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Are you what you eat? A highly transient and prey-influenced gut microbiome in the grey house spider *Badumna longinqua*

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Abstract

Stable core microbial communities have been described in numerous animal species and are commonly associated with fitness benefits for their hosts. Recent research, however, highlights examples of species whose microbiota are transient and environmentally derived. Here, we test the effect of diet on gut microbial community assembly in the spider *Badumna longinqua*. Using 16S rRNA gene amplicon sequencing combined with quantitative PCR, we analyzed diversity and abundance of the spider's gut microbes, and simultaneously characterized its prey communities using nuclear rRNA markers. We found a clear correlation between community similarity of the spider's insect prey and gut microbial DNA, suggesting that microbiome assembly is primarily diet-driven. This assumption is supported by a feeding experiment, in which two types of prey—crickets and fruit flies—both substantially altered microbial diversity and community similarity between spiders, but did so in different ways. After cricket consumption, numerous cricket-derived microbes appeared in the spider's gut, resulting in a rapid homogenization of microbial communities among spiders. In contrast, few prey-associated bacteria were detected after consumption of fruit flies; instead, the microbial community was remodelled by environmentally sourced microbes, or abundance shifts of rare taxa in the spider's gut. The reshaping of the microbiota by both prey taxa mimicked a stable core microbiome in the spiders for several weeks post feeding. Our results suggest that the spider's gut microbiome undergoes pronounced temporal fluctuations, that its assembly is dictated by the consumed prey, and that different prey taxa may remodel the microbiota in drastically different ways.

KEYWORDS

diet analysis, gut microbiome, holobiont, predator–prey interactions, spider

1 | INTRODUCTION

A wealth of studies highlights the intimate association of animals and microbes (Franche, Lindström, & Elmerich, 2009; de O Gaio et al., 2011; Oliver, Moran, & Hunter, 2005; Ricci et al., 2012; Sabree, Kambhampati, & Moran, 2009). A stable and conserved set of core microbes is often presented as a common feature of such associations (Hosokawa, Kikuchi, Nikoh, Shimada, & Fukatsu, 2006; Shade & Handelsman, 2012),

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and is commonly linked with fitness benefits for the host (Hurst, 2017; Margulis & Fester, 1991; Zilber-Rosenberg & Rosenberg, 2008). One particularly well-studied and often highly diverse microbial community, which is frequently attributed with great functional importance, is the gut microbiome (Huttenhower et al., 2012; Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012; Ursell et al., 2012). Aside from their obvious contribution to digestion and detoxification of food (Boone et al., 2013; Brune & Ohkuma, 2010), gut microbes can supply essential nutrients (LeBlanc et al., 2013), mediate defense against pathogens (Kwong & Moran, 2016) and even influence their host's behaviour (Sharon et al., 2010). A disturbance of the gut microbial community is commonly associated with disease phenotypes (Ley, Turnbaugh, Klein, & Gordon, 2006; Slingerland, Schwabkey, Wiesnoski, & Jenq, 2017). The gut microbiome of some taxa has been studied in great detail (Spor, Koren, & Ley, 2011). But, with some notable exceptions (Bourguignon et al., 2018; Raymann & Moran, 2018), surprisingly little is known about the gut microbiota of arthropods (Engel & Moran, 2013), the most diverse group in the animal kingdom (Giribet & Edgecombe, 2012). Current work on the arthropod gut microbiome yields contradictory results, finding host-microbial interactions of widely varying levels of intimacy in different taxa. Some studies suggest a great functional importance of gut microbes for their arthropod hosts, leading to a stable and predictable microbial community (Chu, Spencer, Curzi, Zavala, & Seufferheld, 2013; Engel & Moran, 2013; Reese & Dunn, 2018; Ruokolainen, Ikonen, Makkonen, & Hanski, 2016; Russell et al., 2009; Sanders et al., 2014), whereas other work highlights the instability and transient nature of arthropod gut ecosystems (Engel & Moran, 2013; Ross et al., 2018). The often simple structure of arthropod guts and associated short transit times of ingested material can make them a highly unpredictable environment for microbial colonizers (Paniagua Voirol, Frago, Kaltenpoth, Hilker, & Fatouros, 2018). Some arthropods can even expel their gut microbiota during molting (Moll, Romoser, Modrakowski, Moncayo, & Lerdthusnee, 2001). Environmental factors, diet in particular, can play an important role in arthropod gut microbiome assembly (Chandler, Morgan Lang, Bhatnagar, Eisen, & Kopp, 2011; Wong, Chaston, & Douglas, 2013). Moreover, microbes do not necessarily confer fitness advantages to their host (Hu et al., 2017; Russell, Sanders, & Moreau, 2017), with a notable example recently described in caterpillars (Lepidoptera) whose gut microbiota is largely derived from the surface of food plants and without apparent benefit for the host (Hammer, Janzen, Hallwachs, Jaffe, & Fierer, 2017).

Predatory arthropods tend to have a relatively simple gut, potentially associated with a transient gut microbiota, a prediction borne out by several recent studies (Schmid, Lehman, Brözel, & Lundgren, 2015; Tiede, Scherber, Mutschler, McMahon, & Gratton, 2017). Diet diversity in particular seems to influence microbiome diversity in predatory arthropods (Mrázek, Štrosová, Fliegerová, Kott, & Kopečný, 2008; Tiede et al., 2017). Some prey-derived microbes may even temporarily grow in the predator's gut, thus directly contributing to its microbiome composition (Paula et al., 2015). However, other studies on predatory arthropods suggest a more stable and predictable microbiome composition, largely decoupled from the influence of diet (Anderson et al., 2012; Bili et al., 2016; Funaro et al., 2011). Thus, three alternative hypotheses emerge for the effect of diet on microbial community assembly in the guts of predatory arthropods. Microbial communities could comprise: (a) A resident core microbiome which is resilient against dietary influence on the community (Bili et al., 2016) and remains largely unchanged after feeding. (b) A prey-associated microbiota that is temporarily integrated into the predator's gut (Paula et al., 2015). (c) An environmentally derived microbiota that becomes integrated into the predator's gut due to changing physicochemical conditions caused by the ingestion of prey (Ren, Kahrl, Wu, & Cox, 2016).

To test these three alternative hypotheses, we use the spider *Badumna longinqua* (Araneae, Desidae) as a model. Originating from Australia, this species is a very successful invader of natural and urban habitats in the western United States (Simó et al., 2011). *B. longinqua* is a generalist predator, feeding on various arthropod taxa across a wide range of orders. Their protein-rich diet is digested extra-intestinally and easily metabolized in the gut and may thus not require the presence of specialized microbiota. Also, spiders often feed irregularly, leading to pronounced environmental fluctuations in their gut lumen, which could encumber the establishment of a stable microbiome (Vanthournout & Hendrickx, 2015). However, recent work suggests the presence of typical insect gut microbial taxa in the digestive tract of spiders (Zhang, Yun, Hu, & Peng, 2018), possibly resulting from a temporary recruitment of prey-derived taxa. Considering this background, we expect that the spiders lack a stable core microbiome and instead possess a highly transient microbiome, whose assembly is dictated by dietary influences. If this is true, then we predict the following: (a) As an integration of microbial species associated with different prey taxa, the spider's gut microbiome should be relatively diverse. (b) Due to different microbes entering the guts of different spiders, we expect a high beta diversity of the microbial communities between individual spiders. (c) Spiders that have consumed a similar diet should show a similar gut microbiome.

We performed two experiments to test these predictions. First, we identified the gut microbial communities in wild-caught *B. longinqua*. Using amplicon sequencing of the 16S rRNA gene (hereafter "16S"), we characterized microbial community composition and diversity. In parallel, we evaluated the arthropod diet composition of wild-caught spiders by sequencing arthropod-specific rRNA markers, and tested for similarities of microbial and prey communities. In a second experiment, we provided *B. longinqua* specimens in the laboratory with different prey items and directly estimated the effect of prey on the dynamics and diversity of the spider's gut microbiota using 16S amplicon sequencing. To detect microbial growth in the spider guts, qPCR was used to estimate the microbial load of spiders at different times after feeding.

2 | MATERIALS AND METHODS

2.1 | Dietary and microbial analysis of spiders in nature

A total of 30 *B. longinqua* specimens were collected from a residential neighborhood in North Berkeley, California, USA, in 2017.

These spiders were used both to assess the natural diets and microbial communities of *B. longinqua*, and to serve as a control for the feeding experiments. Specimens were transferred to the laboratory and kept at room temperature in individual 25 ml snap cap vials. The spiders were starved for 60 hr before being sacrificed by freezing in a −80°C freezer, then stored at −80°C until DNA was extracted. The specimens were then washed with water and ethanol to remove surface contaminants, and the opisthosoma (abdomen) was separated from the body with a sterile scalpel blade and finely ground in a 1.5 ml tube with a sterile pestle. While this wash will not entirely remove all surface contamination, this method should yield good representation of the gut microbiota (Hammer, Dickerson, & Fierer, 2015). All DNA was extracted using a single Qiagen Puregene Kit according to the manufacturer's protocol (Qiagen).

We used whole opisthosomas for DNA extraction. Although the opisthosoma contains several organs, including the heart, lungs, silk glands and ovaries, the majority of its volume is occupied by the midgut. Due to its extensive bifurcations, the midgut is not easily dissected out from surrounding tissues. Other organs in the opisthosoma can also contain microbial communities: spiders' ovaries in particular are often occupied by endosymbionts such as *Wolbachia* (Duron, Bouchon, et al., 2008; Duron, Hurst, Hornett, Josling, & Engelstädter, 2008), which can pose a significant challenge to microbial community analysis because they often dominate the community and outcompete other taxa during sequencing (Vanthournout & Hendrickx, 2015; Zhang, Zhang, Yun, & Peng, 2017). However, our preliminary work on *B. longinqua* suggests that this species does not harbour abundant endosymbionts, making it well-suited to explore the gut microbiome. Also, recent work on an Araneid spider (Sheffer et al., 2019) shows that a large proportion of microbial diversity in a spider's opisthosoma is found in the gut lumen, while other organs in the opisthosoma mostly harbour a single dominant microbial species. Thus, the gut microbiome is expected to dominate the opisthosomal DNA extraction.

DNA extractions were quantified using a Qubit spectrophotometer (Fisher Scientific) and diluted to concentrations of 50 ng/µl. We then amplified the variable regions V1–V2 of the microbial 16S rRNA gene using the primer pair MS-27F/MS-338R (Donia et al., 2011) in 10 µl reactions and with 30 cycles using the Qiagen Multiplex PCR kit. PCR was run on all spider samples at the same time to prevent batch effects.

In addition, we ran a multiplex PCR of two amplicons of 18S rRNA (hereafter "18S") and one of 28S rRNA (hereafter "28S") to identify the spiders' prey species (Table S1; Krehenwinkel et al., 2019). These primers contain a 3′-mismatch with spiders, leading to suppression of spiders from amplification, while at the same time amplifying other arthropods very reliably. Although these primers are not well suited to detect spider-on-spider predation, they are still expected to recover reasonably accurate prey communities because *Badumna* feeds primarily on insects rather than spiders (Laing, 1988). The target amplicons are short fragments of <300 bp, which accounts for the degraded condition of prey DNA in the gut. Thus, our primers enable the detection of minute amounts of degraded prey DNA from extractions of whole spiders.

PCR primers for both microbial and dietary analysis contained 5′-tails on which a second round dual indexing PCR of five cycles was performed. Truseq indexing primers were used as described in Lange et al. (2014). The indexed libraries were visualized on a 1.5% agarose gel and then pooled in approximately equal amounts, based on band intensity on the gel. The pooled library was cleaned of residual primers using 1X AMPure Beads XP (Beckman Coulter) and sequenced on an Illumina MiSeq using V3 chemistry with 600 cycles (Illumina). Template-free negative control PCRs were run alongside all experiments and sequenced to allow for the identification and removal of contaminant sequences. In addition to the template-free PCRs, we added a blank DNA extraction control PCR in the microbial analysis to identify potential contaminants in the DNA extraction reagents.

2.2 | Feeding experiments: assessing effects of cricket and fly prey on spiders' microbiomes

In parallel to the control samples, an additional 119 *B. longinqua* specimens were collected in the same residential area and maintained under the same conditions as the previous unfed sample, i.e., kept in individual snap cap vials and starved for 60 hr, until the start of the feeding experiment. We designated two groups, which were fed on either two small tropical house crickets (*Gryllodes sigillatus*) or 10 fruit flies (*Drosophila hydei*). Both crickets and fruit flies are well within the potential natural prey range of *B. longinqua*. The crickets were purchased at a local pet store, while the flies originated from a breeding colony in our own laboratory. All prey remains were removed immediately after feeding. The spiders were then kept at room temperature, individually housed in snap cap vials under identical conditions, for different periods of time—8, 24, 72, 216 and 648 hr—before being sacrificed by freezing at −80°C, and thereafter stored at −80°C until DNA extraction (see Table S2 for sample sizes).

DNA extraction and PCR amplification of the gut microbial community were completed as described above, in the same batch as the unfed spiders. Besides microbial 16S, we also amplified a mitochondrial COI amplicon using the highly degenerate primer combination mlCOIintF/Fol-degen-rev (Leray et al., 2013; Yu et al., 2012). The COI amplicon had a very similar length to the target microbial 16S amplicon and served to trace the degradation of prey DNA over time. Assuming that microbial DNA is simply transferred to the spider from dead and digested prey-associated microbes, the temporal abundance of microbial DNA in the spider should correlate with the abundance of prey DNA. To identify both the microbial communities and the COI sequences of the prey, we also amplified microbial 16S and COI for several pools of the prey items (*Gryllodes* and *Drosophila*) we used. Four pools per prey species, each containing five individuals, were prepared following the same protocols and in the same batches as described for the spiders above. The pooled 16S and COI libraries were then sequenced as described above.

2.3 | Sequence analysis

Demultiplexed reads were merged using PEAR (version 0.9.8; Zhang, Kobert, Flouri, & Stamatakis, 2013) with a minimum quality of 20 and a minimum overlap of 50 bp. Merged reads were then filtered for sequences with >90% of the sequence >Q30 and transformed into Fasta files using the FastX toolkit (version 0.0.13; Gordon & Hannon, 2010). PCR primers were trimmed off using awk. USEARCH v9 (Edgar, 2010, 2013) was used to dereplicate all sequences and then to generate OTU clusters at a similarity threshold of 3% (hereafter: OTUs). Chimeras were removed by the de novo algorithm as implemented in the cluster_otus command in USEARCH. We additionally used the unoise3 command in USEARCH to generate zero radius OTUs (hereafter: zOTUs) for the microbial 16S data, to determine whether microbial sequences observed in spider guts after feeding were exactly identical to those in the respective prey item. Taxonomy for microbial 16S OTUs was assigned using the Sintax command in USEARCH (Edgar, 2018) based on the RDP database (version 11; Cole et al., 2014). Taxonomy for COI, 18S and 28S OTUs was assigned by BLASTn against the whole NCBI database (accessed 02/2018). We used the top 10 BLAST hits for each sequence and assigned taxonomy to the lowest possible level. All nonarthropod sequences were removed and classification at least to the order level was assigned to each OTU, using a minimum similarity of 90%. BLAST results were visually inspected for consistency in taxonomic assignment. As the RDP database does not allow species-level classification for the microbial OTUs, we also used BLASTn to screen microbial 16S OTUs against Genbank to resolve the taxonomy of some unidentified taxa. BLAST was run as described above and taxonomy only assigned if the sequence matched at >97% similarity. OTU tables were then built by mapping all reads back to the OTUs in USEARCH.

Negative controls were included in the 16S OTU and zOTU table construction and all probable contaminant sequences removed from the tables before further processing. The two most predominant contaminants were an unidentified *Brevibacterium* and chloroplast DNA. Despite showing some contaminant sequences, the spider samples' taxonomic composition was clearly distinct from the negative controls (Figure S1). Also, the removal of potential contaminant OTUs did not strongly affect community structure, suggesting a unique microbial community in the samples (Figure S1). The negative controls for the nuclear rRNA did not yield detectable amplification.

For COI, we identified the proportion of recovered prey DNA (e.g., *Gryllodes* or *Drosophila*) in relation to that of the spider across the different time treatments. For nuclear rRNA, we rarefied each OTU table to an equal coverage using GUniFrac (version 1.1; Chen, 2012) and then identified the recovered OTU richness for each marker and specimen as a measure of prey taxonomic diversity. Jaccard and Bray-Curtis dissimilarity were calculated in ECODIST (version 2.0.1; Goslee & Urban, 2007) in R (R Core Development Team, 2016) between spiders as a measure of prey community differentiation.

The microbial 16S OTU tables were rarefied to an equal coverage of 9,000 reads using GUniFrac. This coverage was chosen based on rarefaction analysis, in order to saturate taxon recovery. We then calculated microbial OTU richness and Shannon *H* for each spider specimen as measures of alpha diversity using VEGAN (version 2.5.2; Oksanen et al., 2013) in R. As a measure of community gut microbial differentiation, we calculated Bray-Curtis and Jaccard dissimilarity between different spider specimens and between spider-associated and prey-associated microbiota using ecodist. Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarity was used to visualize community similarity between spiders and the two prey types using vegan (k = 3, trymax = 1,000). To test for a possible association of the spider's diet and gut microbial community composition, we performed Mantel tests (Pearson correlation with 9,999 permutations) comparing microbial and prey community beta diversity for all unfed spiders using vegan.

2.4 | Determination of relative microbial load using qPCR

We used quantitative PCR to estimate the microbial load of each *B. longinqua* specimen used in the previous experiments. Triplicate qPCRs for each sample were run on an ABI 7500 Fast RT PCR System (Applied Biosystems) in 20 µl volumes with 100 ng template DNA and using the Promega GoTaq qPCR Mastermix (Promega) with 45 cycles according to the manufacturer's protocol. Microbial 16S was amplified with the same primer pair we used for the previous community analysis, omitting the Illumina-specific 5′-tails. We used the 18S rRNA gene of the spider host as an internal reference for relative quantification. The V1 region of 18S was amplified using the primer pair SSU_FO4/SSU_R22 (Fonseca et al., 2010), which targets an amplicon of similar length to the 16S primers. PCR efficiency was determined using a 6-fold dilution series using average C_t values of triplicate reactions. Efficiency was determined as 0.90 for the 16S and 0.98 for the 18S amplicon. Using the ΔC_T method (Livak & Schmittgen, 2001), we estimated the relative fold change of copy number between microbial 16S and nuclear 18S for each experimental treatment. If prey-derived microbes survive and grow in the spider, then the microbial load should increase and remain at elevated levels for extended times post feeding. In contrast, microbes that die in the spider may at best cause a short spike of microbial DNA copy number, which then should rapidly decline due to DNA degradation.

3 | RESULTS

3.1 | Prey and gut microbial communities in wild-caught *Badumna longinqua*

We generated prey community data for the wild-caught *B. longinqua* specimens based on three nuclear rRNA amplicons (18Slong, 18Sshort, 28S). After removing residual *B. longinqua* sequences, we

recovered on average 2,146 ± 2,499 prey sequences for the 28S amplicon, 2,319 ± 2,991 for the long and 7,030 ± 5,264 for the short 18S amplicon. The three markers were well correlated in their beta diversity (Bray-Curtis dissimilarity) of prey communities between individual spiders (Mantel test, Pearson correlation, R^2 = .77–.95, p < .001); thus, the prey spectra recovered by the three markers should be comparable. On average, we found 2.36 ± 0.95 prey OTUs for the long 18S, 3.07 ± 1.65 for the short 18S and 2.46 ± 1.17 for the 28S amplicon per spider specimen. *B. longinqua* consumes a diverse diet, consisting of members of 10 different arthropod orders (Figure 1a). The average Bray-Curtis (BC) dissimilarity between the prey communities of different spider specimens was very high ($BC_{18Sshort}$ = 0.86 ± 0.31; $BC_{18Slong}$ = 0.82 ± 0.32; BC_{28S} = 0.84 ± 0.32), suggesting little overlap of the consumed prey taxa between individual spiders.

We recovered on average 40,985 ± 22,997 16S sequences for bacterial community profiling of the *B. longinqua* specimens. After removal of contaminant sequences and rarefication to 9,000 sequences, we recovered a total of 1,770 OTUs and 3,690 zOTUs in our spider data set. Unfed spiders showed an average microbial OTU richness of 55.67 ± 21.92 and Shannon index (*H*) of 2.43 ± 0.83 (Table S2). Similar to the prey communities, the microbial communities between different wild-caught *B. longinqua* specimens varied widely (Bray-Curtis dissimilarity = 0.86 ± 0.12; Figure S2). The high microbial community dissimilarity between unfed spiders was also evidenced by a low number of identified core microbes (Table S2). Only nine microbial OTUs (1.48% of 676 observed OTUs in the unfed group) were present in more than 50% of unfed specimens. The microbial community dissimilarity between different wild-caught spiders was well correlated to the respective prey community dissimilarity. This finding was supported by all three markers we used to score the prey community (Mantel test, $r_{18Sshort}$ = .42, p = .001; $r_{18Slong}$ = .41, p = .002; r_{28S} = .38, p = .002). Thus, spider specimens with a more similar prey community also had a more similar microbiota. This association is visible in Figure 1b. Some coevolved microbial taxa could be directly associated with their respective host in the spider's prey, e.g., *Arsenophonus*, *Hamiltonella*, *Buchnera* and *Sulcia*, which were each found in 1–2 spiders, which fed on aphids, wasps and spittlebugs. Besides *Spiroplasma*, which were present in several wild-caught spiders (28 specimens, 1,049 reads on average), we found very few sequences of potential reproductive parasites such as *Wolbachia* (four specimens, 186 reads on average), *Rickettsia* (two specimens, two reads on average) or *Cardinium* (0 specimens) in the wild-caught spiders.

3.2 | Feeding experiments: the effect of prey species on gut microbial diversity and microbial load

Our feeding experiment showed pronounced effects of diet on the diversity of microbial DNA in *B. longinqua*, with the two different prey taxa influencing the microbiota quite differently (Figure 2; Table S2). A diet of *Gryllodes* resulted in an immediate and significant increase of microbial species richness, detectable 8 hr after feeding (pairwise Wilcoxon test, FDR corrected p < .05; Figure 2a). The microbial species richness continued increasing at the same rate until 72 hr after feeding, started to slowly level off at 216 hr, and finally decreased to prefeeding conditions at 648 hr. A similar and significant trajectory was found for the community's Shannon *H* (Figure S3a), which showed a pronounced increase until 72 hr post feeding (pairwise Wilcoxon test, FDR corrected p < .05).

A delayed response in microbial richness was observed for *Drosophila*-fed spiders. Richness remained constant until 24 hr post feeding (Figure 2b), then significantly increased at 72 and 216 hr, levelling back off to prefeeding values after 648 hr (pairwise Wilcoxon test, FDR corrected p < .05). In contrast, evenness (Figure S3b) did not significantly change post feeding (pairwise Wilcoxon test, FDR corrected p > .05).

The two studied prey taxa showed high microbial community diversities, with *Gryllodes* having a considerably higher microbial richness (271.50 ± 48.68) and evenness (4.35 ± 0.24) than *Drosophila* (70.50 ± 1.73; 2.61 ± 0.03).

We used quantitative PCR to measure the microbial load of *B. longinqua* specimens and prey insects relative to the internal reference gene 18S rRNA. Concurrently with the microbial diversity, we found a significant increase of the microbial load for *B. longinqua* fed with *Gryllodes* (pairwise Wilcoxon test, FDR corrected p < .05; Figure 2c; Table S2). Eight hours after feeding, the microbial load had increased significantly compared to unfed specimens, and remained stable until 72 hr post feeding. The microbial load then increased additionally at 216 hr, while at 648 hr, it had moved back to prefeeding levels. A less clear effect was found for *Drosophila*-fed spiders (Figure 2d; Table S2): no significant change in the average microbial load was detected. Both prey insect taxa carried a considerably higher microbial load than the unfed (control) spiders. The average fold change of microbial 16S copy number between *Gryllodes* and unfed spiders was 3.72×10^6 and between *Drosophila* and unfed spiders 1.77×10^6.

To detect the proportion of prey DNA remaining in gut extracts at different times after feeding, we sequenced a fragment of the mitochondrial COI gene. *Drosophila* and *Gryllodes* DNA showed very similar patterns of degradation (Figure 2e+f; Table S2). The percentage of prey DNA among all COI reads peaked at 8 hr post feeding. A slightly higher amount of *Gryllodes* DNA was detected (0.86 ± 0.98% of *Gryllodes* vs. 0.48 ± 0.41% of *Drosophila* DNA). A significant drop in prey DNA abundance was then found at 24 hr (*Gryllodes*: 0.64 ± 0.75%, *Drosophila*: 0.53 ± 1.01%), and again at 72 (*Gryllodes*: 0.29 ± 0.50%, *Drosophila*: 0.07 ± 0.08%) and 216 (*Gryllodes*: 0.06 ± 0.07%, *Drosophila*: 0.09 ± 0.14%) hours. At 648 hr, prey DNA was no longer detectable, except in two specimens of *Drosophila*-fed spiders (pairwise Wilcoxon test, FDR corrected p < .05). Thus, the DNA of the prey items in the spider's gut followed a noticeably different trajectory from the microbial load, particularly for the *Gryllodes*-fed spiders (Table S2).

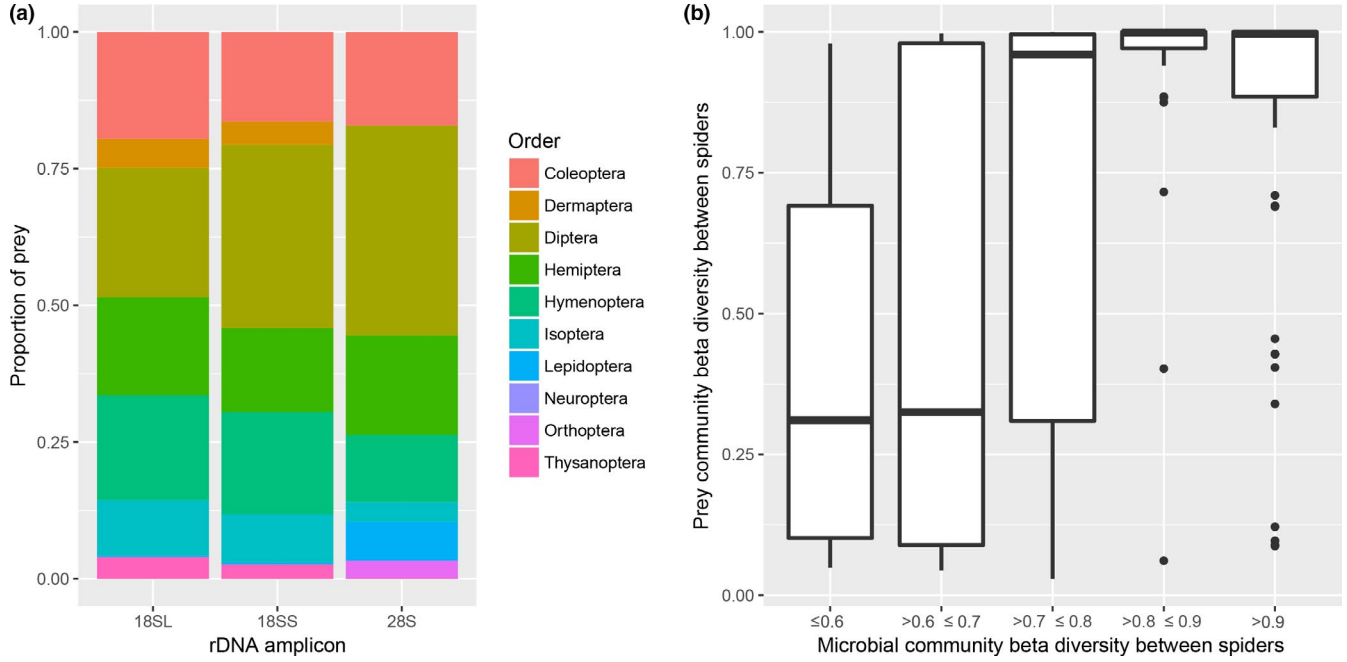

FIGURE 1 (a) Prey community composition of wild-caught *B. longinqua* specimens at the order level, based on three different rRNA gene amplicons (see Figure S2 for taxonomic composition of microbial communities). (b) Bray-Curtis dissimilarity between microbial communities of wild-caught *B. longinqua* specimens in relation to the Bray-Curtis dissimilarity of the insect prey communities of the same specimens. The prey community dissimilarity is based on an average of the three separate rRNA markers. Microbial community dissimilarity is presented in five categories with increments of 0.1. Dissimilarities were calculated from 3% radius OTUs

3.3 | The effect of diet on gut microbial beta diversity

In contrast to wild-caught *Badumna* spiders, the prey insects used in our experiments had a more homogeneous microbial community ($BC_{Gryllodes}$ = 0.58 ± 0.07; $BC_{Drosophila}$ = 0.38 ± 0.134). Feeding the spiders on different prey species significantly affected the microbial community composition of the spiders (Table S2). *Gryllodes*-fed spiders displayed a significant reduction of between-spider community variability, which was observed until 216 hr post feeding (pairwise Wilcoxon test, FDR corrected $p < .05$). Beta diversity then increased back to prefeeding levels after 648 hr. Ordination of microbial community dissimilarity (Bray-Curtis) showed a clear effect of a *Gryllodes* diet on microbial community similarity (Figure 3a). Control (unfed) specimens were widely spread across the plot, suggesting heterogeneous microbial communities. From 8 to 72 hr after feeding, *Gryllodes*-fed spiders clustered together closely and were barely distinguishable from actual *Gryllodes* samples. At 216 hr after feeding, the microbial community slowly transformed back to a prefeeding composition. However, even after 648 hr, the microbial communities of *Gryllodes*-fed spiders differed considerably from those of the unfed spiders. The increasing similarity of *Gryllodes*-and spider-associated microbiota after feeding was also evidenced by a significant drop of Bray-Curtis dissimilarity (Figure 3c). Even 648 hr post feeding, the spider-associated microbiota was significantly more similar to the *Gryllodes* microbiota than before feeding (pairwise Wilcoxon test, FDR corrected $p < .05$).

We observed different effects in spiders fed with *Drosophila hydei*. Feeding of spiders with *Drosophila* was also associated with a significant reduction of microbial community variability between spiders, although not as pronounced as observed under a *Gryllodes* diet (pairwise Wilcoxon test, FDR corrected $p < .05$; Table S2). The drop of community variability between spiders started more gradually and reached its minimum at 216 hr after feeding.

Our NMDS plots also suggest a considerable effect of a *Drosophila* diet on similarity of microbial DNA composition at different times after feeding (Figure 3b). Specifically, microbial communities became well differentiated from the control group at 72 and 216 hr after feeding. In contrast to the *Gryllodes* diet, the *Drosophila* diet did not lead to a homogenization of prey and spider microbial DNA composition. Moreover, the effect of feeding was less predictable in *Drosophila*-fed spiders. The Bray-Curtis dissimilarity between *Drosophila*-associated and spider-associated microbiota was generally very high throughout our feeding experiment, suggesting highly different community composition between the spiders and the prey taxon (Figure 3d). A slight but significant drop of dissimilarity was only observed 8 hr and then again at 648 hr post feeding (pairwise Wilcoxon test, FDR corrected $p < .05$). Similar effects of prey on the microbial DNA composition were detected based on zOTUs (Figure S4).

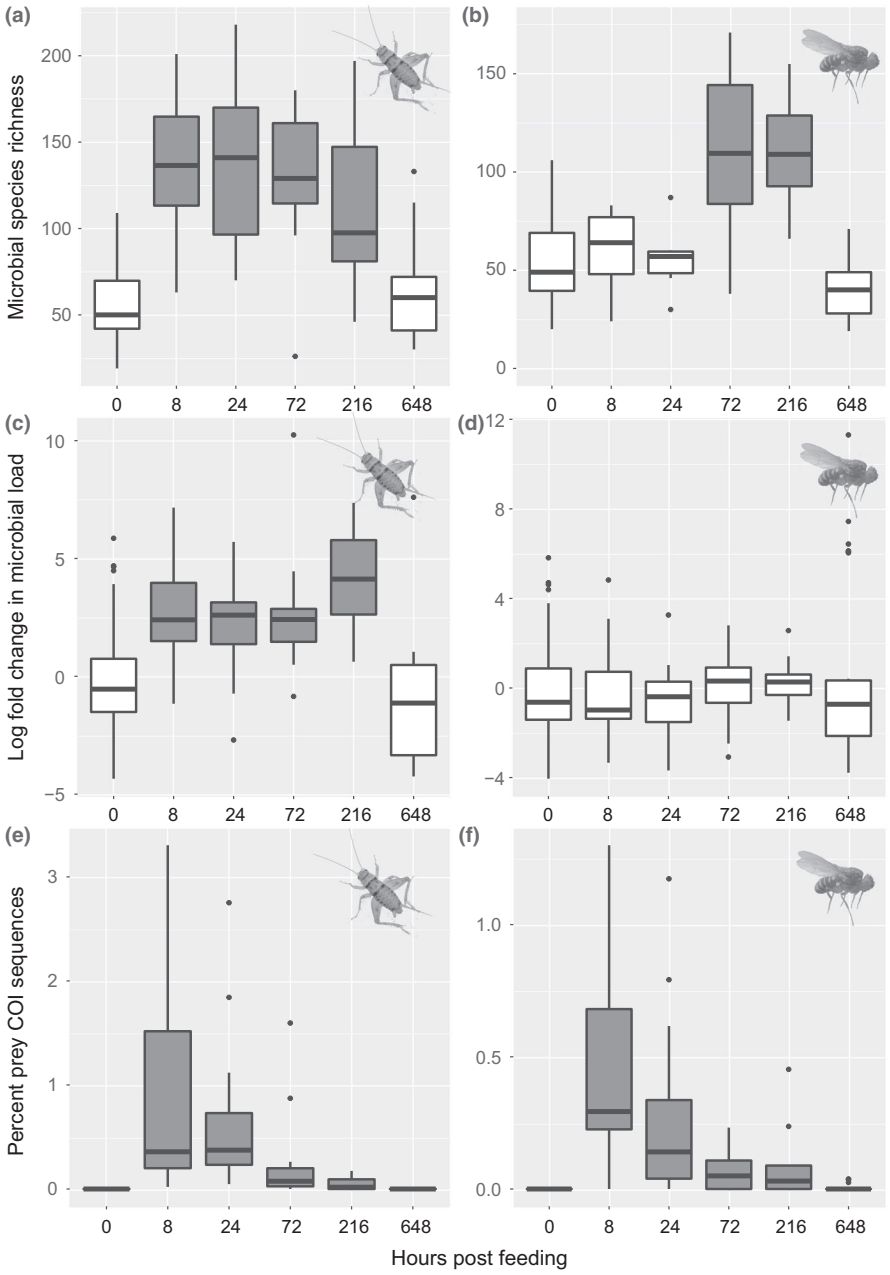

FIGURE 2 (a and b) Microbial species richness (3% OTU radius) of *B. longinqua* at different times after feeding on a diet of (a) *Gryllodes sigillatus* or (b) *Drosophila hydei*. (c and d) qPCR-derived microbial load of *B. longinqua* at different times after feeding on a diet of (c) *Gryllodes sigillatus* or (d) *Drosophila hydei*. The load was calculated as a fold change between microbial 16S and nuclear 18S. (e and f) Proportion of prey COI sequences that can be detected in *B. longinqua* at different times after feeding on a diet of (e) *Gryllodes sigillatus* or (f) *Drosophila hydei*. Significant differences from the control group are indicated by shading (pairwise Wilcoxon test, FDR corrected $p < .05$). On *x*-axis, 0 indicates unfed control group

3.4 | The effect of diet on gut microbial taxonomic composition

In contrast to the unfed control spiders, the consumption of identical prey taxa mimicked the presence of a much larger and more stable core microbiota in our feeding experiment (Figure 4). The proportion of core OTUs increased significantly in both feeding groups (Chi-square test, $p < .05$). The number of core OTUs peaked at 72 hr for both *Gryllodes*-fed (109 OTUs, 22.38%) and *Drosophila*-fed spiders (69 OTUs, 15.83%). Even 648 hr after feeding, both groups carried a considerably elevated proportion of common OTUs compared to the unfed spiders (5.42% for *Gryllodes*- and 4.86% for *Drosophila*-fed).

While the two feeding groups both showed a considerable increase in core OTUs, the possible origin of the observed core OTUs was quite different. For *Gryllodes*-fed spiders, the majority (>60%) of the observed core OTUs were probably cricket-derived (Figure S5a), as they were also found in the extracts of *Gryllodes*. Many microbial taxa that were abundant in *Gryllodes* could be detected in *Gryllodes*-fed spiders, reaching high frequencies and persisting for extended times post feeding. The appearance of *Gryllodes*-derived OTUs in the spiders was decoupled from that of actual cricket DNA (Figures 2e and 5), which peaked at 8 hr post feeding and then rapidly disappeared (linear model, $p > .05$).

Different *Gryllodes*-derived microbial taxa showed very distinct patterns of appearance in the spiders' guts (Figures 5 and S6). This

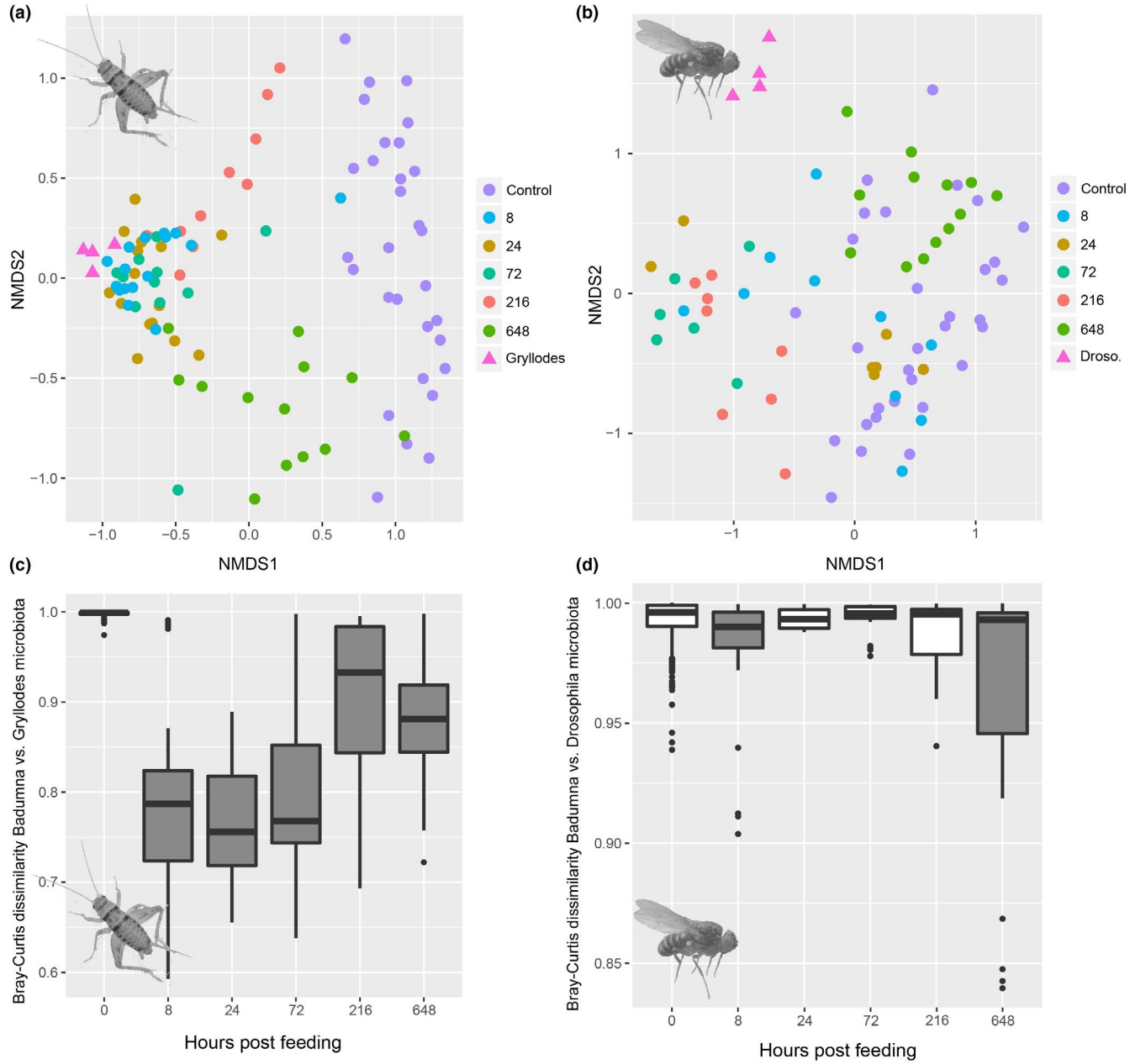

FIGURE 3 (a and b) Non-metric multidimensional scaling plots based on Bray-Curtis dissimilarity (3% OTU radius) between *B. longinqua*-associated and prey-insect-associated microbial communities at different times after feeding on a diet of (a) *Gryllodes sigillatus* (stress = 0.150) and (b) *Drosophila hydei* (stress = 0.166). Prey insects are represented by triangles, spider specimens by circles. See Figure S4 for a zOTU-based analysis. (c and d) Bray-Curtis dissimilarity between spider- and prey insect-associated microbial communities at different times after feeding for *B. longinqua* fed on a diet of (c) *Gryllodes sigillatus* and (d) *Drosophila hydei*. Shading indicates significant differences from the control group (pairwise Wilcoxon test, FDR corrected $p < .05$)

held true for the timing of their appearance as well as their abundances. The most abundant microbial taxon in the crickets (Figure 5a, 9.1% of the total *Gryllodes*-associated microbial community) reached its peak abundance in the spiders 216 hr post feeding at a nearly 100-fold lower abundance than in the crickets. Other abundant *Gryllodes*-associated microbes reached their peak abundances at 24 hr (Figure 5b,d,f) or 72 hr post feeding (Figure 5e) and at nearly 10-fold higher abundances in the spiders than in the crickets. Even one month after feeding, some cricket-derived microbes still made up a considerable proportion of the detected microbial DNA in the spiders' guts (Figure 5b–f).

Analyzing the same data for zOTUs confirmed that the observed microbial sequences in *Gryllodes*-fed spiders were identical to those in the prey organisms (Figure 5). Different zOTUs within an OTU followed very different trajectories of appearance. zOTUs within some OTUs reflected their initial abundances in the crickets very well, with similar proportions of each zOTU detected in the crickets found in the spiders (Figure 5a,c–e). Yet others showed patterns

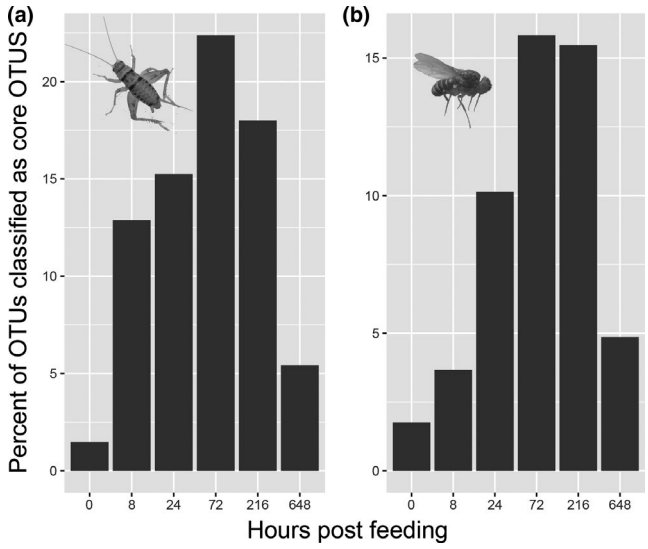

FIGURE 4 Percent of *B. longinqua*-associated microbial OTUs (3% radius) out of the total microbial population that were classified as core OTUs (present in >50% of specimens) at different times after feeding on a diet of (a) *Gryllodes sigillatus* and (b) *Drosophila hydei*. On *x*-axis, 0 indicates unfed control group

of appearance decoupled from the initial abundance in the crickets (Figure 5b,f). For these OTUs, rare zOTUs from crickets reached very high abundances, while some abundant cricket-derived zOTUs appeared in considerably reduced abundances in the spiders.

In contrast, no dominant *Drosophila*-associated OTUs reached high abundances in *Drosophila*-fed spiders (Figure S5b). Most *Drosophila*-derived OTUs only appeared shortly after feeding and quickly disappeared from the spider's digestive tract, quite similarly to the actual DNA of the flies (Figures S7 and 2f). Yet, we detected numerous core OTUs in the *Drosophila*-fed spiders, which persisted for extended times, suggesting a different mode of acquisition. Generally, the appearance of microbial taxa was much less predictable and showed a larger variation in the *Drosophila*-fed spiders than in the *Gryllodes*-fed ones. Most microbes that formed core taxa in *Drosophila*-fed spiders were already present in some of the unfed control spiders at low frequencies. These rare microbial taxa shifted their abundance in response to the *Drosophila* diet (Figure S8).

4 | DISCUSSION

4.1 | A transient and prey-derived microbiome in spiders

We tested several scenarios for the assembly of gut microbial communities of the spider *B. longinqua* in response to diet. First, microbial communities could be stable and largely unaffected by dietary influences (Bili et al., 2016). Based on our results, we reject this hypothesis. Unfed *B. longinqua* did not possess a stable microbiota, a result similar to recent work from caterpillars (Hammer et al., 2017). Instead, the gut microbiome composition of *B. longinqua* appeared to be strongly influenced by diet. Our feeding experiment suggests that diet can affect gut microbiome assembly in the spiders in two drastically different ways, depending on the prey taxon. In the case of *Drosophila*-fed spiders, changing physicochemical conditions in the predator's gut after prey ingestion may have caused microbiome remodelling by environmentally derived taxa (Ren et al., 2016). We detected a considerable change in microbial community composition and diversity in *Drosophila*-fed spiders. This effect was primarily based on unpredictable increases in abundance of microbial taxa. Most *Drosophila*-associated microbes showed a similar temporal pattern of appearance in the spider's gut to the actual DNA of the fruit flies, reaching their peak abundance early after feeding and then quickly disappearing. This is evidenced by the small drop of microbial community dissimilarity between spiders and *Drosophila* flies right after feeding. This pattern is consistent with the prey-associated microbes not surviving the digestion process (Paula et al., 2015) and only being detected as DNA from dead cells immediately after feeding.

As for microbial taxa that appeared in *Drosophila*-fed spiders, but were absent from the *Drosophila* themselves, these newly appearing microbes must have either been acquired from the environment, or were rare taxa already present in the spiders' guts that underwent abundance shifts after feeding (Costello, Gordon, Secor, & Knight, 2010; Ren et al., 2016). Interestingly, many microbes that were later detected as core OTUs in our feeding experiment were already present in some unfed spiders at low frequencies.

A diet of *Gryllodes* led to a rapid increase of microbial diversity and load, as well as a homogenization of the microbiota between individual spiders. This effect lasted for several weeks and was based on the temporal presence of actual *Gryllodes*-associated microbes in the spider's gut. The detection of high abundances of *Gryllodes*-derived microbes has three possible explanations: (a) Microbes could have died during ingestion and their DNA was still present in the gut lumen. (b) Microbial cells may survive ingestion, and persist in the gut in a dormant state. (c) Prey-associated microbes could grow and be temporarily recruited into the spider's microbiota (David et al., 2013; Paula et al., 2015). Considering our result in the *Drosophila*-fed group, the first scenario is unlikely. In contrast to the detected *Gryllodes* microbes, the DNA of dead, *Drosophila*-associated microbes disappeared rapidly from the spider's gut, without leading to a detectable increase in microbial load. Also, the presence of *Gryllodes*-derived microbial taxa in the spider's gut was decoupled from the appearance of actual cricket DNA. While different microbial OTUs showed very different patterns of appearance and decline in the spider's gut, the DNA of crickets degraded rapidly post feeding.

The second scenario may hold true for some microbial taxa, i.e., they may enter a dormant state after ingestion. They would then show an initial increase of abundance after feeding and decline very slowly. However, the extra-intestinal digestion of spiders may impose very strong selection on microbial populations, probably leading to a significant drop in population sizes before they enter the spider's gut. Spider venom, for example, is known to contain antimicrobial peptides, which kill microbes before ingestion (Wang &

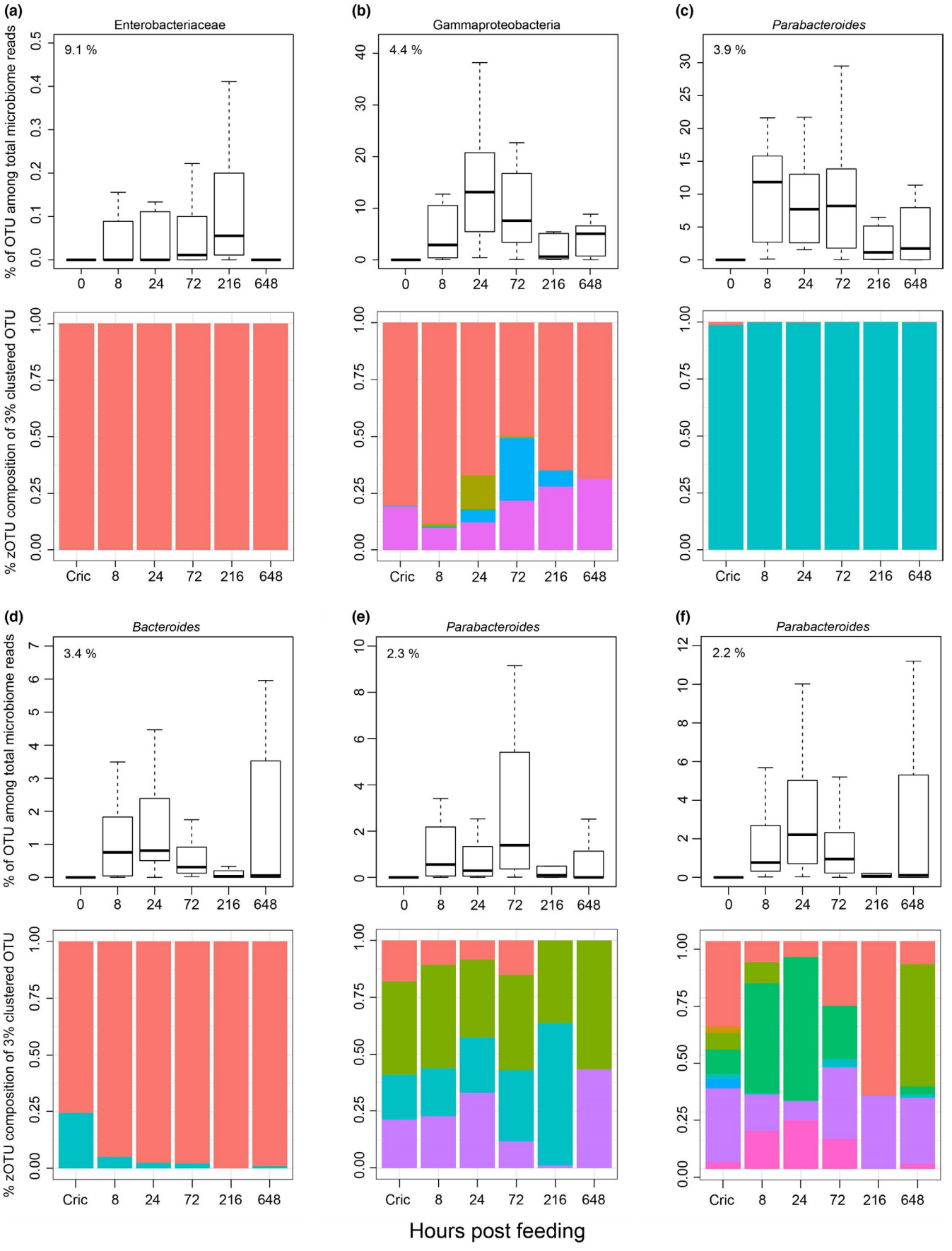
(a)
Enterobacteriaceae
9.1 %
(b)
Gammaproteobacteria
4.4 %
(c)
Parabacteroides
3.9 %
(d)
Bacteroides
3.4 %
(e)
Parabacteroides
2.3 %
(f)
Parabacteroides
2.2 %
% of OTU among total microbiome reads
% zOTU composition of 3% clustered OTU
Cric
0
8
24
72
216
648
Hours post feeding

FIGURE 5 Percentage of six of the nine most abundant tropical house cricket (*Gryllodes sigillatus*)-associated microbial OTUs (3% radius) as they were detected in *B. longinqua* at different times after feeding on a diet of tropical house crickets and the relative abundances of zOTUs detected within each OTU. The OTUs were chosen to display representative patterns of appearance of *Gryllodes*-associated microbes in the spiders (for all nine most abundant OTUs, see Figure S6). Each boxplot shows the taxonomic classification of OTUs to the lowest possible level on top and the percentage of these OTUs in the total cricket microbiome in the upper left. On *X*-axis, 0 indicates unfed control group. "Cric" in the lower panel barplots indicates the zOTU composition of the crickets used as prey. Note that the cricket zOTU panel in (b) actually consists of all five zOTUs, of which three are very rare in the crickets

Wang, 2016; Yan & Adams, 1998). Such reduced population sizes may not cause a significant increase in gut microbial load. Instead, the third possibility, the temporal establishment and growth of *Gryllodes*-derived microbial taxa in the spider's gut, may be relevant. The recruitment of microbes was predictable and replicable between spiders, with different microbial taxa showing very distinct temporal patterns of growth and decline. Some *Gryllodes*-derived microbial taxa reached their peak abundances in the spiders only after 24 hr or even 72 hr or reached secondary abundance peaks a month after feeding. If microbes enter the gut and merely persist in dormant stages, they should reach their peak abundances very early after feeding and their relative OTU and zOTU abundances should roughly mirror those in their cricket hosts. However, microbial OTUs and zOTUs that were very abundant in the crickets (10% of the total microbiome) reached only very low frequencies in the spiders. At the same time, microbes that were very rare in the crickets reached considerably increased abundances in the spiders. A simple take-up and persistence or take-up and degradation of microbes is not well explained by this pattern. Instead, a temporary growth of certain microbial taxa may best explain the considerable turnover of microbial communities observed in our experiment.

The drastically different effects of different prey taxa in our feeding experiment could be associated with their trophic ecologies. Crickets are omnivorous with a protein-rich diet and a microbial community that tends to be taxon-specific (Ng, Stat, Bunce, & Simmons, 2018), while *Drosophila* are adapted to utilize fermenting fruits and have a microbiome that is dominated by bacteria from the diet (Engel & Moran, 2013). The physicochemical conditions of the gut environment of the crickets are probably more similar to those of the spider than those of the *Drosophila* fly, thus making it more likely for cricket-derived microbes to survive and grow in the spider.

Our analysis of unfed wild-caught *Badumna* also supports the assumption of a prey-influenced microbiota. *B. longinqua* is a generalist predator, feeding on a diverse diet of insects. An incubation of prey-sourced microbes or the recruitment of environmental taxa in response to certain prey items may explain the relatively high microbial richness of individual spiders. Moreover, our results show a clear correlation between similarity of spider gut microbial and insect prey communities, suggesting prey taxon-specific effects on microbiome assembly. Indeed, we detected DNA of several arthropod symbionts, which were probably prey-associated, in some of the wild-caught *Badumna* specimens. Among those taxa were *Arsenophonus* and *Hamiltonella*, as well as *Wolbachia*, which are all known to be able to survive and grow in arthropod guts after ingestion (Paula et al., 2015; Pietri, DeBruhl, & Sullivan, 2016). We also discovered DNA of the obligate endosymbionts *Buchnera* and *Sulcia* in a spider specimen that fed on spittlebugs and aphids. These taxa are highly coadapted to their host and will most probably not survive in the spider's gut, so their detection is not indicative of a temporary recruitment into the spider's microbiota. Their DNA should be digested in parallel to that of the prey insect, but DNA traces may still be detectable for a few weeks post feeding (Krehenwinkel et al., 2019; Krehenwinkel, Kennedy, Pekár, & Gillespie, 2017). In a predator with a very low microbial load in its gut, such DNA traces will contribute to the detected community composition. Indeed, the spider specimen in which we found *Buchnera* and *Sulcia* had one of the lowest microbial loads in our control group. Due to the sensitivity of PCR-based community analysis, we thus cannot rule out a contribution of dead microbial cells to the observed patterns. However, considering the results of our feeding experiments, this effect is probably of minor importance here.

4.2 | Are prey-sourced microbes functionally relevant?

Irrespective of the prey taxon, our results suggest that a spider's gut is not a stable ecosystem for microbes and can change profoundly over short periods of time. The spider's prey is digested extra-intestinally and enters the gut largely liquefied. The prey is rich in proteins and should be easily digested and resorbed in the spider's midgut. Gut microbes in many animals contribute to the breaking down of food that is toxic or hard to digest (Kudo, 2009; Vilanova, Baixeras, Latorre, & Porcar, 2016), or provide nutrients that their host cannot synthesize (Ayayee et al., 2016). Spiders probably do not need such aid in processing their diet. The observed microbes in *B. longinqua* spiders may thus not perform any function. Microbial cells may survive the process of prey liquefication, enter the spider's gut intact and possibly grow there for a certain time. Most of these microbes would merely be commensals, feeding on prey remains in the spider's gut. However, even seemingly random and environmentally derived gut microbiomes can still perform essential tasks for their host (Coon, Brown, & Strand, 2016). For example, they could prevent pathogenic bacteria from entering the digestive system by filling available niche space (Dillon, Vennard, Buckling, & Charnley, 2005; Kwong & Moran, 2016). A recent study found an increased lifespan in spiders fed with a diet of crickets (Keiser et al., 2016), suggesting a possible probiotic effect of prey-derived microbes. However, further experiments with germ-free *Gryllodes* will be necessary to explore fitness effects of prey-derived microbes.

Interestingly, many *Gryllodes*-derived microbes that reached high densities in spiders were well-known arthropod gut bacteria, for example *Paludibacter* and *Parabacteroides* (Brune & Dietrich, 2015). *Providencia rettgeri*, the most abundant taxon associated with *Drosophila hydei*, is an actual pathogen (Galac & Lazzaro, 2011). This taxon quickly disappeared from the spider's gut, suggesting a certain selectivity in the uptake of prey-derived microbes. The uptake of pathogens would be highly disadvantageous for a generalist predator such as *B. longinqua*, thus the spider may possess defence mechanisms that control the uptake of microbes. In that regard, it is interesting that we discovered very few species of potential reproductive parasites in our data. A considerable proportion of insect species is infected with such taxa (Duron, Bouchon, et al., 2008; Zug & Hammerstein, 2012). Considering the generalist diet of *Badumna*, reproductive parasites such as *Wolbachia*, *Cardinium* and *Rickettsia* should regularly enter the spider's gut, yet we found them in few spider specimens and at low abundances.

4.3 | Practical implications of prey-derived microbiota for microbiome analysis in predatory arthropods

Our findings have important practical implications for future microbiome studies in spiders and other predatory arthropods. The presence of a stable core microbiome is often taken as evidence for a strong host-microbe association (Shade & Handelsman, 2012). Microbiome studies in wild-caught predatory arthropods could be prone to erroneous assumption of such associations. For example, when a spider population is sampled at a time when a certain prey item is very abundant, e.g., during an insect mass hatching, prey-derived microbes may mimic the presence of a stable core microbiome for extended periods after feeding. Moreover, different populations of a predator, utilizing different locally abundant prey species, could temporarily carry highly similar microbial communities within, but highly divergent ones between, populations. This could be taken as evidence for local adaptation or biogeographic differentiation of microbial communities. To avoid these pitfalls, microbiome analyses in predatory arthropods should be coupled with an exploration of the target taxon's diet, e.g., by DNA barcoding of prey remains in the gut. It may even be advisable to screen the microbial communities associated with the actual prey taxa.

In conclusion, the assembly of microbial communities associated with predatory arthropods is not well understood. Here, we show that the gut microbiome of a spider is transient and largely affected by its insect prey. Feeding on similar prey taxa can mimic the presence of a stable core microbiome that can be detected for extended time periods after feeding. The diet-based remodelling of gut microbial communities can be achieved in very different ways. Depending on the consumed prey taxon, either a predictable set of prey-derived microbial species is incubated in the spider, or the gut microbial community is remodelled by changes in the abundances of existing taxa as well as the possible acquisition of environmentally derived microbes. Our results may be of general relevance for predatory arthropods, highlighting them as a group with an unstable and primarily diet-influenced gut microbiome.

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AUTHOR CONTRIBUTIONS

H.K., S.K., S.T. devised the study, performed laboratory work and data analysis. H.K., S.K., S.T., and R.G. wrote the manuscript.

DATA AVAILABILITY STATEMENT

The metadata of the study is available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.2ngf1vhhz; Krehenwinkel, 2019). Current temporary link: https://datadryad.org/stash/share/-mE_kJFfLBbD58JnQhg2bZB7c6dLs8uafpchOyTTK10.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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