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Changes in Larval Mosquito Microbiota Reveal Non-target Effects of Insecticide Treatments in Hurricane-Created Habitats

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Abstract

Ephemeral aquatic habitats and their associated microbial communities (microbiomes) play important roles in the growth and development of numerous aquatic insects, including mosquitoes (Diptera). Biological control agents, such as *Bacillus thuringiensis israelensis* (*Bti*) or insect growth regulators (e.g., methoprene), are commonly used to control mosquitoes in these habitats. However, it is unknown how commonly used control compounds affect the mosquito internal microbiome and potentially alter their life history traits. The objectives of this study were threefold: characterize the internal microbiota of *Aedes* larvae (Culicidae) in ephemeral forested mosquito habitat using high-throughput amplicon based sequencing, assess how mosquito control treatments affect the internal microbial communities of larval mosquitoes, and determine if changes to the microbiome resulted from direct or indirect treatment effects. The larval microbiome varied in community composition and diversity with development stage and treatment, suggesting potential effects of control compounds on insect microbial ecology. While microbial community differences due to *Bti* treatment were a result of indirect effects on larval development, methoprene had significant impacts on bacterial and algal taxa that could not be explained by indirect treatment effects. These results provide new information on the interactions between pesticide treatments and insect microbial communities.

Keywords Microbial ecology · Microbe · Higher organism interactions · Community genomics · Non-target effects

Introduction

Microbial communities play significant functional roles in ecosystems including primary production, decomposition, and nutrient cycling [1–3]. In addition to their direct ecosystem impacts, aquatic microbial communities influence the growth and development of numerous aquatic insects, including mosquitoes [4, 5]. Mosquito development, survivorship, and behavior are all substantially influenced by the microbial communities they interact with during their life cycle [6, 7]. Similarly, mosquito larvae can alter the microbial community dynamics of their habitat through their feeding behavior [8, 9]. The removal of mosquito larvae from aquatic habitats has been shown to result in marked differences in composition [10] and phenotypic changes [11] in aquatic microbial communities. Larval mosquitoes feed predominantly on microorganisms and detritus using a variety of feeding behaviors including filter feeding, and their internal microbial communities can be influenced by numerous biotic and abiotic factors, such as environmental conditions, leaf litter type, and developmental stage [6, 12–16].

These acquired microbial communities provide numerous services for mosquitoes including assisting with digestion [4], stimulation of immune function [17], and providing resistance to colonization by outside and potentially harmful microbes and viruses [18]. The ability of microbes to reduce the susceptibility of mosquitoes to viral infection has recently fostered great interest for the control of vector-borne diseases including West Nile virus, dengue fever, and more recently Zika virus

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[18–20]. If introduced microbes are to be used effectively as part of an integrated mosquito control program, the impact of other commonly used mosquito control methods on internal microbial communities requires evaluation.

Biological control compounds are widely used in mosquito control and management programs due to their limited non-target effects, cost-effectiveness, and ease of use [21–23]. Two of the most commonly used larval treatments are methoprene and several *Bacillus thurengenesis* serotypes including *Bacillus thurengenesis isrelensis* (*Bti*) [22, 24]. *Bti* and methoprene are considered two of the safest in terms of their limited effects on non-target organisms [21, 22]. These two control agents use different mechanisms to reduce mosquito populations and limit their potential to effectively vector pathogens. *Bti* produces and stores a crystallized endotoxin which, when ingested by mosquito larvae, leads to their death, usually within 24 h of application [25]. Methoprene interrupts the growth cycle of mosquitoes by acting as a juvenile hormone mimic, which during the pupal stage prevents adult emergence [26]. While the effects of these treatments on macroinvertebrates and vertebrates have been well studied, there has been limited research into their effects on mosquito-associated microbial communities under natural conditions [27, 28]. In outdoor microcosms, it has been shown that *Bti* and other pesticides can alter the microbial communities in lentic aquatic habitats, potentially impacting the quality of larval mosquito habitat [28, 29]. In this study, we monitored natural ephemeral pools created when trees were uprooted and blown down during a major hurricane on the eastern coast of the USA in 2012, which for this study we refer to as tree divots (Fig. 1). Understanding how mosquitoes and their associated microbes in storm-associated habitats respond to control treatments may help increase the effectiveness of emergency mosquito control programs.

The goals of this study were to (1) characterize the internal larval mosquito microbial communities in natural ephemeral habitats, (2) test how sub-lethal effects of two commercial treatments, containing either methoprene or *Bti*, impact the internal microbiota of mosquito larvae, and (3) determine if changes in microbial communities were explainable by direct or indirect effects (changes in larval density or development stage) of treatment. Our results demonstrate distinct internal microbiomes of mosquito larvae based on development stage that are impacted by indirect and direct effects of treatment, and that treatment with *Bti* and methoprene-based control compounds impacts the internal microbiota of mosquito larvae differently.

Methods

Study Site

The study site was in Teetertown Nature Preserve, a mostly wooded 59.5 ha preserve located in Teetertown, NJ, USA. All the tree divots used in this experiment were located in a 16 ha section of the preserve, which consisted of a deciduous forest comprised of predominantly oak and poplar trees and a sparse understory (Fig. S2). Twenty-one (21) divots from mature oak (*Quercus* spp.) and tulip poplar (*Liriodendron tulipifera*) trees uprooted during Hurricane Sandy (October 2012) were randomly selected from the total divots (41) in the area. All sites had predominantly oak and tulip poplar leaf litter. The tree divots were divided into three groups of seven: a control, *Bacillus thuringiensis israelensis* (*Bti*) treatment, and a methoprene treatment. Precipitation data was taken from the nearest NOAA weather station (KSMQ) located 12 mi away from the study site.

Fig. 1 Example of aquatic tree divot habitat. All tree divots sites in this study contained oak (*Quercus* spp.) and tulip poplar (*Liriodendron tulipifera*) leaf litter. The mean volume of the tree divots was 0.32 m^3 (SE ± 0.03). Photo credit: Greg Vaccarino

Insecticide Treatment

Two commercial treatments were used at the manufacturer's recommended dosages for unpolluted sites. The *Bti* treatment group was treated with Vectobac G® [11 kg/ha (10 lb/ac), Valent BioSciences Illinois, USA], and the methoprene treatment group was treated with the commercial product Altosid® [1076 briquets/ha (1 briquet/200 ft^2), Central Life Sciences, Dallas, TX]. Treatments were applied (hand broadcast) by licensed personnel from Hunterdon County Vector Control Program (HCVCP) on April 17, 2015. Due to proprietary concerns with obtaining inactive controls for each commercial product, untreated divots were used as the control group, as done previously for evaluating pesticide impacts on aquatic microbial communities [28, 30].

Sample Collection

Larval mosquitoes were collected using a 250-ml mosquito dipper (BioQuip, Ranchero Domingo, CA), and density was determined by taking five dip samples from each divot and

pooling these samples for a total number of mosquitoes per 1250 ml. Larvae were collected at four timepoints during the study (April 3–May 12, 2015), 7 days before treatment (7D Pre), as well as 1 day (1D Post), 7 days (7D Post), and 14 days (14D Post) post-treatment, after which the divots dried and could no longer support larval mosquitoes. At all timepoints, only live mosquito larvae were used for density measurements and collected for microbial community analysis. Larvae were preserved in 100% molecular grade ethanol and returned for identification in the laboratory using a dichotomous key from Darsie and Ward [31]. At each timepoint, water volume, pH, and temperature were measured for each divot using handheld portable probes (Etekcity Handheld pH Meter ± 0.05 and Etekcity infrared thermometer). Larval instar was determined using a dissecting scope and ImageJ software [32].

DNA Extraction and Quantification

Mosquito larvae from 1D Post, 7D Post, and 14D Post were used to determine the impact of larvicidal treatment on the internal microbial communities. Before DNA from the mosquito larvae was extracted, the external surface of the larvae was decontaminated with a 10% hypochlorite wash followed by a triple rinse in sterile water as previously described [33]. To minimize microbial artifacts associated with specimen dissection and potential contamination from other internal tissues, we chose to analyze the whole internal communities rather than perform midgut dissections, as dissection or decontamination of the peritrophic membrane may have influenced the midgut microbial community. DNA extraction was performed using the PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA). Whole larval mosquitoes (5–7 depending on size, from the predominant instar of each sample; Table S1) were extracted according to the manufacturer's protocol with the addition of lysozyme (15 mg ml^{-1}, Invitrogen) before the lysis step [horizontally vortex at maximum speed for 15 min on a MO BIO Vortex Adapter (MoBio)]. The DNA was quantified using a Qubit® dsDNA HS (High Sensitivity) Assay Kit (Invitrogen) and a Qubit 2.0 (Grand Island, NY, USA). All DNA preparations were stored at − 20 °C.

DNA Sequencing

Illumina MiSeq 16S library construction (2 × 250 bp paired-end reads) and sequencing were performed by the Michigan State University Genomics Core Facility using a modified version of the protocol adapted for the Illumina MiSeq, as described by Caporaso et al. [34]. Variable region 4 of the 16S rRNA gene was amplified with region-specific primers, 515F/806R (5′-GTGCCAGCMGCCGCGG-3′, 5′-TACNVGGGTATCTAATCC-3′) according to previously described methods [35, 36]. While these primers are designed to amplify a wide variety of 16S sequences, the possibility remains that amplification and sequencing introduced systemic biases in the microbial taxa observed. The raw fastq files were assembled, quality-filtered, demultiplexed, and analyzed as described previously, using the default settings in QIIME (v 1.9.0) [37, 38]. Reads were discarded if they had a quality score less than 20, contained ambiguous base calls, or were reads with < 75% (of total read length) consecutive high-quality base calls, as suggested by Bokulich and colleagues [39]. After chimeric reads were removed using default settings in QIIME, the remaining sequences were binned into OTUs using UCLUST and a 97% sequence similarity cutoff [40, 41]. Sequences from each OTU cluster were taxonomically assigned using the RDP classifier [42] and identified using BLAST against the 97% Greengenes reference data set (http://greengenes.secondgenome.com) [43–45]. Representative OTU sequences were then aligned to the Greengenes reference alignment using PYNAST [37]. Singleton OTUs and low abundance OTUs, making up less than 0.0005% of reads, were removed from the data set, as recommended for Illumina-generated data [39]. After Archaea and mitochondrial reads were removed, taxa were separated into two groups, chloroplast reads, which were assigned to algal taxa, and bacterial (including other cyanobacterial reads) similarly to Lehmann et al. [46]. Due to limitations of using the 16S gene region (V4) and primers 515F and 806R for algal classification, we only assigned algal reads to the highest classification level available (Order) using the curated Greengenes database (v 13.8). By limiting our algal taxonomic assignment, the algal reads presented were assigned with > 99% confidence using the Greengenes (v 13.8) database. While this taxonomic cutoff does not allow for an exhaustive analysis of the algal results, it provides a compromise that allows an examination of the general trends in the sequences assigned to algal taxa, which in some samples represent up to 40% of the relative abundance. Sequence files for this study have been deposited in the EMBL database under the accession number PRJEB22597.

Statistical Analysis

Temperature, pH, divot volume, depth, and mosquito density met the assumptions of normality, so repeated measures analysis of variance (ANOVA) was used to compare these variables between treatments. The impact of treatment on instar was tested with ANOVA and Tukey's HSD tests within each date. ANOVA and Tukey's tests were conducted using R (v 3.4.1) and the car package (v 2.1.5) [47, 48]. Variation in community composition between sample groups was tested with PERMANOVA, Simpson's evenness and visualized with principal coordinates analysis (PCoA). To determine if high and low larval density resulted in differences in microbial

community composition, densities lower than the first quartile and larger than the third quartile were compared. To test whether treatment differences at 7 and 14 days post-treatment were explainable by indirect effects, *Bti* and methoprene were individually tested against the control group, with treatment added as the second term in a sequential PERMANOVA model already accounting for instar differences. PERMANOVA tests and PCoA ordinations were performed on OTU level abundances and based on a weighted phylogenetic distance metric (UniFrac). PERMANOVA tests were conducted using the adonis function with 999 permutations in the vegan 3.1.0 library in R [49]. PCoA plots and bar plots were created using the ggplots2 (v 2.2.1), phyloseq (v 1.19.1), and vegan libraries in R [50, 51]. To determine if the observed microbial communities could be used to classify samples to each treatment group, a random forest classification was conducted using the randomForest (v 4.6–12) package with 1000 trees in R [52]. Microbial taxa identified as predictors in the random forest classification were then tested to determine if they differed significantly between treatment groups with a Kruskal-Wallis test and a Bonferroni correction in R. Statistical tests were considered significant at $P < 0.05$.

Results

Mosquito Abundance and Environmental Conditions

The only mosquito species documented in the divot habitats during this study was *Aedes stimulans* (Walker) (Diptera: Culicidae). A total of 3205 *A. stimulans* larvae were collected from ephemeral tree divot habitats ($n = 21$) over the duration of the study (April 3–May 12, 2015). The divots had an average hydroperiod of 32 days (SE ± 2 days) from the beginning of the study, and no divot supported mosquito larvae after 39 days. During the study, 3.9 cm of precipitation was observed, with 95% (3.7 cm) of the total precipitation occurring between 1D Post and 7D Post. Over the course of the study, pH and water temperature increased as the volume of the divots decreased (Fig. 2a). There were no significant differences among any of these environmental factors based on treatment group (ANOVA, $P > 0.05$).

Both *Bti* and methoprene treatments reduced the mean density of mosquito larvae compared to the control divot habitats at 7D and 14D Post (Fig. 2b). At 1D Post, treatment did not result in differences in the observed developmental stage (instar) (ANOVA, $F_{2,147} = 0.50$, $P = 0.60$). However, there were significant differences in mean instar at 7D Post (ANOVA, $F_{2,256} = 29.9$, $P < 0.001$; Fig. 2c). *Bti* treatment significantly reduced the mean larval instar by 40% compared to the control (Tukey HSD, $Q = 14.24$, $P = 0.001$), while methoprene did not have a significant impact ($Q = 2.27$, $P = 0.244$).

Internal Bacterial Communities

In total, 2,667,865 16S rRNA gene amplicon sequences were generated from 40 pooled mosquito larval samples (Table S1 and S2), representing 1550 operational taxonomic units (OTUs). Proteobacteria, predominantly Gammaproteobacteria, comprised over 80% of the total bacterial sequence abundance at 1D Post and remained the most abundant phyla and class at 7D Post, but by 14D Post, bacteria from the phylum Firmicutes was the highest relative abundance in both the control and *Bti* treatments. The most abundant families (> 80% relative abundance across all samples and treatments) were Enterobacteriaceae (Proteobacteria), Planococcaceae (Firmicutes), Clostridiaceae (Firmicutes), Staphylococcaceae (Firmicutes), and Streptococcaceae (Firmicutes) (Fig. 3a). Enterobacteriaceae was the most abundant family, representing 37% of the total bacterial relative abundance across all samples. The second highest family was Planococcaceae (Firmicutes), which accounted for 7% of the total abundance. The four most abundant genera, which together comprise over 70% of the total relative abundance across all samples, were *Lysinibacillus*, *Clostridium*, *Staphylococcus*, and an unassigned genus from the family Enterobacteriaceae (Fig. 3b).

Development stage (instar) had the largest impact on the internal microbial communities of the sample variables measured (PERMANOVA, $F_{2,37} = 13.07$, $P < 0.001$). Second instar larvae ($n = 11$) had a distinct microbial community compared to both third ($n = 21$) and fourth instar larvae ($n = 8$) (Fig. 3c, d). Simpson's diversity was significantly lower in second (0.45 SE ± 0.08) compared to third instar larvae (0.74 SE ± 0.05; Kruskal-Wallis, $H = 7.60$, $P = 0.005$) and nearly significantly lower in second compared to fourth instar larvae (0.67 ± 0.04; $H = 3.15$, $P = 0.07$), with no difference between third and fourth instars ($H = 0.77$, $P = 0.37$).

Impacts of Treatment on Microbial Communities

Treatment (PERMANOVA, $F_{2,31} = 2.37$, $P = 0.008$) and sampling date ($F_{2,31} = 4.74$, $P < 0.001$) were determined to have a significant impact on the bacterial communities of mosquito larvae (Table 1). When bacterial community structure between treatments at 1D Post was tested, treatment did not result in significant community differences ($F_{2,5} = 0.47$, $P = 0.80$). As no significant differences due to date were observed between 7D Post and 14D Post ($F_{1,30} = 1.33$, $P = 0.199$), they were combined for subsequent statistical analyses.

Enterobacteriaceae comprised less than 25% of the relative bacterial abundance in the control and *Bti* groups but over 50% in the methoprene treatment at 14D Post. Staphylococcaceae, which comprised less than 1% of the relative abundance in control and *Bti* larvae at 7D and 14D Post, comprised 10% of the abundance in larvae treated with

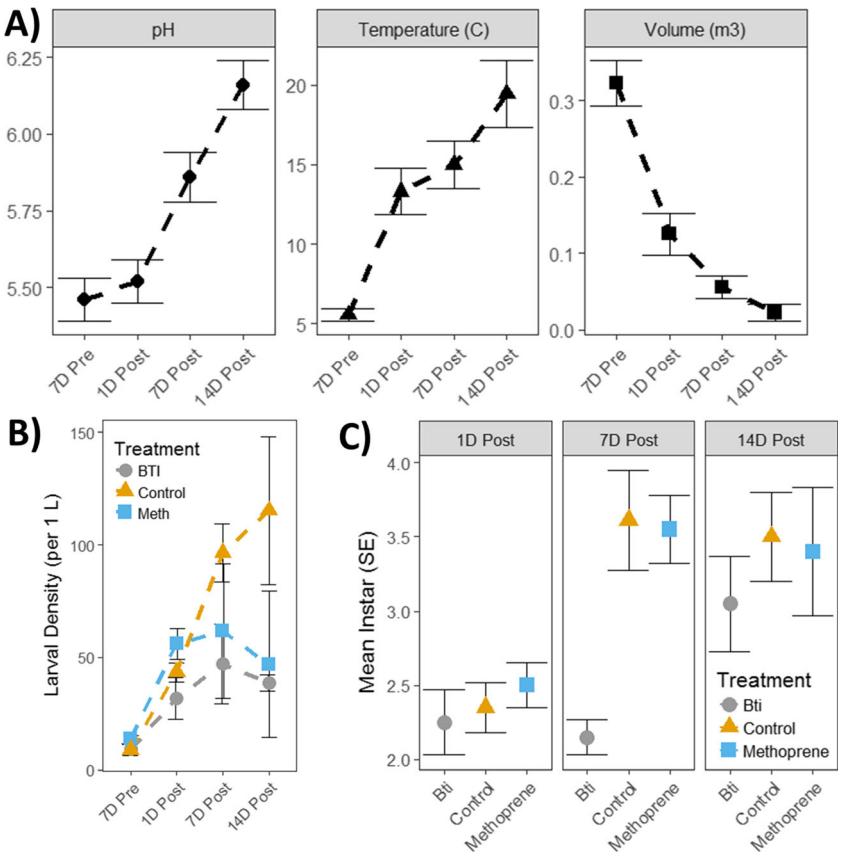

Fig. 2 Environmental conditions and impact of treatment on larval abundance and development. **a** Environmental conditions by sampling date. pH, water temperature (°C), and volume (m^3) are given as means ± SE. **b** Density of *Aedes stimulans* larvae by treatment group (± SE). **c** Mean larval instar by treatment (± SE)

methoprene. While *Bti* treatment did not significantly alter the bacterial communities at greater than 1 day post-treatment ($F_{1,16} = 0.78$, $P = 0.604$), methoprene had significant impacts on the internal bacterial communities ($F_{1,20} = 5.43$, $P = 0.003$). Sampling date ($F_{2,37} = 7.84$, $P < 0.001$; Fig. S1) also had a highly significant effect on microbial communities as well. Larval density did not have a significant impact on the internal bacterial communities ($F_{1,34} = 0.474$, $P = 0.68$).

Impact of Treatment on Photosynthetic Algal Communities

Sequencing revealed the high prevalence of photosynthetic algal taxa within the internal microbial communities, with some larval samples having up to 40% of their reads assigned to algal taxa (Fig. 4a). In order to examine the effects of treatment on photosynthetic algae, we compared the 16S rRNA sequences that corresponded to algal taxa as done previously [46]. Treatment with both *Bti* (PERMANOVA, $F_{1,20} = 7.26$, $P < 0.001$) and methoprene ($F_{1,26} = 4.67$, $P = 0.002$) resulted in significant differences in algal community composition, compared to the control, at greater than 1 day post-treatment with no significant impact of treatment at 1D Post ($F_{2,5} = 1.18$, $P = 0.332$). The composition of algal taxa differed significantly between treatments, with the relative abundance of *Stramenopiles* significantly different between treatments (Kruskal-Wallis, $P = 0.018$; Fig. 4b). All other algal taxa were not significantly different between treatments after correction for multiple comparisons ($P > 0.05$). The relative abundance of *Stramenopiles* in the methoprene treatment (41% ± 5.4% SE) was significantly higher than the *Bti* treatment (Wilcox, $P = 0.005$) and nearly significantly higher than the control treatment ($P = 0.07$).

Direct Vs Indirect Effects of Treatment

To test whether the observed microbial community differences (at 7D and 14D Post treatment) were explainable by indirect effects of treatment, such as changes in development stage, treatment was added as the second term in a sequential PERMANOVA model accounting for instar (larval density, although impacted by treatment, was not included as it did not have a significant impact on the larval microbial communities). After accounting for differences due to larval instar, treatment with *Bti* had no significant impact on bacterial ($F_{1,13} = 0.82$, $P = 0.467$; Fig. 4c) or algal ($F_{1,13} = 1.48$, $P = 0.20$) communities.

Methoprene continued to have significant impacts on bacterial ($F_{1,18} = 2.94$, $P = 0.013$) and algal ($F_{1,18} = 6.15$, $P = 0.002$) communities after accounting for effects of treatment on instar. A random forest model was able to discriminate between control and methoprene samples, at greater than

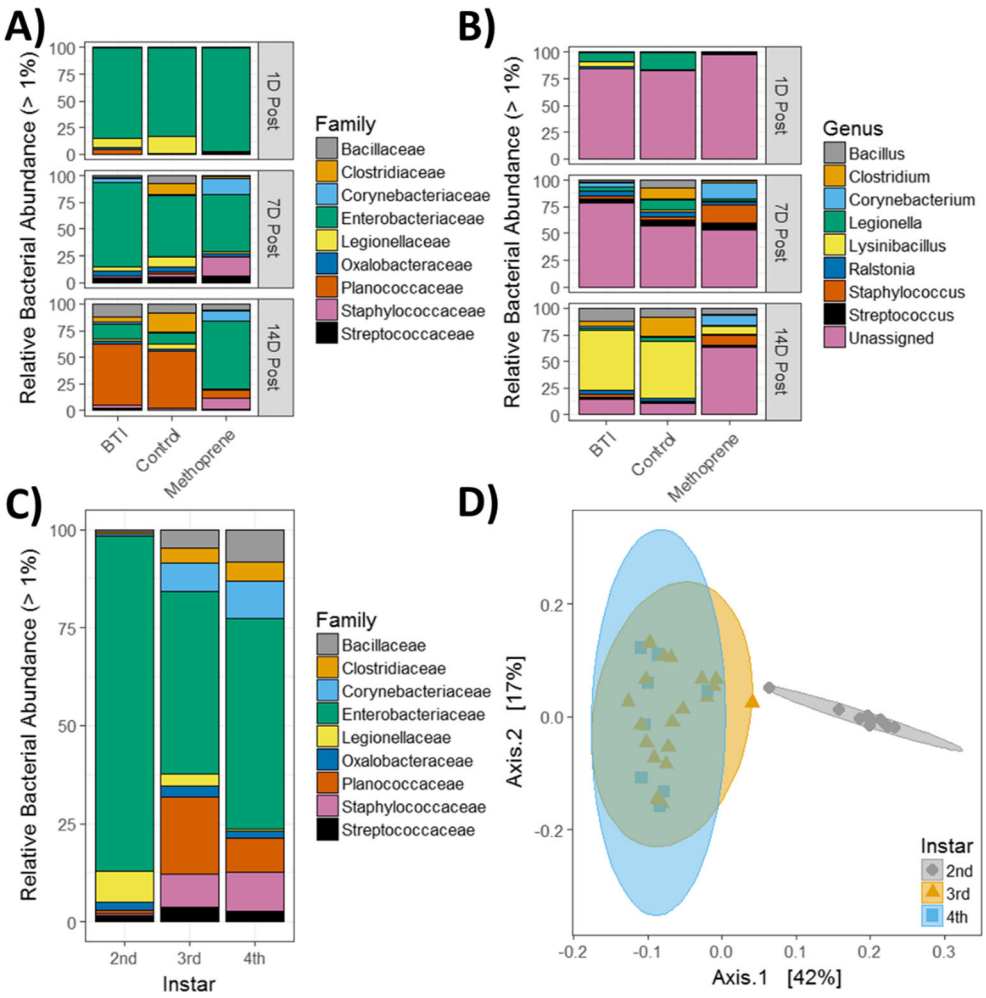

Fig. 3 Bacterial community diversity across instar and treatment. **a** Family level relative bacterial abundance across sampling dates (1D Post = 1 day post-treatment, 7D Post = 7 days post-treatment, 14D Post = 14 days post-treatment). **b** Genus level bacterial abundance, unassigned genus is from the family Enterobacteriaceae. **c** Family level bacterial community structure across instar. **d** Principle coordinate analysis (PCoA) based on weighted phylogenetic distance (UniFrac) matrix for β-diversity across instar. Ellipses represent the 95% CI for the mean of each group

1 day post-treatment, with a 77% success rate. The most important bacterial genera for classifying samples between the two treatment groups were *Staphylococcus*, *Lysinibacillus*, *Legionella*, *Bacillus*, and *Corynebacterium* (Fig. 4d; Table S3). The most important algal taxa for classification were *Chlorophyta*, *Streptophyta*, and *Stramenopiles*.

Table 1 Microbial community differences between treatment groups across sampling date. PERMANOVA results testing bacterial community structure based on weighted phylogenetic distance (UniFrac) matrix among treatment groups across sampling dates. *SS* sum of squares, *MS* mean sum of squares

Factor	*df*	SS	MS	*F*	R^2	*P*	
Date	2	2.04	1.02	4.74	0.19	0.001	*
Treatment	2	1.02	0.51	2.37	0.09	0.008	*
Date/treatment	4	0.85	0.21	0.98	0.08	0.475	
Residuals	31	6.68	0.21	0.63			
Total	39	10.6					

* indicates $P < 0.05$

Discussion

In this study, we present the internal microbial communities of *A. stimulans* larvae, a floodwater mosquito species commonly found in woodland depressions [53], and their changes in response to two common mosquito control treatments. This study contributes to our understanding of the unintended impacts of control compounds on insect microbiota by investigating the sublethal effects of two widely used pesticides on internal microbial communities under natural conditions. The tree divots examined in this study exhibited similar physical and chemical attributes to other ephemeral habitats, with decreasing volume and increasing pH over time [54]. In vernal pool habitats, which these tree divots closely mimic, temporal shifts and pH changes have been shown to cause changes in microbial community structure which may be impacting the larval mosquito microbiota [55]. The shifts in microbial community structure and diversity agree with previous studies that demonstrate abiotic factors that alter bacterial communities within mosquito larvae and in their aquatic habitat [15, 56, 57].

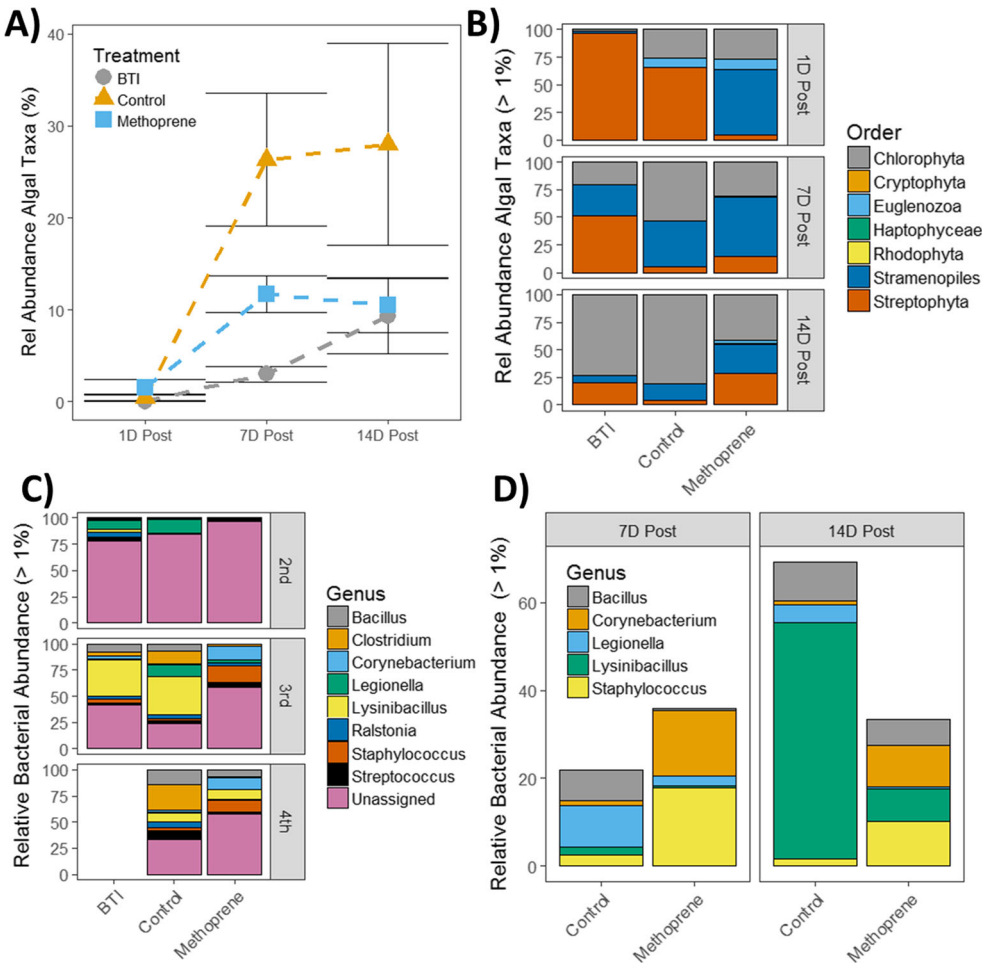

Fig. 4 Algal community response to treatment. **a** Relative percentage of algal reads from each treatment (± SE). **b** Algal community structure across treatment and date. **c** Relative bacterial abundance by treatment and instar. The relative abundance of fourth instar larvae from the *Bti* treatment group is not shown, as less than three samples from this group were sequenced. **d** Most important genera for classifying between methoprene and control treatment groups at greater than 1 day post-treatment using a random forest model. *Corynebacterium* relative abundance was significantly higher in the methoprene treatment group (Kruskal-Wallis, $P = 0.03$), while the other taxa were not significantly different between groups ($P > 0.05$)

The observed increase of larval density over time may have been a result of additional egg hatching as the season progressed. The changing temperature and environmental conditions may have stimulated additional egg hatching, leading to an increase in larval density. While methoprene is not normally lethal to mosquito larvae, it has been shown to reduce egg maturation and hatching which may explain the reduction in larval density compared to the control treatment [58, 59]. *Bti* has also been shown to be less effective in mosquito breeding sites where leaf litter is present [60], providing a possible explanation for the limited lethality of the *Bti* treatment.

The effects of larval instar on internal bacterial communities were similar to previous research into midgut mosquito microbiomes, which also showed changing community structure and diversity over developmental stages with high abundances of Proteobacteria, specifically Gammaproteobacteria and Enterobacteriaceae across all stages [9, 15, 28, 61, 62].

The high relative abundance of algal taxa found internally suggests that algal communities are important food resources for mosquito larvae, similar to previous analyses of mosquito feeding behavior [8]. Since the internal microbial communities of larval mosquitoes are greatly influenced by their habitat [15, 63, 64], the high relative abundance (11% of all assigned taxa) of algae may also suggest that primary production is still occurring in these tree divots as of the last sampling date. While it would be expected to see a reduction in algal taxa as the surrounding trees leaf out and begin to shade the divot, [8], the reduction in the relative abundance of algae in both *Bti* and methoprene treatments was not observed in the control divots, suggesting that the two treatments may be altering primary production, and potentially larval food resources. The impact of these changes on insect life histories and food web interactions will require additional study to determine conclusively.

That both treatments resulted in significant community differences at greater than 1 day post but not at 1 day post-treatment suggests that the internal microbiome of larvae requires time to change, similar to findings assessing water column response to insecticides by Muturi et al. [30]. When tested alone, *Bti* treatment had significant impacts on internal microbial communities; however, it did not have significant effects after accounting for differences in larval instar suggesting that differences were due to *Bti*'s impact on the observed

larval development stage and not a direct effect of treatment application. Methoprene, on the other hand, continued to have significant impacts on bacterial and algal communities after accounting for developmental differences, suggesting that methoprene treatment may have direct effects on the internal microbial communities. Additional studies into the bacterial and algal taxa identified may provide insight on the mechanisms by which methoprene treatment alters the internal microbial communities of mosquito larvae and the implications of these changes for mosquito control programs and aquatic food web interactions.

A better understanding of how these commonly used control compounds impact mosquito larvae and their food resources will have important implications for mosquito and disease control efforts. Whether treatment-induced changes in microbial communities lead to alterations in larval fitness or behavior will greatly improve our knowledge of sub-lethal and non-target effects of these control compounds. Our results have identified microbial taxa impacted by treatment, providing initial data for the development of further studies into the non-target impacts of these compounds on microbial communities and larval development.

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