Microbiome-assisted carrion preservation aids larval development in a burying beetle

Shantanu P. Shukla[a,1], Camila Plata[a], Michael Reichelt[b], Sandra Steiger[c], David G. Heckel[a], Martin Kaltenpoth[d], Andreas Vilcinskas[c,e], and Heiko Vogel[a,1]

[a]Department of Entomology, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany; [b]Department of Biochemistry, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany; [c]Institute of Insect Biotechnology, Justus-Liebig-University of Giessen, 35392 Giessen, Germany; [d]Evolutionary Ecology, Institute of Organismic and Molecular Evolution, Johannes Gutenberg University, 55128 Mainz, Germany; and [e]Department Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, 35394 Giessen, Germany

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The ability to feed on a wide range of diets has enabled insects to diversify and colonize specialized niches. Carrion, for example, is highly susceptible to microbial decomposers, but is kept palatable several days after an animal's death by carrion-feeding insects. Here we show that the burying beetle *Nicrophorus vespilloides* preserves carrion by preventing the microbial succession associated with carrion decomposition, thus ensuring a high-quality resource for their developing larvae. Beetle-tended carcasses showed no signs of degradation and hosted a microbial community containing the beetles' gut microbiota, including the yeast *Yarrowia*. In contrast, untended carcasses showed visual and olfactory signs of putrefaction, and their microbial community consisted of endogenous and soil-originating microbial decomposers. This regulation of the carcass' bacterial and fungal community and transcriptomic profile was associated with lower concentrations of putrescine and cadaverine (toxic polyamines associated with carcass putrefaction) and altered levels of proteases, lipases, and free amino acids. Beetle-tended carcasses develop a biofilm-like matrix housing the yeast, which, when experimentally removed, leads to reduced larval growth. Thus, tended carcasses hosted a mutualistic microbial community that promotes optimal larval development, likely through symbiont-mediated extraintestinal digestion and detoxification of carrion nutrients. The adaptive preservation of carrion coordinated by the beetles and their symbionts demonstrates a specialized resource-management strategy through which insects modify their habitats to enhance fitness.

insect nutrition | gut microbiota | symbiosis | fungus | resource competition

Insects exploit a wide range of diets, and their ability to adapt to challenging environments and to form symbiotic associations with microorganisms has enabled them to occupy specialized niches (1–4). Some specialist insects feed on ephemeral and nutritionally rich diets such as cadavers, dung, and fruits that are susceptible to degradation by microbial competitors (5). Necrophagous beetles (family Silphidae) have evolved several physiological and behavioral adaptations in response to resource competitors (6–8) and host a conserved and characteristic gut microbiome (9, 10). Loss of carrion quality and presence of microbial competitors can have negative effects on insect fitness (11–13), but necrophagous insects also introduce microbes to carrion and greatly accelerate nutrient turnover (14). Unregulated microbial growth can render the carrion unpalatable and toxic through the accumulation of microbial metabolites, nitrogenous products such as putrescine and cadaverine (polyamines that give decomposing meat its characteristic smell), acids, phenols, and alcohols (15–20). It remains unclear how these insects and their antimicrobial strategies of carrion management (10, 11, 21–26) affect carrion properties, which enable the utilization of aging, decomposing carcasses.

The burying beetle *Nicrophorus vespilloides* (Coleoptera; Silphidae) uses small cadavers for breeding and is seemingly immune to any ill effects of feeding on aging, buried carcasses. Beetles prepare carcasses by removing hair or feathers, burying, rolling, smearing them with oral and anal secretions, and creating a feeding cavity for their larvae, thereby modifying the carcass substantially (12, 23, 26, 27). Application of oral and anal secretions is hypothesized to support larval development (27), to transfer nutritive enzymes (21, 28, 29), transmit mutualistic microorganisms to the carcass (10, 21, 22, 30), and suppress microbial competitors through their antimicrobial activity (11, 23, 31–34). The secretions inhibit several Gram-positive and Gram-negative bacteria, yeasts, and molds (11, 31, 35), and the beetles up-regulate transcription of antimicrobial peptides and lysozymes in the presence of the carcass (36, 37). Nevertheless, beetle-prepared carcasses host a diverse bacterial community (Xanthomonadaceae, Enterobacteriaceae, Enterococcaceae, Porphyromonadaceae) and a yeast-dominated fungal community that is transmitted to the larvae (10, 21).

Apart from the proliferation of microbial competitors, other features of carrion degradation (such as loss of nutrients or accumulation of toxic metabolites) can also impact beetle fitness (12, 26). Despite these challenges, burying beetles convert this protein-rich resource into a benign, nourishing nursery for their developing larvae. However, the microbial and biochemical basis of carrion utilization, especially in comparison with untended carcasses, remains poorly understood. Dermal bacterial communities of beetle-smeared

Significance

Ephemeral diets such as carrion are high-quality resources that are susceptible to microbial spoilage. Carrion-feeding insects that breed on decaying carcasses must overcome challenges arising from competing microbes. Here we report that a carrion-feeding burying beetle preserves carcasses by regulating its microbial growth, resulting in changes in its biochemical properties including the reduction of toxic polyamines associated with putrefaction and nutrient loss. The beetle's microbial symbionts form a biofilm-like matrix on carcasses, which is important for optimal larval development. The beetles and their microbiome thus coordinate a specialized adaptive strategy of carrion management, enabling them to preserve carrion quality and support larval growth in a challenging resource such as carrion.

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[1]To whom correspondence may be addressed. Email: sshukla@ice.mpg.de or hvogel@ice.mpg.de.

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carcasses differ from untended carcasses (22). However, early decomposition of carcasses is driven by endogenous microbiota (38), and the full extent of differences in the bacterial and fungal communities between decomposing and beetle-tended carcasses remains unexplored. It is also unknown if (and how) beetles "preserve" carrion to support larval development. The surface of beetle-prepared carcasses comprises a matrix (10), comparable to biofilms on decomposing cadavers (15). Using this matrix, this study compares the microbiome and the transcriptome of beetle-tended and untended carcasses. We also investigate whether preparation of carcasses by the beetles affects levels of toxic polyamines (putrescine and cadaverine), free amino acids, and digestive enzymes. We find that the beetles regulate the microbial community of carcasses by supporting the growth of mutualists that aid optimal larval development, constituting an adaptive carrion management strategy by the beetles.

Results

Characteristics of Tended and Untended Carcasses. To investigate the effect of the burying beetles' preparation of carcasses on their microbial and biochemical properties, we compared tended carcasses (those provided to a pair of breeding *N. vespilloides* beetles) with untended carcasses (that had no contact with the beetles). Tended carcasses housed final instar larvae (*SI Appendix*, Fig. S1) and showed no visual or olfactory signs of degradation. However, the untended carcasses were enveloped with a mold (identified as *Mucor*, *SI Appendix*), appeared relatively liquefied, and emitted a strong putrefactive odor (Fig. 1).

Burying Beetles and the Carcass Microbiome. Bacterial communities in tended and untended carcasses were characterized by high-throughput 16*S* rRNA amplicon sequencing. Utilization of carcasses by the beetles resulted in substantial changes in the phylum-level composition of tended and untended carcasses, especially with regard to Proteobacteria and Firmicutes (*SI Appendix*, Fig. S2). Principal coordinates analysis using three separate metrics (Jaccard's distance, Bray–Curtis dissimilarity, and unweighted UniFrac distances) showed that tended carcasses supported a distinct community compared with untended carcasses and the surrounding soil (Fig. 2*A*). Bacterial community composition of carcasses separated based on their association with beetles [analysis of similarities (ANOSIM) using unweighted UniFrac distances, $R = 0.58$, 999 permutations, $P = 0.002$]. There was no significant difference between alpha diversity measures of tended and untended carcasses: Shannon index ($P = 0.20$), phylogenetic diversity ($P = 0.20$), and evenness ($P = 0.20$) (*SI Appendix*, Table S1). Bacteria assigned to *Dysgonomonas* (Porphyromonadaceae), *Myroides* (Flavobacteriaceae), *Vitreoscilla* (Neisseriaceae), *Providencia* and *Serratia* (both Enterobacteriaceae), and unclassified Lactobacillales that have been previously described from the hindgut community of *N. vespilloides* beetles (9, 21) were found to be overrepresented in the community of tended carcasses but relatively depleted in that of untended carcasses (analysis of composition of microbes; *SI Appendix*, Table S2). Some of these genera were previously identified as part of a core microbiota transmitted by the beetles to the larvae through the carcass surface (10). In contrast, *Pseudomonas* (Pseudomonaceae), *Staphylococcus* (Staphylococcaceae), *Chitinophaga* (Chitinophagaceae), *Aerococcus* (Aerococcaceae), and unclassified Sphingobacteriaceae and Alcaligenaceae had higher abundances in the untended carcasses (Fig. 2*B* and *SI Appendix*, Table S2).

The fungal communities of carcasses were characterized using internal transcribed spacer 1 (ITS1) sequences. Principal coordinates analysis based on Jaccard's distance and Bray–Curtis dissimilarity showed separation between the fungal communities

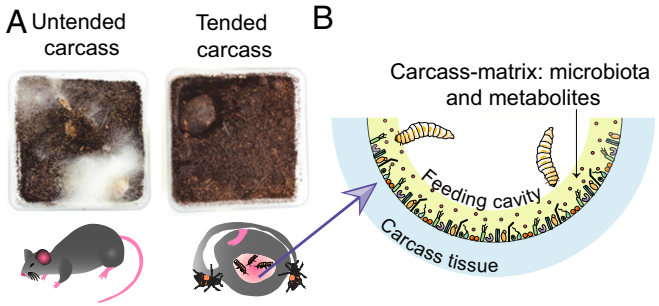

Fig. 1. Comparison of untended and *N. vespilloides*-tended carcasses. (*A*) Untended carcasses showed overt signs of carrion degradation, tissue liquefaction, and fungal (*Mucor*) overgrowth. Tended carcasses, however, did not show signs of carrion deterioration or fungal growth. (*B*) Burying beetles prepare carcasses by creating a feeding cavity (indicated by the arrow) that houses the larvae. We test the hypothesis that the microbiota present in the biofilm-like matrix acts as an interface between the carcass and the beetles and promotes carrion preservation and larval development.

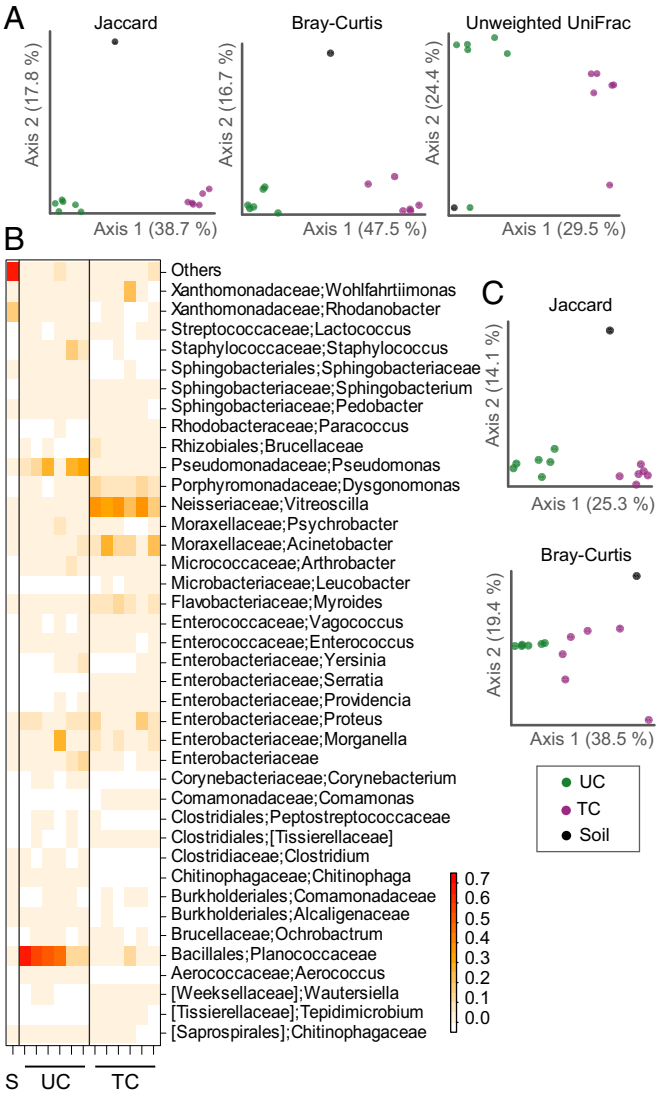

Fig. 2. Beetles alter the microbial communities of tended carcasses. (*A*) Principal coordinates analysis using three separate distances showed no overlap between the bacterial communities of untended carcasses, tended carcasses, and soil. (*B*) Relative proportion of bacterial taxa (summarized at the genus level) for untended carcasses (UC), tended carcasses (TC), and soil (S) plotted as a heatmap. (*C*) Fungal communities (characterized using ITS sequences) also separated in principal coordinate space, indicating that the beetles altered the fungal community of tended carcasses.

of tended and untended carcasses and soil (Fig. 2*C*). The fungal community composition clustered significantly based on the carcass category (ANOSIM using a Jaccard's distance matrix, R = 0.97, 999 permutations, $P = 0.004$), while alpha diversity (Shannon index) was marginally nonsignificant between tended and untended carcasses ($P = 0.054$, nonparametric Kruskal–Wallis test, *SI Appendix*, Table S1). Only one fungal taxon was differentially abundant between the communities of tended and untended carcasses [analysis of composition of microbiomes (ANCOM) procedure, *SI Appendix*, Table S3]: the genus *Yarrowia* had higher abundance in tended carcasses. Sequences assigned to *Yarrowia* were consistently present in all tended carcasses (*SI Appendix*, Fig. S3) and were absent or present at extremely low levels in untended carcasses and soil. The fungal community of untended carcasses was dominated by *Candida*, *Apiotrichum*, *Pseudogymnoascus*, and *Mortierella*, which were also detected in tended carcasses (*SI Appendix*, Fig. S3). Soil supported a unique fungal community that was dominated by *Candida*, *Peziza*, and *Meliniomyces*.

To further investigate differences between the fungal community of tended and untended carcasses, we cloned and sequenced a part of the fungal large subunit (LSU) of the rRNA gene. The fungal community of tended carcasses was dominated by a single yeast species assigned to the genus *Yarrowia*, which formed about 88% of the total fungal sequences ($n = 44$) and was consistently present in all tended samples (*SI Appendix*, Table S4). In contrast, the untended carcass community showed higher abundance of *Candida*, *Scheffersomyces*, *Trichosporon*, and *Apiotrichum*.

Burying Beetles and the Carcass Transcriptome. Tended carcasses supported a highly divergent eukaryotic (polyA$^+$-enriched mRNA) and prokaryotic (after removal of polyA$^+$ mRNAs) transcriptome compared with the untended carcasses (Fig. 3*A*). A majority (90.8%) of differentially expressed transcripts that were significantly up-regulated in tended carcasses were assigned to the yeast *Yarrowia* followed by meager representation of *Mucor*, *Trichosporon*, and *Candida* (Fig. 3*B*). The eukaryotic transcriptome of tended carcasses showed a high abundance of transcripts assigned to nematodes (possibly owing to their high relative biomass), likely originating from *N. vespilloides*-associated carrion-dwelling nematodes (39). In contrast, 90.4% of the differentially up-regulated transcripts in untended carcasses were assigned to the saprophytic mold *Mucor* (Fig. 3*B*), which was also identified as the white mold that covered untended carcasses (Fig. 1*A* and *SI Appendix*). The most abundant bacterial transcripts in the tended carcasses represented the genera *Dysgonomonas*, *Providencia*, *Clostridium*, *Prevotella*, *Morganella*, *Sphingobacterium*, *Bacteroides*, and *Myroides*. In contrast, the transcriptome of untended carcasses showed a high abundance of transcripts assigned to *Pseudomonas*, *Advenella*, *Clostridium*, *Enterobacter*, and *Acinetobacter*, which were relatively depleted in the tended carcasses (Fig. 3*B*).

Gene Ontology (GO) term enrichment analysis indicated that, for the eukaryotic dataset, transcripts assigned to fungal reproduction, biofilm formation, symbiotic interactions, cellular detoxification of nitrogenous compounds, and other cellular and metabolic processes were enriched in the transcriptome of tended carcasses (*SI Appendix*, Table S5). In contrast, the transcriptome of untended carcasses showed enrichment of GO terms associated with carbohydrate, amino acid and organic acid metabolism, xenobiotic compound metabolism, cellular response to stress/starvation, and response to nitrogen compounds (*SI Appendix*, Table S6).

Putrescine, Cadaverine, and Free Amino Acid Levels in Carcasses. To quantify the extent of protein degradation in carcasses, we compared polyamine levels between the carcass matrix of beetle-tended ($n = 13$) and untended carcasses ($n = 14$). Putrescine concentrations in the tended carcasses were significantly lower than in the untended carcasses (mean ± SD = 4.6 ± 6.8 μg/g carcass-matrix biomass) (Wilcoxon rank-sum test, $P = 1.1 \times 10^{-5}$) and were below the detection limit of our assay in all samples of tended carcasses (Fig. 4*A*). Cadaverine concentrations were ~10-fold lower in the tended carcasses (mean ± SD = 1.32 ± 1.8 μg/g carcass-matrix biomass) than in the untended carcasses (11.9 ± 7.3 μg/g carcass-matrix biomass) (Fig. 4*A*, Wilcoxon rank-sum test, $P = 4.6 \times 10^{-5}$). The cavities of tended carcasses had lower levels of free amino acids compared with untended carcasses. Relative abundances (peak area per gram carcass-matrix biomass) were lower in tended carcasses compared with untended carcasses (Wilcoxon rank-sum test, FDR-corrected P value) for valine ($P = 0.007$), leucine ($P = 0.007$), isoleucine ($P = 0.007$), tyrosine ($P = 0.007$), phenylalanine ($P = 0.013$), and tryptophan ($P = 0.007$) (Fig. 4*B*).

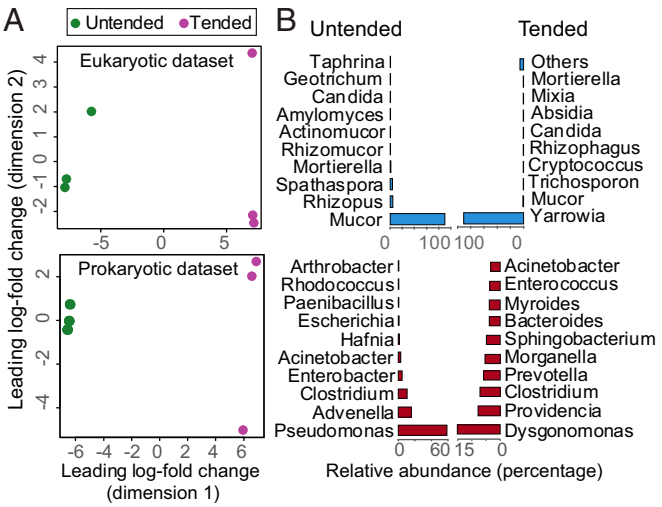

Fig. 3. Metatranscriptomic analysis of carcasses. (*A*) Tended carcasses supported a highly divergent eukaryotic and prokaryotic community compared with the decomposing, untended carcasses (multidimensional scaling based on trimmed mean of M-value–normalized transcript abundances). (*B*) Fungal (blue bars) and bacterial (red bars) community distribution based on top hits of differentially abundant RNA transcripts that are significantly overrepresented in untended and tended carcasses, representing percentage distribution for each genus. Transcripts assigned to *Yarrowia* were highly overrepresented in tended carcasses.

Extracellular Enzyme Levels in Carcasses. We measured proteases and lipases, two classes of enzyme that can influence microbial decomposition of carrion, in the cavities of tended and untended carcasses. Protease concentrations were estimated based on the proteolytic activity of trypsin as a reference (Pierce fluorescent protease assay kit; Thermo Scientific). The biomass in the cavities of untended carcasses had higher protease concentrations (mean ± SD = 85.7 ± 41.6 ng/g carcass-matrix biomass, $n = 6$) than tended carcasses (12.9 ± 6.0 ng/g carcass-matrix biomass, $n = 13$) (Wilcoxon rank-sum test, $P = 2.5 \times 10^{-5}$) (Fig. 4*C* and *SI Appendix*, Fig. S5). In contrast, lipase activity (measured using a Lipase Activity Assay kit III; Sigma) was significantly higher in tended carcasses (53.0 ± 63.7 milliunit/g carcass-matrix biomass, $n = 7$) than in untended carcasses (1.6 ± 4.2 milliunit/g carcass-matrix biomass, $n = 7$) (Wilcoxon rank-sum test, $P = 0.023$), indicating greater potential for lipid breakdown in tended carcasses (*SI Appendix*, Figs. S5 and S6).

The Role of the Carcass Matrix in Larval Growth. We hypothesized that the carcass matrix containing *Yarrowia* and beetle-introduced bacteria forms an interface between the larvae and the carrion that facilitates a more efficient utilization of carrion nutrients (Fig. 1*C*). We therefore compared larval development in matrix-removed carcasses ($n = 17$) and matrix-control carcasses ($n = 15$) containing 206 larvae each. Larvae attained significantly lower biomass per gram of (consumed) carcass in matrix-removed carcasses compared with the control broods (t test, $P = 0.02$; Fig. 5*A*). Larvae produced from matrix-removed carcasses also had lower average weights per brood (t test, $P = 0.03$; *SI Appendix*, Fig. S7) and lower maximum larval weight per brood (t test, $P = 0.03$; Fig. 5*B*) compared with the control carcasses. There was no difference

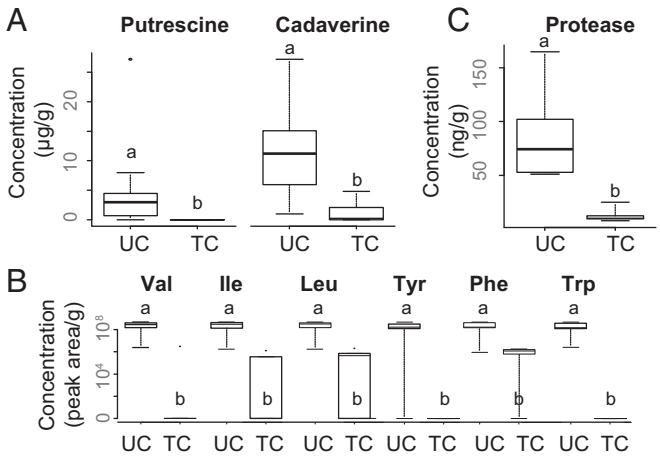

Fig. 4. Preservation of beetle-tended carcasses is associated with changes in their biochemical properties. (*A*) Tended carcasses had significantly lower concentrations of putrescine and cadaverine compared with untended carcasses. Concentrations are expressed per gram of carcass-matrix biomass. (*B*) Tended carcasses also showed reduced levels of free amino acids valine (val), isoleucine (ile), leucine (leu), tyrosine (tyr), phenylalanine (phe), and tryptophan (trp), indicating suppression of protein degradation in tended carcasses. For each amino acid, peak areas per gram of carcass matrix are plotted. (*C*) Protease concentrations (ng/g carcass matrix) were higher in untended carcasses, indicating higher proteolytic degradation in untended carcasses. TC, tended carcasses; UC, untended carcasses. Different letters indicate significant pairwise differences, $P < 0.05$.

in the number of larvae produced per brood between the two groups, compared either per gram of carcass consumed (*t* test, $P = 0.27$, Fig. 5*C*) or when compared directly (*t* test, $P = 0.56$, *SI Appendix*, Fig. S7). Thus, the carcass matrix promoted optimal larval development by enabling higher biomass conversion for the same amount of carcass tissue consumed.

Discussion

Beetle-tended carcasses showed no signs of carrion putrefaction and thus differed markedly from untended carcasses. The utilization of carcasses involved selectively supporting the growth of their gut microbiota while suppressing the endogenous carcass community that would otherwise proliferate on decomposing carcasses. The current characterization of the microbial communities based on 16*S* rRNA amplicon sequences and the metatranscriptome corroborated the earlier characterization of the *N. vespilloides* microbiome, but highlights differences in the microbial communities of tended carcasses, untended carcasses, and soil. Bacterial genera that had higher abundances in tended carcasses, *i.e.*, *Dysgonomonas*, *Vitreoscilla*, *Myroides*, *Providencia*, and *Serratia* have been previously described from the *N. vespilloides* hindgut community and the feeding cavity of tended carcasses (9, 10, 21). *Morganella*, *Providencia*, and *Myroides* had relatively higher transcript abundances in tended carcasses and were previously detected in the *N. vespilloides* larval microbiome (30) in breeding adults, as well as in early stages of carcass preparation (10, 22). Their occurrence in the microbiome of other necrophagous beetles and flies and their ability to produce antimicrobial compounds and to grow on lipid-rich and protein-rich substrates including meat products (9, 40–45) indicate a potential role for these bacteria in regulating carrion quality and its microbial community. Furthermore, major decomposers of naturally decaying mouse cadavers such as *Pseudomonas*, *Chitinophaga*, *Staphylococcus*, and *Aerococcus* (22, 38, 46, 47) proliferated on untended carcasses and were suppressed in tended carcasses. This replacement of the cadaver's incumbent microbiome with the beetles' gut microbiota was likely facilitated through the beetle's antimicrobial secretions (10, 21, 24, 28, 31, 33, 34, 36). However, antibiotic-producing bacteria such as *Myroides* and *Serratia* (40, 48) in tended carcasses could also suppress microbial decomposers. In naturally decomposing carcasses, early decomposition is mediated by the cadaver's native gut community, followed by advanced decomposition by dermal and soil-dwelling microbes (38, 49). This could explain the dissimilarity in the bacterial diversity of untended carcasses and soil.

There was selective inhibition of one fungus (*Mucor*) and the simultaneous growth of another fungus (*Yarrowia*) in tended carcasses, demonstrating a highly specialized antimicrobial strategy of carrion management mediated by the beetles and/or their microbiota. *Yarrowia* (family Dipodascaceae), an aerobic and nonpathogenic yeast that degrades various lipids and proteins (50), was consistently present in all tended carcasses and is known to be transmitted from parents to offspring through the anal secretions (21). Once introduced, *Yarrowia* colonized and almost monopolized fungal metabolism in beetle-tended carcasses. In contrast, *Mucor*, a predominantly saprophytic fungus that produces proteolytic enzymes and is a known agent of meat spoilage (51) was suppressed in tended carcasses. *Yarrowia* appeared underrepresented in tended carcasses using ITS amplicon sequencing, possibly due to biases of the ITS primers arising from mismatches in the primer-binding region (52). However, cloning of the partial fungal LSU rRNA sequences showed that *Yarrowia* was dominant in tended carcasses, constituting about 88% of the total fungal sequences, corroborating the transcript abundances in this study and earlier studies that used transcriptomic and quantitative PCR-based approaches (10, 21). Based on its consistently high prevalence in beetle life stages and in the carcass microbiome and transcriptome, as noted here and in previous studies (9, 10, 21), we infer that *Yarrowia* plays an important role in maintaining the quality of tended carcasses.

The reduction of putrescine and cadaverine have important implications for burying beetle ecology. First, the volatile signals can reveal the carcass' location to competitors and predators (53), and reduced levels can decrease the probability of carcass takeover by intruders (54). Second, the polyamines are toxic at higher concentrations (55, 56), and reduced levels can improve larval survival. Putrescine and cadaverine are generated through the decarboxylation of arginine and lysine, primarily by bacteria belonging to the genera *Pseudomonas*, *Enterobacter*, *Enterococcus*, and *Bacillus* (57). Transcripts from these genera were abundant in the untended carcasses and depleted in tended carcasses (especially *Pseudomonas* transcripts), indicating that beetles suppressed putrescine-producing organisms.

Extracellular proteases secreted by microbial decomposers break down proteins into peptones, polypeptides, and free amino acids (58). Although proteases are digestive enzymes that can aid larval nutrition, excess amino acid content in the diets can lead to nitrogen toxicity caused by accumulation of urea, uric acid, and ammonia

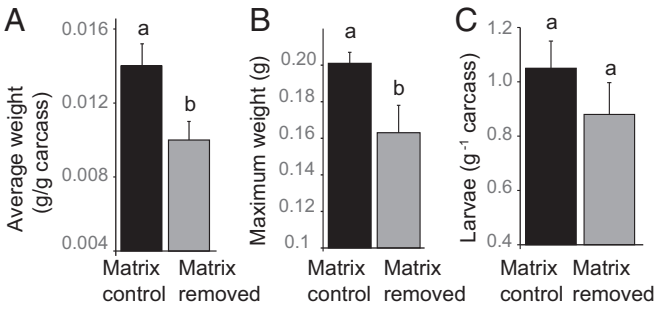

Fig. 5. Effect of the carcass matrix on larval development. (*A*) Matrix-removed carcasses produced significantly smaller larvae for every gram of carcass tissue consumed. Average larval weights per gram of carcass were compared between matrix-control ($n = 15$) and matrix-removed ($n = 17$) carcasses. (*B*) The maximum larval weight per brood was lower in matrix-removed carcasses compared with the matrix-control carcasses. (*C*) There was no difference in the average number of larvae per brood per gram of carcass tissue consumed between the two groups. Bar plots show average with SE values. Different letters indicate significant differences using a *t* test; alpha = 0.05.

(59). We hypothesize that reduced protease levels in tended carcass could facilitate gradual breakdown of proteins, resulting in manageable physiological concentrations of nitrogenous metabolites in the larvae, and further ensure that carcass nutrients last the entire duration of larval development. It is not clear, however, why lipase activity was higher in tended carcasses. Unlike proteins, the breakdown products of triglycerides are relatively less toxic, producing aldehyde and ketones under oxidative conditions (58). Possible causes include the growth of profuse lipase secretors such as *Yarrowia* spp. (50) or the beetles' smearing of lipase-containing oral secretions (29) on the carcass. In addition, fatty acids produced by lipid digestion also exhibit bactericidal properties (60), and thus greater lipase activity might function not only in the digestion of triglycerides but also in providing antimicrobial effects in tended carcasses. Further studies are needed to test the effect of proteases and lipases on beetle fitness. It should also be noted that enzyme levels were measured only from the cavity of tended carcasses, where larvae feed, and thus would not necessarily be representative of the entire carcass.

Removal of the biofilm-like carcass matrix from tended carcasses resulted in lower biomass accumulation in larvae. Without the matrix, the carcass itself was insufficient to support optimal larval development. Since the matrix affected larval biomass conversion but not survival, it likely provided additional nutritional benefits to the larvae through a mixture of mutualistic microbiota, digestive enzymes, and predigested/detoxified nutrients. Matrix-deprived smaller larvae are expected to emerge as smaller adults, which are disadvantaged in overwintering, claiming and defending carcasses, and burying carcasses compared with larger conspecifics (27, 61–64). It is not clear whether the matrix contains beetle-originating enzymes that could partly contribute to this effect. However, any parental transmission of nutrients or enzymes through regurgitations and larval begging (65, 66) is expected to be similar between the two groups. Thus, these data support a microbe-mediated role of the matrix in promoting larval growth. Furthermore, prolonged contact between the microbiota and the carrion surface was required for optimal larval development. If the carrion served as a one-time source of symbionts, then a single event of symbiont acquisition through carcass consumption should have been sufficient for the larvae. Instead, daily removal of the carcass-matrix likely disrupted microbial colonization of the carrion and subsequent metabolic processes that aided larval development. Thus, the carrion potentially acts as an extraintestinal site for nutrient processing mediated through microbial symbionts (such as *Yarrowia* spp.).

Taken together, these data highlight that carrion functions more than a source of nutrients for the larvae: it also serves to transmit and selectively cultivate symbionts that promote larval development. The cultivation of symbionts on external diets is also seen in other insects (67–70). However, what makes the burying beetle system different is the growth of symbionts on a protein-rich substrate that is susceptible to decomposition, rather than a nutritionally imbalanced or recalcitrant diet. The regulation of the carrion microbial community demonstrates how insects can modify their habitats to enhance their fitness and highlights the role of the microbiome in modifying a resource that can facilitate the initiation of symbiosis (71) and enable insects to colonize novel niches.

Methods

Experimental Setup. The burying beetle *N. vespilloides* was reared in laboratory incubators (20 °C, 80% relative humidity, 16-h photoperiod). Mouse cadavers were assigned to either "untended carcasses" that had no contact with the beetles or "tended carcasses" that were provided to a pair of sexually mature beetles. We experimentally buried and cut open untended carcasses to match tended carcasses (*SI Appendix*, Fig. S1). The cavities of tended and untended carcasses were sampled using sterile, DNA-free swabs (Sarstedt) 9 d after the start of the experiment when tended carcasses housed final instar larvae. This made the comparisons ecologically relevant, as tended carcasses were exposed to the full duration of environmental and microbial stress that is expected under natural conditions.

Characterization of the Carcass Microbiome and Metatranscriptome. Genomic DNA was extracted from swabs sampled from tended and untended carcasses using the PowerSoil DNA isolation kit (MoBio) (10). The carcass microbiomes were characterized using the bacterial primers (modified) 515f and 806R, which target the V4 region of the 16*S* rRNA gene, and the fungal primers ITS1f and ITS2, which target ITS1 (the first internal transcribed spacer) in the fungal rRNA operon. Untended carcasses ($n = 6$), tended carcasses ($n = 6$), and soil ($n = 1$) samples were sequenced using paired-end Illumina MiSeq technology (Illumina) at Molecular Research LP. The 16*S* rRNA and ITS amplicon sequence data were analyzed using DADA2 (72) in QIIME2 (73) (*SI Appendix*). We used ANCOM to identify differentially abundant bacterial genera between tended and untended carcasses. Additionally, partial sequences of the LSU of the fungal rRNA gene were amplified (74, 75), cloned, and sequenced to characterize the fungal community of tended and untended carcasses. For characterizing the carcass metatranscriptome, RNA was extracted from a separate set of untended and tended carcasses (three replicates each) using TRIsure (Bioline) (details in *SI Appendix, SI Methods*). Total RNA was depleted for rRNAs and separated into poly(A)$^+$ (eukaryotic) and poly(A)$^-$ (prokaryotic) mRNA fractions. Samples were sequenced at the Max Planck Genome Center (Cologne, Germany) using an Illumina HiSeq2500 Genome Analyzer. Digital gene expression analysis was carried out using CLC Genomics Workbench v9.1 and BLAST2GO v4.1 (https://www.blast2go.com/) was used for gene annotations and to identify the overrepresentation of GO terms among lists of differentially expressed genes (10-fold change or higher) between the tended and untended carcass samples.

Polyamine and Amino Acid Quantification. Filtered carcass swab suspensions were derivatized with ortho-phthaldialdehyde/ethanethiol/fluorenylmethoxycarbonyl chloride and analyzed using an Agilent 1100 series HPLC (Agilent Technologies). Calibration curves were constructed using commercial standards of putrescine dihydrochloride and cadaverine dihydrochloride (Sigma-Aldrich). Amino acid levels were quantified by liquid chromatography–mass spectrometry using a Bruker Esquire 6000 ion trap mass spectrometer (Bruker Daltonics) (*SI Appendix, SI Methods*).

Enzyme Assays. Total lipase activity was estimated using the fluorometric Lipase Activity Assay Kit III (Sigma-Aldrich). Total protease concentrations were estimated using the Pierce Fluorescent Protease Assay Kit (Thermo Fisher Scientific). In each case, fluorescence was measured using a Tecan Infinite200 plate spectrophotometer. Lipase and protease activity were also tested visually by agar diffusion assays (*SI Appendix, SI Methods*).

Effect of Carcass Matrix on Larval Growth. Tended carcasses were divided into two groups. In the matrix-removed group, the carcass matrix lining the feeding cavity was removed daily using sterile swabs. In the matrix-control group, the carcass matrix was not removed. All of the larvae and adults were temporarily removed from the carcass during matrix removal. To maintain consistency, they were also removed for the same duration in the matrix-control group. Larvae were counted and weighed on the day that they migrated from the carcass for pupation. Carcasses were weighed before and after the experiment to estimate the amount of carcass tissue consumed by the brood.

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