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Complete metamorphosis and microbiota turnover in insects

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Abstract

The insects constitute the majority of animal diversity. Most insects are holometabolous: during complete metamorphosis their bodies are radically reorganized. This reorganization poses a significant challenge to the gut microbiota, as the gut is replaced during pupation, a process that does not occur in hemimetabolous insects. In holometabolous hosts, it offers the opportunity to decouple the gut microbiota between the larval and adult life stages resulting in high beta diversity whilst limiting alpha diversity. Here, we studied 18 different herbivorous insect species from five orders of holometabolous and three orders of hemimetabolous insects. Comparing larval and adult specimens, we find a much higher beta-diversity and hence microbiota turnover in holometabolous insects compared to hemimetabolous insects. Alpha diversity did not differ between holo- and hemimetabolous insects nor between developmental stages within these groups. Our results support the idea that pupation offers the opportunity to change the gut microbiota and hence might facilitate ecological niche shifts. This possible effect of niche shift facilitation could explain a selective advantage of the evolution of complete metamorphosis, which is a defining trait of the most speciose insect taxon, the holometabola.

KEYWORDS
beta diversity, evolution of metamorphosis, gut microbiota, holometaboly

1 | INTRODUCTION

Insects are the most diverse animal taxon on earth (Berenbaum, 2017; Mora et al., 2011) and collectively comprise 50–70% of all living animal species (Grimaldi & Engel, 2005; Mora et al., 2011). More than 80% of all described insect species are holometabolous—they undergo complete metamorphosis (Grimaldi & Engel, 2005) that includes a pupal stage intercalated between the larva and the adult. In the pupa, the insect body is radically remodelled. All larval organs, including the gut, are broken down and reconstructed, resulting in distinct and specialized larval and adult life stages (Hall & Martín-Vega, 2019; Hinton, 1948; Rolff et al., 2019; Truman, 2019). Complete metamorphosis is considered a key trait explaining insect diversity (Mayhew, 2007; Rainford et al., 2014, but see Condamine et al., 2016) and only evolved once, hence the holometabola are a monophyletic group (Misof et al., 2014). The diversification of the speciose orders of the holometabolous insects coincides with the diversification of the land plants (Condamine et al., 2016; Misof et al., 2014).

How this radical reorganization of the insect body is related to the astounding radiation of the holometabolous insects is not known (Rolff et al., 2019), but it is one of the key traits of holometabolous insects (Nicholson et al., 2014). One possible explanation could be that intercalating the pupal stage decouples growth and differentiation (Arendt, 1997; Rolff et al., 2019), allowing for efficient and competitive exploitation of ephemeral resources. Another, not mutually

exclusive explanation is, that larvae and adults can occupy distinct niches (Hammer & Moran, 2019). If the niche shift also includes a diet shift, a change in gut microbiota could possibly facilitate such niche shifts (Hammer & Moran, 2019).

One of the major internal reconstructions during the pupal stage includes the replacement of the gut epithelium. From the perspective of the microbes in the gut, the epithelial replacement constitutes a dramatic habitat change. The gut microbiota, that can provide nutrition, defence and other services to the insect host, changes in density and community structure, including the elimination of particular microbes during pupation (Hammer & Moran, 2019; Johnston & Rolff, 2015). These changes may result from a combination of factors including the drastic anatomical and physiological transformations in the replacement gut, host immune effector induction in the metamorphic gut, bacterial competition for continued occupancy of the pupal gut, and ontogenetic habitat and diet shifts of the host. In the lepidopteran *Galleria mellonella*, the absolute abundance of the microbiota can be reduced by several orders of magnitude during metamorphosis, including the elimination of pathogenic bacteria that would otherwise persist in the adult host (Johnston & Rolff, 2015). In *Galleria mellonella*, the host immune system and the symbionts interact with the microbial community in the gut through complete metamorphosis. Observations in other taxa of the Lepidoptera (Rolff et al., 2019 and refs therein) as well as in some Coleoptera (Critchlow et al., 2019) are consistent with partial host control of the microbiota. The replacement of the gut epithelium potentially offers the insect a unique opportunity to significantly alter the gut microbiota, allowing an insect to acquire life stage-specific microbes (Hammer & Moran, 2019; Johnston & Rolff, 2015). Such an opportunity on gut microbiota changes during the pupal stage may facilitate niche shifts (Engel & Moran, 2013).

Some studies have investigated changes in the gut microbiota at different stages of host development. For example, the hemimetabolous insect *Pyrrhocoris apterus* (Sudakaran et al., 2012) hosts a very stable mid-gut community composition with six predominant taxa being consistently abundant throughout development. By contrast in a holometabolous insect, the hymenopteran *Bombus pascuorum*, Parmentier et al. (2018) reported different gut microbial communities within larval and adult specimens of a wild nest. The typical core gut bacteria in the adults were absent in the larvae. Hammer et al. (2014) also found distinct gut microbiota communities in the leaf-chewing larvae and nectar- and pollen-feeding adults in the lepidopteran *Heliconius erato*. Studies of the dipteran *Musca domestica* (de Jonge et al., 2020) and the coleopteran *Phalacrognathus muelleri* (Wang et al., 2020) have found a similar pattern.

The high microbiota turnover observed in some holometabolous species poses the risk of losing beneficial microbes which would result in a cost to both, host and symbiont. Hammer and Moran (2019) suggest that holometabolous insects may be less likely to evolve strictly vertically transmitted symbioses than hemimetabolous insects. To overcome this hurdle a number of strategies have evolved to ensure transmission of obligate symbiont between life stages in holometabolous insects. Stoll et al. (2012) showed vertical transmission of microbes via bacteriocytes in ant species. The relative number of bacteria-filled bacteriocytes increased strongly during complete metamorphosis. Maire et al. (2020) also showed a transmission of microbes via bacteriocytes in weevils by maintaining and relocating bacteriocytes during gut renewal in the pupa. Other specialized structures to transmit symbionts in insects are antennal glands (Kaltenpoth et al., 2012) or crypts (Kikuchi et al., 2011).

The gut microbiota between larval and adult holometabolous insects is also influenced by the diet and the environment. Though not studied specifically in the context of metamorphosis, the influence of diet on gut microbiota composition has been demonstrated in arthropods. Chandler et al. (2011) found that diet in a range of *Drosophila* species, comprising cactus, flower, fruit and mushroom feeding species, shapes the adult gut microbiota within a taxonomically restricted selection of microbes. In the spider *Badumna longinqua*, the gut microbiota composition is strongly influenced by the microbiota of the prey species.

Here, we investigated whether gut microbiota changes during the adult moult, which includes pupation in the holometabolous insects, differ between hemi- and holometabolous insects. Because of the reorganization of the gut in the pupal stage we expect (1) a significant change in bacterial composition resulting in much greater beta-diversity in holometabolous than in hemimetabolous insects. The diversity of gut microbes can be strongly reduced during pupation (Hammer et al., 2014; Johnston & Rolff, 2015). (2) We therefore speculated that greater alpha diversity would be observed in the gut microbiota of hemimetabolous insects, given the lack of gut epithelial replacement and associate host immunity. Also, as the diversity of the gut microbiota scales positively with size across species (Sherrill-Mix et al., 2018), it is possible that alpha diversity is higher in adult than larval insects, especially in hemimetabolous species.

To address these questions, we sampled 18 different species from seven major insect orders across the life stages. To reduce geographic variance, we collected all insect species in Central and Northern Europe. To further reduce variance, we sampled only herbivorous insects from terrestrial habitats and excluded social insects. Additionally, we collected a subsample of those species from laboratory-reared colonies, consisting of five species from four different insect orders (Figure 1) as the gut microbiota may differ between specimens from the field and laboratory (Martinson et al., 2017; Staudacher et al., 2016).

2 | MATERIALS AND METHODS

2.1 | Insect sampling and preparation

Larval and adult specimens of 18 insect species from seven different insect orders, including Orthoptera, Thysanoptera, Hemiptera, Hymenoptera, Coleoptera, Lepidoptera and Diptera, were sampled in Central and Northern Europe between April and October 2018 (see Figure 1 and Table S1). Pupae were additionally sampled for the three hymenopteran species. In total, 16 species across development

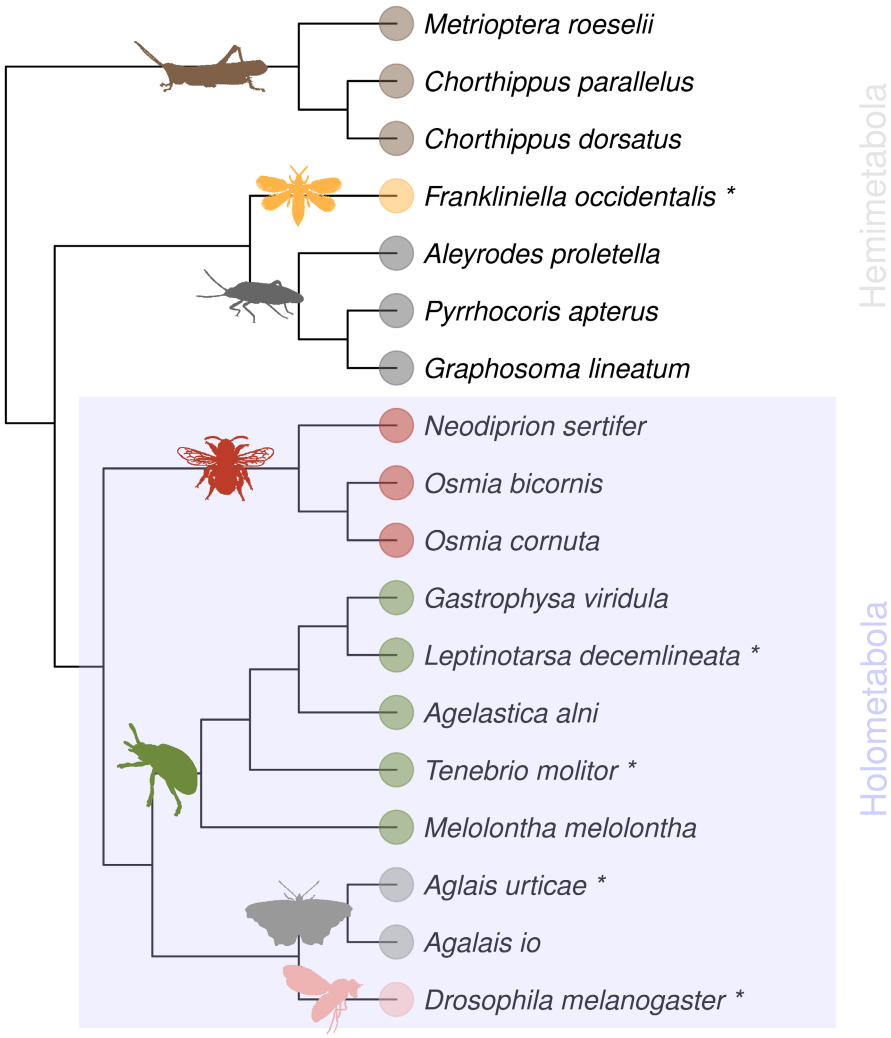

FIGURE 1 Phylogenetic tree of the 18 field-collected species. The five species marked with a star were additionally sampled from laboratory-reared colonies. The 11 highlighted species represent the monophyletic group of the holometabolous insects. The other seven species are hemimetabolous.

were collected in Northern Germany, *Tenebrio molitor* in Croatia and *Neodiprion sertifer* in Finland. Additionally, a subsample of those species was sampled, consisting of five species from four different insect orders, which originated from laboratory-reared colonies (see Table S2). Figure 1 gives an overview of all insect species collected in the field and the subset of those species from laboratory-reared colonies. A total of 643 individual insects were sampled. All species were identified using common identification keys and were confirmed by specialists.

After collection, the insects were stored individually in 50 ml centrifuge tubes (Falcon tubes) with holes for ventilation. In two very small species, *Frankliniella occidentalis* and *Aleyrodes proletella*, individuals were pooled (see Tables S1 and S2). These pools were kept and used as a biological replicate later. After a 24-h starvation period, the insects were sacrificed, and preserved by freezing (−80°C), except two field-collected species which were preserved in ethanol (95%): *Frankliniella occidentalis* and *Neodiprion sertifer*. Hammer et al. (2015) compared two different storage methods, freezing and ethanol, among others, and found that the storage method did not affect microbiota composition assessments. The whole body microbiome as a proxy for the gut microbiome is used in this study. This is in accordance with current literature, such as the studies by Hammer et al. (2015) and other studies on arthropod microbiota (De Cock et al., 2019; Kennedy et al., 2020) that show that such approach provides a robust representation of the gut microbiota.

2.2 | DNA extraction

Samples of the sacrificed insect were processed on ice under sterile conditions. A biological replicate was an individual insect sample, except for samples of three small species: a replicate of *Frankliniella occidentalis* was pooled from 30 individuals, a replicate of *Aleyrodes proletella* from 40 and a replicate of *Drosophila melanogaster* from 10 individuals. The exact number of biological replicates per species and life stage are shown in Tables S1 and S2. After removing the legs and wings (adults only) using sterilized forceps and dissecting scissors, the samples were placed in 2 ml microcentrifuge tubes (Eppendorf Safe-Lock tubes). Then samples were bead-ground using TCBeads and C1 solution from the PowerSoil DNA Isolation Kit (Qiagen) three times for 30 s at 30 Hz in a tissue homogenizer. The insects were not dissected before homogenization in order to process all samples under standardized methods as the Thrips and Whiteflies were too small to dissect guts. Insects were not sterilized prior to

homogenization. Hammer et al. (2015) found no effect on the bacterial communities of not surface sterilized insect species (butterfly, grasshopper, bee and beetle) compared to control specimens that were surface sterilized: samples clustered by species independent of surface sterilization and relative abundances of bacterial genera were similar between sterilized and nonsterilised specimens. Also, surface contaminants derived from handling the specimens were extremely rare in nonsterilised and surface sterilized specimens. As it remains possible that surface sterilization could affect internal bacterial communities, Hammer et al. (2015) recommend omitting surface sterilization from insect microbiota studies.

Total DNA was extracted from 60 µl of tissue homogenate using the PowerSoil DNA extraction kit (Qiagen) under sterile conditions. Tissue homogenates were pretreated with 10 µl Proteinase K and 500 µl Power soil bead solution at 56°C overnight. Subsequent DNA isolation was continued as indicated in the manufacturer's instructions.

Negative extraction controls were included to detect and filter contamination. The negative controls consisted of mock samples, which contained no insect tissue.

2.3 | Primers and PCR amplification of the 16S rRNA gene fragment

PCR amplification of the 16S rRNA gene fragments was performed with MyTaqTM HS DNA polymerase and the forward and reverse 515f-806r primer sequence pairs, targeting the V3–V4 region of the 16S rRNA gene (Thompson et al., 2017). The PCR reaction was conducted using 1 µl sample in a total volume of 25 µl. The PCR amplification program was as follows: 94°C for 1 min, 95°C for 15 s, 50°C for 15 s, two cycles of 72°C for 45 s and 2 min, followed by a final extension step to 4°C. A volume of 5 µl of the PCR product was run on a 1.5% agarose gel stained with Sybr Gold at 160 V for 40 min.

PCR products were purified with CleanNGS CNGS-0050 (GC Biotech B.V.) and dual indices and Illumina sequencing adapters were attached by limited-cycle PCR amplification (initial denaturation at 95°C for 2 min followed by eight cycles of denaturation at 95°C for 20 s, annealing at 52°C for 30 s, extension at 72°C for 30 s, and a final extension cycle at 72°C for 3 min). The enzymes used were Herculase II Fusion DNA polymerase (Agilent Technologies Sales & Services GmbH & Co). PCR products were quantified with Quant-iT PicoGreen dsDNA assay kit (Life Technologies GmbH Thermo Fisher Scientific), measured with Optima Fluostar (BMG Labtech GmbH).

2.4 | Sequencing of bacterial community

Amplicon libraries were sequenced for 600 cycles using an Illumina MiSeq (Illumina) at the Berlin Centre for Genomics in Biodiversity Research (BeGenDiv). The resulting 300-bp paired end reads were analysed using a full-stack R (R Core Team, 2020) pipeline incorporating dada2 (Callahan et al., 2016, p. 2) and phyloseq (McMurdie & Holmes, 2013). Forward reads were trimmed to 240 bp and reverse reads to 160 bp. The reads were truncated at the first instance of a quality score less than two and filtered to a maximum amount of estimated errors of two per truncated read. The remaining forward and reverse reads were dereplicated, and error rate estimates were computed. The developed error model was used to infer exact amplicon sequence variants (ASVs) from the amplicon sequencing data. The resulting denoised read pairs were merged. A sequencing table was constructed with the denoised and merged reads and chimeras were removed. Taxonomy was assigned to the sequence table using the Ribosomal Database Project (Cole et al., 2014) training set, version 16. Contaminant taxa were identified using prevalence-based filtering from the decontam package (Davis et al., 2018). Remaining unknown sequences were identified using the Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990) and taxonomy was assigned using TaxonKit (Shen & Xiong, 2019). Further remaining unknown sequences were renamed with higher taxonomic ranks and eukaryota were removed. See R script for more details on the dada2 pipeline.

2.5 | Statistical analysis

All statistical analyses were performed in R (version 3.6.3; Core Team, 2020). Shannon indices were calculated per developmental stage and species using the microbiome package (Lathi et al., 2019). To adjust for differences in library sizes, Willis (2019) suggests accounting for unobserved taxa instead of rarefying the data. The breakaway estimator (Willis & Bunge, 2016) was used but did not differ from an estimator that does not account for unobserved taxa. Therefore, the simpler approach using proportions was used. The number of reads can be found in (Figure S19, Table S22). After generating a distance matrix for each species, mean differences in alpha-diversity between life stages using the Shannon index were computed using the meandist function from the R package vegan (Oksanen et al., 2008). The generated distance matrix tests each larval specimen against each adult specimen in Shannon diversity by calculating absolute difference values. Shannon means for larvae and adults within each species were computed using the summarySE function from the Rmisc package (Hope, 2013). Bray–Curtis dissimilarity matrices were computed for each species with the distance function from the phyloseq package (McMurdie & Holmes, 2013) and used to calculate mean beta-diversities comparing life stages using the meandist function from the R package vegan (Oksanen et al., 2008). The data were normalized to proportions to control for read depth prior to ordination. We tested for differences between holo- and hemimetabolous insects in beta-diversity, Shannon life-stage difference, and the larval and adult Shannon estimates. Regression analyses controlled for phylogeny were performed to reduce type I error rates. Grafen's branch lengths were generated before modelling the phylogenetic correlation matrix for the models (Grafen, 1989). The models were phylogenetic linear mixed-effects models using the rma.mv function from the R package metafor (Cinar et al., 2021) that incorporates

sampling variance (the square of SE). First, an intercept model with two random effects (species ID and phylogeny) was fitted. The total amount of heterogeneity (I^2) and the heterogeneity explained by differences between species and phylogeny was calculated. I^2 describes the percentage of total variation across samples that is due to heterogeneity rather than sampling variance (Higgins, 2003; Senior et al., 2016). Then a model with the two random effects (species ID and phylogeny) and the type of metamorphosis as a fixed effect that looked at the contrast between hemi- and holometabolous insects was fitted, and the amount of variation in the response attributed to the type of metamorphosis was calculated as marginal R^2 (Nakagawa & Schielzeth, 2013). A third model that specified the variance structure of the two insect groups by modelling heteroscedasticity was fitted and used to visualize the results (Table S12) using the orchaRd package (Nakagawa et al., 2020). The orchard plots display 95% confidence intervals and 95% prediction intervals of the group means for hemi- and holometabolous insects. The prediction interval displays the 95% probability that the response estimate of an insect species in a new study lies within this interval. Further absolute abundances were plotted for all species and life stages using the R package microbiome (Lathi et al., 2019; see Figures S1–S18). The sequences were agglomerated at the genus level for the relative abundance plots. Rare bacterial taxa present <1% of all taxa per species are not shown in the figures. The larval and adult Shannon means per species were compared, according to test assumptions, with a two-sample *t* test, Wilcoxon rank-sum test and Welch test, respectively (see Tables S18 and S19 and Figures S1–S18 for more details). To meet the assumption of normally distributed data for the two-sample *t* test, the response variable was transformed before testing for group differences using logarithm transformation for *Chorthippus parallelus* and *Chorthippus dorsatus* and via reciprocal $1/x^6$ transformation for the data set of *Graphosoma lineatum*. The effect sizes were calculated using the effsize package (Torchiano, 2020). The beta dissimilarity data per species and for all data pooled was analysed by perMANOVA with life stage as a predictor variable, and a dispersion test was fitted using the vegan package (Oksanen et al., 2008; see Tables S13 and S14). Principle coordinate analysis (PCoA) on Bray–Curtis dissimilarity was used to display the beta diversities per species with life stage as a grouping factor using the phyloseq package (McMurdie & Holmes, 2013; see Figures S1–S18) and for all data pooled with species and life stage as a grouping factor (see Figures S20 and S21, and Table S15).

3 | RESULTS

We obtained 18 insect species covering seven major orders, including three hemimetabolous (Orthoptera, Thysanoptera, Hemiptera) and four holometabolous insect orders (Hymenoptera, Coleoptera, Lepidoptera and Diptera; see Tables S1 and S2). We sampled juvenile and adult life stages for all of them (Figure 1). Using 16S rRNA gene metabarcoding (see Supporting Information for more details), we determined the gut microbial compositions per life stage and species (all relative abundance plots can be found in the Supporting Information, Figures S1–S18). The data from *Pyrrhocoris apterus* and *Melolontha melolontha*, which originated from two different locations, were pooled as population did not affect alpha diversity (see Table S16) nor beta diversity (Table S13).

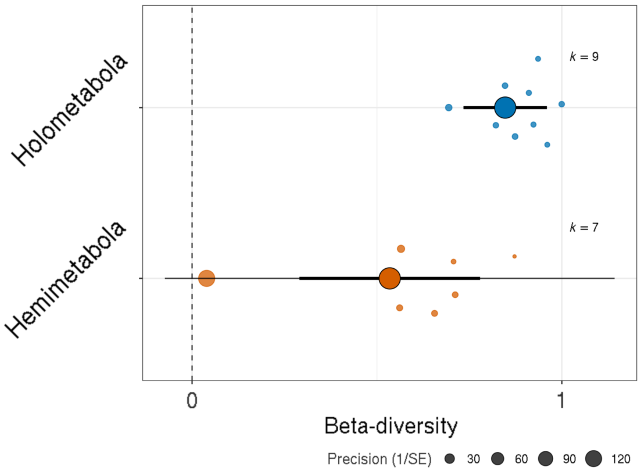

FIGURE 2 The average beta diversity (Bray–Curtis dissimilarity) in larval and adult bacterial communities among hemi- versus holometabolous insects (Beta = 0.3123; 95% CI: 0.0432, 0.5814) with 95% confidence (bold error bars) and prediction intervals (thin error bars). Each point represents the beta diversity between life stages of a particular insect.

3.1 | Microbiota turnover at the larval-adult transition

Microbial beta diversity of holometabolous insects was significantly greater than that of hemimetabolous insects when comparing larval and adult life stages of each species (Beta = 0.3123; 95% CI: 0.0432, 0.5814; Figure 2; see Tables S3–S5 and S17 for more details). The differences in beta diversity are not only driven by differences in abundances as indicated by an analysis on unweighted beta diversity indices (see Figure S22, Table S23). The heterogeneity was high (I^2 = 87.41%), with 52.42% of the variance explained by phylogeny and 34.99% explained by differences between species. The amount of variation in beta-diversity attributed to the type of metamorphosis was 46.29% (R^2). With the exception of *Leptinotarsa decemlineata*, all field-collected holometabolous insect species showed significant differences in beta diversity between larval and adult life stages (Table S13). Within the hemimetabolous species collected in the field, two species differed significantly in beta diversity: *Chorthippus dorsatus* and *Pyrrhocoris apterus*. The other five Hemimetabola did not differ in beta-diversity between life stages. This pattern was consistent in the subset of five laboratory-reared species. To display the differences in beta diversity between life stages for each species, we used principal coordinate analysis (PCoA) ordination (see Figures S1–S18, PCoA plots per species).

3.2 | Alpha-diversity

We calculated Shannon diversity indices per life stage and species and calculated the difference in alpha diversities between life stages per species. Alpha diversity differences between larval and adult life stages did not differ between holo- and hemimetabolous insects (Shannon-difference = 0.2786; 95% CI: −0.4827, 1.04; Figure 3; see Tables S3, S6 and S7 for more details). The heterogeneity was high (I^2 = 94.68%), with 89.32% of the variance explained by phylogeny and 5.36% explained by differences between species. The amount of variation in Shannon-difference attributed to the type of metamorphosis was 12.61% (R^2). The microbial alpha diversity was also not different between holo- versus hemimetabolous larvae (Shannon_Larvae = 0.1586; 95% CI: −0.4614, 0.7787; Figure 4; see Tables S3, S8 and S9 for more details) and adults (Shannon_Adults = 0.1803; 95% CI: −0.6152, 0.9758; Figure 4; see Tables S3, S10 and S11 for more details), respectively. The heterogeneity was high in larval (I^2 = 98.8%) and adult (I^2 = 97.28%) Shannon indices. 46.48% of the variance is explained by phylogeny in the model testing larval Shannon and 36.26% in the model testing adult Shannon group differences. Five species did differ significantly in alpha diversity between life stages within the holometabolous insects. Within the hemimetabolous insects, one species differed significantly in alpha diversity between life stages (see Tables S18 and S19 for more details).

4 | DISCUSSION

We investigated beta and alpha diversity throughout development, comparing 18 insect species from four holo- and three hemimetabolous insect orders. We find a clear pattern: generally holometabolous insects show a strong microbial turnover between larvae and adults, while this is not found in hemimetabolous insects.

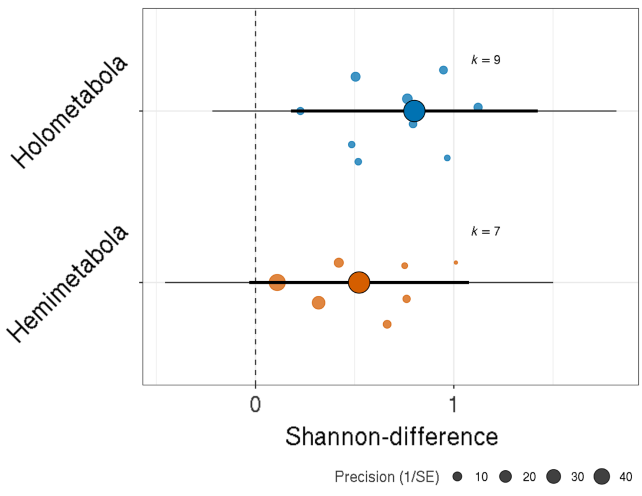

FIGURE 3 Average Shannon-difference of larval and adult bacterial communities with 95% confidence (bold error bars) and prediction intervals (thin error bars) among hemi- and holometabolous insects (Shannon-difference = 0.2786; 95% CI: −0.4827, 1.04). Each point represents Shannon-difference between life stages of a particular insect.

Almost all examined holometabolous insect species showed significantly different gut microbial communities between larval and adult specimens as reflected by the differences in beta diversity. The overall pattern we report is well supported: hemimetabolous insects do not show changes in beta diversity during development. The remaining variation in beta diversity within the holometabolous insects may be partly explained by different ecologies which warrants further investigation. *Neodiprion sertifer*, the European pine sawfly, displayed the lowest beta diversity in our sample of holometabolous insects but still a higher beta diversity than many hemimetabolous insects. Larval and adult generations overlap in *N. sertifer*, and larvae feed pine needles, while adults usually do not feed. The three most abundant bacterial taxa (see Table S21) are *Enterobacteriacae*, *Pseudomonas* and *Yersinia*. Despite the fact that *N. sertifer* as a forest pest is relatively well studied, the role of the microbiota has rarely been studied. A previous paper on a closely related species also found a high abundance of *Yersinia* (Whittome et al., 2007).

The flour beetle *Tenebrio molitor* also showed a very low beta diversity between larvae and adults. Larval and adult *Tenebrio molitor* have overlapping populations, and unusually for holometabolous insects they share the same habitat throughout development, and they are cannibalistic (Staudacher et al., 2016) and could obtain microbes via feeding on conspecifics. Both laboratory- and field-collected individuals were dominated by either *Lactobacillales* or *Enterobacterales* with a small population of *Actinobacteria*.

The highest microbiota turnover in the holometabolous insects was found in the cockchafer (*Melolontha melolontha*). This is the only species in our selection that has a soil-dwelling larval stage, which almost certainly exposes the larvae to a high diversity of soil bacterial during its very long development time that lasts several years. The adults feed on plant leaves. Many of the anaerobic taxa observed in *M. melolontha* are also described from the forest cockchafer *Melolontha hippocastani*, where they inhabit an expanded midgut organ that resembles the termite paunch, which is specialized for anaerobic fermentation (Arias-Cordero et al., 2012). A similar reduction of bacterial diversity also occurs in adult *M. hippocastani*, which may relate to the drastic reduction in size and content of the anaerobic compartment in the adult (Arias-Cordero et al., 2012).

Interestingly, the highest beta diversity in hemimetabolous species was recorded in thrips. They have evolved a neometabolous life-style with two partly quiescent stages between the larva and the adult (Truman, 2019). The stages are also called pupae but their development does not entail the dramatic change in morphology as in holometabolous insects (Truman, 2019). The lowest beta diversity was found in the striped shield bug *Graphasoma lineatum*. *Graphosoma lineatum* harbour beneficial symbionts in midgut crypts, including *Pantoea* (Karamipour et al., 2016), a genus of gram-negative bacteria and the most abundant bacterial taxon in both larval and adult specimens. *Pantoea* is consistently present throughout insect development and accounts for the vast majority of reads in all *G. lineatum* samples.

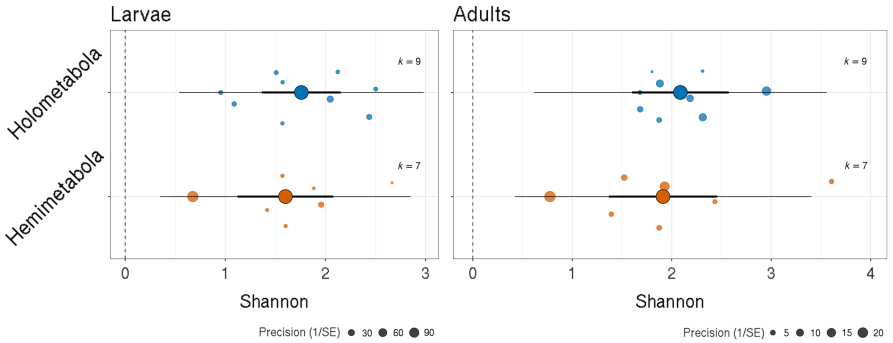

FIGURE 4 Average larval alpha diversity between hemi- and holometabolous insects (Shannon_Larvae = 0.1586; 95% CI: −0.4614, 0.7787, upper graph), and of adults (Shannon_Adults = 0.1803; 95% CI: −0.6152, 0.9758, lower graph). Each point represents the alpha diversity, measured as Shannon difference, of larvae (left panel) or adults (right panel).

Microbiota turnover seems to be a general pattern within holometabolous, but not hemimetabolous insects, independent of the insects' field and laboratory origin. We did not find any consistent patterns of the most abundant bacterial taxa between larvae and adults or holo-and hemi-metabolous insects (Table S21). The microbial composition changes are presumably driven by the intercalated pupal stage in holometabolous insects, which allows a radical remodelling of the hosts' gut, but often is also accompanied by different diet choices of larvae and adults. Prior to pupation a cessation of feeding and purging of the gut contents takes place (Johnston & Rolff, 2015). After that, immune effectors such as lysozyme and AMPs are secreted into the gut (Johnston & Rolff, 2015) followed by anatomical and physiological changes resulting in the replacement of the gut. Competition of the remaining bacteria with possible new colonizers of the adult gut then shapes the adult microbiota. The role of the host immune system is illustrated by a study in *Galleria mellonella*, a species where the stage of gut replacement can be precisely determined in vivo (Johnston et al., 2019). This study revealed that pupal gut delamination coincides with peak immune gene expression in the gut. This is consistent with other observations in other holometabolous insects with lower temporal resolution (Johnston et al., 2019; Russell & Dunn, 1991; Xu et al., 2012). In contrast, no such effect was observed for the hemimetabolous *Gryllus bimaculatus* (Johnston et al., 2019). The upregulation of immune genes during gut renewal is therefore a candidate mechanism contributing to the patterns reported in our study. In principle, the insect host can establish a completely new and distinct adult gut microbiome by this reduction of the gut microbiota and a subsequent change in diet of the emerging adult. A different diet will expose the insects to new microbes that can colonize the gut and potentially also facilitate better digestion of the new diet.

Alpha diversity did not display a pattern related to holo- versus hemimetabolous development. Complete metamorphosis results in a reduction of the microbial absolute abundance by orders of magnitude (Johnston & Rolff, 2015, and references therein) which can be recovered in the adults. In the light of Hammer et al. (2014) this could be explained by a recovery of the microbiota upon adult feeding. They reported that the richness of the microbiota was recovered in the feeding adults of Helioconus, although the composition had changed, strongly suggesting that the new members of the microbiota have been acquired from the diet. Therefore, the gut microbiota is shaped by a niche modification, in which early arriving species change the types of niches available within the local sites (Fukami, 2015).

A simple explanation for the observed higher microbiota turnover in holometabolous insects could be that a niche shift results in the exposition to a different environmental microbiota. This though would also apply to other species without complete metamorphosis. Alternatively, it has been suggested that microbiota turnover during host metamorphosis, which could be caused by the host immune system (Johnston et al., 2019; Johnston & Rolff, 2015), would allow insects to occupy different niches throughout development (Hammer & Moran, 2019), which most probably contributed to the success of holometabolous insects. Our data are consistent with this hypothesis, the clearance of the gut provides the opportunity for a microbiota turnover, an effect not observed in hemimetabolous insects. It seems possible that this observation is directly related to the decoupling hypothesis, which proposes that growth is confined to the larval stage, while most differentiation occurs in the pupa (Arendt, 1997; Rolff et al., 2019). A facilitation of niche shifts by changes in the gut microbiota, if confirmed by experimental studies, could be considered as an important driver of the evolution of complete metamorphosis. Alternatively, the advent of complete metamorphosis, driven by other selective factors such as growth rate (Rolff et al., 2019), facilitated the gut microbiota turnover.

AUTHOR CONTRIBUTIONS

Christin Manthey, Paul R. Johnston and Jens Rolff conceived the study. Christin Manthey obtained the samples. Christin Manthey, Paul R. Johnston and Shinichi Nakagawa analysed the data. Christin Manthey wrote the manuscript, and all authors contributed to editing the manuscript.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

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