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RESEARCH ARTICLE

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Ultraviolet light alters experimental aquarium water microbial communities

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Abstract

The effect of ultraviolet (UV) light exposure, alone and in combination with CO_2 exposure, on the water microbial community composition was tested in replicate experimental aquaria using source water from an established Amazon-themed exhibit housing mixed species of fishes. Total bacterial abundance, α-diversity metrics, and β-diversity metrics were determined 3 weeks and 1 week before, and weekly during 8 weeks of continuous treatment. The UV treatment significantly lowered the overall bacterial abundance while CO_2 treatment had no effect. However, the UV exposure effect was variable across phyla. Some phyla were decreased while others were increased, including some of potential clinical significance. At the genus level, there were no significant differences in the relative abundance of *Mycobacteria* between treatments and an increase in the relative abundance of *Aeromonas* spp. with UV light treatment. Further work is needed to determine if the observed effects are dose-dependent or if different exposure doses produce different results.

KEYWORDS
aquarium, microbial ecology, UV sterilization

1 | INTRODUCTION

The microbial communities of built aquatic environments like zoos and aquaria are being increasingly recognized as important drivers of aquarium and animal health (Cardona et al., 2018; Pinnell et al., 2020; Van Bonn et al., 2015). Characterizing how microbial communities within these artificial systems respond to changes in their environmental conditions will allow aquarists and water-quality managers to make more informed decisions around building and exhibit design, operation, and maintenance (Stephens, 2016). A growing number of studies have examined the role of microbial communities within aquarium life support systems and habitats (Bagchi et al., 2014; Bik et al., 2019; Kim et al., 2017; Smith et al., 2012), but relatively few have investigated how common life support methods alter the microbial community structure and subsequently animal health within aquaria.

One frequent practice is the use of ultraviolet (UV) radiation. It is very common for UV contact chambers to be included in the life support system of aquarium exhibits. The use of UV radiation is to control the proliferation of pathogens in the water column of the system although it is also reported to improve water clarity. To our knowledge, there are no broadly accepted industry engineering standards for UV use. Despite a tremendous amount of anecdotal information in the hobbyist community, the only peer-reviewed scientific literature we could find describing the efficacy of UV light exposure to control aquarium pathogens was published in 1981 (Spotte & Adams, 1981). That report found that UV radiation was ineffective at controlling the spread of water-borne pathogens in recirculating systems like those in aquariums. A more recent publication describes an experiment to evaluate the microbiota associated with multiple sites in a zebrafish (*Danio rerio*) housing system (Ericsson et al., 2021). Although the focus of that study was to characterize the microbiota present during the setup and population

of a commercial housing system, the authors noted that water samples that had passed through the UV disinfection unit revealed very different communities from the other sites sampled. Some inference can be made from the literature describing UV radiation use for drinking water disinfection (Hijnen et al., 2006), but there is clearly a need to better understand its application in aquarium science.

Home aquarists and exhibit managers sometimes infuse aquarium water with carbon dioxide (CO_2) gas reportedly to provide a carbon source for plants and to aid in pH control. Similar to the situation with UV radiation use in aquariums, scientific literature justifying the use of CO_2 infusion is lacking. Infusion of CO_2 gas is frequently used in controlled aquarium systems that also employ UV radiation for disinfection and that is the case in our Amazon-themed exhibit. To explore the efficacy of UV radiation as a disinfection method in our Amazon exhibit, we set up a dedicated experimental system. The purpose of this study was to characterize the effect of UV radiation alone or in combination with CO_2 infusion on the diversity and structure of water-associated microbial communities in a controlled aquarium environment. To our knowledge, these results demonstrate for the first time how UV radiation impacts aquarium, total microbial abundance, microbial community structure, and the relative abundance of microbial taxa of clinical interest.

2 | MATERIALS AND METHODS

2.1 | Experimental design and sample collection

Eight 30-gallon (114 L) rectangular glass aquaria were established on a rack system for the experiments. Two tanks served as controls with no treatment and the other six tanks provided two replicate systems for each of three treatments. An EHEIM ecco pro 200 (EHEIM GmbH & Co. KG) unit on each aquarium recirculated water at 600 L per hour through a canister cartridge filter to provide mechanical filtration. Each system also incorporated a reservoir of EHEIM SUBSTRATpro filter media (EHEIM GmbH & Co. KG) as a substrate for nitrifying bacterial biofilms and an EHEIM Thermocontrol 100 submersible heater (EHEIM GmbH & Co. KG) for temperature regulation. Standard aquarium gravel was placed in 8.22 cm top diameter × 6.35 cm bottom diameter × 11.43 cm deep, open-top plastic containers and one was submerged in each test aquarium to provide a substrate. Treatment systems also incorporated a submersible UV emitting lamp (Sunsun) and a FLUVAL Pressurized CO_2 Kit (Rolf C. Hagen (USA) Corp.) with a 95 g cartridge gas infuser designed for home aquaria. All aquaria were filled with water sourced from an operational, established freshwater exhibit which displays Amazonian fishes, and 30 individual Splash tetras (*Copella arnoldi*) were placed in each, 3 weeks before initiating any experimental treatment (Study Day −21). Fish were of wild capture origin, obtained via a commercial wholesale vendor. Water quality parameter values were maintained within the ranges indicated in Table 1 for the duration of the experiment. To reproduce exhibit husbandry practices, water changes of 40% volume

TABLE 1 Water quality parameter values for the duration of the experiment.

Parameter	Units	Tank (treatment)							
		C1 (CO_2)	C2 (no treatment)	C3 (UV + CO_2)	C4 (UV)	C5 (no treatment)	C6 (CO_2)	C7 (UV)	C8 (UV + CO_2)
pH		6.33–7.63	5.23–7.12	5.21–7.38	6.25–7.40	6.64–7.33	5.91–7.26	6.26–7.49	6.15–7.51
NH_3	ppm	0.01–0.05	0–0.03	0–0.05	0–0.03	0–0.03	0–0.03	0–0.03	0–0.09
NO_2	ppm	0.0035–0.0100	0.0035–0.0237	0.0036–0.0340	0.0035–0.0489	0.0034–0.0155	0.0037–0.0092	0.0037–0.0462	0.0033–0.0341
NO_3	ppm	5.2–37.5	4.8–29.9	4.5–32.0	4.9–33.8	5.3–34.1	5.7–32.9	4.1–34.8	4.1–35.7
Dissolved O_2	ppm	6.01–7.67	3.98–7.45	6.67–8.64	6.58–7.66	6.27–7.31	6.37–7.45	6.34–7.67	6.84–7.59
Temperature	°C	26.7–27.4	26.2–27.2	27.3–29.3	26.4–29.6	27.9–28.3	27.0–27.7	26.1–28.6	29.1–29.4
Conductivity	µS/cm	185.0–1172.7	161.5–340.6	142.4–435.4	150.6–331.6	158.2–309.7	151.8–394.4	190.3–357.4	148.0–597.0
Alkalinity	ppm	31.4–82.0	52.8–69.8	34.4–70.6	41.2–86.2	40.4–79.8	35.0–77.4	48.6–72.4	37.6–71.8

Abbreviation: UV, ultraviolet.

were made to each aquarium simultaneously once per week for the study duration and used the same makeup water as the operational display exhibit.

On Study Day 0, 3 weeks after establishing the test aquaria, the UV units and CO_2 infusers were turned on in the treatment aquaria. The UV units incorporated a 5 W UV-C lamp and circulated water from the aquarium through at a rate of 500 L per hour. Samples were collected once each week (i.e., at 7-day intervals, immediately before water changes) starting 28 days before treatment and ending 56 days after treatment began. Water samples (500 ml) were collected from each aquarium with sterile Nalgene™ bottles (Thermo Fisher Scientific), immediately transported to the onsite laboratory, and pulled through a sterile 0.22 µm membrane filter (Pall Biotech). The filters were then aseptically transferred to cryovials and frozen at −70°C until DNA extraction. DNA was extracted from filter membranes using the MagAttract Power Water DNA/RNA kit (QIAGEN Company) following the manufacturer's instructions. Extractions were carried out in 96-well plates using the KingFisher™ Flex Purification System (Thermo Fisher Scientific). From the extracts, one analysis was to quantitate total bacterial abundance, and one to evaluate community structure. Measurements compared pretreatment values to no treatment, and each of the three treatments, UV, CO_2, or UV + CO_2.

All work described herein was reviewed and approved by the Shedd Aquarium Research Review Committee and conducted in compliance with the US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.

2.2 | 16S rRNA gene total abundance qPCR

To measure changes in total microbial abundance, qPCR was performed on water samples collected on Study Days 0, 28, and 56 of the experiment. Only one sample from each tank at each collection date was analyzed using qPCR. To facilitate statistical comparisons, samples collected on Day 0 were all combined ($n = 8$) and samples collected on Days 28 and 56 were grouped by treatment (no treatment, $n = 4$; CO_2, $n = 4$; UV, $n = 4$; UV + CO_2, $n = 4$). One sample from the UV group failed amplification and was excluded from analyses (UV, final $n = 3$). Triplicate 20 µl reactions were prepared for each sample, containing 10 µl of 2× PowerUp SYBR Green MasterMix (Applied Biosystems), 0.5 µM of primers 331F and 797R (Nadkarni et al., 2002) which target a conserved region of the bacterial 16S rRNA gene, 2 µl of DNA template and nuclease-free water added to equal 20 µl. Standard curves generated by serial dilution from 300,000 to 30 copies of *Escherichia coli* genomic DNA (ATCC 25922) were run concurrently with samples to quantify bacterial load. Thermal cycling was performed on a QuantStudio 3 (Applied Biosystems) in standard cycling mode. UDG activation for 2 min at 50°C was followed by denaturation at 95°C for 10 min and 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min.

2.3 | 16S rRNA PCR amplification and sequencing

Bacterial and archaeal DNA was amplified using primer constructs (515f/806rB) targeting the V4 region of the 16S rRNA gene (Walters et al., 2016). The constructs contain Illumina-specific adapters followed by 12 bp Golay barcodes on each forward primer, primer pads, and linkers as well as the template-specific PCR primer at the 3′ end. PCR was performed in replicate 25 µl reactions containing 12.5 µl Phusion Hot-Start Flex 2× MasterMix (New England Biolabs), 0.2 µM final concentrations of forward primer 515f and reverse primer 806rB, 2 µl of template DNA and nuclease-free water to equal 25 µl. Mock microbial community DNA standards (Zymo Research) and negative controls containing no template DNA were prepared with each PCR replicate. Thermal cycling conditions were carried out as follows: 98°C for 30 s, 30 cycles at 98°C for 10 s, 55°C for 30 s and 72°C for 30 s, with a final extension of 5 min at 72°C. After PCR, replicate amplicons were combined and 5 µl of each were electrophoresed in 1.8% agarose gels to confirm amplification of the V4 region. Twenty-five microliters of each amplicon library was then cleaned and normalized using the SequalPrep™ Normalization Plate Kit (Applied Biosystems), and equal volumes of each normalized library were pooled together. The pooled amplicon library was quantified using a Qubit™ 3.0 fluorometer and Qubit™ dsDNA HS Assay Kit (Life Technologies), then further cleaned and concentrated using the UltraClean® PCR Clean-Up Kit (MO BIO Laboratories). The molarity of the pooled library was calculated and diluted to 2 nM before denaturation and further dilution to a loading concentration of 6 pM. Paired-end sequencing for a total of 500 cycles was conducted on the Illumina MiSeq platform using custom sequencing primers described previously (Caporaso et al., 2012) with the addition of a 10% PhiX Control library (Illumina) to increase sequence diversity. Across all samples, the average sequencing depth was 39,034 reads per sample, and the median sequencing depth was 40,819 reads per sample. Samples from the pretreatment group had significantly greater read depth (29,156 reads per sample ± 931 SEM) than each of the four groups of samples collected after treatment began. However, the read depths from samples in the no treatment (21,578 reads per sample ± 1042 SEM), CO_2 (21,359 reads per sample ± 1003 SEM), UV (20,701 reads per sample ± 1084 SEM), and UV + CO_2 (20,276 reads per sample ± 885) treatment groups were very similar and did not differ significantly from each other.

2.4 | Microbial community structure analysis

To facilitate the analysis of changes in microbial community structure over time within the experimental aquariums, samples were parsed into five groups; samples from before the start of treatment (Study Day −28, Day −7) were considered pretreatment, and samples from the day treatment started and beyond (Study Days 0, 7, 14, 21, 28, 35, 42, 49, and 56) were grouped into four groups (no treatment, CO_2, UV, and UV + CO_2). The total number of samples analyzed using 16S rRNA gene sequencing was 136, which were divided into these

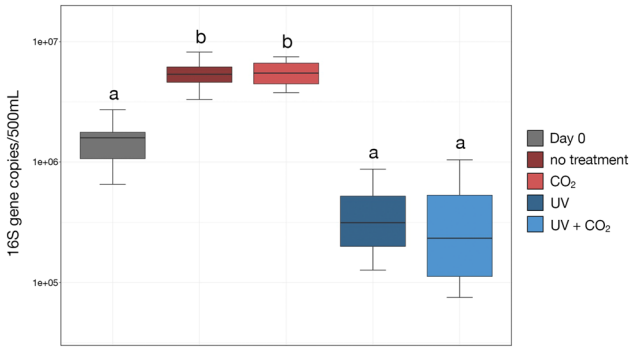

FIGURE 1 Boxplot showing differences in natural log-transformed 16S gene copy numbers per 500 ml of water between groups (Day 0, $n = 8$; no treatment, $n = 4$; CO_2, $n = 4$; UV, $n = 3$, UV + CO_2, $n = 4$). Statistically significant differences in total abundance between groups signified by different letters (ANOVA with Tukey post hoc test and Westfall adjusted p values; $p < .05$). Pretreatment values represent those from study Day ≤0. Treatment values (including control = no treatment) include all values from Days 28 and 56. The boxplots indicate minimum, maximum, mean, and first and third quartiles of the values for each group. [Color figure can be viewed at wileyonlinelibrary.com]

five groups (pretreatment, $n = 16$; no treatment, $n = 30$; CO_2, $n = 30$; UV, $n = 30$; UV + CO_2, $n = 30$).

Demultiplexed paired-end reads were imported into QIIME2 version 2019.7 (Caporaso et al., 2010) and processed as described previously (Pinnell & Turner, 2020). Forward reads were truncated at 237 bp and trimmed from 2 bp, while reverse reads were truncated at 214 bp and trimmed from 7 bp. Data were then imported into phyloseq version 1.26.1 (McMurdie & Holmes, 2013). Two samples had less than 1000 amplicon sequence variant (ASVs) and were discarded from the analysis. This resulted in 134 total samples being analyzed (pretreatment, $n = 15$; CO_2, $n = 30$; UV, $n = 30$; UV + CO_2, $n = 29$). Richness, Shannon's diversity, and Faith's phylogenetic distance were calculated for all remaining samples using phyloseq and the "estimate_pd" function within the package btools. Samples were then normalized using total sum scaling based on the lowest number of per-sample ASVs (5353) and beta-diversity was analyzed using generalized UniFrac values (Chen et al., 2012; Lozupone et al., 2011). From these distances, a principal coordinates analysis (PCoA) was calculated and plotted, and a permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences between communities using the "vegan" (Oksanen et al., 2019) and "pairwiseAdonis" (Arbizu, 2017) packages in R version 3.6.1 (R Core Team, 2017). To ensure differences in microbial communities were not due to unequal dispersion of variability among groups, permutational analyses of dispersion (PERMDISP) were conducted for all significant PERMANOVA outcomes with the "vegan" package in R. The relative abundances of ASVs within each sample were calculated and plotted for the 10 most abundant phyla across all samples. Further, the relative abundance of various taxa known to include pathogens of fish was compared between treatment groups. These included the class Gammaproteobacteria, the family Enterobacteriaceae, and the genera *Mycobacterium*, *Aeromonas*, *Salmonella*, *Campylobacter*, *Francisella*, *Plesiomonas*, *Edwardsiella*, *Renibacterium*, *Photobacterium*, *Vibrio*, *Flavobacterium*, and *Flexibacter*.

2.5 | Statistical analyses

Unless specified otherwise, R version 3.6.1 was used for the statistical analysis of data. Shapiro–Wilk tests were used to test data for normality. A one-way analysis of variance (ANOVA) with a Tukey post hoc test and Westfall adjusted p values was used to test total bacterial abundance. Differences in α-diversity were tested using pairwise Wilcoxon rank-sum tests with a Benjamini–Hochberg correction for multiple comparisons (Benjamini & Hochberg, 1995). Differences in β-diversity were tested using PERMANOVA with a Benjamini–Hochberg correction and 999 permutations. Additionally, a PERMDISP with 999 permutations was used to test for differences in dispersions.

3 | RESULTS

3.1 | Total microbial abundance

Figure 1 compares total microbial abundance values pretreatment to the values measured post treatment in the control (no treatment) and treatment tanks. Exposure to UV radiation, with or without exposure to CO_2, significantly lowered total microbial abundance when compared to no treatment (Figure 1; ANOVA with Tukey post hoc test and Westfall adjusted p values; $p < .05$). The decrease was not significant when compared to pretreatment values. However, all posttreatment samples were taken at the same time points and microbial abundance did increase significantly over time in the control no-treatment tanks (Figure 1; ANOVA with Tukey post hoc test and Westfall adjusted p values; $p < .05$). The infusion of CO_2 gas had no effect on microbial abundance on its own or in combination with UV radiation (Figure 1).

3.2 | Community richness and diversity

The comparison of observed ASVs demonstrated that microbial communities exposed to UV radiation were significantly richer (550 observed ASVs ± 23.2 SEM) than pretreatment communities (285 observed ASVs ± 15.1 SEM), communities in the no-treatment group (202 observed ASVs ± 11.5 SEM), and communities exposed to CO_2 alone (190 observed ASVs ± 11.3 SEM) (Figure 2; pairwise Wilcoxon rank-sum test with Benajmini–Hochberg correction; $p < .05$). Communities exposed to UV + CO_2 (589 observed ASVs ± 18.4 SEM) had a similar richness to those exposed to just UV. Similarly, Shannon's diversity was significantly higher in communities exposed to UV

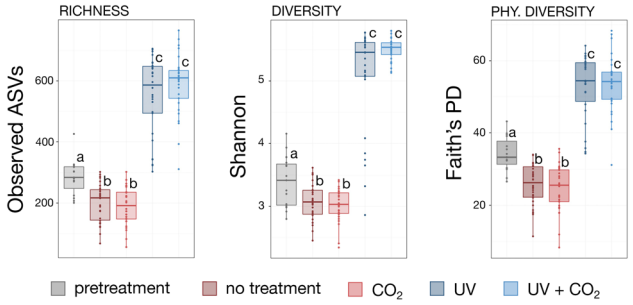

FIGURE 2 Boxplots showing differences in richness (total number of ASVs), Shannon (nonphylogenetic diversity metric), and Faith's PD (phylogenetic diversity metric) between groups (pretreatment, $n = 15$; no treatment, $n = 30$; CO_2, $n = 30$; UV, $n = 30$; UV + CO_2, $n = 29$). Statistically significant differences in total abundance between groups signified by different letters (pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$). ASV, amplicon sequence variant. [Color figure can be viewed at wileyonlinelibrary.com]

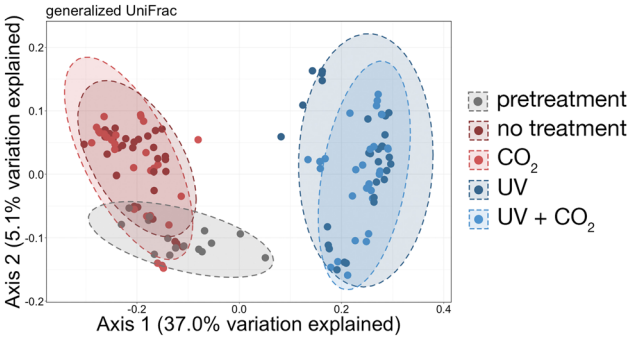

FIGURE 3 Principal coordinates analysis (PCoA) of generalized UniFrac distances illustrating the differences in microbial community composition across treatment groups. The PCoA demonstrates clustering of 16S rRNA sequences from the pretreatment, no-treatment, UV, CO_2, and UV + CO_2 treatment groups. Dashed lines and shaded areas represent 95% confidence ellipses for each treatment group. Each point is a community. The closer together the points, the more similar the community. [Color figure can be viewed at wileyonlinelibrary.com]

radiation (UV alone 5.1 ± 0.15 SEM; UV + CO_2 5.5 ± 0.03) compared to pretreatment communities (3.4 ± 0.11), or no-treatment (3.1 ± 0.05) and CO_2 communities (3.0 ± 0.05) (Figure 2; pairwise Wilcoxon rank-sum test with Benajmini–Hochberg correction; $p < .05$). Faith's phylogenetic diversity followed the same pattern, with communities exposed to UV (UV alone 52.7 ± 1.7; UV + CO_2 53.6 ± 1.5) being significantly more phylogenetically diverse than pretreatment communities (34.2 ± 1.2), no-treatment communities (25.6 ± 1.0), and communities exposed to CO_2 alone (24.7 ± 1.2) (Figure 2; pairwise Wilcoxon rank-sum test with Benajmini–Hochberg correction; $p < .05$).

3.3 | Microbial community composition

Differences in community structure were identified between all groups (PERMANOVA with Benjamini–Hochberg correction, $p < .05$), but a PERMDISP ($p < .05$) indicated that the significant PERMANOVA could be the result of different community structures or different dispersions. However, the PCoA clearly illustrated that treatment with UV radiation significantly impacted community structure, while CO_2 had little effect (Figure 3). The community composition also shifted over time without treatment (Figure 3).

Relative abundance plots of the 10 phyla that accounted for an average greater than 1% of the microbial communities across all samples are presented in Figure 4. For Proteobacteria no significant difference was seen when comparing pretreatment to treatment groups, or between treatment groups.

UV radiation resulted in significant decreases in the relative abundance of Bacteroidetes (~15% loss) and Actinobacteria (~15% loss), which were the second and third most abundant phyla across all samples. Conversely, exposure to UV resulted in significant increases in the abundance of Planctomycetes, Chloroflexi, Patescibacteria, Verrumicrobia, Cyanobacteria, Acidobacteria, and Firmicutes (Figure 4; pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$). Of the 10 most abundant phyla, only Actinobacteria were significantly impacted by the infusion of CO_2 alone compared to no treatment. Treatment with UV radiation combined with CO_2 infusion lessened the decrease in Bacteroidetes abundance caused by the exposure to UV alone. No treatment or treatment with CO_2 infusion alone significantly reduced the relative abundance of Cyanobacteria and Acidobacteria when compared to pretreatment values (Figure 4; pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$).

3.4 | Relative abundance of taxa of clinical interest

Of the 13 taxa investigated, *Salmonella*, *Campylobacter*, *Francisella*, *Plesiomonas*, *Edwardsiella*, *Renibacterium*, *Photobacterium*, *Vibrio*, and *Flexibacter* were not detected in any sample. There were no significant differences within the genus *Mycobacterium* across any treatment. The class Gammaproteobacteria comprised approximately 18% of the total microbial community across all treatment groups and there were no significant changes following any treatment (Figure 5). However, there was a significant increase in the relative abundance of Enterobacteriaceae, a family within Gammaproteobacteria, following exposure to UV radiation (Figure 5; pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$). Enterobacteriaceae abundance, when exposed to UV alone, was not significantly different than pretreatment but was significantly higher in the UV + CO_2 group compared to the UV group (Figure 5; pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$). This was the result of a single tank within the UV + CO_2

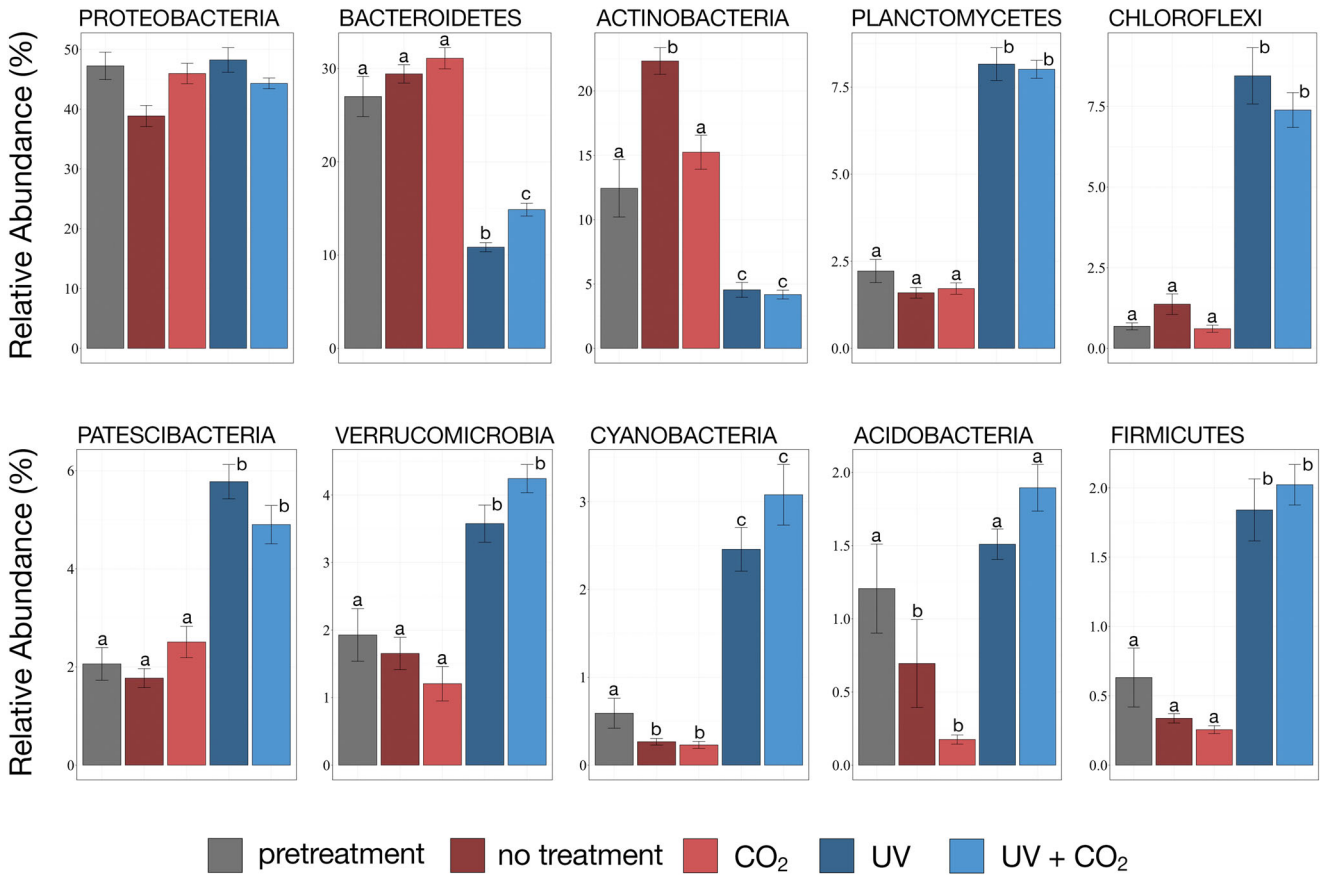

FIGURE 4 Bar plot demonstrating differences in the relative abundance of all phyla with a mean relative abundance of >1% between treatment groups with standard error bars (pretreatment, $n = 15$; no treatment, $n = 30$; CO_2, $n = 30$; UV, $n = 30$; UV + CO_2, $n = 29$). Statistically significant differences in relative abundance between treatment groups signified by different letters (pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$). Phyla are displayed in order of decreasing abundance. [Color figure can be viewed at wileyonlinelibrary.com]

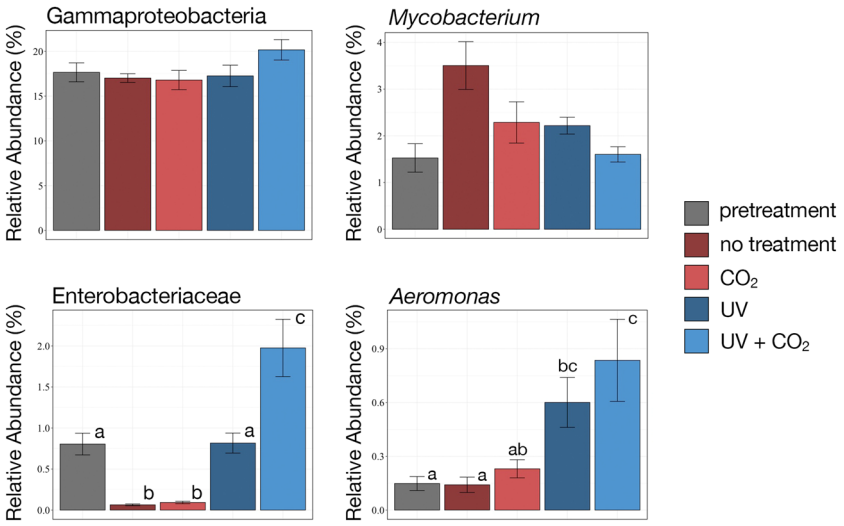

FIGURE 5 Bar plots showing the differences in relative abundance of various microbial taxa of clinical interest (Gammaproteobacteria, Enterobacteriaceae, *Mycobacterium*, and *Aeromonas*) between treatment groups with standard error bars (pretreatment, $n = 15$; no treatment, $n = 30$; CO_2, $n = 30$; UV, $n = 30$; UV + CO_2, $n = 29$). Statistically significant differences in relative abundance between treatment groups signified by different letters (pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$). [Color figure can be viewed at wileyonlinelibrary.com]

group having very high Enterobacteriaceae abundance. The relative abundance of Enterobacteriaceae was significantly higher in both treatments exposed to UV as compared to no treatment or CO_2 alone. The genus *Aeromonas* was also significantly more abundant following exposure to UV radiation, alone or with CO_2, when compared to the no-treatment or pretreatment group. (Figure 5; pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$).

4 | DISCUSSION

Our experimental setup was intentionally designed to enable us to evaluate the influence of UV radiation and CO_2 gas infusion on microbial communities in the water of an Amazon-themed exhibit where both are used as a routine system management practice. Interestingly, the richness and diversity of microbial communities significantly increased when exposed to UV radiation, while CO_2 had no effect. This may be the result of ecological niches previously occupied by dominant community members (i.e., Bacteroidetes and Actinobacteria) being made available to a wider variety of taxa after UV radiation reduced the abundance of the predominant taxa. However, this increased richness and diversity may also be the result of a reduced abundance of 16S rRNA gene sequences from predominant taxa within amplicon libraries from samples exposed to UV radiation, which resulted in the sequencing of a greater number of rare taxa within these samples. Further work targeting the absolute abundance of differentially abundant taxa via qPCR will help elucidate the true nature of the increased alpha-diversity following UV radiation described here.

The absolute microbial abundance decreased significantly when water was exposed to UV radiation and CO_2 had no effect. The decrease is not surprising, as it is well documented that UV radiation causes microbial cell death (Elasri & Miller, 1999; Shaban et al., 1997). In the context of aquarium life support systems, UV radiation is used to specifically control water-borne pathogens, so it would be insightful to characterize if UV radiation shifts microbial community diversity and structure and if it results in decreased abundance within taxa of clinical interest. The majority of the decreased microbial abundance was the result of significant decreases in the levels of Bacteroidetes and Actinobacteria, whose relative abundances dropped a combined ~30% following exposure to UV radiation. The loss of Bacteroidetes is particularly interesting as we have observed a decrease in Bacteroidetes within microbial communities over time exposed to aquarium life support systems across multiple data sets (Patula et al., 2021; Pinnell et al., 2020). Further, a study examining the impact of UV radiation on municipal drinking water noted that Bacteroidetes were particularly sensitive to UV (Pullerits et al., 2020), suggesting that the loss of Bacteroidetes is likely occurring within all aquariums utilizing a UV radiation chamber as part of their life support system.

Concurrent with the decrease in the relative abundance of Bacteroidetes and Actinobacteria, the relative abundance of remaining phyla increased following exposure to UV. While they make up a larger proportion of the community, we do not believe this is representative of an absolute increase in abundance of these phyla because of the decreased total microbial abundance. However, the higher abundance of Cyanobacteria, a potentially harmful toxin-producing phyla, is both interesting and alarming. Cyanobacteria have efficient radiation protection mechanisms and can withstand exposure to UV (Sinha & Häder, 2008), and our results suggest UV exposure may provide Cyanobacteria with a competitive advantage compared to other microbial taxa. Further research needs to be completed to determine how UV radiation impacts the absolute abundance of Cyanobacteria and address the effectiveness of UV radiation within aquarium life support systems in reducing Cyanobacteria and the potential for toxin production.

Similarly, UV radiation displayed mixed results in decreasing the relative abundance of microbial taxa of clinical interest. The relative abundance of Enterobacteriaceae, a family of Gammaproteobacteria that contains *E. coli* and *Enterobacter* along with a multitude of multi-drug resistant pathogens (Denton, 2007), was higher when treated with UV. While the relative abundance of Enterobacteriaceae was significantly higher in the UV + CO_2 group compared to the UV group (Figure 5; pairwise Wilcoxon rank-sum test with Benjamini–Hochberg correction; $p < .05$), this was the result of a single tank within the UV + CO_2 group having very high Enterobacteriaceae abundance while the other was very similar (see Supporting Information: Table S1). As a result, we do not believe the difference in Enterobacteriaceae abundance between UV and UV + CO_2 to be of biological significance. The genus *Aeromonas*, which includes strains recognized as fish pathogens, was in significantly higher relative abundance following UV exposure. *Aeromonas* has been shown to be resilient to UV radiation in Nile River water (Shaban et al., 1997), which may suggest UV radiation is an ineffective method to deal with these known fish pathogens. UV radiation had no impact on the relative abundance of the genus *Mycobacterium*. The lack of significant treatment effect on the genus *Mycobacterium* is certainly of clinical interest, as UV radiation is a strategy used by aquarium managers to attempt to reduce *Mycobacterium*. Our results clearly show that while UV radiation significantly alters the microbial community of the system water, it may not be effective at controlling microbial taxa of clinical interest.

The terms disinfection and sterilization are often indiscriminately used when referring to aquarium design and operation. For example, aquarium operators and managers often refer to "UV sterilization" as a component of life support systems when sterilization is not the goal, and the actual effect is unknown. Some inferences can be made from water treatment for potable water supplies, but it is important to acknowledge that the goal of their use in aquaria is different than their use in municipal potable water systems. Natural aquatic ecosystems teem with a robust community of microscopic organisms. Eliminating them all, thus truly sterilizing the water, is contrary to trying to replicate healthy ecosystems to support display animals. Even within municipal potable water systems, scientific evidence also suggests sterilization is not possible (Haig et al., 2020).

We acknowledge there were limitations to our study. We did not test for the effect of temperature on bacterial community structure, but it is noteworthy that the temperature of the experimental tanks treated with UV tended higher than those without. This is likely due to the radiant energy from the bulbs. Despite the tendency of elevated temperature, the absolute abundance of bacteria in tanks under UV treatment was significantly lower than in those without (Figure 1). The effective dose of UV irradiation depends upon the size (output) of the lamp, the flow rate of the water through the contact chamber, and the amount of organic matter in the water column. Lamp performance is also known to vary with time. Despite knowing that the test UV units incorporated a 5 W UV-C lamp and circulated

water from the aquarium though at a rate of 500 L per hour, it was not possible to scale the UV radiation dose rates to match the system in use on exhibit. The manufacturer specifications supplied with the unit did not indicate the effective UV radiation output or expected performance of the bulb. The life support system of the display exhibit this study sought to inform incorporates a 120 W UV emitting bulb for a total system volume of 5678 L circulating at approximately 22,740 L per hour but the actual flow rate through the UV contact chamber at any given time is unknown. The exhibit bulb is changed on an irregular schedule. It is possible that our exposure dose was either higher or lower than that of operational exhibit systems. We were unable to compare specifically to the recent zebrafish housing unit study (Ericsson et al., 2021) either. In that study, the UV units were described only as "a standard 40-W ultraviolet disinfection unit." Total volume of the system and flow rate through the UV unit were not included. That study also extracted and amplified DNA from samples of only 1 ml unfiltered water rather than the 500 ml filtered samples in our protocol. Further experiments to control for a dose will be needed to clarify the efficacy of UV radiation in controlling water-borne pathogens within the context of aquarium habitats.

Our findings are not meant to imply that disinfection, specifically the use of UV irradiation units, should be abandoned. Some means of managing the microbial community structure within built aquatic environments is very likely required to ensure optimal environments for display animals and UV will remain a viable option. Instead, we hope to have illustrated that the effects of UV radiation on microbial communities are varied and poorly understood in aquarium life support systems. There remains a tremendous opportunity for learning and improvement in our ability to provide for the health and well-being of aquatic animals.

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DATA AVAILABILITY STATEMENT

All sequence reads were made available through BioProject PRJNA728482 at the NCBI's Sequence Read Archive.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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