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Microbiota Composition Associates With Mosquito Productivity Outcomes in Belowground Larval Habitats

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ABSTRACT

Vector mosquitoes are well-adapted to habitats in urban areas, including belowground infrastructure such as stormwater systems. As a major source of larval habitat in population centers, control of larval populations in stormwater catch basins is an important tool for control of vector-borne disease. Larval development and adult phenotypes driving vectorial capacity in mosquitoes are modulated by the larval gut microbiota, which is recruited from the aquatic environment in which larvae develop. Laboratory studies have quantified microbe-mediated impacts on individual mosquito phenotypes, but more work is needed to characterise how microbiota variation shapes population-level outcomes. Here, we evaluated the relationship between habitat microbiota variation and mosquito population dynamics by simultaneously characterising microbiota diversity, water quality, and mosquito productivity in a network of stormwater catch basins in the Chicago metropolitan area. High throughput sequencing of 16S rRNA gene amplicons from water samples collected from 60 basins over an entire mosquito breeding season detected highly diverse bacterial communities that varied with measures of water quality and over time. In situ measurements of mosquito abundance in the same basins further varied by microbiota composition and the relative abundance of specific bacterial taxa. Altogether, these results illustrate the importance of habitat microbiota in shaping ecological processes that affect mosquito populations. They also lay the foundation for future studies to characterise the mechanisms by which specific bacterial taxa impact individual and population-level phenotypes related to mosquito vectorial capacity.

1 | Introduction

Mosquito-transmitted diseases are a major cause of mortality and morbidity worldwide, with up to 700 million people infected each year (Caraballo and King 2014). Viruses transmitted by mosquitoes include Dengue, Yellow Fever, Zika, and West Nile Virus, and these all rely on a bite from an infected mosquito for transmission to a new host. Before they reach the biting adult stage, all mosquitoes begin their life cycle as eggs laid in water. Larvae hatch and moult through four aquatic instars, feeding on detritus and environmental microbes (Merritt, Dadd, and Walker 1992; Clements and Clements 2008) before pupating and emerging from the water's surface. Larvae utilise a wide range of habitats, including wetlands, pools, tree holes, and artificial containers such as water tanks and discarded tires (Yee et al. 2012). However, most widespread vector species, such as *Aedes aegypti* and *Culex pipiens*, have a particular affinity for container habitats. A great deal of attention has therefore been

given to examining aboveground container habitats for their characteristics relating to mosquito productivity (Leisnham et al. 2007; Noori, Lockaby, and Kalin 2015; Karki et al. 2016; Omolade 2018; Reinhold, Lazzari, and Lahondère 2018; Hessou-Djossou et al. 2022).

In urban environments, mosquitoes also take advantage of larval habitat in belowground infrastructure, and a growing body of literature supports a role for belowground habitats as important niches in the shifting global distribution of mosquito species (Haba and McBride 2022). Compared to conditions outside, belowground habitats maintain less extreme temperature ranges and stay warmer during the winter (Arsenault-Benoit, Greene, and Fritz 2021). In this way, the sheltered environments provided by underground structures can sustain local mosquito populations through dry or cold periods when conditions on the surface are less favourable and/or serve as reservoirs for species expanding into areas with surface temperatures outside of their normal range (Harbison, Metzger, and Hu 2011; Arsenault-Benoit, Greene, and Fritz 2021). Such habitats further support stable populations of belowground-adapted ecotypes (Haba and McBride 2022) and may even confer an advantage to invading species that are more common in belowground habitat than native species (Metzger, Harbison, and Hu 2011).

A key belowground structure supporting mosquitoes is stormwater catch basins (Calhoun et al. 2007; Manrique-Saide et al. 2013; Arana-Guardia et al. 2014; Hamer et al. 2014; Harbison et al. 2014; Ocampo et al. 2014; Gao et al. 2018; Valdelfener et al. 2019). Stormwater systems carry runoff water from roads and other impervious surfaces to outflows to a body of water. Catch basins collect the water into sumps, from which excess water flows into mains that lead to discharge points. In addition to the water necessary for oviposition (i.e., egg-laying), basins also retain debris and sediment, and this organic matter provides forage for both mosquito larvae and the bacteria that larvae eat. Additional nutrients and microbes are also assembled to catch basins from the various origins of runoff water (McLellan, Fisher, and Newton 2015) or, in municipalities with combined stormwater and sewer systems, from sewage lines during periods of high flow. However, the assembly and diversity of catch basin-associated microbial communities and how they associate with different basin characteristics and mosquito productivity are unknown. The potential for basin-associated microbiota to shape the efficacy of larvicide-based mosquito control, which is commonly employed by urban municipalities to reduce mosquito populations in catch basins and the surrounding environment, is also largely unexplored.

The microbiota associated with different larval habitats have been demonstrated to have profound impacts on mosquito biology (reviewed in Cansado-Utrilla et al. 2021). Habitat microbiota, in addition to serving as a primary food resource for developing larvae (Cansado-Utrilla et al. 2021), are the source of the larval gut microbiota (Cansado-Utrilla et al. 2021), which provision photosensitive B vitamins and other metabolic products that affect larval growth and consequently the size and quality of emerging adults (Valzania et al. 2018; Wang, Eum et al. 2021). Habitat microbiota derived via transstadial transmission from the larval stage and/or feeding from the water's surface after emergence are also a strong determinant of the adult gut microbiota, which modulate adult traits related to pathogen transmission (Cansado-Utrilla et al. 2021). There is therefore a growing interest in improving our understanding of how microbiota variation shapes individual and population fitness outcomes in mosquitoes. However, almost all studies to date have focused on studying phenotypes in individuals colonised by only one or several lab-derived microbial isolates that are not representative of the taxonomic or functional diversity of microbiota present in the field (Cansado-Utrilla et al. 2021).

Here, we evaluated the relationship between habitat microbiota variation and mosquito population dynamics—including the success of mosquito control treatments designed to interfere with larval development to the adult stage—by simultaneously characterising microbiota diversity, water quality, treatment outcomes, and overall mosquito productivity in a network of public health-monitored stormwater catch basins in the Chicago metropolitan area. 16S rRNA gene amplicon sequencing was used to characterise bacterial communities in basins at eight time points throughout a single mosquito breeding season. Models were then employed to identify associations between patterns of microbiota assembly and community composition and weekly in situ measurements of water quality and mosquito abundance in the same basins.

2 | Materials and Methods

2.1 | Environmental Variables, Larval Abundance, and Microbiota Sampling

Sixty catch basins were monitored weekly during an entire mosquito season (June–September 2021) in the jurisdiction of the Northwest Mosquito Abatement District in Wheeling, IL, in the Chicago metropolitan area (Figure 1 and Table S1). Thirty of the monitored basins are in combined sewer systems, and the remaining 30 are in separated sewer systems. Basins were assigned to flow groups based on connectivity of the storm sewer mains each basin flows into, as assessed by the Village of Arlington Heights using the GIS Water Utility Tool. Basin water conditions (pH, temperature, conductivity, dissolved oxygen, salinity) were measured using a Horiba U-10 water quality checker. Rainfall measurements were obtained from the NOAA National Environmental Satellite, Data, and Information Service station at Chicago O'Hare International Airport (41.9602°N, −87.9316°W), 13 km away from the study area.

Basins were treated with Altosid extended release 150-day briquets (Wellmark International, Schaumburg, IL, USA) containing methoprene, an insect growth inhibitor that limits populations by preventing larvae from developing to later stages and emerging as adults. Basins were treated once for the season between 6/7 and 6/24. Treatment outcomes in each basin were subsequently monitored weekly by dip sampling as described in (Harbison et al. 2019). If late instar larvae or pupae were not present, the basin treatment was automatically scored as a "pass". Late instar larvae or pupae present in basins during the monitoring period may indicate unsuccessful treatment or movement from a different location after reaching the observed developmental stage. To test treatment efficacy in these basins, water and at least 10 larvae/pupae were sampled and monitored

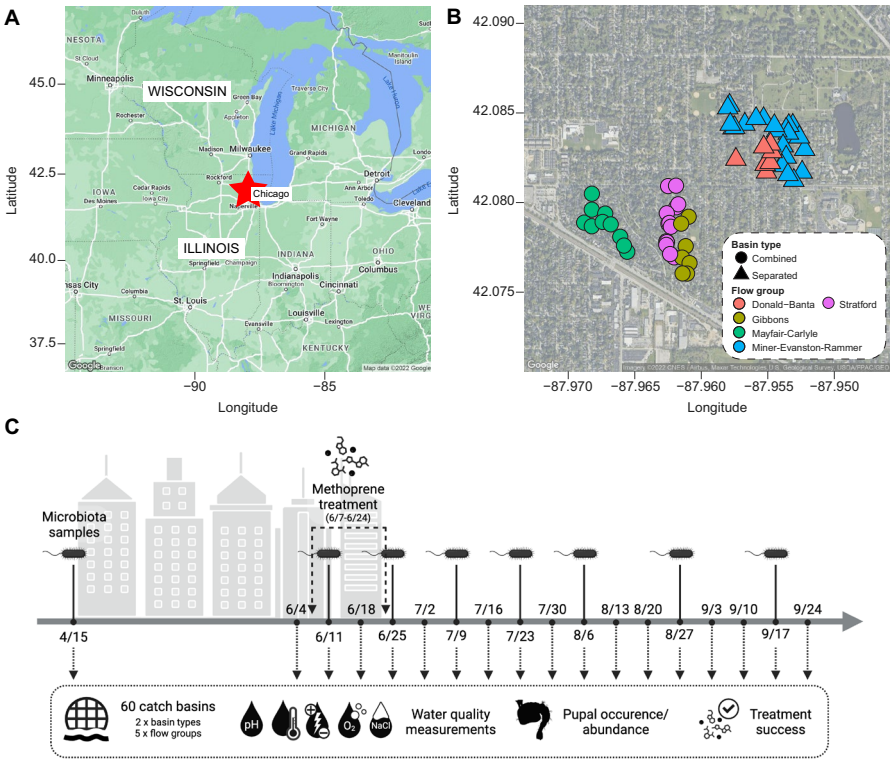

FIGURE 1 | Locations of collection sites and overall study design. (A) Sixty catch basins were monitored weekly throughout an entire mosquito breeding season (June-September 2021) in the jurisdiction of the Northwest Mosquito Abatement District in Wheeling, IL USA, in the Chicago metropolitan area. (B) Thirty of the monitored basins are in combined sewer systems, and the remaining 30 are in separated sewer systems. "Combined" sewer basins collect stormwater runoff and sewage water (wastewater) in a shared system, while "Separated" sewer basins carry surface run-off and wastewater separately. Flow group indicates the type of connection of the storm sewer mains each basin flows into, as assessed by the Village of Arlington Heights using the GIS Water Utility Tool. Symbols are coloured by flow group (Donald-Banta, red; Gibbons, yellow; Mayfair-Carlyle, green; Miner-Evanston-Rammer, blue; Stratford, pink). Basin type is designated by symbol shape ("Combined", circles; "Separated", triangles). (C) Basins were visited on a total of 18 sampling dates—one prior to the start of the mosquito breeding season (4/15) and at ~7-day intervals thereafter from 6/4 through 9/24. At the beginning of the season (between 6/7-6/24), most basins (= 59/60) were also visited to apply a briquet formulation of the insect growth inhibitor methoprene to control mosquito populations. Basin water conditions (pH, temperature, conductivity, dissolved oxygen, salinity), as well as the occurrence and abundance of any mosquito pupae, were measured on all sampling dates, while water was collected from methoprene-treated basins on only a subset of sampling dates (occurring between 6/11-9/24) to assess treatment efficacy via standard laboratory assays (see "Materials and Methods"). Water was also collected for 16S rRNA gene amplicon sequencing to characterise bacterial communities in all basins (both methoprene-treated and untreated) on eight sampling dates before and throughout the season (4/15, 6/11, 6/25, 7/9, 7/23, 8/6, 8/27, 9/17). Figure created with BioRender.com.

in the lab at 22°C for 4 days. Treatment was scored as a "pass" if adults did not emerge from the basin sample and scored as a "fail" if any adults emerged.

The occurrence and abundance of pupae in each basin was monitored with two dips of a standard 350 mL dipper. We chose to focus on the presence and abundance of pupae as the closest indicator of overall productivity for three reasons. First, the abundance of pupae in catch basins has previously been shown to be associated with adult abundance (Harbison et al. 2014). Second, since many larvae do not survive to the pupal and/or adult stage due to competition, larval abundance at a particular source is not reflective of the adults that will be produced. Third, the basins in our study area were treated with methoprene, which as previously mentioned controls mosquito populations by preventing larvae from developing to the pupal and/or adult stage. Because methoprene-affected larvae remain in the habitat but do not continue development, the number of larvae does not reflect the number of viable adults that will develop from said pool of larvae. In contrast, individuals that are not impacted by treatment are able to develop to pupal and adult stages; thus, pupal (but not larval) abundance in aquatic habitat more directly reflects adult abundance in the surrounding area.

2.2 | Microbiota Sample Preparation and Sequencing

Basin-associated microbiota were isolated from 50 mL of water collected in sterile 50 mL centrifuge tubes dipped into the basin. The middle of the water column was sampled because these are the communities the resident larvae, predominantly *Culex* spp., are most exposed to as they filter feed (Merritt, Dadd, and Walker 1992). A total of 240 water samples from 20 separated and 22 combined basins were processed for microbiota sequencing: 204 collected from 39 to 42 basins on each of five sampling dates (6/11, 6/25, 8/6, 8/27, 9/17), and 36

collected from a subset of 12 basins on each of three additional sampling dates (4/15, 7/9, 7/23) (Figure 1; Table S2). A pre-season sampling date (4/15) was included to provide a baseline characterisation of bacterial communities present in the network of catch basins prior to any influence of mosquitoes, and to capture baseline measures of environmental variables (described above) (Figure 1).

To pellet cells, water samples were centrifuged at high speed (21,130 × *g*) for 20 min. Total genomic DNA was then isolated from each cell pellet using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA) following the manufacturer's protocol for purification of total DNA from animal tissues, with pre-treatment for Gram-positive bacteria and a final elution volume of 40 mL. DNA concentrations were quantified using a Quantus fluorometer (Promega, Madison, WI, USA) prior to storage at −20°C. Negative controls for DNA extraction followed the same protocol using only extraction reagents.

The V4 region of the bacterial 16S rRNA gene was amplified using a one-step PCR protocol from (Kozich et al. 2013) with 25 mL reactions containing ~10 ng of template DNA, 12.5 mL of 2X HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), and 5 pmol of each primer, and the following cycling conditions: 95°C for 3 min; 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. PCR reactions without the addition of DNA served as negative controls. PCR amplification was confirmed by visualising 5 μL of each product on 1% agarose gels prior to purification using a MagJET NGS Cleanup and Size Selection Kit (Thermo Scientific, Waltham, MA, USA). Concentrations of purified libraries were quantified using a Quantus fluorometer and the resulting purified libraries were pooled in equimolar amounts prior to paired-end sequencing (2 × 250 bp) on an Illumina MiSeq at the DNA Sequencing Facility at the University of Wisconsin-Madison.

2.3 | Analysis of 16S rRNA Gene Amplicon Sequences

Demultiplexed sequences were processed using DADA2 (Callahan et al. 2016) in QIIME 22021.2 (Bolyen et al. 2019) and default parameters to filter for quality, merge paired end reads and remove chimeras. Taxonomy was assigned at a percent identity cut-off of 98% using TaxAss (Rohwer et al. 2018), which assigns taxonomy from an aquatic-specific bacterial database and Silva (v138) (Quast et al. 2012). The following ASVs and samples were then filtered from the dataset: (i) ASVs flagged as likely contaminants following both prevalence and frequency-based procedures in the R package "decontam" (Davis et al. 2018), (ii) ASVs classified as Archaea, chloroplasts, or mitochondria, and (*iii*) samples with fewer than 1000 reads. After denoising, read merging, and filtering, a total of 9,463,114 reads from 19,840 ASVs in 233 samples were retained. A phylogenetic tree was then built using FastTree (Price, Dehal, and Arkin 2009) from a multiple sequence alignment made in MAFFT (Katoh and Standley 2013), and the ASV table, taxonomy, and phylogeny outputs from QIIME, along with all sample metadata, were finally imported into R (http://www.r-project.org/) using the package "phyloseq" (McMurdie and Holmes 2013) for downstream analyses.

Estimates of alpha diversity (measured as ASV richness and Shannon's *H* index) were calculated in "vegan" (Oksanen et al. 2020), while beta diversity (measured as the Euclidean distances between samples in PhILR transformed space) was calculated using the R package "philr" (Silverman et al. 2017). The significance of sample clustering by sampling date, fixed basin characteristics (basin type, flow group), and different water quality and mosquito productivity measures (temperature, pH, salinity, conductivity, dissolved oxygen, pupal occurrence/abundance) was analysed by permutational multivariate analysis of variance (PERMANOVA) or permutational distance-based redundancy analysis (dbRDA) with basin identifier as a blocking factor, using the vegan function *adonis()*. Permutational analyses with fixed basin characteristics were performed using data aggregated by basin (i.e., reads summed over the entire season for each basin), while analyses with water quality and mosquito productivity measures used community composition from each basin observation without aggregation. The instance of variance-driven significant results was then assessed using PERMDISP (permutational analysis of multivariate dispersions) using the vegan function *betadisper()*. Bacterial community biotypes were identified by PAM clustering using the R package "BiotypeR" (Arumugam et al. 2011). Decision trees were constructed using the R package "tree" (Ripley 2022). Taxa associated with fixed basin characteristics (basin type, flow group) and different water quality and mosquito productivity measures were identified using Wilcoxon Rank Sum tests implemented using the R package "ALDEx2" (Fernandes et al. 2013).

2.4 | Model Estimation

Simple linear regression models were used to identify relationships between microbiota diversity, water quality, and mosquito productivity using (i) data aggregated by basin (i.e., measures averaged over the entire season for each basin), (ii) data aggregated by sampling date (i.e., measures averaged across basins for each sampling date), or (ii) unaggregated data including all individual observations of basins. Linear mixed-effects models were used to identify relationships between microbiota diversity, water quality, and mosquito productivity using (i) unaggregated data with basin observations, or (ii) unaggregated data with observations only included from basin visits where pupae were present. Exchangeable autocorrelation structures were used to account for autocorrelation between samples taken at different times from the same basin, while maximising use of the available data. For models including water quality and mosquito productivity measures, the resulting datasets included up to 18 weeks of monitoring records from up to 60 basins. For models including microbiota diversity and mosquito productivity measures, the resulting datasets included up to eight microbiota samples from 42 basins. Linear regressions were fit using base R, while linear mixed-effects models were fit using R packages "nlme" (Pinheiro et al. 2022), "lme4" (Bates et al. 2015), and "lmerTest" (Kuznetsova, Brockhoff, and Christensen 2017). ANOVA tables from a subset of linear and linear mixed-effects models were generated using the base R function *anova()*, with associated statistics reported as results from one-way or repeated measures ANOVAs, respectively. No spatial autocorrelation

was detected for any of the response variables we examined (Table S3).

2.5 | Other Statistical Analyses

One-way analysis of variance (ANOVA) was used to compare aggregated measures of microbiota diversity and mosquito productivity across sampling dates and basins assigned to different basin types (combined versus separated) and flow groups (Donald-Banta, Gibbons, Mayfair-Carlyle, Miner-Evanston-Rammer, Stratford). Logistic regression (implemented using the lme4 function *glmer()* and the base R function *glm()*) was used to model the relationship between basin type and biotype assignment across all individual basin observations. Relationships between different mosquito productivity measures were estimated via Pearson correlations.

3 | Results

3.1 | Mosquito Productivity Is Shaped by Water Quality

Pupae were detected in the vast majority (59/60) of the catch basins we monitored, although both the occurrence and abundance of pupae among basins fluctuated throughout the season (Figure S1 and Table S1). Basins with high average pupal abundance were those that had the highest frequency of pupae over time (Pearson's correlation; $r = 0.79$, $p < 0.0001$) (Table S1). Over the course of the season, peaks in pupal abundance generally corresponded to when pupae were widespread among basins (Figure S1; Table S1). Pupal abundances were also generally higher later in the season (repeated measures ANOVA; $F_{1,913} = 5.53$, $p = 0.019$) (Table S1).

Fixed basin characteristics (basin type, flow group) did not determine mosquito productivity. In both combined and separated basins, larvae were present during less than 50% of the season and both the season-wide frequency and abundance of pupae did not significantly differ between basins as a function of basin type (one-way ANOVA; pupal occurrence: $F_{1,58} = 2.91$, $p > 0.05$, pupal abundance: $F_{1,58} = 0.56$, $p > 0.05$) or flow group (one-way ANOVA; pupal occurrence: $F_{4,55} = 0.73$, $p > 0.05$, pupal abundance: $F_{4,55} = 0.30$, $p > 0.05$) (Figure S2A,B and Table S1). Combined and separated basins further had similar changes in productivity through time (Figure S2C and Table S1).

How frequently pupae appeared in a given basin over the entire season correlated with pH and conductivity, with more pupae present in more acidic basins and basins with higher measures of conductivity (Tables 1A and S1). Seasonal dynamics in the occurrence and abundance of pupae across all basins over time were further predicted by measures of dissolved oxygen, conductivity, and salinity (Tables 1B and S1). Among individual basin observations where pupae were present, pupal abundance was also greater at higher conductivity (Table 1C and S1).

3.2 | Methoprene Treatment Reduces Mosquito Productivity

As expected, treatment success predicted area-wide and season-wide mosquito productivity (Table S4) and most observations with pupae present were when treatment failed (Table S1). However, pupae still appeared in some basins scored as successfully treated based on laboratory assays, with pupae present in 89 of the 496 successfully treated observations (227 of the 293 observations of treatment failure had pupae present) (Table S1). While season-wide treatment success did not differ between combined and separated basins (one-way ANOVA; $F_{1,57} = 2.12$, $p > 0.05$) or among basin flow groups (one-way ANOVA; $F_{4,54} = 1.05$, $p > 0.05$) (Table S1), the season-wide success rate of treatment in individual basins was predicted by pH, with basins with higher average pH values associated with higher treatment success rates (Tables S1 and S5).

3.3 | Catch Basin Microbiota Are Highly Diverse, and Cluster Into Early- and Late-Season Biotypes

Bacterial 16S rRNA gene amplicon sequences from catch basins represented 19,840 ASVs from 1515 genera, 728 families, and 59 phyla. The most abundant phyla in the catch basins included those that have previously been reported in natural aquatic environments harbouring mosquito larvae, such as Proteobacteria, Cyanobacteria, Bacteroidota, Verrucomicrobia, Actinobacteria, and Firmicutes (Coon, Brown, and Strand 2016). Among these, Proteobacteria, Bacteroidota, Actinobacteria, and Firmicutes are also common in wastewater sludge (McLellan et al. 2010) and have been found to be dominant in other stormwater systems (Lee et al. 2020). Other abundant phyla in the catch basins included Campilobacterota, members of which are commonly detected in sewage (Domínguez et al. 2021), Spirochaetota, members of which have previously been detected in mosquito larvae (Scolari et al. 2021), and Desulfobacterota, members of which are widespread across freshwater and terrestrial systems (Murphy et al. 2021). Proteobacteria was the most dominant phylum across all basins and sampling dates and was the most abundant phylum in 91.85% of basin water samples (Figure 2A and Table S2), consistent with previous studies of catch basin microbiota (Zaheer et al. 2019). Other phyla that dominated individual basin samples included Campilobacterota (most abundant in 3.43% of samples), Firmicutes (2.58%), and Bacteroidota (2.15%) (Figure 2A and Table S2). The most dominant genera were C39 (family Rhodocyclaceae; most abundant genus in 33.05% of samples), betI-A (order Burkholderiales; 10.30%), Pseudarcobacter (family Arcobacteraceae; 9.87%), and betVII-A (order Burkholderiales; 9.44%) (Figure 2B and Table S2).

Early-season basin microbiota were characterised by high diversity, with rare genera (< 1% relative abundance) accounting for an average of 53.18% relative abundance in aggregate per sample (Table S2). Late-season communities contained high relative abundances of genus C39, which accounted for up to 96.94% relative abundance per sample (Figure 2C,D and Table S2).

TABLE 1 | Effects of water quality on mosquito productivity.

Outcome	Predictor	Estimate	Standard error	*p* value
(A) Linear regression analyses with data aggregated by basin				
Pupal occurrence (presence/absence)	pH	−0.16	0.05	0.0052*
	Temperature	−0.00	0.03	0.99
	Conductivity	0.12	0.12	0.31
	Dissolved oxygen	−0.03	0.10	0.78
	Salinity	1.16	1.46	0.43
Pupal abundance (pupae per dip)	pH	−1.68	0.67	0.015*
	Temperature	−0.08	0.34	0.82
	Conductivity	3.51	1.40	0.015*
	Dissolved oxygen	−0.45	1.20	0.71
	Salinity	29.43	17.40	0.096
(B) Linear regression analyses with data aggregated by sampling date				
Pupal occurrence (presence/absence)	pH	0.21	0.17	0.23
	Temperature	−0.03	0.03	0.38
	Conductivity	0.35	0.25	0.18
	Dissolved oxygen	1.65	0.76	0.046*
	Salinity	1.33	2.49	0.60
	Rainfall	−0.15	0.17	0.39
Pupal abundance (pupae per dip)	pH	1.22	1.43	0.41
	Temperature	−0.35	0.24	0.17
	Conductivity	6.47	1.38	0.00029*
	Dissolved oxygen	16.80	5.71	0.010*
	Salinity	60.38	13.73	0.00052*
	Rainfall	−0.71	1.41	0.62
(C) Linear mixed-effects models with unaggregated data (observations only where pupae were present)				
Pupal abundance (pupae per dip, square-root transformed)	pH	−0.01	0.11	0.94
	Temperature	−0.04	0.04	0.25
	Conductivity	0.70	0.16	< 0.0001*
	Dissolved oxygen	0.11	0.20	0.57
	Salinity	1.16	1.11	0.30

*p value significant at the ≤ 0.05 level.

Catch basin microbiota grouped into two biotypes based on PAM clustering using PhILR community distances calculated at the ASV level (hereafter referred to as "Biotype A" and "Biotype B") (Table S2). Early-season basins had a distinct community type, while late-season basins tended to have a different community type but occasionally reverted to the early season community structure (Table S2). All 89 samples from the 4/15, 6/11, and 6/25 sampling dates were categorised as Biotype A, while samples from 7/9 and later were 77.08% Biotype B and 22.92% Biotype A (Table S2). Sample microbiota categorised as Biotype A were characterised by higher overall diversity, with on average 47.31% relative abundance accounted for by rare genera (< 1% relative abundance), and a higher relative abundance of taxa within the Firmicutes as compared to those categorised as Biotype B (Figure 3; Table S2). In contrast, sample microbiota categorised as Biotype B′ were characterised by higher relative abundances of the genus C39 as compared to those categorised as Biotype

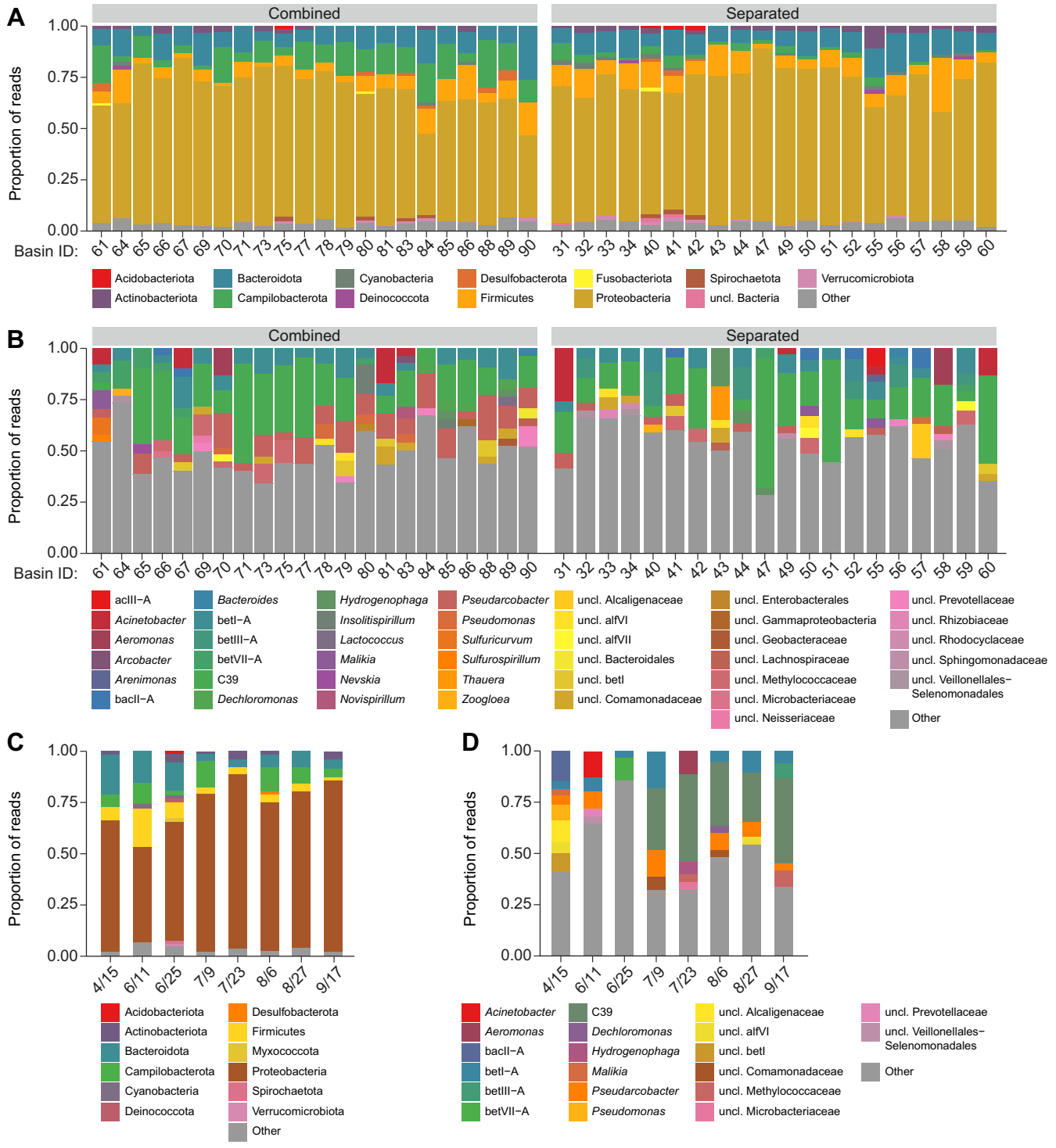

FIGURE 2 | Bacterial diversity in water sampled from study catch basins. (A) Relative abundance of bacterial phyla. Each bar presents the proportion of sequencing reads assigned to a given bacterial phylum. Low abundance phyla (< 1%) are represented by the "Other" category. (B) Relative abundance of bacterial genera. Each bar presents the proportion of sequencing reads assigned to a given bacterial genus. Low abundance genera (< 3%) are represented by the "Other" category. Water libraries for each basin were pooled to produce the bar graphs in (A) and (B). Panels (C) and (D) present the same data pooled by sampling date. Basin IDs and sampling dates (*x*-axes) follow those provided in Tables S1 and S2.

A (Figure 3 and Table S2). Classification tree analysis further revealed that microbiota biotype assignment was driven primarily by sampling date, followed by dissolved oxygen and pH (Figure S3 and Table S2). Late-season samples largely fell into Biotype B, unless dissolved oxygen levels were greater than 0.74 mg/L or dissolved oxygen was below 0.74 mg/L and pH was less than 6.115 (Figure S3 and Table S2). Basin type did not affect biotype assignment, as most basins switched between biotypes at some point in the season (binomial glm; estimate = −0.10, $p > 0.05$) (Table S2).

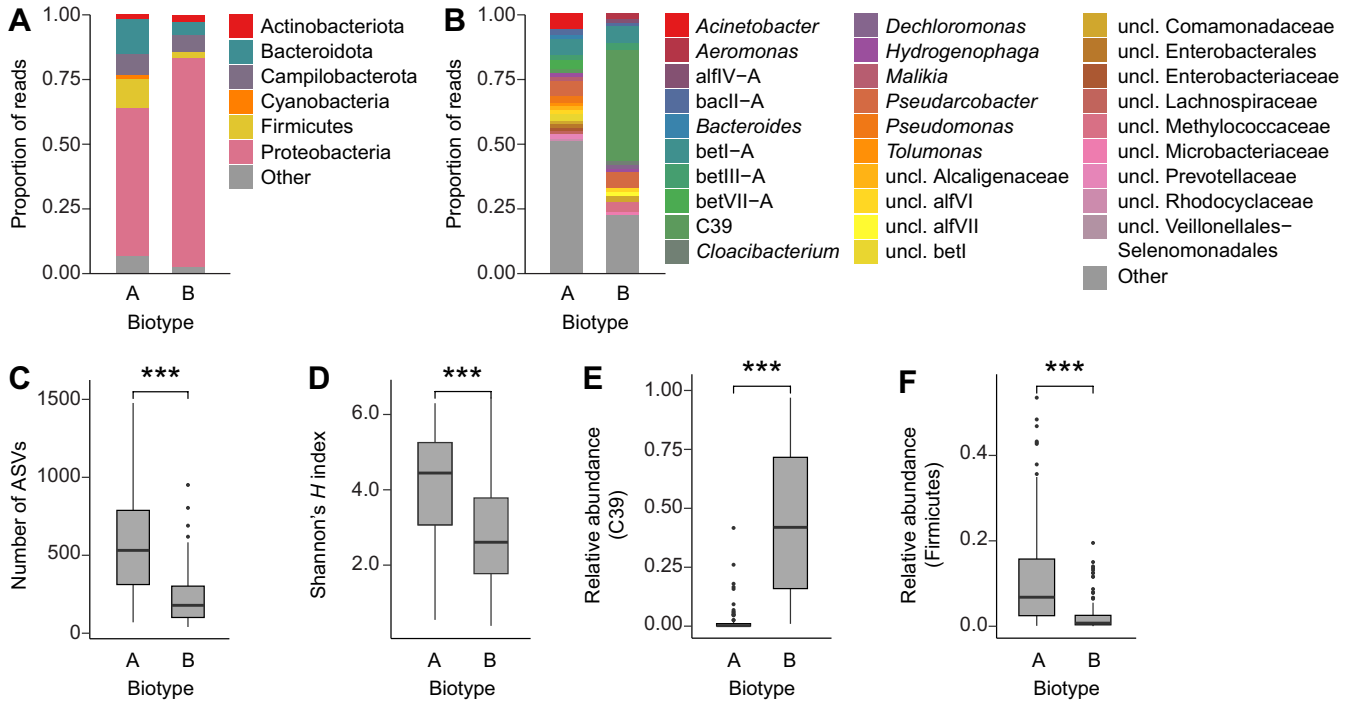

FIGURE 3 | Catch basin microbiota biotypes identified by PAM clustering. (A) Relative abundance of bacterial phyla in sample microbiota categorised as Biotype A versus Biotype B. Each bar presents the proportion of sequencing reads assigned to a given bacterial phylum. Low abundance phyla (< 1%) are represented by the "Other" category. (B) Relative abundance of bacterial genera in sample microbiota categorized as Biotype A versus Biotype B. Each bar presents the proportion of sequencing reads assigned to a given bacterial genus. Low abundance genera (< 1%) are represented by the "Other" category. Samples were pooled by biotype to produce the bar graphs in Panels A & B, irrespective of basin type of sampling date. (C, D) Alpha diversity in sample microbiota categorized as Biotype A versus Biotype B as measured by ASV richness (C) and Shannon's *H* index (D). Box-and-whisker plots show high, low and median values, with lower and upper edges of each box denoting first and third quartiles, respecitvely. Asterisks (***) indicate significant differences between biotypes (repeated measures ANOVA; ASV richness: $F_{1,190} = 33.00$, $p < 0.0001$, Shannon's *H* index: $F_{1,190} = 43.30$, $p < 0.0001$). (E, F) Relative abundance of bacterial taxa principally responsible for the separation of biotypes. Box-and-whisker plots show high, low and median values, with lower and upper edges of each box denoting first and third quartiles, respectively. Asterisks (***) indicate significant differences between biotypes (repeated measures ANOVA; C39: $F_{1,190} = 45.58$, $p < 0.0001$, Firmicutes: $F_{1,190} = 42.00$, $p < 0.0001$).

3.3.1 | Basin Conditions Predict Both Alpha and Beta Diversity of Associated Microbial Communities

Alpha diversity in basins (as measured by Shannon's *H* index) differed among sampling dates (repeated measures ANOVA; $F_{7,49} = 11.88$, $p < 0.0001$), with diversity increasing from the beginning of the season until 6/25, and late-season diversity being lower on average than at the beginning of the season (Figure S4 and Table S2). Certain water quality metrics (pH and conductivity) also significantly predicted Shannon index values among individual basin observations (Table S2 and Table S6). In contrast, both separated and combined basins, as well as basins assigned to different flow groups, harboured bacterial communities characterised by Shannon index values that varied somewhat unpredictably but were overall statistically similar to one another over the season (repeated measures ANOVA; by basin type: $F_{1,6} = 0.74$, $p > 0.05$; by flow group: $F_{4,3} = 0.99$, $p > 0.05$) (Figure S4; Table S2).

With respect to inter-basin beta diversity, early season sampling dates (April–June) and late season sampling dates (July–September) exhibited different dynamics, with early-season samples clustering more strongly by date than late-season samples (Figure S5). Climatic conditions likely contributed to the community shift mid-season. Air and water temperatures increased steadily until 6/18, after which temperatures fluctuated through the rest of the season (Figure S6). A June sampling date (6/25) was the highest rainfall day of the season (Figure S6), and this heavy rainfall event likely flushed away any accumulated sediment or nutrients that supported the existing bacterial community. Biotic drivers of the early-season assemblage would have also been flushed away, initiating a new process of succession and the assembly of divergent communities.

Community composition also varied by environmental variables and basin characteristics. Community beta diversity differed significantly by sampling date, temperature, conductivity, and salinity, as indicated by PERMANOVA and dbRDA tests using PhILR Euclidean distances as a measure of beta diversity and basin identifier as a blocking variable to account for correlation of community composition between samples collected from the same basin over time (Table 2A and Table S7). Fixed basin characteristics also differentiated basin communities aggregated across the whole season, with communities differing between combined and separated basins but not among flow groups (Table 2B and Table S7). Combined basins were enriched in Campilobacterota compared to separated basins (Figures 2A and 4), reflecting a greater presence of human gut-associated bacteria where sewer systems are connected. In contrast, separated basins were enriched in Patescibacteria, members

TABLE 2 | Effects of sampling date, water quality, and mosquito productivity on microbiota diversity. Results from PERMDISP analyses of significant explanatory variables can be found in Table S7.

	(df1, df2)	R^2	Pseudo-*F*	*p* value
(A) PERMANOVA and dbRDA tests using PhILR distances between samples (unaggregated data)				
Sampling date	(7, 222)	0.46	27.06	0.0010*
pH	(137, 92)	0.60	1.01	0.47
Temperature (°C)	(75, 154)	0.39	1.29	0.0060*
Conductivity	(196, 33)	0.90	1.51	0.0020*
Dissolved oxygen	(77, 152)	0.35	1.05	0.37
Salinity	(19, 210)	0.14	1.78	0.0010*
Pupal occurrence (presence/absence)	(1, 215)	0.07	17.25	0.0010*
Pupal abundance (pupae per dip)	(22, 194)	0.17	1.85	0.0010*
(B) PERMANOVA tests using PhILR distances between samples aggregated by basin				
Basin type (combined/separated)	(1, 40)	0.05	2.18	0.041*
Flow group (Donald-Banta, Gibbons, Mayfair-Carlyle, Miner-Evanston-Rammer, Stratford)	(4, 37)	0.14	1.54	0.053

*p value significant at the ≤ 0.05 level.

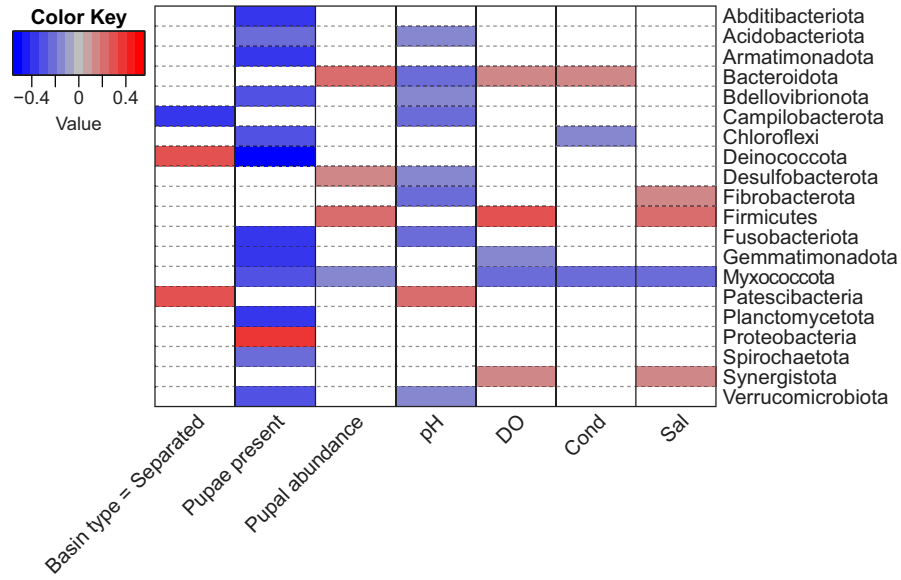

FIGURE 4 | Bacterial taxa significantly associated with different catch basin variables. Heatmap showing bacterial phyla that significantly changed in relative abundance in response to basin type, pupal presence/abundance, and water quality variables that were significantly associated with mosquito productivity (pH; dissolved oxygen, DO; conductivity, Cond; salinity, Sal) (ALDEx2, $p < 0.05$; FDR, $p < 0.05$). Colours represent standardised effect sizes for each phylum (red, high; blue, low).

of which are commonly detected in groundwater and lake environments (Herrmann et al. 2019; Tian et al. 2020) and, in this system, were also associated with basins with high pH (Figure 4). Basins with low dissolved oxygen measures were enriched in Gemmatimonadota and Myxococcota (Figure 4). Several common lake phyla were overrepresented in low-pH and low-conductivity basins, including Acidobacteria, Bacteroidota, Bdellovibrionota, Campilobacterota, Chloroflexi, Desulfobacterota, Fibrobacterota, Fusobacteriota, Myxococcota, and Verrucomicrobiota. Among these, members of the Acidobacteria, Bacteroidota, Chloroflexi, and Verrucomicrobiota are also commonly detected in sewer environments, lakes and wastewater systems (Newton et al. 2011; Zhang, Shao, and Ye 2012). Basins with high dissolved oxygen and salinity measures were also enriched in Firmicutes, which appear in high abundance in influent sewage (Cai, Ju, and Zhang 2014).

3.4 | Microbial Diversity Associates With Mosquito Productivity

Community profiles from sampled catch basins supported a relationship between habitat microbiota and mosquito productivity.

TABLE 3 | Effects of microbiota diversity on mosquito productivity.

Linear regression analyses with data aggregated by basin				
Outcome	**Predictor**	**Estimate**	**Standard error**	***p* value**
Pupal occurrence (presence/absence)	Shannon's *H* index	−0.12	0.04	0.012*
	ASV richness	−0.00	0.00	0.051
	Relative abundance (C39)	0.52	0.20	0.013*
	Relative abundance (Proteobacteria)	0.28	0.34	0.41
Pupal abundance (pupae per dip)	Shannon's *H* index	−2.11	1.04	0.049*
	ASV richness	−0.00	0.01	0.47
	Relative abundance (C39)	9.21	4.65	0.054
	Relative abundance (Proteobacteria)	−0.27	7.67	0.97

*p value significant at the ≤ 0.05 level.

Basins with lower estimates of alpha diversity (measured by both ASV richness and Shannon's *H* index) were more likely to have pupae present and tended to have higher pupal abundances (Tables 3 and S2 and Figure 5A,B). Community beta diversity also significantly differed by pupal occurrence and abundance, as indicated by PERMANOVA and dbRDA tests (Table 2A and Table S7).

Proteobacteria were abundant in samples across basins and over the season, and they were enriched in basins with pupae present (Figure 5C and Table S2). In basins with pupae, a large proportion of the Proteobacteria present were from the genus C39 (Figure 5D and Table S2). C39 was widespread in basins throughout the season but was particularly abundant in the second half of the season (Figure 2C,D and Table S2), and season-wide relative abundances of C39 predicted season-wide measures of pupal occurrence across individual basins (Table 3 and Table S2). However, the highest pupal counts appeared with low C39 (Figure 5E and Table S2), indicating that multiple factors were instrumental in supporting high pupal abundance in addition to C39 abundance. Several strains (ASVs) of C39 were also present throughout the season, but the late-season C39 that accounted for on average greater than 75% relative abundance was represented by a single strain (Figure 5F). Several other genera in the Proteobacteria were significantly associated with pupal occurrence, including *Thorsellia* (Wilcoxon Rank Sum test; Benjamini-Hochberg-corrected $p = 0.0079$), *Nevskia* ($p = 0.024$), and *Aeromonas* ($p = 0.00090$). *Thorsellia* spp. are dominant members of the adult *Anopheles gambiae* gut microbiota (Briones et al. 2008), and *Thorsellia* and *Nevskia* spp. have been reported from the water surface of rice paddies and freshwater ditches, respectively (Wotton and Preston 2005). *Aeromonas* strains have been detected in *Culex* and *Anopheles* mosquitoes (Terenius et al. 2008). Taxa associated with basins without pupae also included several phyla commonly reported in lake epilimnia and/or wastewater effluent, namely Acidobacteria, Chloroflexi, Fusobacteriota, Gemmatimonadota, Planctomycetota, and Spirochaetota (Figure 4) (Newton et al. 2011; Zhang, Shao, and Ye 2012).

4 | Discussion

The existing literature on microbial communities associated with mosquito habitats largely consists of descriptive studies of bacterial diversity in aboveground habitats that do not connect measures of bacterial diversity with measures of mosquito fitness. Recent studies further support that belowground habitats such as stormwater catch basins can serve as important reservoirs for urban mosquito populations. However, the microbiota associated with different belowground habitats and their relationship with mosquito productivity has to date been unexplored. In this study, we addressed these knowledge gaps by characterising microbiota diversity, water quality, and mosquito productivity in a network of catch basins treated with methoprene over an entire mosquito breeding season. We then used these data to assess whether specific bacterial taxa and/or measures of total alpha and beta microbial diversity were associated with the presence and abundance of pupae over time.

As in previous studies in Chicago area catch basins, we found that mosquito productivity was higher later in the season (Jackson et al. 2013; Harbison, Runde et al. 2018) and impacted by several factors, including methoprene treatment and water quality. Methoprene treatment during the present study was effective 62.5% of the time, which is consistent with previously reported rates of 36%–75% (Harbison, Nasciet et al. 2018; Harbison, Runde et al. 2018) and the inability of monitoring methods to distinguish between larvae/pupae resulting from newly colonised populations and those potentially dispersed from other basins, derived from late instar larvae that were not methoprene-sensitive at the time of treatment, or already present in the basin pre-treatment. In addition to treatment impacts, basins with higher dissolved oxygen, conductivity, and temperature measures were associated with higher pupal occurrence and abundance values, while basins with higher pH measures were associated with lower measures of mosquito productivity. This is overall consistent with previous field studies reporting similar relationships between mosquito population outcomes and dissolved oxygen (Dejenie, Yohannes, and Assmelash 2011; Pinault and Hunter 2012; Botello et al. 2013; Vanlalruia, Senthilkumar, and Gurusubramanian 2014;

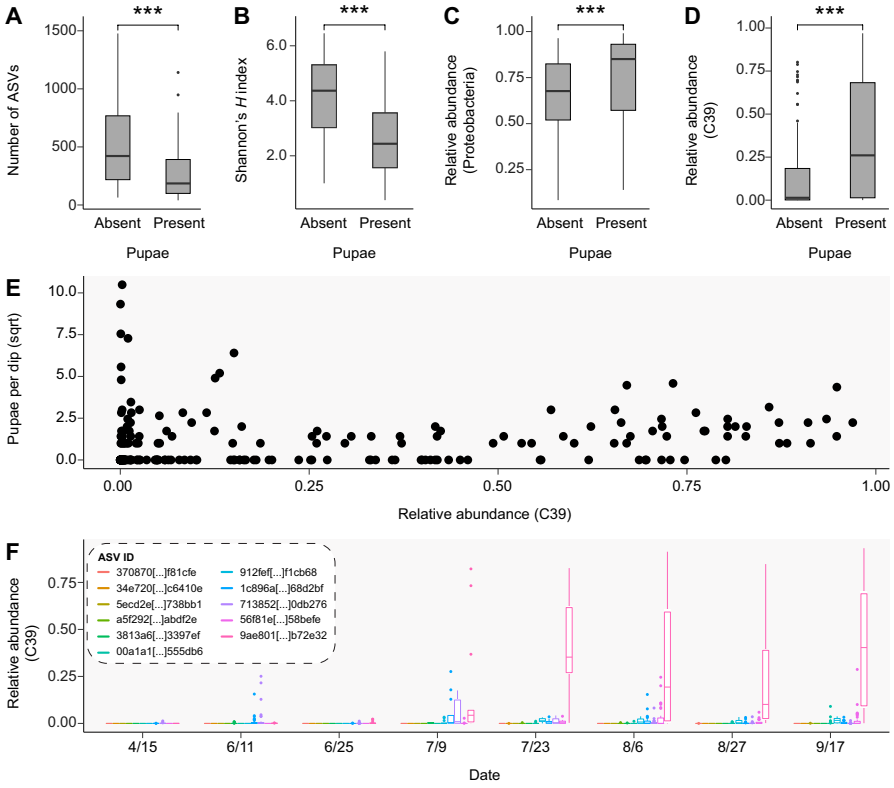

FIGURE 5 | Bacterial community differences by pupal occurrence. (A, B) Alpha diversity of microbiota in basins in the absence and presence of pupae as measured by ASV richness (A) and Shannon's *H* index (B). Box-and-whisker plots show high, low and median values, with lower and upper edges of each box denoting first and third quartiles, respectively. Asterisks (***) indicate significant differences as a function of pupal occurrence (repeated measures ANOVA; ASV richness: $F_{1,177} = 31.32$, $p < 0.0001$, Shannon's *H* index: $F_{1,177} = 57.52$, $p < 0.0001$). (C, D) Relative abundance of bacterial taxa principally responsible for the separation of basins by pupal occurrence (ALDEx2, $p < 0.05$; FDR, $p < 0.05$). Box-and-whisker plots show high, low and median values, with lower and upper edges of each box denoting first and third quartiles, respectively. Asterisks (***) indicate significant differences as a function of pupal occurrence (repeated measures ANOVA; Proteobacteria: $F_{1,177} = 5.92$, $p = 0.016$, C39: $F_{1,177} = 20.62$, $p < 0.0001$). (E) Pupae per dip in each sampled basin (square-root transformed, *y*-axis) by the relative abundance of genus C39 in the same basin (*x*-axis). (F) Relative abundance of each of 11 C39 ASVs detected across all sampled basins (*y*-axis) by sampling date (*x*-axis). Box-and-whisker plots show high, low and median values, with lower and upper edges of each box denoting first and third quartiles, respectively.

Cepeda-Palacios et al. 2017; Ranathunge et al. 2020), conductivity (Low et al. 2012; Yadav et al. 2012; Kipyab et al. 2015; Arcos et al. 2018), and temperature (Gardner et al. 2012; Kipyab et al. 2015; Cepeda-Palacios et al. 2017; Zimmerman 2019; Ranathunge et al. 2020) and the observation that low oxygen levels and temperatures reduce larval survival and developmental rates in the lab (Ciota et al. 2014; Silberbush, Abramsky, and Tsurim 2015; Reinhold, Lazzari, and Lahondère 2018). In contrast, previous field studies have reported variable relationships between mosquito productivity and pH (Leisnham et al. 2007; Low et al. 2012; Gardner et al. 2013; Vanlalruia, Senthilkumar, and Gurusubramanian 2014; Bashar et al. 2016; Garcia-Sánchez, Pinilla, and Quintero 2017). However, lab-based studies indicate that, while pH does not directly impact development, it can shape other water quality variables and biological assemblages in the habitat that affect larval growth and moulting, including microbial communities (MacGregor 1929). In sewer pipes, both pH and dissolved oxygen levels are shaped by heterotrophic activity by bacteria (Gudjonsson, Vollertsen, and Hvitved-Jacobsen 2002), so productivity changes associated with pH and/or oxygen level differences may be an indirect product of processes modulating microbial community assemblages and metabolic interactions.

Bacterial communities sampled from catch basins were diverse, and included taxa previously described in mosquito habitats, aquatic environments, and sewer systems. Catch basin samples were comprised of 40–1477 ASVs per sample, which is slightly less diverse than discrete household containers reporting 981–3408 ASVs per sample (Scolari et al. 2021). The catch basin dataset further contained a total of 1516 genera, which is higher than the 693 (Caragata et al. 2022) and 254 (Zouache et al. 2022) genera reported in studies of container water with mosquito larvae present, although per-basin diversity may be more comparable, since this study included a larger number of samples and more opportunities to find rare genera. Prior work has shown overlap in taxa present in stormwater and untreated sewage influent (Fisher et al. 2015), and the present study recovered many of the same taxa.

Catch basin-associated bacterial communities varied most notably over time, with alpha diversity and community composition differing significantly by sampling date, and the two community biotypes splitting mainly into early and late season. Seasonal successional dynamics have been reported in other aquatic environments (Jeffries et al. 2016; Scolari

et al. 2021) and in container larval habitats (Shelomi and Lin 2021). Here, diversity increased early in the season then, following a heavy rainfall event, remained lower in the late season. Rainfall has been shown to shift microbial communities of rivers connected to stormwater flows (Chaudhary et al. 2018). Changes in precipitation have also been found to affect the relative abundances of specific taxa in stormwater after heavy rainfall (Lee et al. 2020). Elevated flow rates reset residence time in water bodies (Tang et al. 2020), which can differentially affect the relative abundances of persistent versus transient taxa (Bouchali et al. 2022), and residence time has been shown to shape community variation in rivers and lakes (Tang et al. 2020). In catch basins, residence times would be highly variable throughout the season, as inputs to the system are dependent on precipitation. The 2 km radius around the study area reported here has a total elevation change of only 15 m. Owing to this flat topography, runoff management in this area depends heavily on the stormwater system and any rainfall would likely affect all basins in the area equally. In contrast, catch basins in more topographically variable areas may experience greater variability in water residence time, leading to differences in successional dynamics across elevation gradients. For this reason, future studies in the catch basin system may benefit from tracking flow rate or turnover in the basins. Shifts in biotype and mosquito productivity from early to late season could also be related to shifts in the concentration of bioactive methoprene present in individual catch basins, due to degradative capacities of associated bacterial communities, dilution from rainfall, and/or the impact of other abiotic factors that we measured, including pH. Future studies are therefore also needed to better understand how different biotic and abiotic factors shape the long-term persistence and efficacy of methoprene against different mosquito species.

While differences in alpha diversity were linked to pH and conductivity measures in individual basins, community composition (i.e., beta diversity) was found to differ by basin type, basin flow group, salinity, and conductivity. Previous studies have found that dissolved oxygen, pH, conductivity, salinity, dissolved organic carbon, and silica levels affect microbial composition in lakes (Núñez Salazar et al. 2020; Somers, Strock, and Saros 2020). Salinity in particular has been characterised as an important environmental variable for microbial community composition among lakes (Yang et al. 2016), and at a global scale (Lozupone and Knight 2007). pH has cross-cutting effects on community composition by altering the efficiency of metabolic processes because of its impact on the kinetics of reactions and bioavailability of nutrients (Núñez Salazar et al. 2020). Taxa in this study associated with low pH included Actinobacteria, Proteobacteria, and Bacteroides, all of which are dominant in stormwater (Lee et al. 2020). Basins with high pH and separated basins were enriched for Patescibacteria, which are associated with oligotrophic conditions (Herrmann et al. 2019) and have been reported in *Aedes* (Qing et al. 2020).

Both pupal occurrence and abundance were positively associated with basins with lower alpha diversity. A study surveying microbiota in natural and man-made mosquito breeding sites found that lower bacterial diversity was associated with higher densities of *Culex pipiens* and *Aedes albopictus* larvae, but lower densities of *Culex tritaeniorhynchus* larvae (Wang, Wang et al. 2021), indicating that the relationship with microbial diversity may differ by the assemblage of mosquitoes present. Heterogeneity in microbial impacts among different mosquito species is important to keep in mind for vector control, as differential effects on larval development in shared habitats may impact the relative proportion of species in adult populations in addition to total abundance, and human contact rates with vectors of different degrees of competence for various pathogens. In the present study, we focused on bulk pupal occurrence and abundance without separating by species primarily because accurate species identification at the pupal stage is difficult, and even at the larval stage, 86% of larvae were not able to be identified to species. Secondarily, because pupal occurrence is low, splitting records by species would further reduce statistical power. However, future studies would benefit from comparison of microbiota effects on population fitness of sympatric mosquito species, both with respect to competitive outcomes in the field and physiological outcomes through laboratory experiments.

Basin microbiota composition (or beta diversity) also differed by pupal occurrence and abundance, as has been reported in other environments (Dada et al. 2013; Mosquera et al. 2021; Hessou-Djossou et al. 2022). For example, the presence of larvae has been demonstrated to alter the relative abundance of certain bacterial taxa in domestic water containers, while other taxa, including members of the Proteobacteria, appear unaffected (Nilsson et al. 2018). In this study, Proteobacteria were present in basins with and without pupae, but were enriched in basins with pupae present. This pattern was most notable for the genus C39, which has previously been reported from mosquito larvae (Alfano et al. 2019) and highly polluted aquatic environments, including early in the process of phytoremediation of sewage from lake water (Chen, Huang et al. 2019), in association with higher levels of perfluoroalkyl acid pollution in seawater (Chen, Tsui et al. 2019), and in polluted urban river water (Zhou et al. 2017). The results herein do not provide information to definitively support a causal relationship between mosquito productivity and the relative abundance of C39 and/or other members of the Proteobacteria; however, follow-up laboratory assays to characterise the effects of C39 isolates on mosquito physiology may provide valuable insight into processes connecting sewage contamination and mosquito productivity in urban infrastructure.

Variation in basin-associated microbial communities may itself be caused by larval processes selecting for different taxa or altering different water quality variables (Murrell and Juliano 2008). Mosquitoes alter the microfauna of their habitat by grazing (Walker, Kaufman, and Merritt 2010), and both field studies and mesocosm experiments have shown that the presence of larvae shifts the bacterial composition of water (Kaufman et al. 1999; Xu et al. 2008; Muturi, Dunlap, and Cáceres 2020; Scolari et al. 2021). The highly alkaline conditions in the midgut of larval stage mosquitoes (Boudko et al. 2001), which aid in digestion of the tannin-rich plant detritus that larvae feed on, also have the potential to inhibit growth of many bacteria and may further influence water nutrient levels (Clements 2013; Muturi, Dunlap, and Cáceres 2020) and microbial metabolism of nitrogen (Kaufman et al. 1999). Adult female mosquitoes may further act as a source of microbial dispersal among aquatic habitats,

by introducing microbes into water during oviposition (Coon et al. 2014; Arellano and Coon 2022; Mosquera et al. 2023) and/or selecting oviposition sites that harbour specific bacteria (Trexler et al. 2003; Lindh et al. 2008).

Habitats in the field are also affected by additional variables that we did not include in this study. Previous studies have found larval abundance associated with dissolved nutrients including nitrate, phosphate, and ammonium (Leisnham et al. 2007; Gardner et al. 2013; Noori, Lockaby, and Kalin 2015; Onchuru et al. 2016; Rydzanicz et al. 2016). The assemblages of fungi, microscopic metazoa, algae, and macrofauna such as fish and other insect juveniles are also important to consider as competitors, prey, and pathogens of mosquito larvae (Ranasinghe and Amarasinghe 2020). Furthermore, relationships identified in this study may be particular to the conditions in the study area. Catch basin sites in this study were relatively uniform with respect to shade cover, distance to buildings, and land use, which all have been found to affect mosquito density (Bashar et al. 2016; Dida et al. 2018; Wang, Wang et al. 2021). Mosquito habitat water has also been found to lead to different developmental outcomes in laboratory experiments compared to the field (Wang, Zhou et al. 2021) and can show similar effects on mosquito development despite very different microbial and water characteristics (Chitolina et al. 2016). Yet unknown is how context-dependent water quality and microbial effects on mosquito populations are, and the geographic and temporal scales at which this varies. However, our results suggest that future studies on mosquito-microbe-environment dynamics would be best equipped to maximise power to capture variation in each variable by targeting deep sampling at one time point, or finer temporal monitoring. Effects of the microbiota from disparate sites could also be isolated from location-specific environmental conditions by testing their effects in laboratory settings. Such efforts will undoubtedly be facilitated by our recently developed approaches for microbiota isolation, cryopreservation, and transplantation in mosquitoes, which have been validated using microbiota derived from both the laboratory and field (Coon, Hegde, and Hughes 2022; Zhao, Hughes, and Coon 2022). Finally, community-wide metagenomic and metatranscriptomic surveys–both in situ and under controlled conditions in the laboratory–could provide insights into mechanisms underlying patterns in mosquito productivity observed in the field, and identify candidate pathways for manipulating mosquito habitats, microbiota, phenotypes, and populations for novel innovations in vector control.

5 | Conclusions

In this study, we investigated the relationship between habitat microbiota variation and mosquito population dynamics by simultaneously characterising microbiota diversity, water quality, and mosquito productivity in a network of urban stormwater catch basins over an entire mosquito breeding season. Our results support roles for both water quality and microbiota in shaping mosquito population fitness outcomes within belowground habitats in the field. Abiotic effects on mosquito productivity appear to be driven—at least in part—by impacts on microbiota diversity. Even more novel is our identification of specific microbial taxa that are associated with positive mosquito population outcomes, and that may be enriched in habitats over time by mosquito-mediated impacts on microbial community assembly. Altogether, these findings highlight the importance of microbiota as an environmental factor shaping host ecology in natural populations. They also have important implications for efforts to optimise efficacy of larval-based mosquito control strategies in the field.

Author Contributions

Serena Y. Zhao and Kerri L. Coon conceived of the study. Serena Y. Zhao, Andrew J. Sommer, Justin E. Harbison, Patrick Irwin, and Kerri L. Coon contributed to the study design. Serena Y. Zhao, Andrew J. Sommer, Dan Bartlett, Justin E. Harbison, Patrick Irwin, and Kerri L. Coon collected the study data. Serena Y. Zhao carried out the data analysis. Serena Y. Zhao wrote the initial manuscript, and Kerri L. Coon contributed to revisions. All authors read and approved the manuscript prior to submission to the journal.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw Illumina reads are available in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under BioProject ID PRJNA1127658. Scripts used for analysis and figure generation are available in the Coon laboratory's GitHub repository (https://github.com/kcoonlab/catch-basin-microbes). All other data generated by this study are available as Supporting Information herein.

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Supporting Information

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