



# An Acute Permethrin Exposure Causes Significant Microbial Shifts in *Montastraea cavernosa*

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Insecticide use is common in developed tropical regions where it may enter coastal reef ecosystems through land-based sources. This local introduction of contaminants could affect ecosystem health as corals can better withstand global stressors more readily if local pressures are reduced. The present study investigated the microbial community and photochemical efficiency of the reef building coral, *Montastraea cavernosa*, when exposed to the commercially applied insecticide, permethrin. *Montastraea cavernosa* was exposed to an acute concentration of permethrin for 24 h in a controlled laboratory setting. Fourteen fragments were integrated across four treatments (2 or 0.325  $\mu\text{g/l}$  of permethrin, acetone control, and saltwater control) with three to four replicates per treatment. Photosynthetic efficiency was measured by quantifying the maximum photochemical yield and maximum electron transport rate (ETR), which were recorded for each fragment before exposure and 24 h after exposure to permethrin. The microbial communities of *M. cavernosa* tissue was measured using 16S rRNA sequencing for each fragment. Permethrin exposure at the tested concentrations suggested no significant effect on the *M. cavernosa*'s photochemical parameters measured during the 24-h permethrin exposure. Microbial communities were significantly different between permethrin treated (2 and 0.325  $\mu\text{g/l}$ ) and non-treated conditions (acetone and saltwater controls). In permethrin treated coral, this study documented a significant increase in *Burkholderia pyroccinnia* and *Bacillus* sp., bacteria groups known to bioremediate insecticides. Exposure of permethrin also decreased the relative abundance of *Mesorhizobium* sp., *Sediminibacterium* sp., *Sphingorhabdus* sp., and *Chondromyces* sp., which are known to inhibit pathogen colonization and provide essential macromolecules. Therefore, although the symbiotic relationship between the host and the intracellular algae remained intact after the corals were exposed to permethrin, the significant shift in the microbiome indicate permethrin may destabilize the microbial composition of the holobiont.

**Keywords:** permethrin, *Montastraea cavernosa*, PAM, microbiome, coral, dysbiosis, bioremediation, microbial shift

## INTRODUCTION

Corals are frequently threatened by both global and local stressors that are causing significant mortality worldwide (Bruno et al., 2007; Eakin et al., 2010; Kennedy et al., 2013). Considerable research has focused on global issues such as high water temperatures from climate change, ocean acidification, and disease (Hoegh-Guldberg et al., 2007; Hughes et al., 2016). However, it is imperative to investigate other existing anthropogenic and natural influences affecting reefs on the local scale, especially because those threats could be alleviated through local management action. Previous research suggest that corals may be able to withstand some global pressures more readily if local threats are reduced (Carilli et al., 2009; Kennedy et al., 2013; Donovan et al., 2021). One potential local threat that has had little attention includes the applications of pesticides within nearshore environments. Permethrin [(3-phenoxyphenyl)methyl(+)*cis,trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-carboxylate] is a mix of synthetically produced *cis* and *trans* isomer pyrethroids, which functions to paralyze the nervous system of insects (Pierce, 1998). Often, permethrin is sprayed from a mounted sprayer attached to the back of a moving vehicle, typically applied in the summer rainy season (May–October) to target when adult mosquitos hatch after a rainfall (Monroe County Mosquito Control) (Ross et al., 2015). Permethrin has been detected in surface water canal systems throughout the Florida Keys (5.1–9.4  $\mu\text{g/l}$ ) and within the Florida Keys National Marine Sanctuary ranging from 0.5 to 50.1  $\mu\text{g/m}^2$  due to aerial drift and runoff (Pierce, 2005). Permethrin is highly toxic to aquatic invertebrates, with reported 96-h LC-50 to be 0.02  $\mu\text{g/l}$  for mysid shrimp, 0.2  $\mu\text{g/l}$  for penaeid shrimp, and 2.2  $\mu\text{g/l}$  for silverside minnows (Clark et al., 1989; Tietze et al., 1995). Permethrin typically has a half-life of 14 days in water and sediment (Lee et al., 2004).

Very few investigations have studied the effects of insecticides on corals. However, one study showed that the mosquito pesticide naled, which is typically sprayed intermittently with permethrin, has a negative effect to larval settlement in *Porites astreoides* by reducing survivorship (Ross et al., 2015). Permethrin, however, did not affect larval settlement success under ambient conditions nor was there a negative response when corals were exposed to permethrin in concert with increased water temperatures. Low concentrations of permethrin (0.3–1.0  $\mu\text{g/l}$ ), however, showed reduced settlement and metamorphosis on the larvae of *Acropora millepora* (Markey et al., 2007), suggesting species and life-stage specific responses. *Acropora cervicornis* corals also showed specific transcription of stress-associated genes after being exposed to low doses of permethrin (Morgan et al., 2001). To date, studies have focused on the effects of insecticides on the coral host and its associated algal symbiont although a suite of microbes exist to create the coral holobiont (Morgan et al., 2001; Ritchie, 2006). The effects of the widely commercialized insecticides on the microbiome of corals are generally unknown.

A healthy coral microbiome can exhibit antibiotic properties to defend the coral from invasive bacteria (Ritchie, 2006). However, as the health of reef ecosystems have shifted from fully functioning to disturbed, microbial shifts within corals

often occur with these environmental stressors that result in the introduction of opportunistic bacteria (Sunagawa et al., 2009). Known stressors that elicit a bacterial shift include high water temperatures, excessive nutrients, coastal development, and overfishing, which all influence coral health (Bruno et al., 2003, 2007; Brandt and Mcmanus, 2009) as well as others. The present study aimed to further investigate the effects of anthropogenic effects on corals by characterizing the microbial community within corals as well as a suite of photochemical efficiency parameters after exposure to two different concentrations of permethrin within a controlled setting. We hypothesized that exposure to permethrin would reduce photochemical efficiency within the coral holobiont, which would suggest a disruption in the coral-algal symbiosis. Additionally, we hypothesized that permethrin exposure would result in a dysbiosis of the microbial community through identifiable shifts in bacterial members. Quantifying the response of the holobiont physiology and the microbiome community characterized the effects of this short-term contaminant exposure on a major reef building coral of Florida's Coral Reef.

## MATERIALS AND METHODS

### Coral Collection

*Montastraea cavernosa*, the great star coral, was originally collected from the National Oceanic and Atmospheric Administration (NOAA) Rescue Nursery located off of Key West, Florida using permit FKNMS-2015-082 and transferred to Mote Marine Laboratory's research facility in Sarasota, Florida. Ten parent colonies of *M. cavernosa* were cut into fourteen 1.5"  $\times$  1.5" fragments and allowed to recover for 14 days within a flow-through raceway prior to random fragment assignment in the subsequent exposure experiment. The raceway was equipped with a scheduled 12-h activated T5 light (Wave Point). The temperature-regulated raceway provided consistent water parameters by circulating water with additional water jets. To conduct the exposure experiment, 14 glass beakers (1 L) were distributed within the water bath of the raceway. A randomly selected single fragment of coral was placed within each beaker and allowed to further acclimate to the controlled system for 7 days. Treatments were then assigned to beakers as controls or permethrin treatments (**Supplementary Figure 1**). Each glass beaker was assigned a water hose for flow through water exchange during acclimation and an air stone to provide additional aeration and to eventually aid in the mixing of permethrin. Water quality parameters, including temperature, pH, dissolved oxygen, and salinity were recorded daily. Temperatures were maintained at 27°C ( $\pm 0.006$ ), pH averaged 7.94 [ $\pm 0.001$ ; National Bureau of Standards (NBS)], salinity averaged 36.35 ppt ( $\pm 0.005$ ), and dissolved oxygen averaged 5.98 mg/L ( $\pm 0.003$ ).

### Permethrin Exposure

Permethrin (Chem Service Inc., West Chester, PA, United States, 99.25% purity) concentrations for exposure to *M. cavernosa* were 2 (high) and 0.325  $\mu\text{g/L}$  (low), plus acetone and seawater controls,

for a total of four treatments. Acetone (5  $\mu\text{l/L}$ ) was utilized to increase the solubility of the permethrin treatments. The target doses of permethrin were selected due to the limited solubility of permethrin in acetone, which precipitates in excess of 10  $\mu\text{g/L}$  (Ross et al., 2015). Low permethrin and acetone treatments had four replicate aquaria each and high and saltwater controls both had three replicate aquaria. The high permethrin treatment and saltwater control had one less replicate due to sample processing error at the end of the exposure and subsequent contamination, which warranted their removal from the analysis.

The corals were dosed with treatment conditions on October 12, 2016, a week after the fragments were placed into the 1-L beakers for acclimation. Water samples (500 ml) were collected for analysis of permethrin concentration 30 mins and 24-h after dosing occurred. Permethrin was recovered from water samples by liquid-liquid extraction into dichloromethane (DCM). The DCM was replaced with MeOH by rotary evaporation for DCM solvent recovery. The MeOH containing permethrin extract was reduced to 1 ml volume for HPLC-MS/MS analysis of both the *cis*- and *trans*-permethrin isomers.

Permethrin analysis was conducted using a Thermo Electron Quantum Access HPLC system, interfaced with a Quantum Access triple quadrupole MS/MS. A Phenomenex Kinetex 2.6  $\mu\text{m}$  particle size, 100 mm  $\times$  2.1 mm analytical column was used with mobile phase gradient of 10 mM Ammonium Formate in Methanol, 10 mM Ammonium Formate in HPLC water, initial ratio 20:80 for 5 min, then to 99:1 for 8 min, back to 20:80 for 2 min, total sample run time 15 min at a flow rate of 200  $\mu\text{l/min}$ . Analytical standards, *Trans*- and *Cis*-Permethrin solutions were purchased from Chem Service.

## Pulse Amplitude Modulation Fluorometer

To determine the state of the coral-algal symbiosis the photochemical efficiency on each colony was measured using a Walz Imaging Pulse Amplitude Modulator (PAM) Fluorometer. The imaging PAM measures the spatial distribution of chlorophyll fluorescence of photosystem II. *Montastraea cavernosa* colonies were dark acclimated (30 min) prior to conducting light curves. Light curves provided information on photochemical yield ( $F_v/F_m$ ) and electron transport rate (ETR). The photochemical yield ( $F_v/F_m$ ) is a normalizing ratio created by dividing the variable fluorescence ( $F_v$ ) by the maximum fluorescence ( $F_m$ ). By dark acclimating the colony, this relaxes the photosystem II reaction centers. When the colony was exposed to a pulse of light, the calculated yield represented the maximum potential quantum efficiency of photosystem II if all capable reaction centers were open. The maximum ETR represents the photosynthetic capacity of an organism after which photoinhibition occurs where the organism cannot process additional photosynthetic active radiation (PAR) that is being emitted by the PAM. The ETR represents photosynthetic capacity because it is informative of the number of electrons passing through photosystem II where four electrons pass through photosystem II for every  $\text{O}_2$  produced during photosynthesis (Ritchie and Bunthawin, 2010). The PAM measurements were taken prior to permethrin exposure and 24 h after exposure. After passing the assumptions for normality using

the Shapiro-Wilk test and homoscedasticity, tested using the Levene's test, a paired *t*-test, and the multiple comparison's false discovery rate (FDR) test compared replicates within treatments before exposure and 24 h after exposure to permethrin in R v1.2.5033 (R Core Team, 2019).

## Microbial Community

After 24 h of exposure, tissue samples were collected from each of the *M. cavernosa* fragments for DNA isolation by scraping off a single polyp using a sterile scalpel. DNA extraction was performed on the coral tissue using a Powersoil DNA isolation kit (Qiagen, Germantown, MD, United States). The extracted DNA was sent to MR DNA for sequencing of the 16S rRNA gene V1-V3 region using PCR primers 27 F and 519 R. Polymerase chain reaction protocol required a 28 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, United States) under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, after which a final elongation step at 72°C for 5 min. Samples were purified using Ampure XP beads and then sequenced at MR DNA<sup>1</sup> (Shallowater, TX, United States) on a Miseq following the manufacturer's standard guidelines. Sequence data was processed using MR DNA analysis pipeline. In summary, sequences were stitched together to assemble contigs, and sequences <150 bp or with ambiguous nucleotide identification were removed. Operational taxonomic units (OTUs) were categorized by clustering at 97% sequence similarity (Supplementary Table 1). Using the 16S barcoding region, 3,311 of the final OTUs were taxonomically classified using the nucleotide basic local alignment search tool (BLASTn) against a database derived from the Ribosomal Database Project (RDPII)<sup>2</sup> and the National Center for Biotechnology Information (NCBI)<sup>3</sup> and organized into each taxonomic level.

A permutational analysis of variance (PERMANOVA) tested for differences in the bacterial community between water samples and coral tissue samples. A PERMANOVA was also used to test for differences in the bacterial community within the coral fragments among treatments. Dominant bacterial classes were identified as those with greater than 3% average relative abundance in any treatment condition. Classes were compared among treatments using a Kruskal Wallis test. A similarity percentage (SIMPER) analysis was used to determine the dissimilarity between treatments and provided the percent dissimilarity caused by each bacterial OTUs. The top six OTUs that contributed to dissimilarity were tested for differences among treatments using the Kruskal Wallis test and *post-hoc* analysis was performed using a Dunnett's test. Bacterial OTUs for each sample were processed through a non-metric multidimensional scaling (NMDS) plot, which provided a visual representation of the bacterial community in multidimensional space in a two-dimension visual plot. The relative abundance of bacterial classes and genera of coral tissue and water samples were also graphed for visualization.

<sup>1</sup>www.mrdnalab.com

<sup>2</sup>http://rdp.cme.msu.edu

<sup>3</sup>www.ncbi.nlm.nih.gov



Simpson diversity, Pielou's richness, and beta diversity of the bacterial community was calculated at the bacterial species level for each sample (**Supplementary Figure 2** and **Supplementary Table 2**). Using an analysis of variance (ANOVA) test, the differences in diversity and richness among treatments for each sample was applied after passing assumptions of normality using the Shapiro-Wilk test and homoscedasticity using the Levene Test.

## RESULTS

### Permethrin Adsorption

The high treatment dose of permethrin ( $n = 3$ ) ranged between 1.60 and 2.20  $\mu\text{g/L}$  with an average of 2.0  $\mu\text{g/l}$  ( $\pm 0.122$   $\mu\text{g/l}$  SE) (**Supplementary Figure 3**). The low permethrin treatments ( $n = 4$ ) received a dosing concentration between 0.20 and 0.40  $\mu\text{g/L}$  with an average of 0.325  $\mu\text{g/l}$  ( $\pm 0.046$   $\mu\text{g/l}$  SE). No permethrin was found in the saltwater control ( $n = 3$ ) or acetone controls ( $n = 4$ ). Measurements after 24 h showed an absence of permethrin for all but one sample within low concentration treatment, which generated an average of 0.0125  $\mu\text{g/l}$  ( $\pm 0.0125$   $\mu\text{g/l}$  SE). The loss of permethrin from exposure solutions was most likely a result of the hydrophobic nature of permethrin causing it to adsorb to surfaces when dispersed in seawater or potentially the biological accumulation of permethrin within the coral tissue itself. However, identifying where the permethrin adsorbed to during the depuration process was outside of the scope of the present project.

### Photochemical Parameters of the Algal Symbiont

Within each treatment, there were no significant differences between the initial and post photochemical yields ( $p > 0.1$  for all comparisons; **Supplementary Figure 4**). The photochemical yield decreased for corals exposed to permethrin in high and low treatments, but this was not significant ( $t = 1.0182$ ,  $df = 6$ ,  $p\text{-value} = 0.3479$ ,  $\text{FDR padj} = 0.80$ ; **Supplementary Figure 4**). The ETR had a significant increase in post permethrin exposure relative to before permethrin exposure in only coral exposed to permethrin, however the *post-hoc* FDR test showed no significant differences ( $t = -3.2609$ ,  $df = 6$ ,  $p\text{-value} = 0.01723$ ,  $\text{FDR padj} = 0.075$ ; **Supplementary Figure 5**).

### Microbial Community

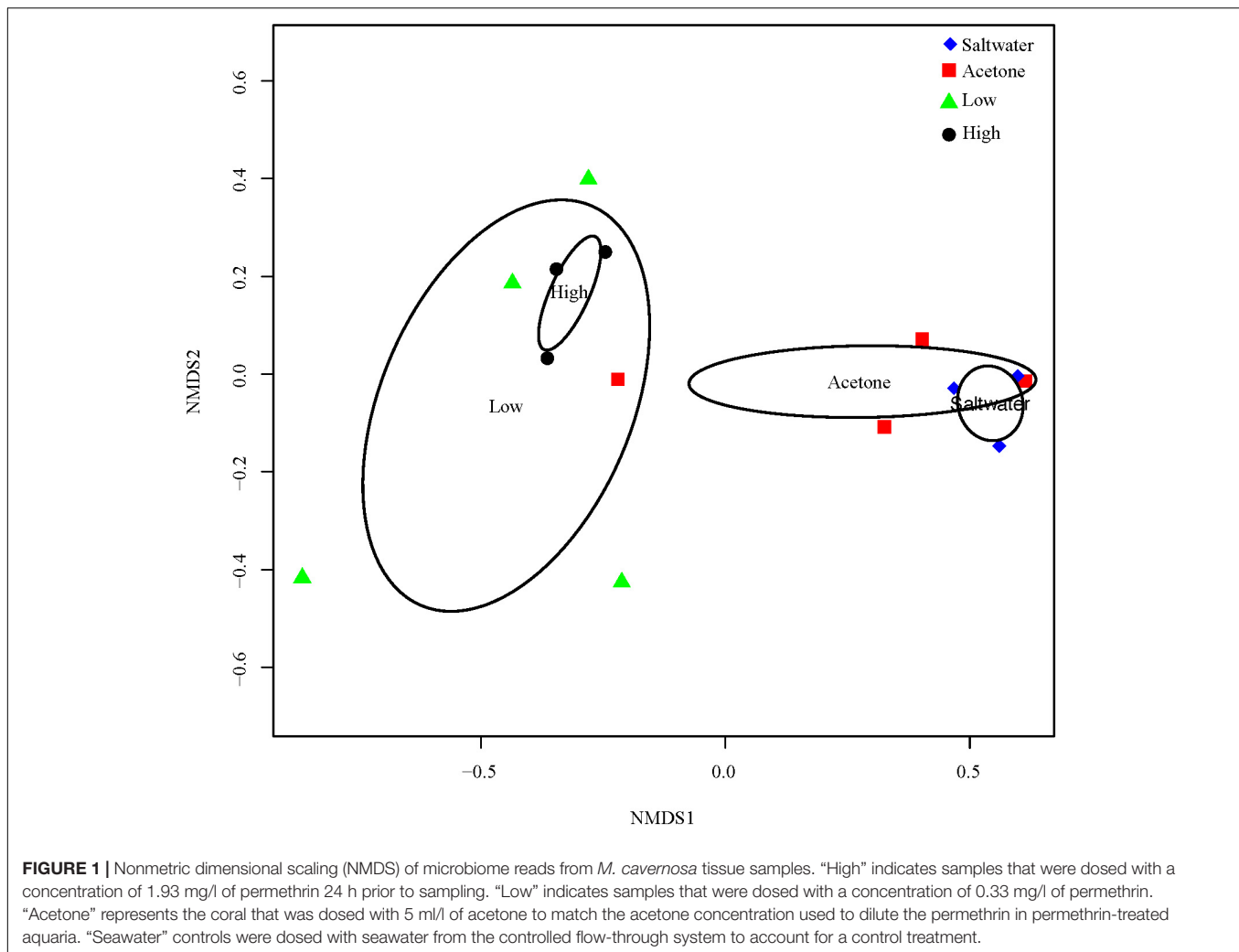
The 16S rDNA sequencing resulted in an average of 47,993 ( $\pm 5,633$  SE) reads per coral tissue sample and the water samples had an average of 108,899 ( $\pm 7,453$  SE) reads. There were a total number of 1,350 distinct OTUs within the coral tissue samples, whereas there was a total of 2,771 distinct OTUs within the water samples. A permutational ANOVA showed a significant difference between the bacterial community within the water compared to the bacterial community of the *M. cavernosa* tissue, suggesting the tissue samples indeed represent the community associated with the living coral tissue ( $F = 9.5354$ ,  $df = 1$ ,  $p\text{-value} < 0.001$ ; **Supplementary Table 3**

and **Supplementary Figures 6, 7**). There was also a significant difference in the bacterial community of *M. cavernosa* among treatments ( $F = 5.272$ ,  $df = 3$ ,  $p\text{-value} = 0.0028$ ; **Figure 1**). However, pairwise PERMANOVAs detected no significance between treatment pairs (**Supplementary Table 4**) due to the conservative approach of the test.

The coral tissue bacterial community of the seawater control treatment was dominated by two classes: Alphaproteobacteria (41.7%) and Sphingobacteria (33.9%). The acetone control tissue samples were dominated by three bacterial classes, Alphaproteobacteria (24%), Betaproteobacteria (34.5%), and Sphingobacteria (22.5%). In seawater controls, Alphaproteobacteria (41.7%) was primarily composed of *Sphingopyxis* sp. (16%), *Mesorhizobium* sp. (11.4%), and *Bradyrhizobium* sp. (6.8%) (**Figure 2C**). Alphaproteobacteria (24%) abundance was lower in acetone control corals but was still primarily composed of *Sphingopyxis* sp. (3.6%), *Mesorhizobium* sp. (8.6%), and *Bradyrhizobium* sp. (5%) (**Figures 2B,C**). Betaproteobacteria was dominated by *Burkholderia pyrrocinia* (**Figure 2D**), and Sphingobacteria was dominated by *Sediminibacterium* sp. (**Figure 2E**) within the acetone and saltwater control colonies.

In low permethrin treatment coral, Betaproteobacteria contributed to 67% of the coral's microbiome, nearly exclusively composed of *B. pyrrocinia* (66.4%) (**Figure 2D**). Betaproteobacteria was higher in abundance in low-dosed coral compared with the other treatments and the bacterial community of this treatment also showed significantly lower ( $p = 0.006$ ) abundances of the bacterial classes that were dominant in the control coral tissue. Alphaproteobacteria (14%) and Sphingobacteria (0.9%) were lower in the low dose treated corals compared with controls (**Figure 2A**). Alphaproteobacteria was dominated by *Sphingopyxis* sp., *Mesorhizobium* sp., and *Bradyrhizobium* sp. in the controls, but these genera were nearly absent in the low-dosed tissue. Rather, this class was composed primarily of *Novosphingobium* sp. (3.6%) and *Acidocella* sp. (3.3%) (**Figure 2C**). The relative abundance of Sphingobacteria (0.9%) remained dominated by *Sediminibacterium* sp. (0.9%) in low-dosed coral (**Figure 2E**).

In the high-dosed coral bacterial community, Betaproteobacteria (64.8%), dominated by *B. pyrrocinia* (62.7%), comprised most of the microbiome. Alphaproteobacteria (5.6%) abundance in the high-dosed treatment was less than in the controls and low-dosed coral. *Sphingopyxis* sp. and *Mesorhizobium* sp. were significantly reduced ( $df = 3$ ,  $p\text{-value} < 0.05$ ;  $df = 3$ ,  $p\text{-value} < 0.05$ ; **Figure 3**) in permethrin treated microbiomes and the Alphaproteobacteria was represented by different bacterial genera and primarily composed of *Acidocella* sp. and *Novosphingobium* sp. (**Figure 2C**). Sphingobacteria was relatively less abundant in high-dosed tissue (1.2%) than in the controls (24%) and was similar in abundance compared with the low-dosed tissue (0.9%) (**Figure 2E**). Bacilli (13.8%), dominated by *Bacillus* sp., increased in abundance in high-dosed tissue (**Figure 2F**). In comparison, Bacilli was observed at decreased abundance in low-dosed (2.3%), acetone control (0.8%), and seawater controls (0.14%).



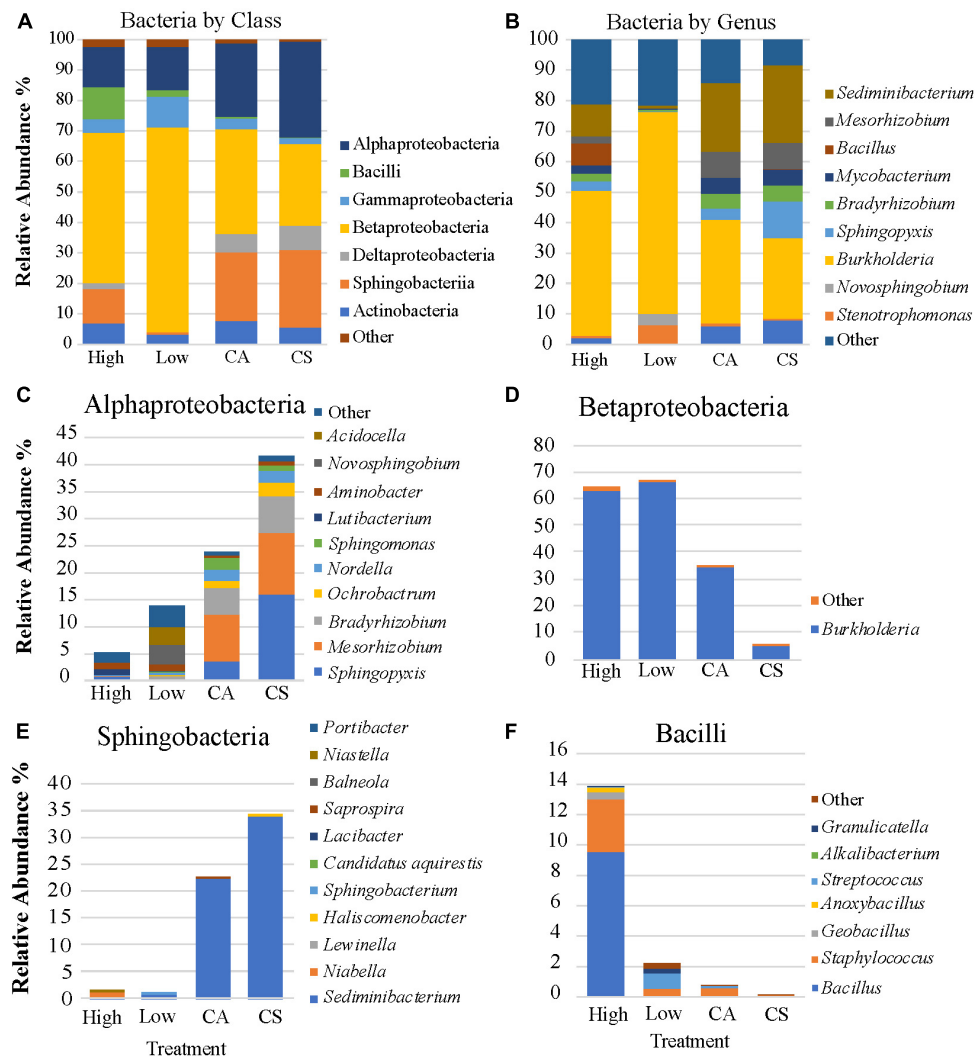
## Dissimilarity Among Treatments

The SIMPER analysis showed that the six OTU's were responsible for the greatest dissimilarity among treatments included: (1) *B. pyrrocinia* (homology identified at 99%; **Figure 3**), (2) *Sediminibacterium* sp. (homology identified at 96%; **Figure 3**), (3) *Sphingopyxis* sp. (homology identified at 99%; **Figure 3**), (4) *Chondromyces* sp. (homology identified at 97%; **Figure 3**), (5) *Bacillus* sp. (homology identified as 99%; **Figure 3**), and (6) *Mesorhizobium* sp. (homology identified as 98%; **Figure 3**). Comparisons of these genera among treatments using non-parametric Kruskal-Wallis tests presented significant differences in the relative abundance (**Supplementary Table 5**) for *B. pyrrocinia* ( $x^2 = 8.3476$ ,  $df = 3$ ,  $p = 0.039$ ; **Figure 3**), *Sediminibacterium* sp. ( $x^2 = 8.7238$ ,  $df = 3$ ,  $p = 0.033$ ; **Figure 3**), *Mesorhizobium* sp. ( $x^2 = 10.799$ ,  $df = 3$ ,  $p = 0.012$ ; **Figure 3**), *Sphingopyxis* sp. ( $x^2 = 9.8762$ ,  $df = 3$ ,  $p = 0.02$ ; **Figure 3**), and *Chondromyces* sp. ( $x^2 = 10.933$ ,  $df = 3$ ,  $p = 0.012$ ; **Figure 3**). The multiple comparison *post-hoc* Dunnett's test showed that *B. pyrrocinia* was significantly higher in the high permethrin treatment compared to the saltwater control ( $t = 3.957$ ,  $p = 0.007$ ). This

conservative *post-hoc* test did not detect any difference in *Bacillus* sp. among treatments. *Sediminibacterium* sp., *Mesorhizobium* sp., *Sphingopyxis* sp., and *Chondromyces* sp. were significantly more abundant in seawater control corals compared to low permethrin exposure conditions ( $p < 0.037$  for all comparisons). *Sediminibacterium* sp., *Mesorhizobium* sp., and *Sphingopyxis* sp. were also significantly more abundant in seawater control corals compared with the high permethrin exposure conditions ( $p < 0.005$  for all comparisons).

## Microbial Diversity and Richness

Statistical analyses showed no significant differences in the alpha diversity of the bacterial community among treatments (**Supplementary Figures 2A–C** and **Supplementary Table 2**). Interestingly, both the Simpson's diversity and the Pielou's Evenness metrics showed a trending linear decrease among treatments from controls to the high treatment exposure (**Supplementary Figure 2C**). Beta diversity of the microbial community was not significantly different among treatments (**Supplementary Figure 2B**) with no apparent trends visible.



**FIGURE 2 |** The average relative abundance of the bacterial community members by class (A), genus (B), and the genera that comprise the four dominant classes (C–F).

## DISCUSSION

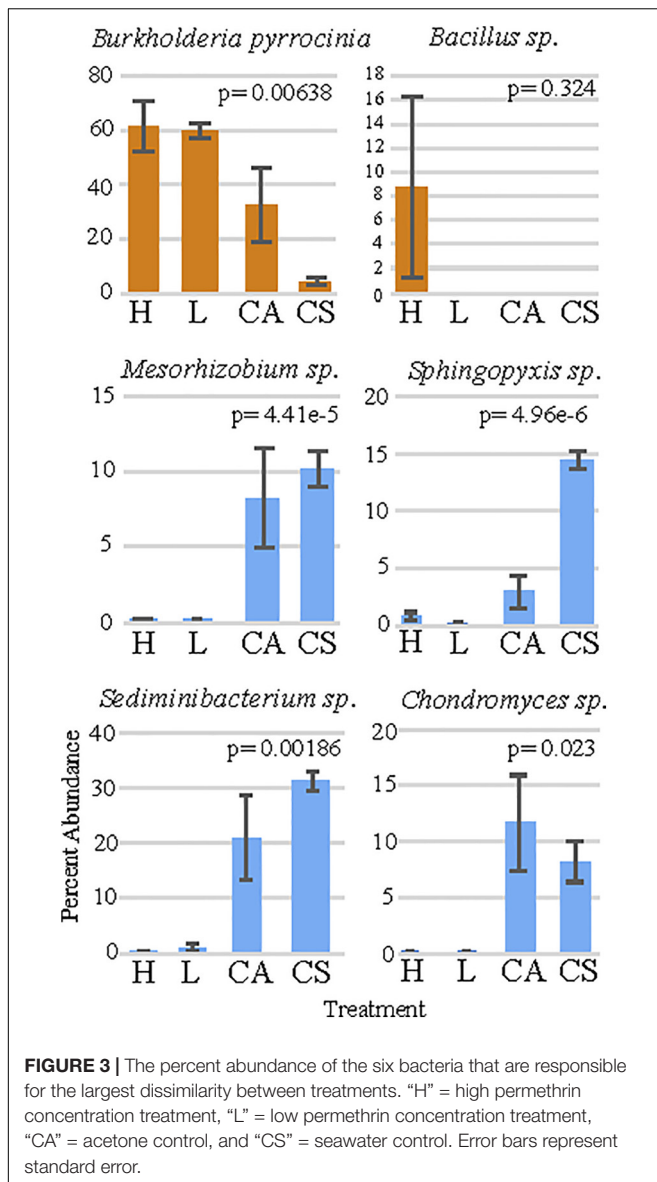
### Stable Photochemical Efficiency

Previous investigations on the effects of pesticide exposure on coral have documented a decline in settlement, survivorship, stunted metamorphosis, and transcriptional stress response in the larvae of *Acropora* sp. (Morgan et al., 2001; Markey et al., 2007; Ross et al., 2015). However, Ross et al. (2015) showed no significant effects of permethrin exposure on *Porites astreoides* larvae settlement and survival. The present study measured the photochemical and microbial community response in adult *M. cavernosa* to an acute permethrin exposure. The maintenance of similar maximum ETR and maximum photochemical yield suggests the algal-coral symbiosis of adult *M. cavernosa* exposed to an acute permethrin dose over 24 h was not negatively affected. The lack of an effect on the coral-algal symbiosis suggests that the dose may have been too low to elicit

a response, the exposure may have been too short, or the corals tested may be resistant to permethrin exposure. Further studies that incorporate repeated or long-term exposure, increased levels of dosing, and additional species are needed to quantify the level of resistance corals may have to this commonly used insecticide.

### Microbiome Shifts May Mediate Insecticide Resistance

*Burkholderia pyrrocinia*, a nitrogen fixing bacteria, was the most abundant bacteria within the bacterial community of the fragments exposed to permethrin, consisting of approximately 60% of the bacterial community. On the other hand, the presence of *Burkholderia* contributed to less than 10% of the bacterial community within the control corals. *Burkholderia* has been identified as an insecticide resistant bacterium found in the soybean bug *Riptortus pedestris*, which allowed the bean bug to survive fenitrothion spraying (Kikuchi et al., 2012). *Burkholderia*



hydrolyzes fenitrothion, an organophosphorous insecticide, into 3-methyl 4-nitrophenol and metabolizes the degradation product as a carbon source for *Burkholderia*'s growth. *Burkholderia* has also been identified with insecticide degradation properties toward bifenthrin, a pyrethroid that includes permethrin (Lee et al., 2004). The increased relative abundance of this particular bacteria within corals exposed to permethrin suggests it may function in a similar way within corals. However, further study is needed to target the function of the bacterial group that becomes dominant within the microbiome of *M. cavernosa* exposed to acute levels of permethrin.

*Bacillus sp.*, which was also abundant within permethrin exposed corals, has the ability to hydrolyze pyrethroids, particularly even permethrin into non-insecticidal compounds (Maloney et al., 1992, 1993; Chen et al., 2014). *Bacillus* shifted to an 8.76% abundance in the high dosage of permethrin treatment

compared to the near 0% of *Bacillus* in other treatments. These results may suggest bioremediation, or a breakdown of permethrin is occurring by the microbiome to mitigate the insecticide presence. However, *Bacillus* is a large genus with hundreds of species with different functional roles. Further exploring how the increase of this bacterial genera may affect the coral holobiont could be an important research topic for follow-up studies. Alternatively, the exposure could have simply resulted in a selection for insecticide resistant bacteria, thus changing the community to have more representation of bacteria that can survive and even break down the compounds within insecticides.

### Permethrin Reduced Potentially Stabilizing Bacteria

The exposure of corals to permethrin caused a significant shift in the bacterial community, perhaps reducing beneficial bacterial found within healthy corals. For example, *Sediminibacterium sp.* was highest in abundance in control corals (up to 32%) and completely lost in permethrin exposed coral. This bacteria has been identified in healthy coral tissues in previous studies (Lesser and Jarett, 2014). Similarly, *Chondromyces sp.* was approximately 10% of the bacterial community in acetone and seawater control coral tissues. However, permethrin treated coral had close to 0% relative abundance of *Chondromyces sp.* This bacteria species has been identified in sponges and produce the bacterial metabolite Apicularen A isolates, which have strong inhibition of angiogenesis, or blood vessel development associated with tumor growth (Kwon, 2002). While *Sediminibacterium sp.*'s function in coral is unknown in corals, it is a well-recognized plant growth promoting rhizobacteria and reported to be responsible for subduing soil borne pathogens, suggesting at least some species within this genus may have probiotic properties (Han et al., 2019). Additionally, the reduced abundance of *Chondromyces sp.*'s to nearly absent as a consequence of permethrin exposure implicates a functional loss in opportunity for this bacteria species when corals interact with this contaminant.

The loss of *Sphingopyxis sp.* bacterium in the permethrin exposed fragments may also have negative effects to the host. *Sphingorhabdus litoris* (previously *Sphingopyxis litoris*) is the closest phylogenetically related bacterium within *Sphingopyxis sp.* that has been observed in coral and isolated from the gorgonian *Eunicella labiate*. Genome annotation of *S. litoris* presented genes coding for polyphosphate kinase and exopolyphosphatase, which mitigates the synthesis of energetic polyphosphatases (Silva et al., 2018). This likely provides phosphorous for the host, an important requirement for stony corals, soft corals, and sponges. Further annotation of the *S. litoris* genome presents genes that are associated with homeostasis with metals, and even resistance to antibiotics. Some strains (EL 138) of *S. litoris* contain genes with homology to astaxanthin, which has anti-inflammatory properties (Silva et al., 2018). Finally, *Sphingopyxis sp.* in general can extrude porphyrin-like growth factors that promote the growth of other bacteria that otherwise grew poorly (Bhuiyan et al., 2015). The beneficial properties of this bacteria within other similar marine organisms



suggest that the loss of this genus within corals exposed to permethrin could have larger implications on coral health by the insecticide exposure resulting in the loss of beneficial microbes within the holobiont.

Finally, *Mesorhizobium* sp. showed a similar result as *Sediminibacterium* sp., *Chondromyces* sp., and *Sphingopyxis* sp. *Mesorhizobium* composed approximately 8.6% of the relative abundance in controls only to decline to 2.25% in high permethrin treatment and 0.1% in low permethrin treated coral tissues. The nitrogen fixing *Mesorhizobium* sp. have been identified in previous coral studies (Kaneko et al., 2000; Carlos et al., 2013), but little is known about this bacterial group. However, a bacterial isolate completed Koch's postulate to induce white plague type II had the highest homology to *Mesorhizobium mediterraneum* with 92% similarity, and so this isolate was given a new genus and species name to become the more well-known, *Aurantimonas coralicida* (Denner et al., 2003). While *Aurantimonas coralicida* and *Mesorhizobium* sp. possess phylogenetic proximity, their homologous functional contributions to the host are unknown. They are still both Rhizobiales bacteria, and likely have a meaningful prokaryotic-eukaryotic relationship suggesting that the reduced abundance of *Mesorhizobium* sp. functional proclivity to fix nitrogen in permethrin exposed coral may have negative impacts to the health of the coral holobiont.

## CONCLUSION

The present 24-h study, designed to mimic an acute permethrin exposure that a coral may experience under natural circumstances, suggests the algal-coral symbiosis and particularly the photochemical parameters of the algal symbionts within the coral host are not significantly impaired under this scenario. Rather, it appears the microbiome of the host significantly shifts after just a single acute exposure to permethrin. While the observed shift in the microbiome toward the potential for insecticide bioremediating bacteria may be beneficial for the coral to mitigate the immediate permethrin exposure, it is unclear if these bacteria indeed bioremediate and if this microbial shift is optimal for the stability of the microbiome to mitigate other stressors. Shifts in the microbiome may be detrimental to the health of the coral as opportunistic bacteria colonize the host during times of microbial dysbiosis. The increased abundance of *Burkholderia* and *Bacillus* along with the reduced abundance in *Sediminibacterium* sp., *Chondromyces*, *Sphingopyxis* sp., and *Mesorhizobium* sp., in permethrin exposed coral suggest significant shifts in the bacterial community are occurring, which could leave the host more vulnerable to secondary threats

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such as infectious disease. However, the functional changes in the coral holobiont associated with this microbiome shift are largely unknown. Further investigations would benefit from determining the functional role of these shifting bacteria within the coral holobiont and conducting chronic insecticide exposures to determine if these microbial shifts return to pre-exposure communities or settle into a community dominated by opportunistic bacteria.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accession: PRJNA747979).

## AUTHOR CONTRIBUTIONS

EM and RP were responsible for compiling the intellectual merit behind the research objectives, design, and acquiring funding for this original research. NM, LA, and AC recorded pulse amplitude modulator measurements and extracted DNA. MH prepared permethrin concentrations and recorded actual permethrin results. EM designed the statistical analysis and implemented by NM created all the figures. NM wrote the manuscript with considerable editorial input from EM with additional revisions from all authors. All authors contributed to the experimental permethrin exposure and submitted the approved version.

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## SUPPLEMENTARY MATERIAL

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