by 7.62 cm each) swabbed for 30 s each and care was taken not to resample the same areas. All swabs were stored at −20 °C until DNA isolation. Skin samples were no longer collected once maggot masses expanded to cover the sampling region.

In addition to the external microbiome of the carcass, the internal larval microbiome (microbes within the larvae) and the microbial communities of the larval mass (external microbes of the larval aggregate, the maggot mass microbiome) were also characterized. At each sampling period, ~20–30 blow fly larvae were collected using a sterile DNA-free cotton-tipped swab from three different areas of a single larval mass located on the carcass, and stored at −20 °C. Third instars were identified to at least family level (Stojanovich et al. 1962) and then stored in 100% molecular grade ethanol. Three third-instar specimens of the most abundant species collected, *Phormia regina* (Meigen) (Diptera: Calliphoridae), from each carcass at each sampling time point were used for internal microbiome analysis. The maggot mass microbiome was sampled every 12 h, for each mass present during decomposition, using a single sterile DNA-free cotton-tipped swab placed into three different areas of a single larval mass. Resulting samples were stored at −20 °C until DNA isolation.

DNA Isolation and Amplicon Sequencing

Samples from seven time points (0, 593, 1557, 1845, 2469, 3384, and 3962 ADH or 0, 24, 60, 72, 96, 132, and 156 h after carcass placement, respectively) were selected for microbial analysis due to sample quantity and progression of decomposition; all phases of decomposition were represented in the microbial analysis (Supp. Table 1[online only]). Time point 0 represents when the carcasses were placed in the field and insects were allowed access, ~4 h postmortem. Genomic DNA was isolated using the PureLink Genomic DNA Mini Kit (Grand Island, NY) according to the manufacturer's protocol with the modification of adding 15 µl lysozyme (15 mg/ml) prior to incubation to improve microbial cell lysis. Skin and maggot mass microbiome swab samples were extracted individually while larval specimens were surface sterilized and pooled ($N = 3$) for characterization of the internal larval microbiome. For surface sterilization, specimens were rinsed in 10% bleach followed by three rinses in sterile deionized water (Pechal and Benbow, 2016). DNA was quantified using the Quant-iT dsDNA HS Assay kit and a Qubit 2.0 (Grand Island, NY). Samples were sequenced at the Michigan State University Genomics Core facility (East Lansing, MI) using Illumina MiSeq with amplification of the variable region 4 (V4) of

the 16S rRNA gene using 515F/806R primers (5′-GTGCCAGCMGCCGCGG-3′, 5′-TACNVGGGTATCTAATCC-3′) (Claesson et al. 2010, Caporaso et al. 2011, Caporaso et al. 2012, Pechal and Benbow 2016). Raw sequencing data were assembled, quality-filtered, demultiplexed, and analyzed using the default settings in QIIME 1.9.0 (Caporaso et al. 2010) and consistent with Pechal and Benbow (2016). The relative abundance at the phyletic and family levels, or the microbial community profiles, was determined and analyzed over decomposition time and between locational communities.

Statistical Analysis

Analysis of variance (ANOVA) and a paired *t*-test were conducted using SAS Studio 3.5 (SAS Institute Inc., 2016, Cary, NC) in order to determine potential significant differences in maggot mass and ambient temperatures. A Friedman test was performed in SAS to evaluate blow fly species relative abundance over decomposition time and a Kruskal Wallis test was performed to evaluate microbial taxa relative abundance between different sampling communities and over time. A Spearman Rank test was used to evaluate correlations in the relative abundance of the predominant taxa among the microbial communities over decompostion. All p-values were considered significant with alpha at or below 0.05.

Results

Maggot Mass Communities

Maggot masses were first observed on the anterior region of all carcasses (primarily in the buccal and nasal cavities) as well as on the posterior region of three carcasses at 1557 ADH (60 h). By 2469 ADH (96 h) each mass had expanded and combined into one large mass that, by visual assessment, covered the majority, if not all, of each carcass's surface area. All masses had dispersed by 3962 ADH (156 h) with the exception of carcass 1 (Table 1; Fig. 1). Maggot mass temperatures over the course of decomposition were not significantly different among carcasses ($F = 0.26$, df = 5, $P = 0.931$), but were significantly different ($t = 16.45$, $P = < 0.0001$) from the ambient air temperature which averaged 9.29 °C (± 0.565 °C SE) lower than larval masses (Fig. 2).

Identification of third instar diptera collected from masses at five time points (1557, 1845, 2469, 3384, 3962 ADH) revealed multiple species were present within each mass and their relative abundances changed over time. Of the 643 larval specimens collected, three species were identified: *P. regina*, *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae), and *Lucilia coeruleiviridis* (Macquart) (Diptera: Calliphoridae). During the formation of the initial maggot masses (by 1557 ADH), *C. macellaria* was the most predominant species (52.0 ± 6.19% SE) on four carcasses, but as decomposition progressed, it was replaced by *P. regina* as the most predominant species (reaching 85.3 ± 4.53% SE) for all carcasses. The relative abundance of *L. coeruleiviridis* remained low (reaching 22.3% ± 9.59% SE) throughout decomposition (Fig. 3). Changes in relative abundance throughout decomposition were significant for *C. macellaria* ($F = 4.40$, df = 9, $P = 0.008$) and *P. regina* ($F = 4.94$, df = 9, $P = 0.005$), but not for *L. coeruleiviridis* ($F = 1.22$, df = 9, $P = 0.360$; Supp. Table 2 [online only]).

Microbial Community Composition

The predominant microbial phyla (>3% relative abundance) of the skin and maggot mass microbial communities were Proteobacteria, Firmicutes, and Bacteroidetes, while the internal larval microbiome was most represented by Proteobacteria and Firmicutes. For all three communities, the most prevalent class of Proteobacteria was Gammaproteobacteria. There was an inverse relationship between the relative abundance of Proteobacteria and Firmicutes over decomposition time, which was reflected in a Proteobacteria to

Table 1. Maggot mass locations, mean mass temperature, and ambient temperature for each carcass throughout decomposition

ADH	Carcass								
	Mass location(s)	Mean maggot mass temperature (C°)	Ambient temperature (C°)	Mass location(s)	Mean maggot mass temperature (C°)	Ambient temperature (C°)	Mass location(s)	Mean maggot mass temperature (C°)	Ambient temperature (C°)
	1			2			3		
1557	Anterior Posterior	34.14	22.37	Anterior	38.73	26.99	Anterior Abdomen Posterior	30.70	24.85
1845	Anterior Abdomen Posterior	35.13	26.35	Carcass	38.63	25.77	Anterior Posterior	37.23	26.99
2469	Carcass	28.23	22.18	Carcass	28.47	22.59	Carcass	28.90	22.63
3384	Anterior	35.83	20.79						
3962	Posterior	28.87	18.44						
	4			5			6		
1557	Anterior	38.70	30.55	Anterior	37.30	30.22	Anterior Posterior	36.50	30.72
1845	Carcass	39.53	23.23	Anterior Posterior	38.83	23.40	Carcass	38.70	25.43
2469	Carcass	27.80	22.63	Carcass	28.53	22.23	Carcass	31.83	22.25
3384	Abdomen	37.40	21.75	Anterior	29.13	20.72			
3962									

Blank areas represent times when maggot masses were no longer present.

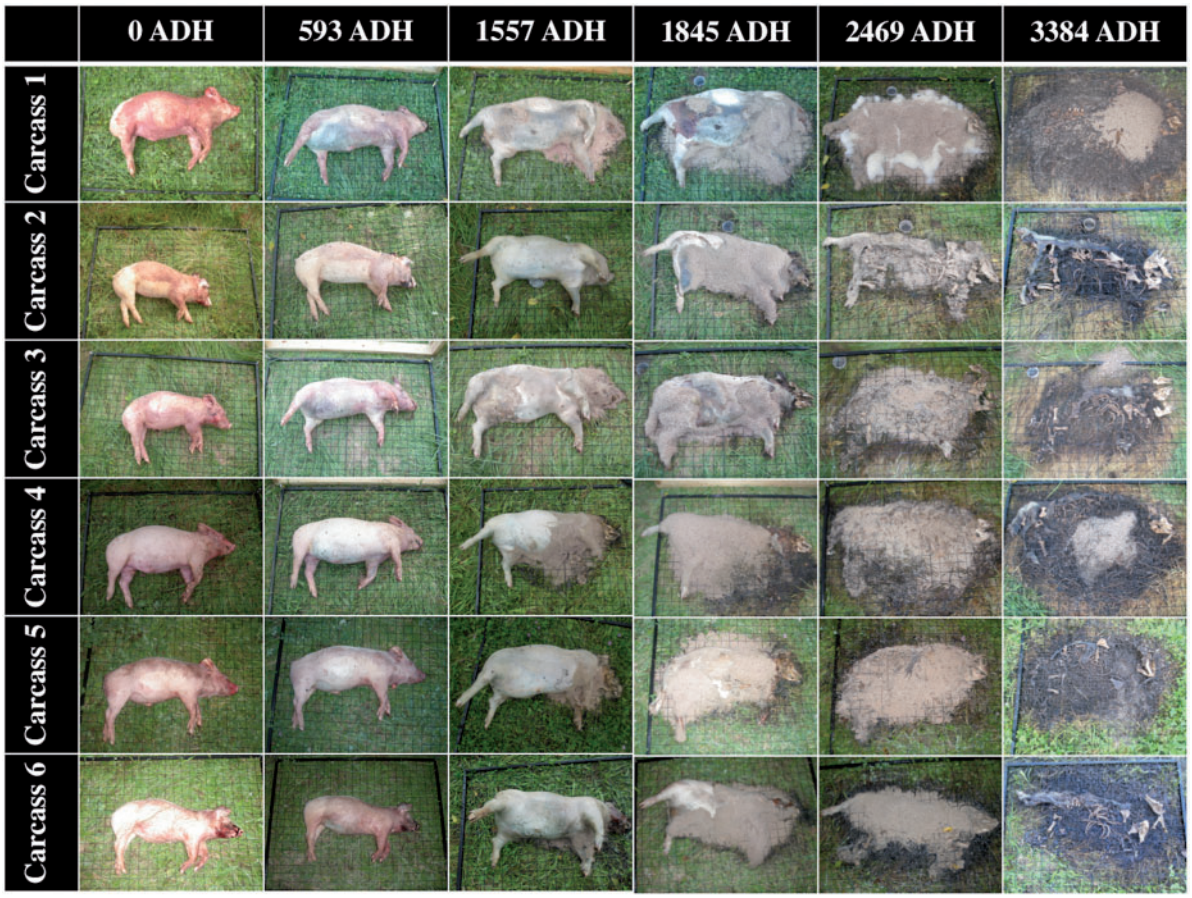

Fig. 1. Taphonomic changes of individual carcasses at each sampling point over decomposition time expressed as accumulated degree hours (ADH). For scale, the average length of the carcasses was ~81.0 cm.

Firmicutes (P/F) ratio that changed during decomposition from 0.38 P/F (0 ADH) to 1.02 P/F (1845 ADH) on the carcass surface, 1.01 P/F (1557 ADH) to 1.33 P/F (3962 ADH) in the maggot mass, and 0.91 P/F (1557 ADH) to 4.43 P/F (3962 ADH) for the internal larval microbiome (Figs. 4 and 5). Early in the decomposition process Firmicutes relative abundance was higher (65.6 ± 6.44% SE) than Proteobacteria (25.0 ± 7.21% SE) on the carcass surface; however, as indicated by the P/F ratios, Firmicutes decreased while Proteobacteria increased over time. Near the end of decomposition (3962 ADH) the relative abundance of Firmicutes and Proteobactera was 35.3% and 47.1% in the maggot mass, respectively, while these phyla were 18.2% and 80.6% in the larval interal microbiome, respectively.

There was a significant corrleation between Firmicutes and Proteobacteria relative abundance throughout decomposition for the carcass surface ($\rho = -1.0$, $P = <0.0001$) and the internal larval microbiome ($\rho = -0.9$, $p = 0.037$), but not for the maggot mass microbial communities ($\rho = 0.8$, $P = 0.104$). The relative abundances of Firmicutes and Proteobacteria were significantly different for the carcass surface ($\chi^2 = 9.46$, $df = 1$, $P = 0.002$) and maggot mass ($\chi^2 = 5.62$, $df = 1$, $P = 0.018$), but not for the internal larval microbiome ($\chi^2 = 3.53$, $df = 1$, $P = 0.060$). For the predominant phyla (>3% overall relative abundance), between the maggot mass and internal larval communities there was a significant correlation over time of Proteobacteria ($\rho = -0.9$, $p = 0.037$), but not Firmicutes ($\rho = 0.8$, $P = 0.104$) and Bacteroidetes ($\rho = -0.2$, $P = 0.747$). Differences in relative abundance between the maggot mass and internal larval microbiome were not significantly different for any of the predominant phyla (Firmicutes: $\chi^2 = 0.061$, $df = 1$, $P = 0.805$; Proteobacteria: $\chi^2 = 0.002$, $df = 1$, $P = 0.963$; Bacteroidetes: $\chi^2 = 1.475$, $df = 1$, $P = 0.225$). In addition, when maggot masses co-occurred with the skin communities (both sample locations were still present before the masses covered the entire carcass surface area) at 1557–1845 ADH, the relative abundances of dominant microbial phyla were similar across all three sampled communities: carcass surface, maggot mass, and larval internal. Ultimately, Proteobacteria abundance increased overall while Firmicutes decreased throughout decomposition in all of the microbial communities (Figs. 4 and 5; Supp. Table 3 [online only]).

At the familial level, there were comparable results as the phyletic level, including an inverse relationship between the rare taxa (individual family relative abundances that were <3%) and Xanthomonadaceae. Immediately following carcass placement (0 ADH) skin microbial communities were dominated by rare taxa that collectively represented 39.80% (± 6.49% SE) of the community. At the same time (0 ADH), Xanthomonadaceae relative abundance was the lowest (0.22 ± 0.04% SE) until it increased at 1557 ADH (the same time maggot masses initially formed) to become the predominant family (41.7 ± 6.40% SE) of the skin communities. This increase in Xanthomonadaceae was also the case for the maggot mass and internal larval microbiomes, suggesting a mixing among these communities as decomposition progressed. For the predominant families (>3% overall relative abundance), there was a significant correlation of a Lactobacillales family ($\rho = 0.9$, $P = 0.037$) between the maggot mass and internal larval communities, but not for Xanthomonadaceae ($\rho = -0.7$, $P = 0.188$),

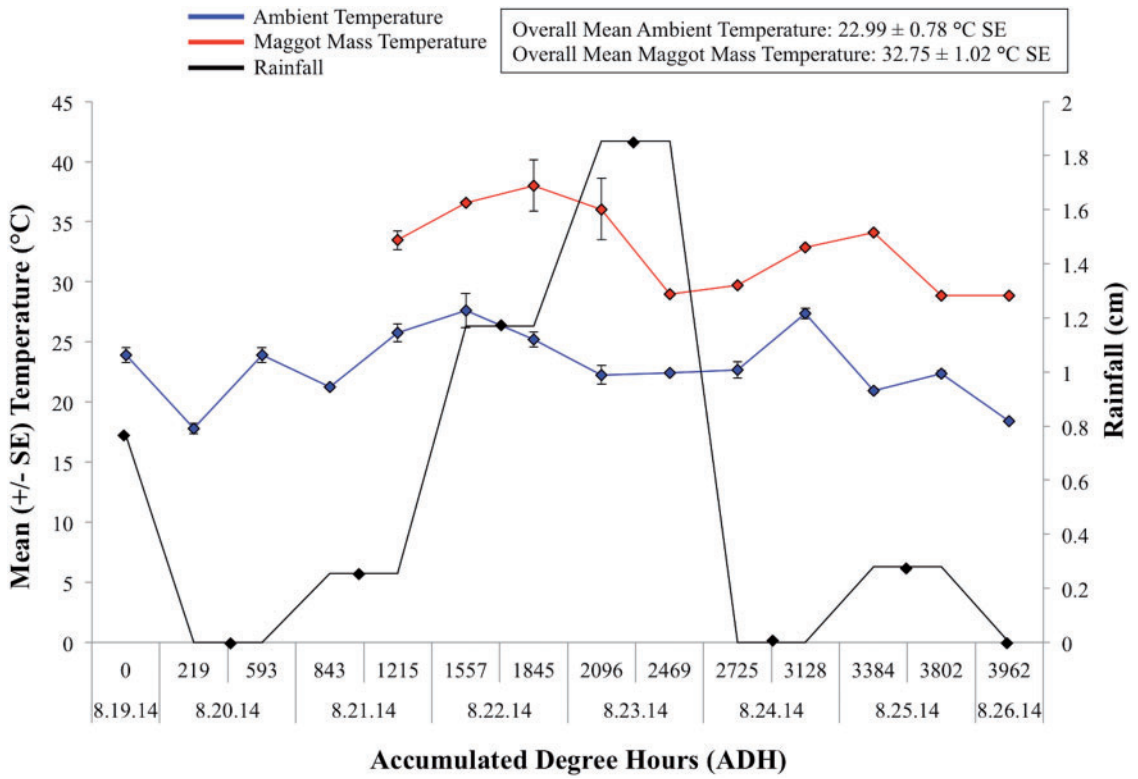

Fig. 2. Rainfall and mean (SE) ambient and larval mass temperatures over decomposition time. The accumulated degree hours (ADH) are given above the calendar date along the x-axis. Rainfall was recorded daily while ambient and maggot mass temperatures were recorded for each carcass at each sampling point (every 12 h) and then averaged.

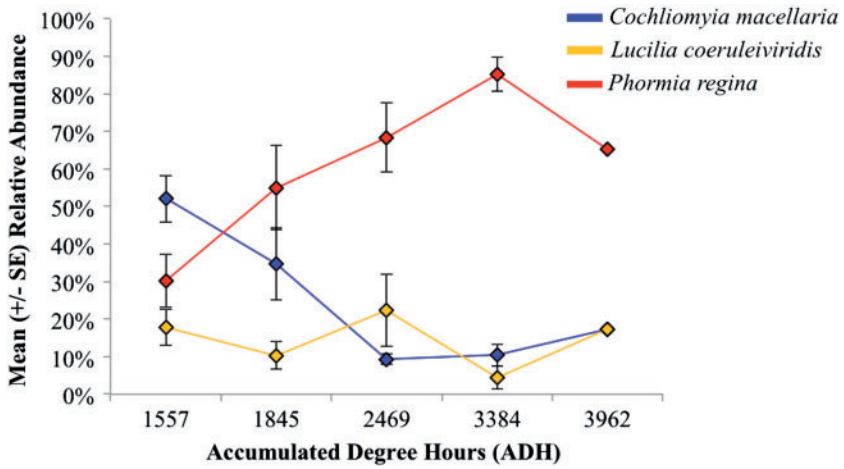

Fig. 3. Mean (SE) relative abundance of third-instar calliphorid larvae over decomposition time expressed as accumulated degree hours (ADH).

Tissierellaceae ($\rho = -0.1$, $P = 0.873$), Clostridiaceae ($\rho = -0.3$, $P = 0.624$), and Planococcaceae ($\rho = 0.1$, $P = 0.873$). However, relative abundances between the two communities were significantly different for all predominant families except Xanthomonadaceae (Clostridiaceae: $\chi^2 = 8.61$, df = 1, $P = 0.003$; Lactobacillales family: $\chi^2 = 6.02$, df = 1, $P = 0.014$; Planococcaceae; $\chi^2 = 18.9$, df = 1, $P = <0.0001$; Tissierellaceae: $\chi^2 = 5.29$, df = 1, $P = 0.021$; Xanthomonadaceae: $\chi^2 = 0.210$, df = 1, $P = 0.647$). Similar to the results at phyla level, family-level relative abundances were similar across communities when the carcass surface, maggot mass, and internal larval communities were all present simultaneously (1557–1845 ADH; Figs. 5 and 6). Additionally, the increase in relative abundance of *P. regina* larvae corresponded to the increase of Proteobacteria, specifically Xanthomonadaceae, in all of the microbial communities (Figs. 4, 5, and 6).

Discussion

Past research has shown insects will colonize decomposing remains in a predictable succession (Payne 1965, Byrd and Castner 2001, Kreitlow 2009, Benbow et al. 2013), but there are few published findings of maggot mass species compositional changes throughout decomposition. Gruner et al. (2007) reported the co-occurrence of

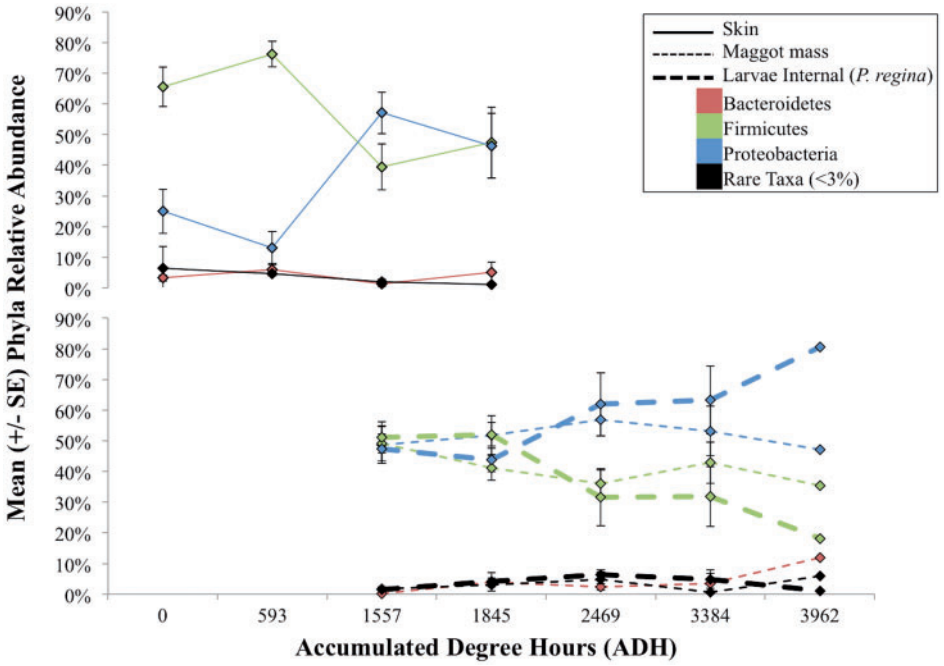

Fig. 4. Mean (SE) relative abundance of dominant microbial phyla for carcass skin, maggot mass, and internal larval microbial communities over decomposition time expressed as accumulated degree hours (ADH). The top panel represents the skin microbial communities that were only present until 1845 ADH. The bottom panel shows the maggot mass external microbial communities as well as those for individual larvae after the mass had formed.

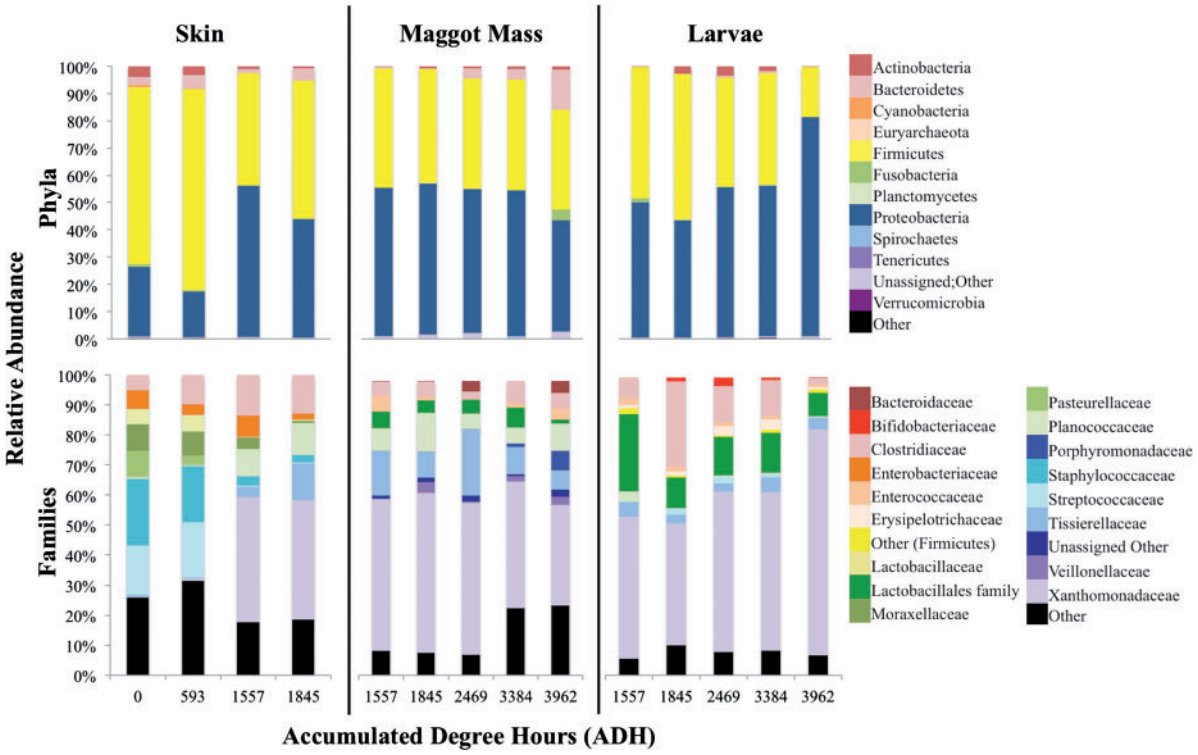

Fig. 5. Relative abundance of the top 10 most abundant microbial phyla (top panel) and families (bottom panel) for each community location over decomposition time expressed as accumulated degree hours (ADH).

L. coeruleiviridis, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), *Chrysomya megacephala* (F.) (Diptera: Calliphoridae), *P. regina*, and *C. macellaria* larvae within masses located on swine carcasses during the summer in Florida. The authors found that *L. coeruleiviridis* was initially the most abundant blow fly species but decreased over time while *P. regina* increased throughout decomposition. However, they did not characterize the microbial communities associated with the carcass, larval mass, or individual larvae. Numerous studies have now shown the epinecrotic postmortem microbiome changes over time for a variety of

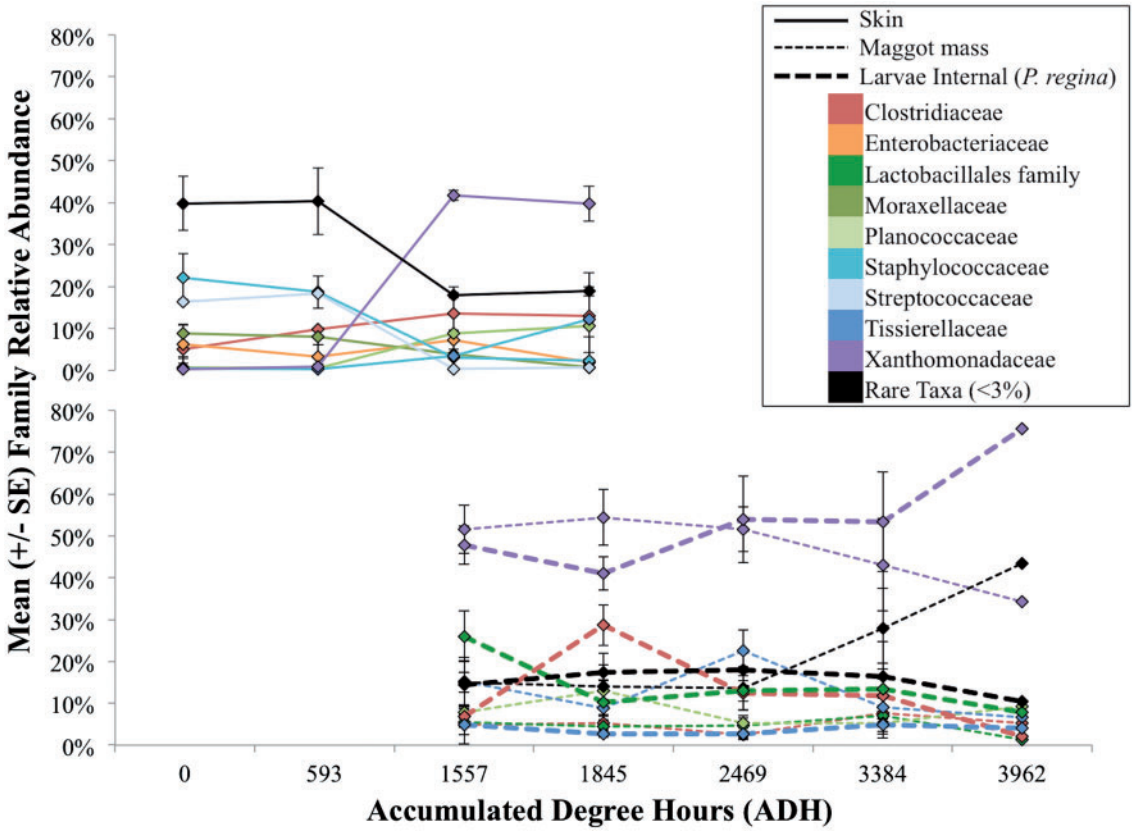

Fig. 6. Mean (SE) relative abundance of dominant microbial families for carcass skin, maggot mass, and larval microbial communities over decomposition time expressed as accumulated degree hours (ADH). The top panel represents the skin microbial communities that were only present until 1845 ADH. The bottom panel shows the maggot mass external microbial communities as well as those for individual larvae after the mass had formed.

animals including swine (*Sus scrofa* L.) (Benbow et al. 2015a, Pechal et al. 2013, 2014a), mice (*Mus musculus* L., strain B6C3F1) (Metcalf et al. 2013, 2016), salmon (*Oncorhynchus keta* Walbaum) (Pechal and Benbow 2016), and humans (*Homo sapiens* L.) (Metcalf et al. 2016, Hyde et al. 2014).

Additionally, recent research has explored how insects are attracted to decomposing resources based on the volatile organic compounds (VOCs) produced by epinecrotic microbial communities (Zheng et al. 2013, Ma et al. 2012, Jordan et al. 2015). It is possible different necrophagous dipteran species prefer specific microbial communities and their emitted VOCs for their offspring and therefore arrive at different times following death (Jordan et al. 2015). This is consistent with Rosati (2014) who demonstrated that *P. regina* preferred to oviposit on a resource that had already been colonized by other calliphorid species. Similarly, in our study there was a large increase in *P. regina* larval abundance later in decomposition, after other species had previously colonized. The successional shifts in relative abundance of each blow fly species over time might be explained by concurrent shifts in the microbial community of the carcass surface (epinecrotic microbial communities); however, this is an area where additional research into interkingdom interactions associated with microbial and insect community succession on carrion is needed.

One of the most notable findings related to the microbial communities was the inverse relationship between the relative abundance of Firmicutes and Proteobacteria over the course of decomposition. This relationship was not unexpected, as previous studies have documented similar results from terrestrial and aquatic swine carrion decomposition (Pechal et al. 2014b, Benbow et al. 2015a), to the human gut (Del Chierico et al. 2015), and even inside the Ocean Cubiculum of the Roman Catacombs where the effect of blue light exposure on microbial communities was monitored over years (Urzi et al. 2014). The reason behind this relationship, however, is not yet fully understood and would be a fruitful area of future study.

There were also differential trends in taxa relative abundance among microbial communities on the carcass—skin, maggot mass, and larval internal. When the maggot masses first formed, between 1557–1845 ADH, the internal and external larval microbiomes were similar to the skin microbial community composition. This suggests the larval internal microbes may have been acquired from their habitat and food source (i.e., the carcass), and that microbial communities can be trophically transferred during the decomposition process. It has already been shown that this microbial transfer is the case between the human diet and gut microbiome (Turnbaugh et al. 2009), and calliphorid and aquatic insect larvae in an aquatic habitat associated with high densities of decomposing salmon carcasses (Pechal and Benbow 2016).

Our results also suggest the carcass microbiome is influenced by insect colonizers. The sudden decrease of Firmicutes and increase of Proteobacteria relative abundances on the carcass surface did not occur until the first sampling point when maggot masses were initially present (1557 ADH). It has been well established that filth flies, such as Calliphoridae, can carry microbes and most notably transfer bacteria and disease causing pathogens to humans or food (Doud et al. 2014, Lilia et al. 2008, Graczyk et al. 2001, Blaak et al. 2014). If blow flies are bringing their own community of microbes to a carcass, it is probable that they are also taking microbes from

the carcass and further dispersing them into the environment. Again, this is another area of interesting future research important to understanding carrion ecology with applications in disease ecology.

There is still a great need for further study in the area of microbe and insect community ecology of carrion, but our findings provide an important foundation to understanding the function of postmortem microbial communities and how they interact with the associated insect colonizers. A single larval mass is often composed of a single family (e.g., Calliphoridae), but can contain multiples species with abundances that change throughout decomposition. Our finding that multiple necrophagous blow fly species co-occur as larvae on decomposing vertebrates has relevance in forensic entomology, where often only a few larvae are collected as evidence during death investigations; thus, there is the potential to miss additional species with different developmental characteristics that could affect period of insect activity estimates (Byrd and Castner 2001, Benbow and Tomberlin 2015). The finding that Xanthomonadaceae relative abundance increases throughout decomposition also has the potential to aid in estimating periods of insect activity and microbial succession. Typically Xanthomonadaceae are considered environmental organisms, including many plant pathogens and a few human pathogens (Pieretti et al. 2009, LaSala et al. 2007). However, our findings, as well as those of similar studies, suggest that this family also plays an important role in decomposition (Metcalf et al. 2013, Pechal and Benbow 2016, Guo et al. 2016). Additionally, the microbiome of the carcass surface, the larval mass microbiome, and internal larval microbiome change temporally, potentially driving the changes in blow fly species abundance and transferring from one community to another becoming integrated into an ecosystem.

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Supplementary Materials

Supplementary data are available at *Annals of the Entomological Society of America* online.

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