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Microplastic ingestion perturbs the microbiome of *Aedes albopictus* (Diptera: Culicidae) and *Aedes aegypti*

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Microplastics (MPs) are common environmental pollutants; however, little is known about their effects after ingestion by insects. Here we fed *Aedes* (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* (Skuse) mosquito larvae 1 µm polystyrene MPs and examined the impacts of ingestion on adult emergence rates, gut damage, and fungal and bacterial microbiota. Results show that MPs accumulate in the larval guts, resulting in gut damage. However, little impact on adult emergence rates was observed. MPs are also found in adult guts postemergence from the pupal stage, and adults expel MPs in their frass after obtaining sugar meals. Moreover, MPs effects on insect microbiomes need to be better defined. To address this knowledge gap, we investigated the relationship between MP ingestion and the microbial communities in *Ae. albopictus* and *Ae. aegypti*. The microbiota composition was altered by the ingestion of increasing concentrations of MPs. Amplicon sequence variants (ASVs) that contributed to differences in the bacterial and fungal microbiota composition between MP treatments were from the genera *Elizabethkingia* and *Aspergillus*, respectively. Furthermore, a decrease in the alpha diversity of the fungal and bacterial microbiota was observed in treatments where larvae ingested MPs. These results highlight the potential for the bacterial and fungal constituents in the mosquito microbiome to respond differently to the ingestion of MPs. Based on our findings and the effects of MP ingestion on the mosquito host micro- and mycobiome, MP pollution could impact the vector competence of important mosquito-transmitted viruses and parasites that cause human and animal diseases.

Key words: pollution, microbiota, microplastic ingestion

Introduction

Plastic pollution remains one of the most ubiquitous and menacing environmental threats worldwide. While larger plastic particles cause concerns when ingested by animals, little is known about the effects of much smaller microplastics (MPs) and nanoplastics (NPs), which can be produced intentionally for abrasion applications or formed as degradation products via weathering and degradation of larger plastics by sunlight, abrasion, and tire wear (Zhang et al. 2021, Wu et al. 2022, Liu et al. 2023). MPs and NPs are polymer particles, which range in size from 5 mm to 1 µm in diameter and 1–0.001 µm, respectively (Wang et al. 2021b). These plastic particles of a few microns in size or less are difficult to characterize and remove from the environment and are easily ingested by animals, particularly invertebrates. Recently, it was demonstrated MPs up to 2 µm in size can be ingested by mosquito larvae and transferred ontogenically from the larval stage to the adult stage (Al-Jaibachi et al. 2019). In addition, *Culex tritaeniorhynchus* and *Aedes aegypti* were collected from household drainage pits and examined for the presence of MPs in their guts and other tissues. Both species were found to ingest MPs, and particle size analyses demonstrated 42% of MPs found in the mosquitoes were less than 5 µm in size, and the remaining MPs were ≥5 µm (Gopinath et al. 2022). Container-inhabiting mosquito species such as *Ae. albopictus* and *Ae. aegypti* are vectors of important disease-causing viruses such as dengue, Zika, and chikungunya

and live and breed in small artificial containers such as discarded tires, food containers, soda/water bottles, and plastic flowerpots, particularly in highly urbanized areas (Champion and Vitek 2014). These containers are weathered by a number of chemical, mechanical, and biological factors resulting in their breakdown and release of MPs of different shapes, sizes, and source materials (Duis and Coors 2016, Fadare et al. 2020). Based on the indiscriminate filter-feeding behaviors of *Aedes* mosquito larvae, the ingestion of MPs is likely a common occurrence in container habitats (Merritt et al. 1992).

The ingestion of MPs has been demonstrated to alter the gut microbiota of honeybees, collembola, and other soil-dwelling invertebrates resulting in gut dysbiosis (Zhu et al. 2018, Fackelmann and Sommer 2019, Wang et al. 2021a). Cui et al. (2022) demonstrated the transfer of MPs from the mosquito *Culex quinquefasciatus* to the wolf spider *Paradosa pseudoannulata* via predation. Ingestion of *C. quinquefasciatus* adults that had been fed MPs as larvae by *P. pseudoannulata* altered the intestinal bacterial composition, caused gut damage, impacted growth and development, and caused behavioral changes in the spiders (Cui et al. 2022). While the mechanism(s) of how MP ingestion may be impacting microbiota in arthropods is not known, one hypothesis is that MPs could be impacting the microbiome directly by providing additional carbon to the gut microenvironment as well as increased surface area for microbial and pathogen growth and distribution (Lear et al. 2021). Additionally, changes in the bioavailability of nutrients due to the presence of plastic leachate can impact bacterial growth (Sheridan et al. 2022). As plastics degrade in the environment or experience environmental weathering, dissolved organic matter or carbon is produced, providing energy for microbial growth (Sheridan et al. 2022). As carbon balance is highly regulated in ecosystems, carbon inputs can interfere with homeostasis. Also, commercial plastic is often treated with additives and plasticizers to enhance its durability and longevity, which can leach into the surrounding environment under normal weathering conditions, salinity, turbulence, and UV radiation (Sheridan et al. 2022). These additives and plasticizers could play a part in altering the microbiome composition in the insect gut and somatic tissues by inhibiting or promoting bacterial growth. Additionally, MPs can also act as a sink for organic and inorganic pollutants that may result in toxic effects due to bioaccumulation, which could also impact microbiota composition (Menendez-Pedriza and Jaumot 2020, Song et al. 2022).

Understanding the impacts of MP ingestion on the mosquito microbiome is important because the composition and, subsequently the interactions of microbes that reside within a mosquito host can influence the host phenotype, including impacts on the vector competence of pathogens (Carissimo et al. 2015, Gao et al. 2020, Gabrieli et al. 2021). Previous studies of bacterial microbiomes in mosquitoes have demonstrated that the microbiome can be highly variable yet contain a low diversity of bacteria (Charan et al. 2013). The observed variability appears to be the result of the influence of multiple intrinsic and extrinsic factors, including environmental influences, diet, species, sex, host genetics, development stage, and contaminants or antibiotic exposure (Novakova et al. 2017, Short et al. 2017, Cansado-Utrilla et al. 2021). This observed variability can also impact pathogen transmission. The bacterial microbiome in *Anopheles* mosquitoes has been observed to affect *Plasmodium* infection (Dennison et al. 2014, Romoli and Gendrin 2018, Martinez-de la Puente et al. 2020) and also has been demonstrated to impact arboviral vector competence (Novakova et al. 2017, Cansado-Utrilla et al. 2021). Previous studies examining the effects of the microbiome on vector competence have used antibiotics to disrupt or eliminate bacteria members of the microbiome. The resulting dysbiosis is enough to result in alterations in vector competence. For example, when *Ae. aegypti* was provided antibiotics and dengue virus (DENV) virus titers decreased (Xi et al. 2008). Similarly, when bacterial isolates of *Chomobacterium*, *Proteus*, and *Paenibacillus* were reintroduced into mosquitoes, DENV replication was inhibited. In contrast, bacteria isolates such as *Serratia odorifera* have been demonstrated to increase DENV and chikungunya virus infection rates (Apte-Deshpande et al. 2012, 2014). Other studies have examined different *Ae. aegypti* mosquito lines with different microbiota profiles, all of which demonstrated a varying level of DENV susceptibility (Charan et al. 2013, Hill et al. 2014). While many studies of mosquito-associated microbiota have focused on bacteria, fungal microbial partners have been largely neglected (Guegan et al. 2018). Mosquito fungal communities (mycobiota) are mainly composed of Ascomycota (yeasts) and are an important component of the microbiome as they represent an average of 19–47% of the mosquito microbiota, suggesting the need to investigate multiple components of mosquito microbiomes and how changes to the microbiome can impact insect host phenotypes (Muturi et al. 2016, Luis et al. 2019).

To investigate the effects of MP ingestion on the *Ae. aegypti* and *Ae. albopictus* fitness and microbiome composition, we fed larvae of both species 1 µm fluorescent tagged polystyrene beads at different concentrations in glass beakers and examined for gut damage, changes in adult emergence rates, and bacterial and fungal microbiome changes. We hypothesized that due to the indiscriminate feeding behavior of *Ae. aegypti* and *Ae. albopictus* in small containers, these species would ingest MPs as larvae, which would remain in the gut before subsequent egestion, resulting in gut damage and a perturbation of the adult fungal and bacterial microbiome. Our results indicate that exposure to MPs during larval development is associated with changes in the bacterial microbiota and fungal mycobiota of *Ae. aegypti* and *Ae. albopictus* adults.

Materials and Methods

Mosquito Rearing and MP Ingestion Experiments

Aedes albopictus and *Ae. aegypti* used for MP feeding experiments originated from a lab-acclimated colony started from eggs collected in Lubbock, TX, USA, and the Rockefeller strain obtained from BEI resources, respectively. Both species were reared in incubators held at 28 ± 2 °C, 80 ± 5% RH, and a 16:8 light–dark cycle. Larvae were fed a 60 g/liter bovine liver powder (MP Biomedicals, Santa Ana, CA) slurry ad libitum. Glass containers were utilized for all food and rearing supplies to avoid the risk of unintended microplastic exposure. Adults were provided a 10% sucrose solution. Adult females were fed heated bovine blood using an artificial blood-feeding apparatus. All eggs were collected in a 140 ml cup lined with seed germination paper (Anchor Paper Company, St. Paul, MN, USA) submerged in an oviposition attractant composed of 1:1 fermented water:DI water. All eggs were allowed to mature for at least five days prior to hatching and hatched in a 1:1 fermented water:DI water seeded with an inoculate from larval habitat collected in Lubbock, TX, USA. The same inoculate water source was used for all larval rearing. To setup MP feeding experiments, 30 first-instar larvae were transferred to a 500-ml glass beaker containing 200 ml of distilled water and different concentrations (0, 100, 10,000, and 100,000 MP/ml) of 1 µm polystyrene carboxylate-modified fluorescent polystyrene beads, and 1 ml of 60 g/liter liver powder slurry (Sigma Item #L4655) using a glass pipettor (Fig. 1a). MP concentrations were chosen based on previous studies, and with a realistic

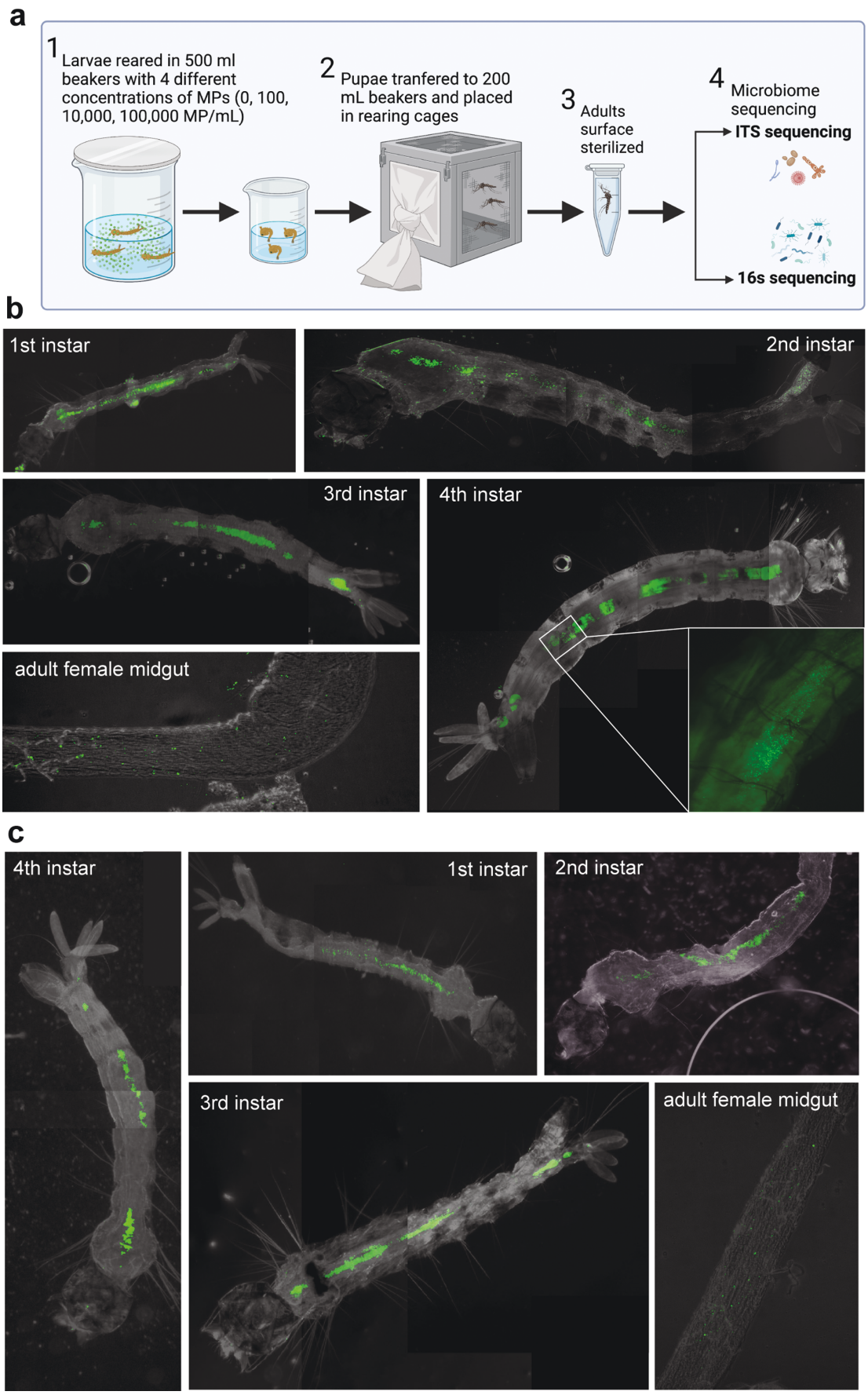

Fig. 1. a) Experimental design to examine the effects of MP ingestion on the adult mosquito microbiome. Created with BioRender.com, b) images of *Ae. albopictus* larvae (first–fourth instar) and adult female midgut dissections demonstrating the presence of 1 µm MP beads in the insect gut, and c) images of *Ae. aegypti* larvae (first–fourth instar) and adult female midgut dissections demonstrating the presence of 1 µm MP beads in the insect gut. All images of larvae and adult females are from the 10,000 MP/ml treatment.

concentration to what mosquitoes may encounter in natural and artificial containers (100 MPs/ml) and higher concentrations (10,000 and 100,000 MPs/ml) similar to what mosquitoes may encounter as an extreme biological stressor (Al-Jaibachi et al. 2019, Thormeyer and Tseng 2023). Each treatment consisted of three replicates. MP concentrations were determined by using a hemocytometer and a DM5 inverted microscope with an integrated camera and GFP filter (Leica Microsystems, Wetzlar, Germany). After the first sign of pupation, beakers were checked every 24 h for pupae, and pupae were subsequently transferred to a 150 ml glass beaker containing 50 ml of distilled water and placed into a 14 cm^3 Bugdorm insect rearing cage (MegaView Science Co., Taichung, Taiwan). After all adults had emerged, the contents of the cage were aspirated and anesthetized on wet ice to determine their sex and calculate adult emergence rates. Adults were then processed for 16s and ITS sequencing by freezing for approximately 10 min at –20 °C and surface sterilized using successive washes of 70% ethanol, 10% sodium hypochlorite, and sterile water and subsequently preserved in DNA/RNA shield (Zymo Research, Irvine, CA). DNA was isolated from a total of 40 *Ae. albopictus* and 24 *Ae. aegypti* adult mosquitoes as a random sample from each replicate treatment using a ZymoBIOMICS–96 MagBead DNA kit (Zymo Research, Irvine, CA).

MP Characterization

The morphologies of the 1 µm polystyrene carboxylate-modified fluorescent polystyrene beads were observed with an FEI Quanta 600 field-emissionSEM. For imaging, the MP dispersion was vigorously mixed and drop cast on a silicon wafer and letting it dry in a vacuum overnight. The acceleration voltage used for the imaging was 20 kV (Supplementary Figure 1). XRD patterns of the MPs were obtained using Bruker D8 powder X-ray diffractometer fitted with LynxEye detector, in a Bragg Brentano geometry with CuKa (l = 1.5418 A) radiation source. The XRD was performed with a scan rate of 1.5 s per step. The MP particle dispersion was deposited on the glass slide via drop-casting deposition and left to dry overnight.

Imaging of Immatures and Adult Guts

Larval ingestion and the presence of MPs in adult guts were observed using a DM3500 inverted microscope (Leica microsystems, Wetzlar, Germany) and GFP filter and pE-300 ultraviolet (UV) light (CoolLED, London, England) with a DFC3000-G (Leica microsystems, Wetzlar, Germany) attached camera. Multiple normal light and UV images of larvae were taken at 50× and merged into one image using Photoshop (Adobe, Mountain View, CA, USA). Twenty of each larval instar from each MP treatment type were examined for MPs in the gut using microscopy.

Gut Damage

In a separate experiment, 500-ml glass beakers containing 0, 100, 10,000, and 100,000 MP/ml, 30 *Ae. aegypti* or *Ae. albopictus* first-instar larvae and 200 ml of DI water were set up as described in the mosquito rearing and MP experiments methods section. Immediately after introducing the larvae to the beakers, 300 µl of trypan blue was added to each beaker. Trypan blue stain is commonly used to determine between viable cells and cells with damaged membranes. Larvae were then removed from the trypan blue solution as early fourth instars and placed in 200 ml of DI water with 10 drops of 60 g/liter liver powder slurry for approximately 12 h. The larvae were then removed and placed on a microscope slide for imaging using an S9i stereo microscope with an integrated camera (Leica Microsystems, Wetzlar, Germany). Ten larvae from each treatment were imaged to show similar patterns of gut damage.

16s and ITS Sequencing

Illumina high-throughput sequencing of 16S rRNA and ITS gene amplicons was used to examine the bacterial and fungal community composition and diversity of *Ae. albopictus* and *Ae. aegypti* samples. Bacterial 16S ribosomal RNA gene-targeted sequencing was performed using the *Quick*-16S NGS Library Prep Kit (Zymo Research, Irvine, CA) using the bacterial 16S primer mix, which amplified the V3–V4 region of the 16S rRNA gene. These primers have been custom-designed by Zymo Research to provide the best coverage of the 16S gene while maintaining high sensitivity (Supplementary Table 1). Fungal ITS gene targeted sequencing was performed using the *Quick*-16S NGS Library Prep Kit with custom ITS2 primers substituted for 16S primers (ITS primers: ITS3f—5′-GCATCGATGAAGAACGCAGC-3′; ITS4r—5′-TCCTCCGCTTATTGATATGC-3′). The ZymoBIOMICS Microbial Community Standard (Zymo Research, Irvine, CA) was used as a positive control for each DNA extraction and used as a positive control for each targeted library preparation. Negative controls (i.e., blank extraction control) were also included to control for the library preparation and contamination during the extraction process. The sequencing library was prepared using a library preparation process in which PCR reactions were performed in real-time PCR instruments to control cycles and therefore limit PCR chimera formation. The final PCR products were quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned with the Select-a-Size DNA Clean & Concentrator (Zymo Research, Irvine, CA), then quantified with TapeStation (Agilent Technologies, Santa Clara, CA) and Qubit (Thermo Fisher Scientific, Waltham, WA). The ZymoBIOMICS Microbial Community Standard (Zymo Research, Irvine, CA) was used as a positive control for each DNA extraction and each targeted library preparation. Negative controls (i.e., blank extraction control, blank library preparation control) were included to assess the level of bioburden carried by the wet-lab process. The final library was sequenced on Illumina MiSeq with a v3 reagent kit (600 cycles). The sequencing was performed with a 10% PhiX spike-in.

Data Analysis

Differences in adult emergence rates were determined using a nonparametric Kruskal–Wallis test for multiple comparisons, and pairwise comparisons of adult emergence rates were conducted using Bonferroni corrected Wilcoxon tests with a P value of 0.01 using JMP Pro16 (SAS, Cary, NC, USA). Unique amplicon sequence variants (ASVs) were inferred from raw reads using the DADA2 pipeline (Callahan et al. 2016). Potential sequencing errors and chimeric sequences were also removed with the DADA2 pipeline. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1 with the Zymo Research Database, a 16S and ITS database that is internally designed and curated, as a reference. Database details and access can be requested by contacting Zymo Research. Composition visualization, alpha-diversity, and beta-diversity analyses were performed with Qiime v.1.9.1 (Caporaso et al. 2010). Differences in Simpson and Shannon indices between treatments were determined using an ANOVA in JMP Pro16 (SAS, Cary, NC, USA). If applicable, taxa with significant abundance among different groups were identified by LEfSe (Segata et al. 2011) using default settings. Other analyses, such as Taxa2ASV Decomposer and PCoA plots, were performed with internal Zymo scripts. Composition stacked bar plots based on

proportions of ASVs were composed using the ggplot2 package in R. Venn diagrams were composed using the R package VennDiagram. Multivariate analysis of similarity (ANOSIM) was used to measure Bray–Curtis dissimilarity and corresponding PCoA plots were generated using R to identify whether MP ingestion had an effect on microbial communities (Clark 1993).

Results

MP Ingestion in Immature and Adult *Ae. albopictus* and *Ae. aegypti*

Scanning electron microscope (SEM) images showed a spherical morphology, and we determined the size of the MPs to be 0.99 ± 0.04 µm in diameter (Supplementary Fig. 1) (Hwang et al. 2020, Zong et al. 2021, Donkers et al. 2022). In X-ray diffraction analysis (XRD), patterns revealed the broad peak around 20° showing the amorphous nature of MPs, consistent with polystyrene-based MPs (Sudha and Sivakala 2009, Zong et al. 2021). Ingestion and internalization of the labeled 1 µm MPs were observed in all 20 (20/20) samples *Ae. aegypti* and *Ae. albopictus* examined for each larval stage (Fig. 1b and c). In addition, MPs were also found in adult male (8/8 adults examined had MPs) and female (10/10 adults examined had MPs) guts of both species suggesting some carry over to adults. In these experiments, no significant differences were observed in adult emergence rates in *Ae. albopictus* and *Ae. aegypti* (Kruskal–Wallis chi-square = 1.58, df = 3, P = 0.66; *Ae. aegypti*, Kruskal–Wallis chi-square = 7.03, df = 3, P = 0.07) (Fig. 2a and b). We also collected frass from adult *Ae. aegypti* and *Ae. albopictus* to examine for the fate of MPs after ingestion by larvae. Results suggest some MPs remain in the adult gut for up to 4 days, but a majority of the MPs are expelled in the frass when mosquitoes feed on sucrose (Fig. 2c–f). In a separate experiment, *Ae. albopictus* and *Ae. aegypti* larvae (8/10 – 100 MP/ml, 10/10, 10,000 MP/ml, and 9/10 MP/ml) show staining in their guts, suggesting MP ingestion may be associated with gut damage (Fig. 2g and h).

MP Ingestion Effects on the Bacterial Microbiome

After 16s rRNA sequencing and quality filtering, a total of 3,120,599 qualified paired-end reads with an average count per sample of 48,759 reads were divided into 379 bacteria ASVs (Supplementary File 1). Any ASVs accounting for <2% of the relative abundance of reads were excluded from the analyses (Supplementary File 2). No contamination was observed in the blank DNA extraction controls in samples used for library preparation. Rarefaction curves indicated that mosquito samples sequenced were sequenced to sufficient depth (Supplementary Fig. 2).

Alpha diversity of bacterial communities for *Ae. albopictus* was assessed using the Shannon and Simpson indices at the species level. There were no observed differences in Shannon and Simpson diversity of bacterial taxa assessed at the species level for males or females (ANOVA, Simpson; males, $F_{3,16}$ = 0.8, P = 0.51, females $F_{3,16}$ = 0.51, P = 0.8, Shannon; males, $F_{3,16}$ = 1.4, P = 0.3, females $F_{3,16}$ = 0.42, P = 0.7) (Fig. 3a). The bacterial microbiome of *Ae. albopictus* and *Ae. aegypti* samples sequenced were dominated by alphaproteobacteria and gammaproteobacteria. The most common ASV found in *Ae. albopictus* was *Wolbachia pipientis* accounting for >47% of all ASVs in male and female treatments (Fig. 3b and c) (Supplementary File 2). Second to *Wolbachia* in terms of ASV abundance was *Elizabethkingia* (Fig. 3b and c). A higher proportion of ASVs from the genus *Elizabethkingia* was observed in the MP treatments for females (Control—5%, 100 MP/ml—28%, 10,000 MP/ml—6%, and 100,000 MP/ml—20%) and males (Control—7%, 100 MP/ml—3%, 10,000 MP/ml—7%, and 100,000 MP/ml—11%). The high abundance of *Wolbachia* and *Elizabethkingia* ASVs suggests that these taxa may be driving the observed diversity profiles detected. Other bacterial genera, including *Pseudomonas*, *Staphylococcus*, *Undibacterium*, *Propionibacterium*, *Serratia*, *Siphonobacter*, *Propionibacterium*, *Rothia*, and *Gloeocalita* did not change in relative abundance when comparing different MP treatments (Fig. 3c).

Beta diversity of bacterial communities was assessed using multidimensional scaling (MDS) of Bray–Curtis dissimilarity performed at the genus level (Fig. 3d). Data suggests there is an overlap in the characteristic bacterial community between all treatments in the females (ANOSIM, R = 0.34, $P \geq 0.05$). The characteristic bacterial community between all treatments in the males suggested there is less similarity between the MP treatments and the untreated control treatments (ANOSIM, R = 0.69, $P \leq 0.05$). Beta diversity was further characterized by the assessment of shared species level ASVs between treatment groups (Fig. 3e), revealing that 64% and 57% of the total bacterial species level ASVs were unique to each treatment group for females and males, respectively. Conversely, only 20% and 19% were shared amongst all four treatment groups for females and males, respectively. The relative abundance of unique bacterial ASVs for females was notably higher in the control and 10,000 MP/ml groups, at 31% and 29%, compared to 3% and 1% in the 100 and 100,000 MP/ml treatment groups (Fig. 3e). The relative abundance of unique bacterial ASVs for males was notably higher in the control and 100 MP/ml groups, at 11% and 43%, compared to 2% and 0% in the 10,000 and 100,000 MP/ml treatment groups (Fig. 3e).

Alpha diversity of bacterial communities for *Ae. aegypti* was assessed using the Shannon and Simpson indices at the species level. There were no observed overall differences in Shannon and Simpson diversity of bacterial taxa between treatments assessed at the species level for males or females (ANOVA, Simpson; males, $F_{3,8}$ = 1.8, P = 0.2, females $F_{3,8}$ = 1.7, P = 0.2, Shannon; males, $F_{3,8}$ = 2.4, P = 0.1, females $F_{3,8}$ = 1.6, P = 0.3) (Fig. 4a). However, a higher diversity of bacteria was observed in the 100,000 and 10,000 MP/ml treatments when compared to the untreated control group in females and lower diversity in males (Bonferroni corrected, Tukey–Kramer post hoc tests [$P \leq 0.0$]) (Fig. 4a).

The most common ASV found in *Ae. aegypti* samples was *Elizabethkingia meningoseptica* accounting for 39.3 ± 21% (mean ± STDV) of all ASVs in male and female treatments (Fig. 4b and c) (Supplementary File 2). A higher proportion of ASVs from the genus *Elizabethkingia* was observed in the MP treatments for females(Control—0.2%, 100 MP/ml – 73.5%, 10,000 MP/ml—42.8%, and 100,000 MP/ml—36.9%) and males (Control—19.1%, 100 MP/ml—52.7%, 10,000 MP/ml—43.4%, and 100,000 MP/ml—45.8%). A higher proportion of ASVs from the genus *Filimonas* was observed in untreated controls compared to MP treatments in females (Control—8.9%, 100 MP/ml—0%, 10,000 MP/ml—1.4%, and 100,000 MP/ml—0.2%) and males (Control—11%, 100 MP/ml—0.5%, 10,000 MP/ml—0.2%, and 100,000 MP/ml—0%) (Fig. 4b and c). Other bacterial genera did not change in relative abundance when comparing different MP treatments (Fig. 4c).

Beta diversity of bacterial communities for *Ae. aegypti* analyses suggest there is a difference in the characteristic bacterial community between all the treatments in females and males (females, ANOSIM, R = 0.84, $P \leq 0.05$; males, ANOSIM, R = 0.65, $P \leq 0.05$). Beta diversity was further characterized by assessment of shared species level ASVs between treatment groups (Fig. 4d), revealing that 62% and 35% of the total bacterial species level ASVs were unique to

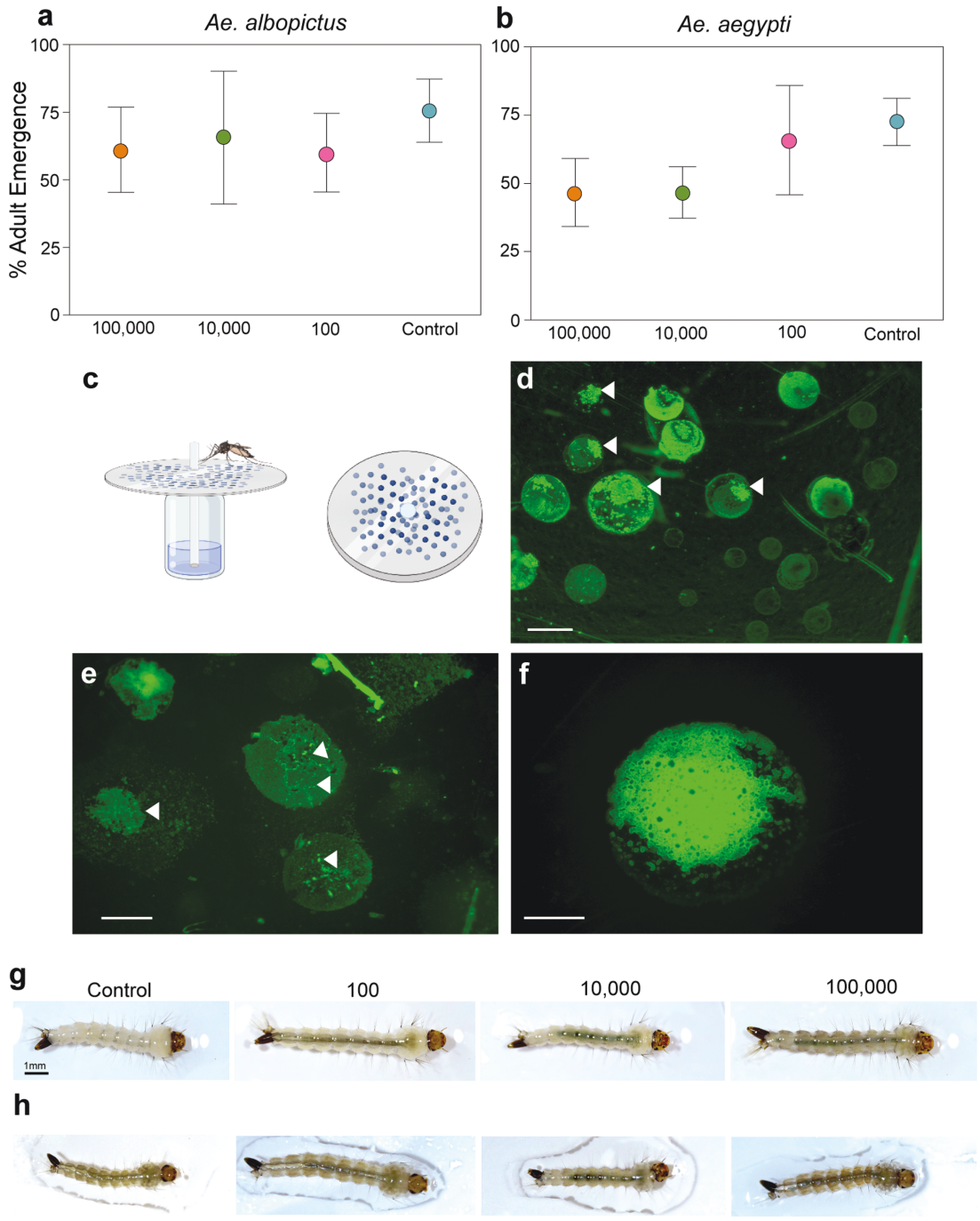

Fig. 2. a) Adult emergence rates of *Ae. albopictus,* and b) *Ae. aegypti* when exposed as larvae to 100,000, 10,000, 100 MP/ml, and untreated controls. All measurements are shown as mean ± SE and are based on three replicates of each treatment type (30 mosquito larvae per treatment). No difference in adult emergence rates were observed using a Bonferroni corrected Wilcoxon pairwise tests ($P \geq 0.01$), c) frass collection device composed of a 50 ml glass scintillation vial filled with 10% sucrose and blue food coloring with a cotton dental wick and transparent plastic disk to collect deposited mosquito frass after sugar feeding for subsequent fluorescent imaging, d) 50×, e) 100×, and f) 200× image of frass droplets under fluorescent light showing the presence of fluorescent-tagged 1 µm MP beads (triangles indicate MP accumulations in the frass) (scale bars represent 0.1 mm), g) images of *Ae. albopictus*, and h) *Ae. aegypti* larvae exposed to trypan blue in rearing water. Staining of the gut cell lining demonstrates gut damage associated with ingesting MPs.

each treatment group for females and males, respectively (Fig. 4e). Conversely, only 13% and 26% were shared amongst all four treatment groups for females and males, respectively (Fig. 4e). The relative abundance of unique bacterial ASVs for females was notably higher in the 10,000 MP/ml and 100,000 MP/ml groups, at 30% and 18%, compared to 8% and 2% in the 100 MP/ml and control treatment groups (Fig. 4e). The relative abundance of unique bacterial ASVs for males was also notably higher in the 10,000 MP/ml and 100,000 MP/ml, at 15% and 11%, compared to 9% and 0% in the 100 MP/ml and control treatment groups (Fig. 4e).

MP Ingestion Effects on the Fungal Mycobiome

After ITS sequencing and quality filtering, a total of 994,941 qualified paired-end reads with an average count per sample of 15,546 reads were divided into 64 fungal ASVs (Supplementary File 3). Any ASVs accounting for <2% of the relative abundance of reads were

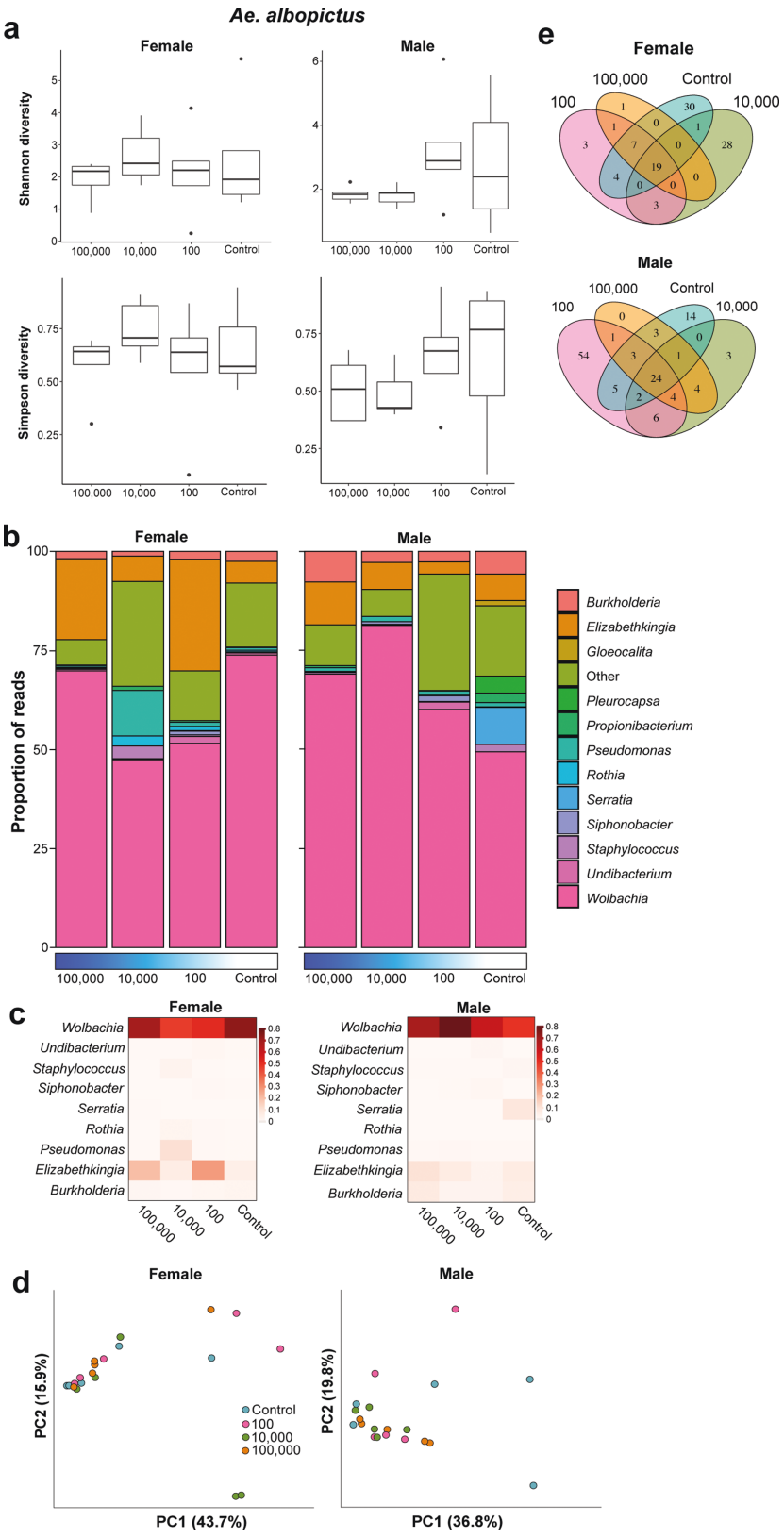

Fig. 3. Analysis of bacteria microbiome diversity over the four MP treatments for *Ae. albopictus* females and males. a) Shannon and Simpson diversity plots used to compare levels of diversity between treatments. Pairwise comparisons between treatment types revealed no significant differences in bacterial diversity (Bonferroni corrected Tukey–Kramer post hoc tests, $P \geq 0.01$), b) mean relative abundance of bacterial taxa in mosquito samples from 4 treatment types (control $n = 10$, 100 MP/ml $n = 10$, 10,000 MP/ml $n = 10$, and 100,000 MP/ml $n = 10$). All ASVs with <2% abundance were grouped into the "other category", c) heat map illustrating the observed changes in abundance of the notable bacterial taxa in *Ae. albopictus* females and males associated with MP ingestion. All values in the heat map are mean ASV abundance for each bacterial genera, d) principal coordinate analysis (PCoA) plot with Bray–Curtis dissimilarity reveals clustering suggesting that the bacterial microbial community is unique between MP treatments and untreated controls for males and females, and e) Venn diagrams of shared groups of bacterial taxa among the four treatment groups.

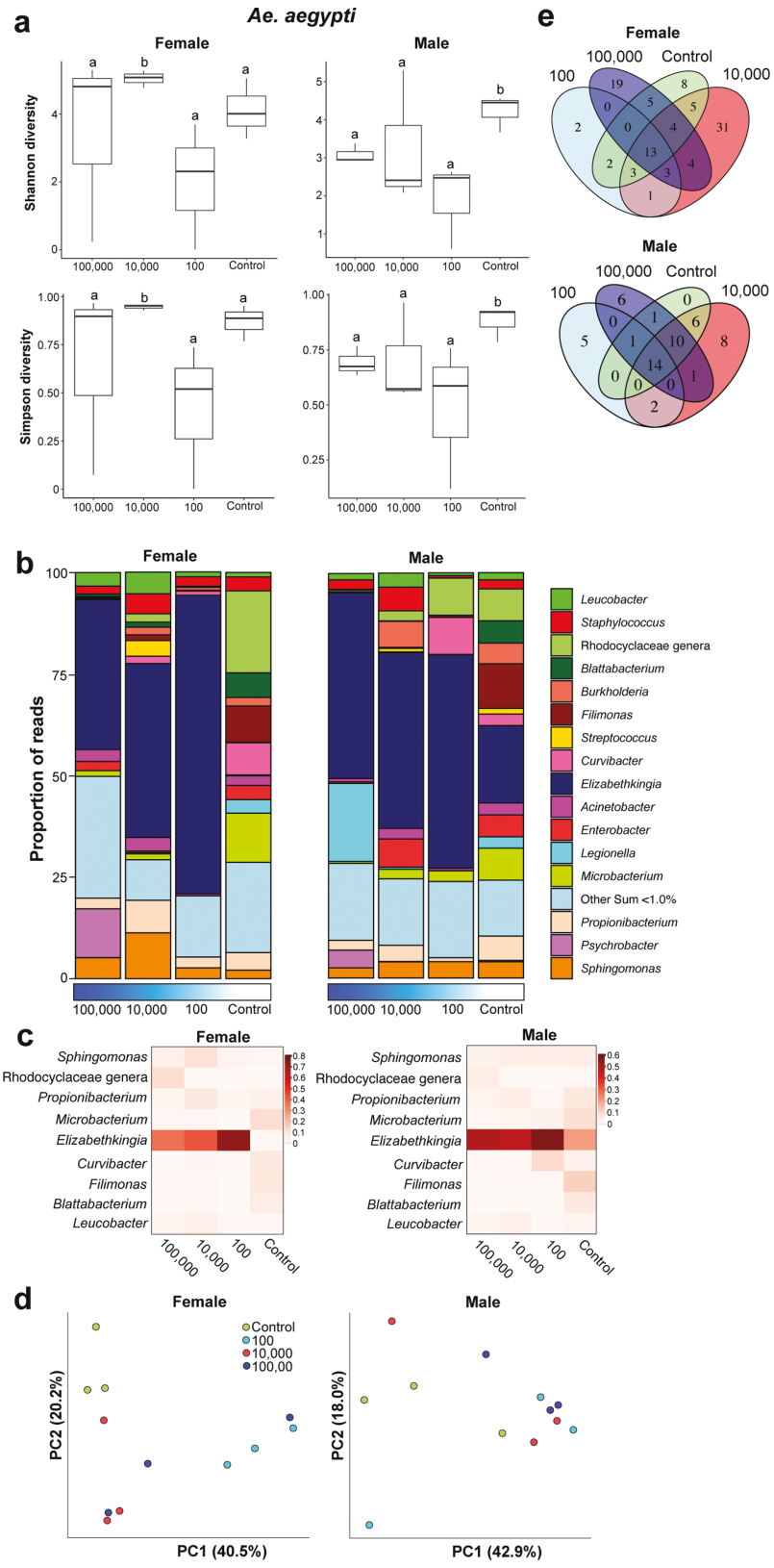

Fig. 4. Analysis of bacteria microbiome diversity over the four MP treatments for *Ae. aegypti* females and males. a) Shannon and Simpson diversity plots were used to compare levels of diversity between treatments. Pairwise comparisons between treatment types revealed significant differences in bacterial diversity in males and females between MP treatments and controls indicated by different letters above each box plot (Bonferroni corrected Tukey–Kramer post hoc tests, $P \leq 0.01$), b) mean relative abundance of bacterial taxa in mosquito samples from the four treatment types (control $n = 6$, 100 MP/ml $n = 6$, 10,000 MP/ml $n = 6$, and 100,000 MP/ml $n = 6$). All ASVs with <2% abundance were grouped into the "other category," c) heat map illustrating the observed changes in abundance of the notable bacterial taxa in *Ae. albopictus* females and males associated with MP ingestion. All values in the heat map are mean ASV abundance for each bacterial genera, d) principal coordinate analysis (PCoA) plot with Bray–Curtis dissimilarity reveals clustering suggesting that the bacterial microbial community is unique between microplastic treatments and untreated controls for males and females, and e) Venn diagrams of shared groups of bacterial taxa among the four treatment groups.

excluded from the analyses (Supplementary File 4). Rarefaction curves indicated that mosquito samples sequenced were sequenced to sufficient depth (Supplementary Figure 3).

Ascomycota and Basidiomycota made up all members of the *Ae. albopictus* fungal mycobiome. For *Ae. albopictus*, there were no overall observed differences in Shannon and Simpson diversity of fungal taxa assessed at the species level for males or females when comparing treatment groups. (ANOVA, Simpson; males, $F_{3,16} = 0.8$, $P = 0.5$, females $F_{3,16} = 0.8$, $P = 0.5$, Shannon; males, $F_{3,16} = 0.7$, $P = 0.6$, females $F_{3,16} = 0.9$, $P = 0.4$) (Fig. 5a). However, a lower diversity of fungi was observed in the 100,000 and 10,000 MP/ml treatments when compared to the untreated control group in males using post hoc pairwise comparisons (Bonferroni corrected, Tukey–Kramer post hoc tests [$P \leq 0.01$]) (Fig. 5a).

The *Ae. albopictus* fungal mycobiome was largely composed of *Alternaria*, *Aspergillus*, *Cladosporium*, *Malassezia*, and *Saccharomyces* genera. The two most common ASVs found in the samples were *Aspergillus* and *Cladosporium,* accounting for 9.5 ± 5% and 10.6 ± 8.1%, respectively (mean ± STDV) of all ASVs in male and female treatments (Fig. 5b and c) (Supplementary File 4). A higher proportion of ASVs from the genus *Cladosporium spp.* was observed in the control treatments for females (Control—9.7%, 100 MP/ml—5.9%, 10,000 MP/ml—1.8%, and 100,000 MP/ml—6.4%) and males (Control—21.5%, 100 MP/ml—24.9%, 10,000 MP/ml—7.8%, and 100,000 MP/ml—7.2%) (Fig. 5b and c). A higher proportion of ASVs from the genus *Aspergillus spp.* was observed in untreated controls compared to MP treatments in females (Control—10.2%, 100 MP/ml—9.0%, 10,000 MP/ml—1.3%, and 100,000 MP/ml—11.8%), but not in males (Control—4.8%, 100 MP/ml—19.2%, 10,000 MP/ml—7.9%, and 100,000 MP/ml—11.7%) (Fig. 5b and c).

Beta diversity of fungal communities was assessed using multidimensional scaling (MDS) of Bray–Curtis dissimilarity performed at the genus level (Fig. 5d). Data suggests there is no significant difference in the characteristic fungal community between all the treatments in females and males (females, ANOSIM, $R = 0.31$, $P \geq 0.05$; males, ANOSIM, $R = 0.36$, $P \geq 0.05$). Beta diversity was further characterized by the assessment of shared species level ASVs between treatment groups (Fig. 5e), revealing that 45% and 76% of the total bacterial species level ASVs were unique to each treatment group for females and males, respectively. Conversely, only 1.8% and 6.7% were shared amongst all four treatment groups for females and males, respectively. The relative abundance of unique fungal ASVs for females was notably higher in the control and 100 MP/ml groups, at 24% and 26%, compared to 19% and 15% in the 10,000 and 100,000 MP/ml treatment groups (Fig. 5e). The relative abundance of unique fungal ASVs for males was similar for all treatment groups (Control—20%, 100 MP/ml—18%, 10,000 MP/ml—16%, and 100,00 MP/ml—22%) (Fig. 5e).

Similar to *Ae. albopictus*, members of Ascomycota and Basidiomycota, made up a majority of the members of the *Ae. aegypti* fungal mycobiome. A total of 64 fungal ASVs were identified in the 24 *Ae. aegypti* samples sequenced. There were no observed differences in Shannon and Simpson diversity of fungal taxa assessed at the species level for males or females (ANOVA, Simpson; males, $F_{3,8} = 0.4$, $P = 0.8$, females $F_{3,8} = 0.5$, $P = 0.7$, Shannon; males, $F_{3,8} = 0.3$, $P = 0.8$, females $F_{3,8} = 0.5$, $P = 0.7$) (Fig. 6a).

The *Ae. aegypti* fungal mycobiome was primarily composed of *Alternaria, Aspergillus, Cladosporium, Malassezia*, and *Saccharomyces* genera. The two most common ASVs found in the samples were *Aspergillus* and *Malassezia,* accounting for 11.1 ± 8.1% and 29 ± 12.2%, respectively (mean ± STDV) of all ASVs in male and female treatments (Fig. 6b and c) (Supplementary File 4). A higher proportion of ASVs from the genus *Malassezia spp.* was observed in the control treatments when compared to MP treatments for males (Control—54.1%, 100 MP/ml – 25.4%, 10,000 MP/ml – 25.4%, and 100,000 MP/ml – 19.4%) but not females(Control – 15.7%, 100 MP/ml – 40.0%, 10,000 MP/ml – 27.0%, and 100,000 MP/ml – 27.2%) (Fig. 6b and c). A higher proportion of ASVs from the genus *Aspergillus spp.* was observed in untreated controls and the 100 MP/ml treatment compared to the 10,000 and 100,000 MP/ml treatments in females (Control – 15.1%, 100 MP/ml – 21.9%, 10,000 MP/ml – 5.4%, and 100,000 MP/ml – 4.3%) and males (Control – 9.1%, 100 MP/ml—23.2%, 10,000 MP/ml – 2.5%, and 100,000 MP/ml – 9.2%) (Fig. 6b and c).

Beta diversity of fungal communities was assessed using multidimensional scaling (MDS) of Bray–Curtis dissimilarity performed at the genus level (Fig. 6d). Data suggests there was a significant difference in the characteristic fungal community between all treatments in females and males (females, ANOSIM, $R = 0.86$, $P \leq 0.05$; males, ANOSIM, $R = 0.71$, $P \leq 0.05$). Beta diversity was further characterized by the assessment of shared species level ASVs between treatment groups (Fig. 6e), revealing that 72% and 83% of the total bacterial species level ASVs were unique to each treatment group for females and males, respectively. Conversely, only 2% and 0% were shared amongst all four treatment groups for females and males, respectively. The relative abundance of unique bacterial ASVs for females was notably higher in the control, 100 MP/ml, and 10,000 MP/ml groups, at 17%, 22%, and 22% compared to 11% in the 100,000 MP/ml treatment group (Fig. 6e). The relative abundance of unique fungal ASVs for males was higher in the control and 100 MP/ml groups at 25% and 27% respectively, compared to the 10,000 and 100,000 MP/ml treatment groups at 15% and 17%, respectively (Fig. 6e).

Discussion

Here we demonstrate that container-inhabiting mosquitoes *Ae. aegypti* and *Ae. albopictus* ingest 1 µm diameter MPs as larvae, and MPs are found in the gut at all larval stages and in low numbers in adult guts. Results suggest larvae egest at least some of the MPs prior to pupation, but they do not expel all of the MPs they ingested as larvae based on the observation that some MPs are still found in the adult gut. The MPs found in the adult gut may imbedded in host gut tissue, and this hypothesis may explain the observed gut damage in mosquito larvae. In contrast, there is the potential these remaining MPs could be residuals left in the gut after the remodeling process, and the remodeling process resulted in MPs being embedded in the newly formed adult gut. Previous studies have demonstrated that gut myco- and microbiota remain in the gut as well as other mosquito anatomical areas, and gut remodeling does not always result in complete microbiome reorganization between the larval and adult stages (Frankel-Bricker et al. 2020, Galeano-Castaneda et al. 2020). Therefore, it is not unrealistic to expect that MP ingestion as larvae may impact the larval as well as the adult microbiome. The results from this study agree with previous studies that have also demonstrated that MPs are found in the adult stage when ingested as larvae (Al-Jaibachi et al. 2019, Gopinath et al. 2022). These MPs in the larval and adult gut could be harboring diverse biofilms and disrupting microbiota and mycobiota homeostasis. The observed compositional variation in the microbiome and mycobiome of *Ae. aegypti* and *Ae. albopictus* within and between treatments could be explained by males or females harboring greater numbers of MPs in the adult stage, or more specifically, the age of the adults

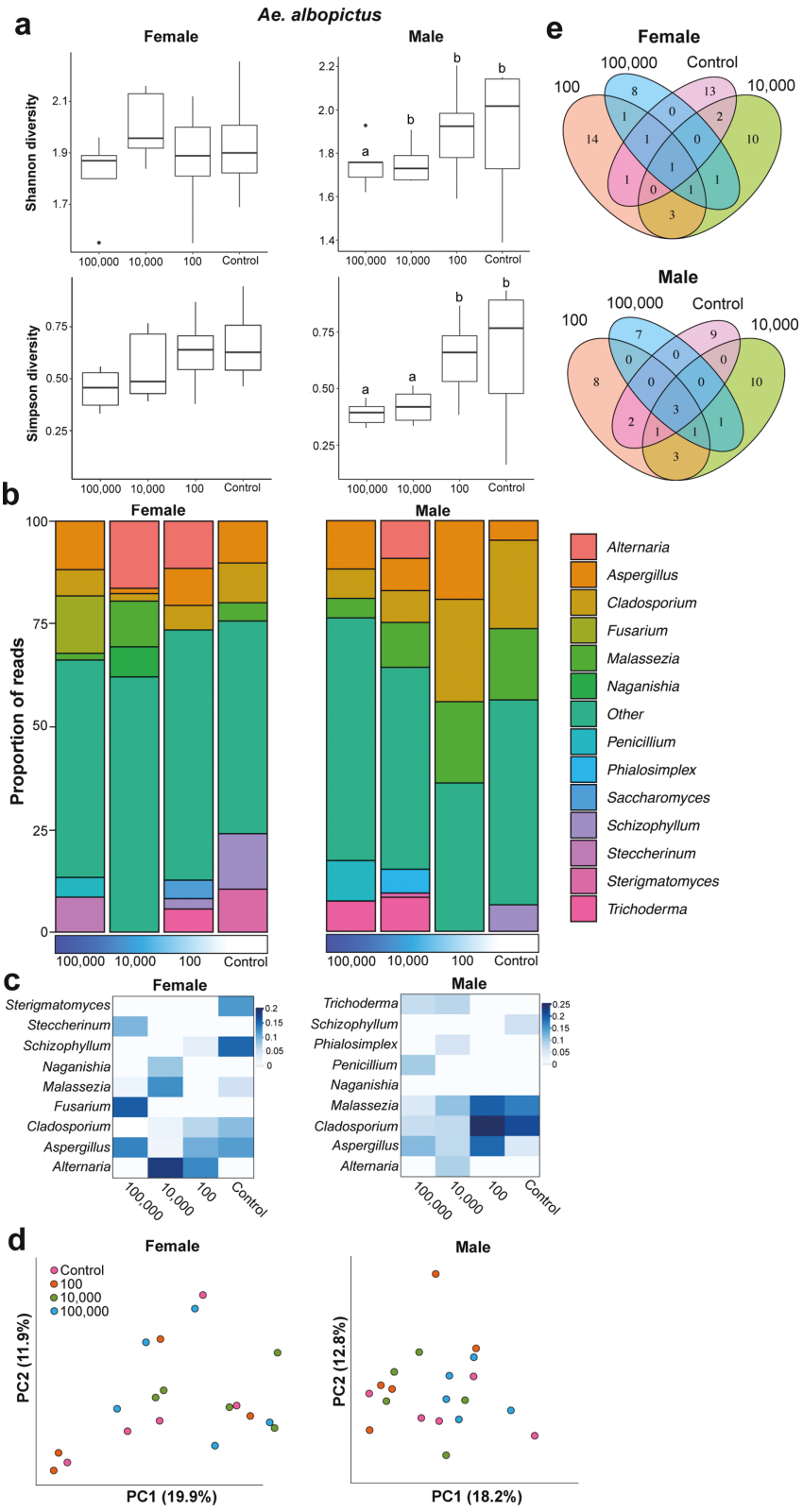

Fig. 5. Analysis of fungal mycobiome diversity over four MP treatments for *Ae. albopictus* females and males. a) Shannon and Simpson diversity plots used to compare levels of diversity between treatments. Pairwise comparisons of fungal alpha diversity between treatment types revealed significant differences between 100,000 and 10,000 MP/ml treatments and 100 MP/ml and control treatments in males indicated by different letters above each box plot (Bonferroni corrected Tukey–Kramer post hoc tests, $P \leq 0.01$), b) mean relative abundance of fungal taxa in mosquito samples from the four treatment types (control $n = 10$, 100 MP/ml $n = 10$, 10,000 MP/ml $n = 10$, and 100,000 MP/ml $n = 10$). All ASVs with <2% abundance were grouped into the "other category", c) heat map illustrating the observed changes in abundance of the notable fungal taxa in *Ae. albopictus* females and males associated with MP ingestion. All values in the heat map are mean ASV abundance for each bacterial genera, d) Principal coordinate analysis (PCoA) plot with Bray–Curtis dissimilarity reveals little clustering suggesting that the fungal microbial community is not unique between MP treatments and untreated controls for males and females, and e) Venn diagrams of shared groups of fungal taxa among the four treatment groups.

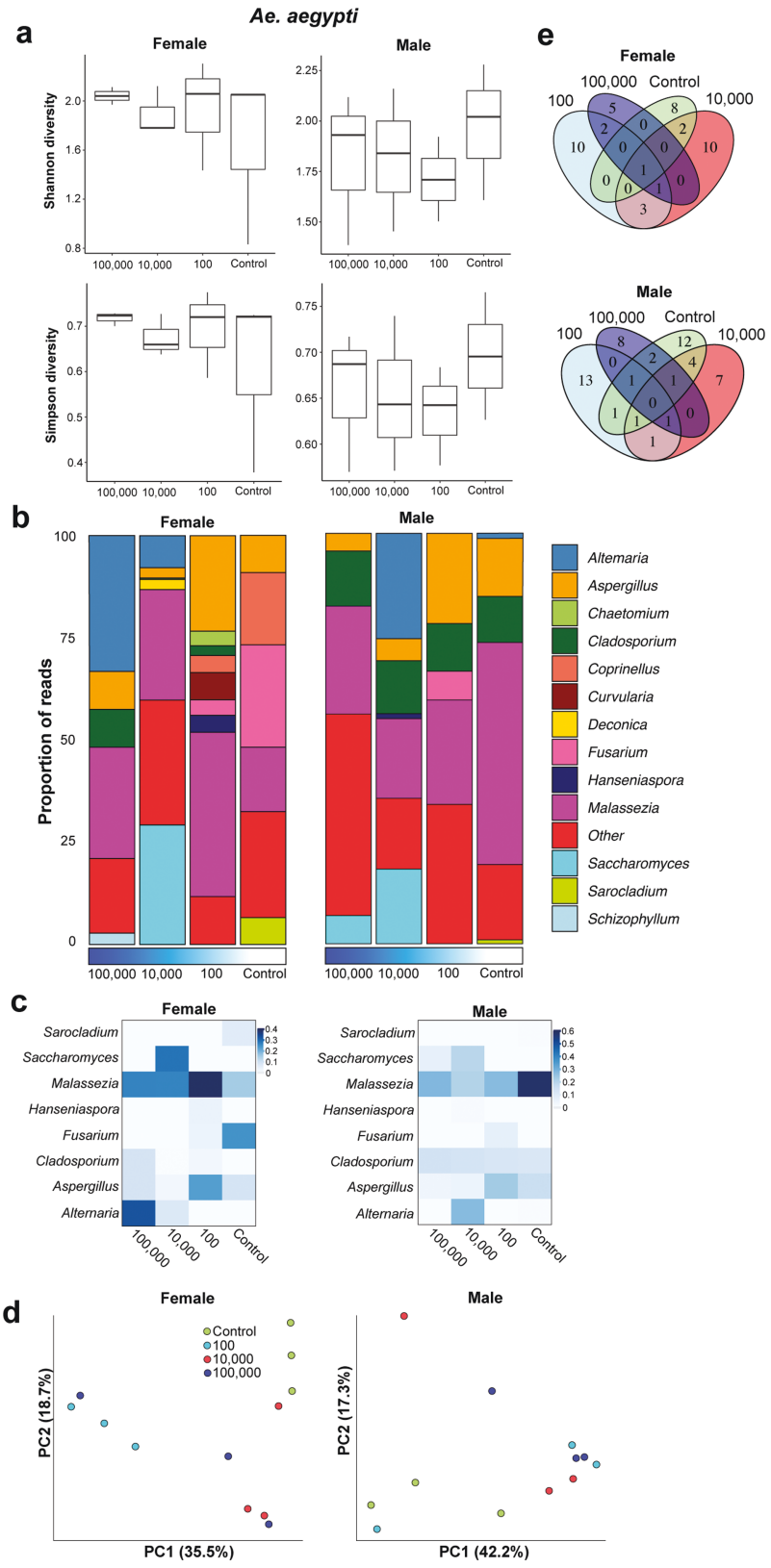

Fig. 6. Analysis of fungal mycobiome diversity over the four treatments for *Ae. aegypti* females and males. a) Shannon and Simpson diversity plots used to compare levels of diversity between treatments. Pairwise comparisons of fungal alpha diversity between treatment types revealed no significant differences (Bonferroni corrected Tukey–Kramer post hoc tests, $P \geq 0.01$), b) mean relative abundance of fungal taxa in mosquito samples from 4 treatment types (control $n = 6$, 100 MP/ml $n = 6$, 10,000 MP/ml $n = 6$, and 100,000 MP/ml $n = 6$). All ASVs with <2% abundance were grouped into the "other category", c) heat map illustrating the observed changes in abundance of the notable fungal taxa in *Ae. aegypti* females and males associated with MP ingestion. All values in the heat map are mean ASV abundance for each bacterial genera, d) principal coordinate analysis (PCoA) plot with Bray–Curtis dissimilarity reveals clustering suggesting that the fungal microbial community is unique between MP treatments and untreated controls for males and females, and e) Venn diagrams of shared groups of fungal taxa among the four treatment groups.

post-emergence (i.e., males typically emerge 24 h before females) could be impacting microbiota composition, particularly if adults are egesting MPs after acquiring a sucrose meal. Further quantification of MPs in adult gut tissues would be needed to confirm this hypothesis. While not examined in this study, it would not be surprising to see MPs in frass samples collected post blood feeding. Similar to previous studies that suggested predation of mosquitoes could be a route of ontogenic transfer to other invertebrates or vertebrates (Al-Jaibachi et al. 2019, Cui et al. 2022), the observation of MPs in frass suggests an additional route of MP translocation to other environments. Since male and female mosquitoes often visit nectar sources for a sugar meal, they could potentially translocate MPs to nectar sources when expelling frass containing MPs after a sugar meal, providing an additional exposure source to other insects or invertebrates that come into contact with excreta. Additionally, females and males could act as environmental transporters for MPs and NPs, potentially transferring MPs to aquatic sites when visiting for oviposition or seeking mates at oviposition locations.

In this study, we did not see any significant effects on adult emergence rates after ingestion of MPs, which may suggest there are limited effects of MPs on adult mosquitoes. This result agrees with previous studies that show no effect of MP ingestion as larvae on adult mosquito survivorship (Al-Jaibachi et al. 2019, Gopinath et al. 2022). From imaging, lower amounts of MPs compared to what we observed in the larvae guts remained in the adult gut, which may be correlated with the observed minor MP effects on adult emergence. However, further experimentation is needed to investigate the effects of MP ingestion on other adult life history traits (e.g., survivorship, longevity, fecundity, and egg hatch rates). Most likely, the effects of MP ingestion originate in the immature stages, where MP concentrations are highest in the gut. When larvae were fed MPs and reared in water with trypan blue, there was an observed increase in cell staining, suggesting cellular damage associated with exposure to increasing concentrations of MPs in *Ae. aegypti* and *Ae. albopictus* larvae. Similar to our results, Cui et al. 2022 (2022) observed damage to the gut tissue of *Parados pseudoannulata* (Wolf spiders) after ingesting adult mosquitoes that fed on up to 1,000 NPs/ml as larvae.

Our results suggest that the ingestion of MPs as larvae perturbs the microbiome of *Ae. aegypti* and *Ae. albopictus*. In the higher MP concentration treatments for both *Ae. aegypti* and *Ae. albopictus* males, the diversity of the bacterial microbiota was generally lower than the untreated controls. Microbiota composition changes were also observed when comparing MP treatments to controls in *Ae. aegypti* and *Ae. albopictus*. These composition changes were more evident in *Ae. aegypti* than *Ae. albopictus*. However, compositional changes in *Ae. albopictus* may be diluted by a large proportion of the microbiota consisting of *Wolbachia pipientis* and *Elizabethkingia*, and measures of alpha diversity between treatments may be largely affected by the dominance of these two taxa. Interestingly, in both *Ae. aegypti* and *Ae. albopictus,* there was an observed increase in *Elizabethkingia* abundance in the MP treatment groups. Bacteria from the genera *Elizabethkingia* are gram-negative, rod-like, aerobic, nonfermenting, nonmotile, and non-spore-forming flavobacterium and widely found in wild and lab-reared *Aedes* and *Anopheles* mosquitoes (Terenius et al. 2012, Perrin et al. 2017). *Elizabethkingia anophelis* has been demonstrated to affect *Plasmodium* development in A*nopheles* mosquitoes and Zika virus infection rates in *Ae. albopictus* (Bahia et al. 2014, Onyango et al. 2021). *Elizabethkingia* density changes as the result of MP ingestion suggest the potential for MPs to indirectly affect arbovirus transmission by *Ae. albopictus* and *Ae. aegypti*. Also, the number of bacterial ASVs shared by all groups for females (19) and males (24) was lower than the total number of bacterial ASVs for females (42) and males (71), suggesting a small core bacterial community that was not altered by the presence of MPs in the gut.

The observed fungal mycobiota in *Ae. aegypti* and *Ae. albopictus* is mainly composed of Ascomycota and Basidiomycota, similar to previously reported studies (Chandler et al. 2015, Luis et al. 2019). The decrease in fungal diversity and composition changes in samples from the MP treatments, particularly in male *Ae. aegypti* and *Ae. albopictus* suggests that the fungal mycobiota in both species are susceptible to MP perturbations and are associated with exposure to higher concentrations of MPs as larvae. Interestingly, yeasts from the genus *Malassezia* were negatively associated with higher concentrations of MPs. *Malassezia* has been demonstrated to metabolize fructose in males and females (Guégan et al. 2020). Concerningly, *Aspergillus* and *Cladosporium* genera have been identified as involved in fructose metabolism and showed minor increases in MP treatments (Guégan et al. 2020). Thus, members of the mycobiota may also be impacted by MPs, and compositional changes of these mycobiota could impact mosquito metabolism. Unfortunately, the taxonomic resolution is still relatively low for insect fungal communities, as many fungal ASVs were unspecified or uncategorized due in part to the lack of a well-defined database of fungal ITS sequences and the convolutions of fungal taxonomy (Gdanetz et al. 2017). Furthermore, insect microbiome research has been biased towards the investigation of the bacterial microbiome rather than the characterization of the complete insect microbiome. Hence, there may be other members of the mycobiota that were not identified that could be impacted by the ingestion of MPs.

Taken together, the observed perturbation of the bacterial and fungal micro- and mycobiota associated with MP ingestion could impact key roles in the biology of mosquitoes. The gut microbiota of adult mosquitoes is critical for blood meal digestion and egg production (Gaio Ade et al. 2011, Coon et al. 2016). When *Ae. aegypti* was fed antibiotics to reduce gut bacteria, there was a reduced digestion of blood proteins and egg production (Gaio Ade et al. 2011). Data from empirical studies have also shown us that several mechanisms are involved in the interaction of members of the microbiome with that of the host mosquito, which can impact arbovirus transmission. The activation and maintenance of basal immune activity in mosquitoes can influence vector competence to pathogens. Recent work has demonstrated that the ingestion of MPs by dipteran larvae induces the production of an enzyme critical to melanogenesis, phenoloxidase, affecting innate immunity and causing oxidative damage, which could impact pathogen transmission (Silva et al. 2021b, 2021a). Fungal infections have also been recently demonstrated to affect the immune gene expression of *An. stephensi* and Anopheles *gambiae* (Wei et al. 2017). *Anopheles gambiae* and *A. arabiensis* microsporidia stimulate immune responses that impact *Plasmodium* spp. oocysts production (Bargielowski and Koella 2009, Cirimotich et al. 2011). Multiple reports have demonstrated the harmful effects of MP ingestion on terrestrial and aquatic organisms (Clark et al. 2016, Rochman et al. 2017, Cole et al. 2019, 2020, Muhammad et al. 2021, Romano and Fischer 2021, Silva et al. 2021b, Cao et al. 2022). These include adverse effects at the cellular level, which can result in altered gene expression levels of the immune system, metabolic and metamorphosis signaling pathways, impacts on life history traits, and reactive oxygen species induced upregulation of oxidative stress genes in aquatic organisms and insects (Malafaia et al. 2020, Romano and Fischer 2021, Silva et al. 2021b). In addition to activating the mosquito immune response, midgut bacteria

can also influence insect mosquito competence by impairing pathogen infection through competition for resources or secretion of anti-pathogen molecules and or metabolites (Perera et al. 2012, Caragata et al. 2014, Mendez et al. 2021). For example, changes to or removal of a majority of midgut bacteria through antibiotic treatment results in greater susceptibility of the mosquitoes *Anopheles gambiae* and *Ae. aegypti* to malaria parasite transmission and Dengue virus, respectively (Beier et al. 1994, Xi et al. 2008, Dong et al. 2009).

While this study does not examine stress and immune response pathways or the removal of particular members of the micro- and mycobiota, it suggests the need for further studies that will investigate the physiological and molecular mechanism associated with the ingestion of MPs impacting the microbiome at different developmental stages of mosquitoes. Specifically, additional studies could investigate whether MPs directly provide additional carbon to the gut microenvironment and an increase in surface area for microbial and pathogen growth in the insect gut (Lear et al. 2021). MPs may also act as a sink for organic and inorganic pollutants and chemical additives added to plastics that may result in toxic effects due to bioaccumulation, which could impact microbiota composition (Menendez-Pedriza and Jaumot 2020, Song et al. 2022). Furthermore, studies are needed to examine the effects of different MP polymers and sizes on the mosquito host. Here, only 1 µm size polystyrene beads were examined. Nanoplastic particles, various polymer types, and larger sizes of MP particles have been observed to impact the microbiomes of multiple invertebrate as well as vertebrate species of animals (Rochman et al. 2017, Silva et al. 2019). The 10,000 and 100,000 MP/ml concentrations of MPs in this study were extreme doses, which are likely not realistic concentrations found in natural and artificial container habitats but could correspond to a large accidental release of MPs and a polluted environment. The proposed future studies should also include MPs of different chemical compositions and mixtures of different sizes and shapes, as well as co-exposure scenarios, which may be more realistic to mixtures larvae ingest in natural aquatic habitats. Furthermore, additional studies on the sizes and types of microplastics could be expanded to include examining other life history traits (e.g., fecundity, immature survivorship, adult longevity, egg hatch, and mating competitiveness). Moreover, this project focused on the whole micro- and mycobiome of *Ae. aegypti* and *Ae. albopictus*, investigating only the gut microbiome may help to determine specific interactions of MPs and the gut microbiota. It would also be interesting to examine mosquito container habitats and characterize the type, size, and density of MPs and complete similar experiments presented here with larvae collected from natural conditions.

In this work, we demonstrated *Ae. aegypti* and *Ae. albopictus* larvae ingest and accumulate MPs in their guts during larval stages, and some of these MPs remain in the adult guts. Also, this accumulation of MPs in the mosquito gut results in perturbations of the micro-and mycobiomes. These results provide a strong basis to further investigate the mechanisms behind MP ingestion altering the mosquito host micro- and mycobiome and whether MP-driven perturbation of the mosquito micro- and mycobiota will impact vector competence of important mosquito-transmitted viruses and parasites that cause human and animal diseases.

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Author Contributions

CE: validation; investigation; visualization; writing – original draft. GM: validation; investigation; visualization; writing – original draft. DR: investigation. YG: investigation. KDA: investigation; visualization. MG; investigation; visualization. JC: conceptualization; supervision; project administration; methods; funding acquisition; writing – review & editing CB: conceptualization; funding acquisition; supervision; project administration; methods; visualization; writing – review & editing

Data Availability

Data is available upon a reasonable request. Please contact the corresponding author Corey L. Brelsfoard (corey.brelsfoard@ttu.edu) with data requests.

Supplementary Material

Supplementary material is available at *Journal of Medical Entomology* online.

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