

# Periphyton Response to Additions of Glucose and Hydrogen Peroxide as Control Measures of Harmful Algal Blooms

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**How to cite this paper:** Romanski, A.K., Partridge, C. and Steinman, A.D. (2025) Periphyton Response to Additions of Glucose and Hydrogen Peroxide as Control Measures of Harmful Algal Blooms. *Journal of Water Resource and Protection*, 17, 47-67.

<https://doi.org/10.4236/jwarp.2025.172004>

**Received:** December 28, 2024

**Accepted:** February 16, 2025

**Published:** February 19, 2025

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## Abstract

A mesocosm-based study was conducted to assess the effect of glucose and hydrogen peroxide on periphyton communities. These chemicals have been found to be effective at controlling cyanobacteria blooms in the water column but their impact on attached communities is unknown. The experimental design included a total of 4 treatments: control (no chemicals; 3 replicates); hydrogen peroxide (3 replicates); glucose alone (3 different concentrations [no replicates]); and additive glucose (3 replicates). After 34 days, mean values of chlorophyll *a* were lower in all experimental treatments compared to the control; mean AFDM values were lower in all treatments except the unreplicated high glucose alone treatment. In contrast, mean autotrophic index values (AFDM/chlorophyll *a*) were greater in all treatments compared to the control, indicating heterotrophs were more resistant to the chemical treatments than autotrophs. Periphyton community biodiversity was much lower in the additive glucose and moderate glucose alone treatments than the hydrogen peroxide and control treatments. The relative abundance of the bacteria *Asticcacaulis* and *Sphingorhabdus* responded positively to the glucose treatments, whereas relative abundance of *Nevskia* and *Caenimonas* declined in both the hydrogen peroxide and glucose treatments. In terms of relative abundance, no cyanobacteria taxa were detected among the top 20 taxa. We conclude that the autotrophic component of periphyton communities is especially vulnerable to hydrogen peroxide and glucose treatments, and that any management strategy employing these chemicals should be aware of this potential impact.

## Keywords

Periphyton, Glucose, Hydrogen Peroxide, Mesocosm, Cyanobacteria

## 1. Introduction

Limnological studies examining lake algal structure and function have historically focused on the plankton community as opposed to the attached community. However, there is a growing recognition that periphyton plays an important role in lake ecosystems [1]-[4]. Stressors such as eutrophication and climate change potentially threaten periphyton, as increased nutrients and warming conditions will stimulate plankton growth, resulting in less light transmission through the water column and to the periphyton.

It is unclear if algal blooms in lakes are increasing over time, with evidence both for no overall change [5] as well as for an increase in median bloom frequency [6]. Hallagraeff *et al.* [7] found that on a global basis there was not a consistent trend in the number of harmful algal events and their distribution over time, once the data were adjusted for regional variations in monitoring effort. They recommended that patterns and trends needed to be considered on regional and species-specific bases.

Regardless of whether the trend is increasing or not, it is clear that harmful algal blooms are a significant ecological and human health issue [8] and there is a need to mitigate them. One solution is to reduce the nutrient load reaching water bodies, but this often requires considerable time and money [9]. Consequently, short-term solutions are frequently implemented to reduce the bloom, essentially treating the symptom (bloom) instead of the disease (nutrient runoff). Common short-term controls include aeration [10], fish removal [11], and chemicals, such as hydrogen peroxide and copper salts [12] [13]. Aeration mixes the water column, preventing surface scums from forming and also keeping the sediment-water interface oxidized and thereby preventing the desorption of phosphorus from the iron oxyhydroxide precipitate [14] [15]. Fish removal can be labor intensive and costly but it reduces nutrient excretion from the fishes, as well as prevents bioturbation (e.g., by carp or gizzard shad) that otherwise would increase internal nutrient loading. Copper sulfate is also a common treatment for cyanobacterial blooms because cyanobacteria are more susceptible to it than other phytoplankton taxa [16]; copper inhibits cyanobacterial growth by replacing the magnesium in the chlorophyll complex [17] and disrupting the thylakoid stacks in chloroplasts, inhibiting photosystem II [18].

Hydrogen peroxide is a strong oxidant that produces reactive oxygen species, which inhibit cyanobacteria and associated cyanotoxin production as it kills the cyanobacteria, preventing additional bloom formation [19]-[22]. Cyanobacteria are more susceptible to hydrogen peroxide than eukaryotic algae because they lack ascorbate peroxidases that otherwise would scavenge the hydroxyl radicals negatively impacting cellular metabolism [23]. Although these chemicals can be effective, they introduce potential toxins into the water body as cells lyse, as well as result in the transfer of nutrients in the cells to the water column and sediments as they mineralize, leading to future blooms.

Another approach to limit cyanobacterial growth and toxin production is the addition of glucose [24] [25]. Rather than eliminating cyanobacteria, glucose is added to help prevent cyanobacterial blooms from forming. When added to the

water column prior to bloom formation, the added carbon stimulates growth of other microorganisms, allowing them to outcompete the cyanobacteria by preferential uptake of nutrients. While shown to be effective at the benchtop scale, it is uncertain if those results translate well to larger, more complex systems. Linz *et al.* [26], using mesocosm tanks, found that hydrogen peroxide and glucose were effective, at least in the short term, in controlling cyanobacteria blooms. The studies addressing bloom control have neglected to assess the response of the periphyton community to these chemicals.

Periphyton communities play a critical role in lakes [1]. They have served as biological indicators of lake health [27] [28], important food resources for higher trophic levels [29], habitat for invertebrates [30], and cycle and transform nutrients [31]-[33]. In some aquatic ecosystems, these primary producers are responsible for generating critical energy to power system dynamics [1] [34].

In the current study, we attempt to fill this knowledge gap by examining the response of periphyton to hydrogen peroxide and glucose additions. We hypothesized that both additions would negatively impact the cyanobacterial community but that the glucose treatment would stimulate the growth of heterotrophs due to the added dissolved organic carbon.

## 2. Materials and Methods

### 2.1. Experimental Design

The experiment was conducted using Muskegon Lake water that was pumped through a series of 300  $\mu\text{m}$  in-line filters to a head tank, where it was gravity fed into twelve 1325 L fiberglass tanks. Each tank was illuminated with a 1000 W metal halide bulb that is suspended from above and provides full-spectrum photosynthetically active radiation ( $\sim 300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) set at a photoperiod of 16:8 L:D.

The experimental design and treatment terminology followed the prior approach used by [24] to be consistent with their benchtop study. The design is unbalanced following the prescription from EPA, which wanted to know if their prior benchtop study results were replicable at the mesocosm scale. The design consisted of four treatments, randomly assigned to the tanks: a control (3 replicate tanks), hydrogen peroxide addition (3 replicate tanks), standard glucose addition (3 tanks, each with a different concentration, so no replicates), and an additive glucose treatment (3 replicate tanks).

All tanks were filled to the 1000 L mark at the start of the experiment (except for the additive glucose treatments which started at the 100 L mark). Each week filtered lake water was added to the tanks in order to account for the small degree of evaporative loss. The 34-day experiment started on 17 August 2023 and ended on 20 September 2023.

On 14 August 2023, 1000 L of filtered (300  $\mu\text{m}$ ) Muskegon Lake water was added to each mesocosm. The water in the mesocosms was left to equilibrate overnight. On 15 August, three mesocosms were untreated and designated as controls. Three other mesocosms were each treated with hydrogen peroxide at 4 mg/L. An

additional three mesocosms were treated with glucose at 0.03 g/L, 0.15 g/L, or 0.30 g/L, which were repeated at 2 and 4 weeks. These glucose additions hereafter are referred to as “straight glucose additions” (to differentiate from “additive” glucose treatments). The three additive glucose tanks each received initial concentrations of 0.30 g/L. These tanks started at 100 L and an additional 100 L was added each week while a 0.30 g/L glucose addition was made every two weeks until the end of the experiment. The water in each tank was hand-stirred with individual wooden dowels assigned to each treatment and rinsed with deionized water between tanks to avoid any possible cross-contamination. Additional details on experimental set up are provided in [26].

The density of *Microcystis* in Muskegon Lake near the water intake to the mesocosms was measured weekly starting in mid-June (2023) using qPCR [35]. By mid-August, *Microcystis* levels had reached  $1 \times 10^4$  cells/L, which was still too low to detect a visible bloom but high enough to cross the threshold for starting the experimental treatments [24].

## 2.2. Water Quality Measurements

Grab samples were taken weekly from each mesocosm for measurement of soluble reactive phosphorus (SRP), total phosphorus (TP), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ), ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), and total Kjeldahl nitrogen (TKN) using standard methods [36]. Also, biweekly (2 times per week) measurements were made of temperature, dissolved oxygen (DO), pH, specific conductivity, total dissolved solids (TDS), and turbidity using a YSI EXO2 sonde (Yellow Springs Instruments, Yellow Springs, OH), following the manufacturer’s instructions. Biweekly glucose concentrations measurements were made in the glucose mesocosms with Glucose Test Strips (Precision Laboratories Inc, Cottonwood, AZ), following the manufacturer’s instructions.

## 2.3. Periphyton Set-Up

Plastic polyacrylic strips (cut to 10 cm  $\times$  3 cm) were used as a substrate for periphyton colonization and growth (Figure 1). A single strip was suspended in each mesocosm tank by fishing line threaded through its top and attached to adhesive tape, and kept vertical by attaching a weight at the bottom of the strip. Strips were harvested after 34 d when visual observation indicated significant growth in the control tanks. Strips were lifted carefully from each tank with large, plastic weigh boats placed below each strip to collect any sloughed material.

## 2.4. Periphyton Analysis

The periphyton on each side of the strip was carefully removed by razor blade; one side was used for biomass determination and the other side for genetic analysis of the periphyton. Each periphyton sample was sonicated using a QSonica Q55 sonicator for 30 seconds at an amplitude of 120 micrometers to disrupt the mats. After sonication, a 10 mL subsample of the slurry was used for chlorophyll *a* analysis and

a separate 10 mL subsample was used for ash-free dry mass (AFDM) analysis.

Chlorophyll *a* subsamples were filtered through a 0.45 µm GF/F filter (Whatman®). Filters were ground and steeped in 90% buffered acetone for 24 hours in the dark. After centrifuging, Chl *a* was analyzed using a Shimadzu UV-1601 spectrophotometer [37]. AFDM was measured gravimetrically, first drying at 90°C until constant mass and then ashed at 550°C for 1 hr, following the procedure in [37]. An autotrophic index (ratio of AFDM to chlorophyll *a* in mg/cm<sup>2</sup>) was calculated for each treatment, which provides an indication of how much of the periphyton biomass is composed of living algae [37].

The periphyton material for genetic analysis was placed into centrifuge tubes filled with DI water and then stored at –20°C until DNA extraction. DNA was extracted using the Qiagen All-Prep DNA/RNA extraction kit (Qiagen, Valencia, CA). A two-step PCR process was used following protocols outlined in Illumina MiSeq Systems 16s Metagenomic Sequencing Library Preparation. The 16S rRNA v4 region was amplified using the 515F/806R primer set [38]. Libraries were normalized to 4 nM using a QIAseq® normalization kit per their instructions (Qiagen Hilden, Germany). The quality of the individual libraries was checked on an Agilent Bioanalyzer (Agilent Santa Clara, CA). Libraries passing the quality check were pooled, and the amplicons were sequenced using a 2 × 250 bp format, along with a 20% spike-in of Phi-X, on the Illumina MiSeq System (Illumina, San Diego, CA).

Sequence reads were filtered based on quality scores, sequencing errors were estimated, paired reads were merged, chimeras were removed, and amplicon sequence variants (ASVs) were identified using the package dada2 in R studio (2022) version 1.30.0 [38]. Taxonomic assignments were based on aligning merged paired reads to the CyanoSeq 1.1.2 SILVA 138.1 database [39] [40]. ASVs were identified as potential contaminants using the “prevalence” method in the R package decontam R package version 1.22.0 [41]. Genetic information from the 0.3 mg/L glucose treatment was highly degraded and did not pass our quality assessment criteria, so those data were not included in our analyses.

## 2.5. Data Analysis

Analysis of variance was used to compare chlorophyll *a* and AFDM biomass in the control, hydrogen peroxide, and glucose additive treatments. Data normality was tested using the Shapiro-Wilk goodness of fit test. If the ANOVA model was statistically significant, a Holm-Sidak multiple comparison test was applied to assess differences. Assimilation index values were assessed with a Kruskal-Wallis test due to their non-normal distribution. Analyses were conducted using R software [42]. Because the straight glucose treatments were not replicated, we qualitatively examined the responses but made no inferences regarding significance.

Biodiversity was calculated at the genus level for the ASVs. Prior to calculating diversity estimates, samples were rarified to 0.9x the lowest read depth (31,968 reads). Shannon’s diversity index was used to estimate periphyton alpha diversity

for each treatment.

### 3. Results

#### 3.1. Water Quality Data

Temperature remained constant in all treatments and over time ( $\sim 22^{\circ}\text{C} - 23^{\circ}\text{C}$ ) (Table 1, supplemental Figure S1). DO and pH concentrations also remained relatively constant ( $\sim 8.5$  mg/L and 8.5 - 8.6, respectively) throughout the experiment in the control and hydrogen peroxide treatments, whereas DO declined about 3 mg/L in the glucose additive treatments and about 4.5 mg/L in the straight glucose treatments (Table 1, supplemental Figure S2). pH declined by  $\sim 0.5 - 1$  unit in the glucose additive treatment and about 1 - 1.5 units in the straight glucose treatments, and similar to DO, the decline was related to the glucose concentration (Table 1, supplemental Figure S3). Specific conductance increased slightly over time in the control, declined slightly in the hydrogen peroxide treatment, and increased from  $\sim 375$  to 425  $\mu\text{S}/\text{cm}$  in the glucose addition treatment (Table 1, supplemental Figure S4). TDS changes were very similar to those of specific conductance (Table 1, supplemental Figure S5), while turbidity declined over time in all treatments; the greatest turbidity declines were in the control and hydrogen peroxide treatments (Table 1, supplemental Figure S6).

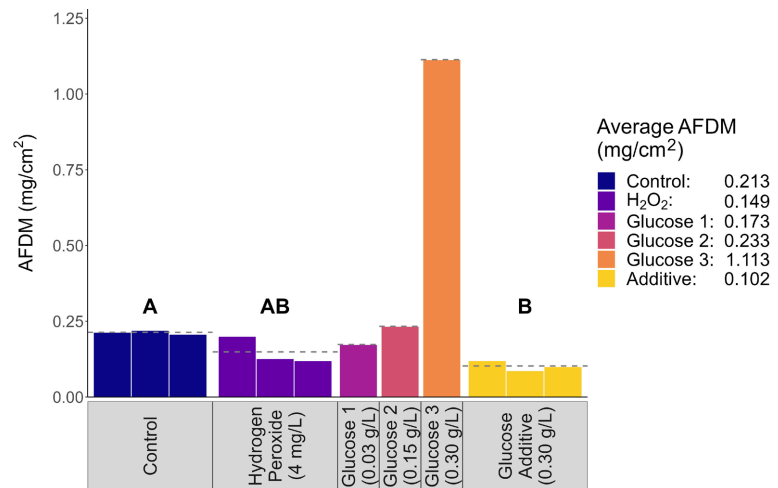
**Table 1.** Environmental data from the start and termination (day 34) of the mesocosm experiment. Data represent means when replicates are present; otherwise values are based on  $n = 1$  (glucose: high, moderate, and low columns). Changes from day 0 to day 34 that exceeded 10% are bolded.

Parameter	Control		Hydrogen Peroxide		Glucose—high		Glucose—moderate		Glucose—low		Additive Glucose	
	Day 0	Day 34	Day 0	Day 34	Day 0	Day 34	Day 0	Day 34	Day 0	Day 34	Day 0	Day 34
<b>Environmental</b>												
Temperature	22.4	22.6	22.5	22.7	22.5	22.7	22.7	23.0	22.0	21.8	21.9	22.8
DO	8.7	8.8	8.6	8.9	<b>8.6</b>	<b>3.0</b>	<b>8.4</b>	<b>4.3</b>	<b>8.4</b>	<b>5.2</b>	<b>8.6</b>	<b>6.6</b>
pH	8.6	8.7	8.5	8.7	<b>8.7</b>	<b>7.3</b>	8.5	7.7	8.5	7.9	8.5	8.2
Spec Cond	378	379	377	368	383	421	<b>379</b>	<b>421</b>	<b>378</b>	<b>421</b>	<b>377</b>	<b>432</b>
TDS	246	246	245	240	249	273	<b>246</b>	<b>274</b>	<b>246</b>	<b>274</b>	<b>245</b>	<b>281</b>
Turbidity	<b>3.0</b>	<b>0.2</b>	<b>3.4</b>	<b>0.1</b>	<b>2.6</b>	<b>1.1</b>	<b>2.6</b>	<b>0.3</b>	<b>2.7</b>	<b>0.5</b>	<b>1.8</b>	<b>0.7</b>
<b>Nutrients</b>												
SRP	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD
TP ( $\mu\text{g}/\text{L}$ )	<b>31</b>	<b>7</b>	<b>28</b>	<b>11</b>	<b>28</b>	<b>11</b>	<b>24</b>	<b>BD</b>	<b>27</b>	<b>13</b>	<b>26</b>	<b>13</b>
Nitrate ( $\mu\text{g}/\text{L}$ )	<b>140</b>	<b>50</b>	<b>130</b>	<b>50</b>	<b>140</b>	<b>100</b>	<b>140</b>	<b>60</b>	<b>130</b>	<b>60</b>	<b>160</b>	<b>BD</b>
Ammonia ( $\mu\text{g}/\text{L}$ )	<b>20</b>	<b>BD</b>	<b>20</b>	<b>BD</b>	<b>20</b>	<b>BD</b>	<b>20</b>	<b>BD</b>	<b>20</b>	<b>BD</b>	<b>20</b>	<b>BD</b>
TKN ( $\mu\text{g}/\text{L}$ )	<b>650</b>	<b>1070</b>	700	710	<b>710</b>	<b>1530</b>	<b>670</b>	<b>430</b>	<b>730</b>	<b>590</b>	<b>650</b>	<b>750</b>

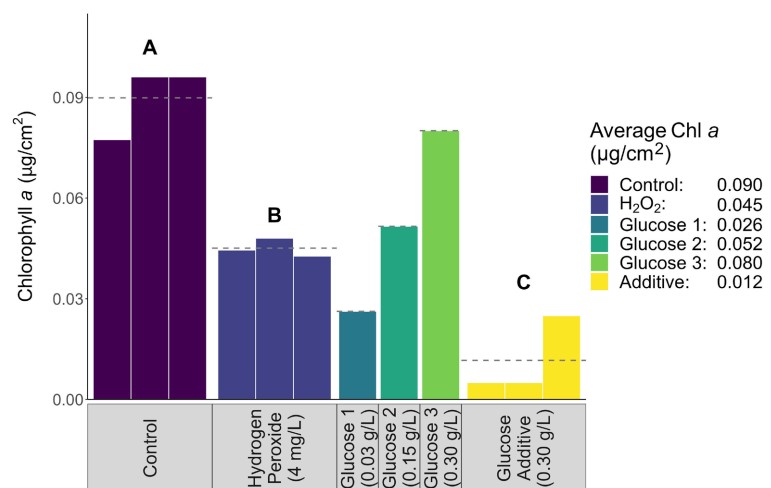
DO = dissolved oxygen, Spec Cond = specific conductance ( $\mu\text{S}/\text{cm}$ ), TDS = total dissolved solids, SRP = soluble reactive phosphorus (mg/L), TP = total phosphorus (mg/L), BD = below detection.



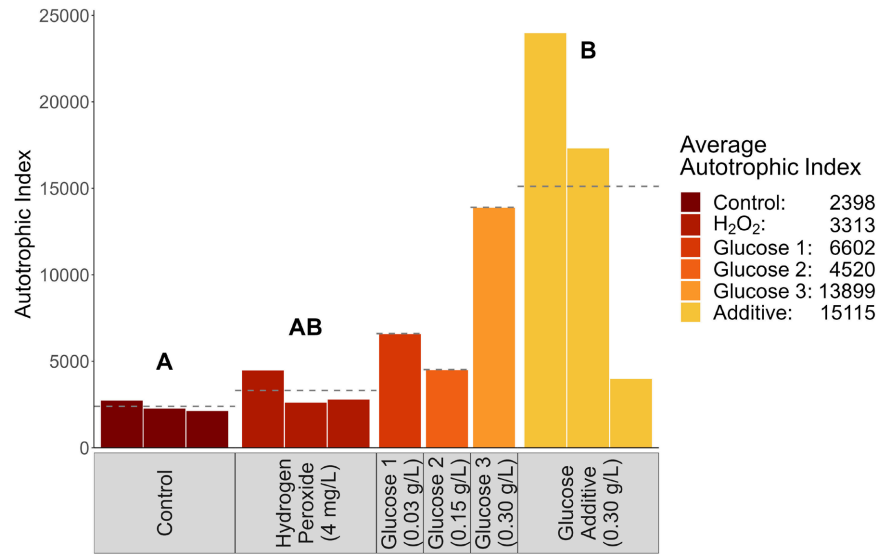
**Figure 1.** Left: AWRI mesocosm facility showing tanks and metal halide lamps suspended above them; right: periphyton-colonized strip after two weeks of deployment in the control treatment.



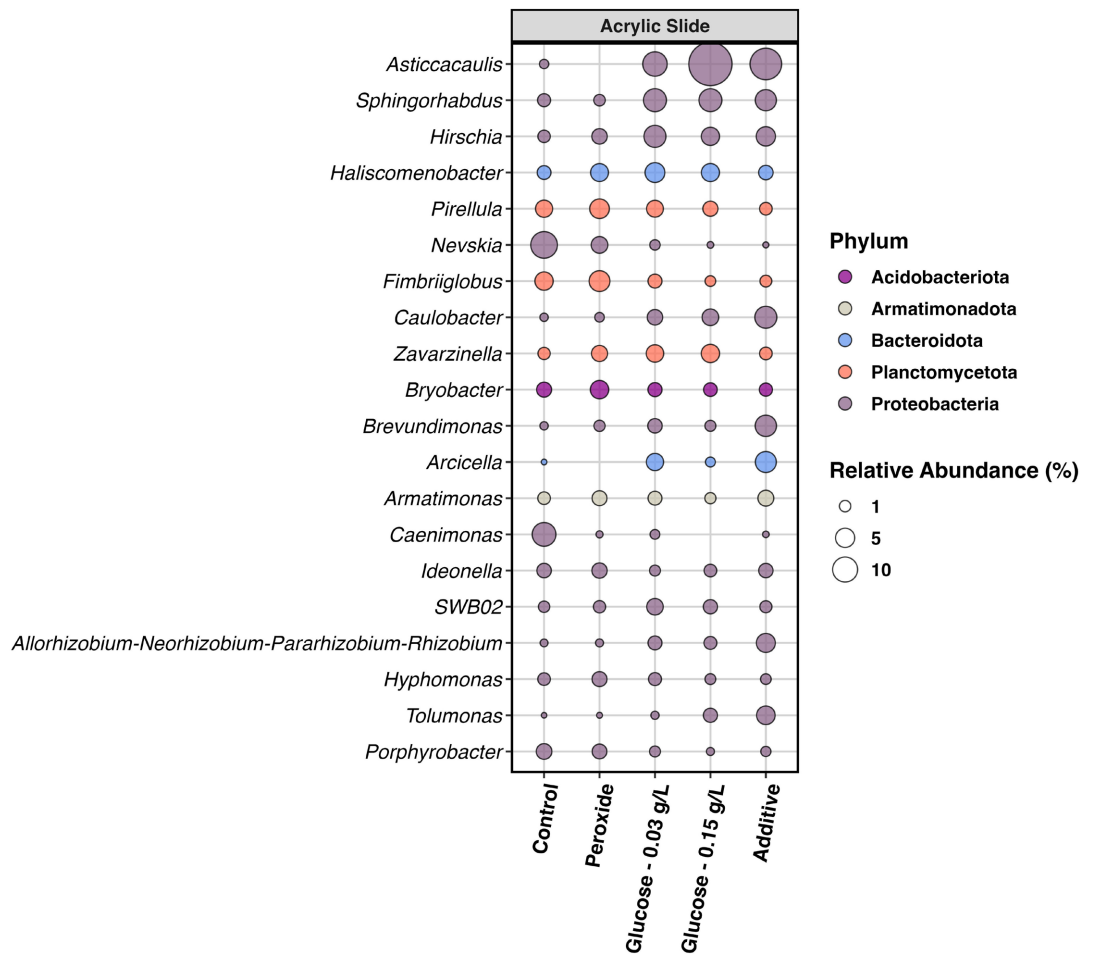
**Figure 2.** AFDM values (mg/cm<sup>2</sup>) from individual tanks within each treatment. Dashed line indicates mean value for each treatment.



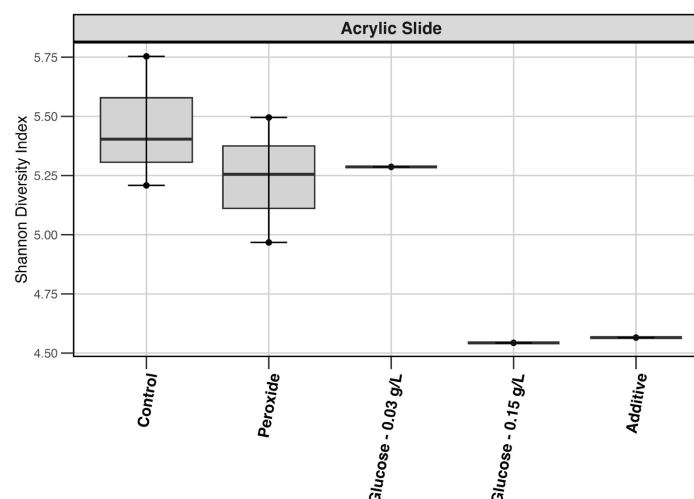
**Figure 3.** Chlorophyll *a* values (µg/cm<sup>2</sup>) from individual tanks within each treatment. Dashed line indicates mean value for each treatment.



**Figure 4.** Autotrophic Index values (ratio of AFDM to chlorophyll *a* in mg/cm<sup>2</sup>) from individual tanks within each treatment. Dashed line indicates mean AI value for each treatment.



**Figure 5.** Bubble plot of the 20 taxa with the highest relative abundance listed in order from most abundant at top to least abundant (of the 20) at the bottom. Diameter of bubble refers to abundance and color refers to phylum.



**Figure 6.** Shannon diversity index for five of the six treatments. DNA from the high concentration glucose (0.3 g/L) treatment was not interpretable.

### 3.2. Periphyton Data

Mean AFDM in the control treatment was  $0.213 \text{ mg/cm}^2$ , which was significantly greater ( $p = 0.008$ ) than mean AFDM in the glucose additive treatment and marginally greater ( $p = 0.057$ ) than in the hydrogen peroxide treatment (Figure 2). The largest increase was measured in the high glucose tank, where AFDM was  $1.113 \text{ mg/cm}^2$ ; however, this was a single observation (unreplicated treatment) and it is unclear how much of that biomass was associated with living organisms vs. mucopolysaccharide excretion (see below). AFDM in the two lower concentration glucose treatments were in the same range as that for the control treatment (Figure 2).

Relative to the controls, mean chlorophyll *a* concentrations in both the hydrogen peroxide and glucose additive treatments were significantly lower ( $p = 0.004$  and  $p < 0.001$ , respectively; Figure 3). The chlorophyll *a* concentration in the high glucose treatment was greater than the other two straight glucose treatments, but unlike the AFDM response, it was very similar to the mean chlorophyll *a* value in the controls, suggesting at least some of the stimulation of AFDM in the 0.3 mg/L glucose treatment was in the form of non-photosynthetic organic matter.

The autotrophic index (AI) confirmed that the periphytic biomass response in the glucose-associated treatments was driven largely by non-photosynthetic organic matter (Figure 4). The glucose additive treatment had a significantly greater AI than the control ( $p = 0.045$ ), although control vs. hydrogen peroxide was not significant ( $p = 0.458$ ).

The genetic analysis revealed no photoautotrophic genera among the 20 most abundant periphyton genera at the end of the experiment (Figure 5), although both *Limnothrix* and *Microcystis* were identified within the top 100 relative abundances (supplemental Figure S7). These largely planktonic taxa likely settled onto the periphyton matrix and should be considered more as metaphyton than true periphyton. Due to the degraded DNA in the high glucose treatment, it is unclear which taxa were responsible for the very high AFDM and moderate levels of chlorophyll *a*

on the substrate in that treatment; it is likely, based on the appearance and texture of the material on the strips that a significant amount of the AFDM was composed of extracellular polymeric substances. Periphytic bacteria were identifiable in all other treatments, with Proteobacteria being the most abundant phylum; the two most relatively abundant genera, *Asticcacaulis* and *Sphingorhabdus*, were found primarily in the treatments that included glucose additions. The other most common genera were from four phyla (Acidobacteriota, Armatiomonadota, Bacteroidota, and Planctomycetota) but at much lower relative abundances (Figure 5).

Biodiversity declined with increasing glucose concentrations (Figure 6). Mean diversity was highest in the control treatment, followed by the hydrogen peroxide and the low glucose treatments.

#### 4. Discussion

Considerable attention has been placed on the control and management of harmful algal blooms on a global scale [43] [44], including biotic, chemical, and physical approaches. Each approach has its own set of advantages and disadvantages, with the focus, understandably, on the phytoplankton. However, control measures can have secondary impacts beyond reducing or eliminating blooms, some of which may have unanticipated or undesirable effects. In the current experiment, we examined one of those potential secondary impacts: the response of the attached microbial community, referred to as biofilm or periphyton, to glucose and hydrogen peroxide.

Prior studies have shown that hydrogen peroxide has varying effects on controlling cyanobacteria, with both the hydrogen peroxide concentration level and cyanobacterial density important factors in determining control [20] [22] [35]. Linz *et al.* [26] found that hydrogen peroxide quickly reduced planktonic populations of cyanobacteria with little effect on the heterotrophic plankton community. In contrast, the effect of glucose on cyanobacteria took weeks to manifest, and the bacterioplankton became dominated by Proteobacteria and Bacteroidota. They speculated that the decline in cyanobacteria was because they were outcompeted for nutrients by the other bacterioplankton, although an increase in Proteobacteria may also be related to the presence of catalases, which protect them from oxidative stress [45] [46].

Our periphyton data examined changes only at the end of the experiment, precluding the evaluation of changes at a finer temporal scale. Exposure to hydrogen peroxide led to a decline in, but not elimination of, periphytic chlorophyll *a*. It is possible that the periphyton matrix, with its associated EPS, provided protection to the autotrophic community from the hydrogen peroxide [47] [48]. The effect of glucose on periphytic chlorophyll *a* was seemingly counterintuitive; we hypothesized that higher glucose concentrations would stimulate heterotrophic growth, resulting in higher nutrient sequestration, and lower autotrophic growth as a consequence. Instead, the higher-concentration straight glucose treatments had more chlorophyll *a* than the lower-concentration straight glucose treatments. We suggest several

possible explanations for this unexpected result. First, it is possible that the predicted competitive interactions between heterotrophic and autotrophic organisms were negated by the high glucose concentrations, resulting in sufficient amounts of bioavailable nutrients for all the organisms in the tanks. Second, stimulation of heterotrophic growth may lead to leakage of nutrients from their matrix, stimulating chlorophyll production (but see [49]).

Hydrogen peroxide exposure reduced AFDM by ~30% compared to the ~50% reduction in chlorophyll *a*, suggesting the periphytic autotrophic community in this experiment was more sensitive to this chemical than heterotrophs. The increasing trend in AFDM biomass with increasing glucose concentration followed the same pattern as observed for chlorophyll *a*, although to a much greater degree at the 0.3 mg/L glucose treatment. Interestingly, this same glucose concentration used in the additive treatment resulted in a reduction, not stimulation in AFDM. Unfortunately, we were unable to compare the community structure of the straight 0.3 mg/L glucose treatment with the additive glucose treatments due to the degraded genetic material in the former treatment. It is possible that there was a disproportionate amount of EPS in the straight high-glucose treatment that accounted for the AFDM levels, which would not be in competition with autotrophs for nutrients, and therefore also account for the highest chlorophyll *a* levels in this treatment.

We had no *a priori* expectations on how hydrogen peroxide would influence community structure. Compared to the control treatments, the two taxa with the greatest reductions in relative abundance after exposure to hydrogen peroxide were *Nevskia* and *Caenimonas*. These two taxa also declined in response to the glucose treatments. *Nevskia* is a widespread bacterium that excretes slime stalks, especially when supplied with excess carbon [50] [51], which is consistent with the phenotypic expression observed on the substrates. This taxon was abundant in the controls but by day 34 its relative abundance was substantially reduced, perhaps due to the reduced nitrogen levels in the tanks [50]. *Caenimonas* also declined substantially in relative abundance throughout the experiment. This taxon has been isolated from within blooms of *Microcystis* [52]; its decline in our tanks corresponds with the loss of *Microcystis*, suggesting that these two taxa may be interacting in some way.

We anticipated that glucose additions would stimulate taxa that have competitive superiority for dissolved organic carbon. The two periphytic taxa that responded most positively to glucose in relative abundance were *Asticcacaulis* and *Sphingorhabdus*. *Asticcacaulis* can metabolize organic materials, even at extremely low concentrations, and can tolerate prolonged periods of low nutrient concentrations [53]. Sphingomonads are typically found as members of freshwater bacterioplankton communities [54]; specifically, *Sphingorhabdus planktonica* exhibits its metabolic plasticity in its utilization of substrates [55].

Other studies have found that Planctomycetes are negatively impacted by hydrogen peroxide [21] [46]. Our results did not confirm this as both *Pirellula* and

*Fimbrioglobus* relative abundances increased slightly in the presence of hydrogen peroxide, and declined in the presence of glucose (Figure 5); some taxa in this phylum have the ability to mineralize organic carbon [56] but if this was the case with these taxa, it did not benefit their growth. In contrast, increased *Zavarzinella* relative abundance in the presence of glucose may have been a response to availability of dissolved organic carbon.

The decline in Shannon diversity with increasing glucose concentrations is related mostly to the loss of a number of taxa that were observed in very low relative abundances in the control, hydrogen peroxide, and low glucose treatments (supplemental Figure S7). Hence, it is likely these taxa had limited ecological significance although taxon-specific studies would be needed to be certain.

Our study had several limitations which need to be considered when applying our results elsewhere. First, the species pool was limited to the original inoculum in all treatments except the additive tanks. In such cases, priority effects [57] can strongly influence community structure. Second, the degraded DNA in the high-concentration straight glucose treatment limited our ability to determine what was responsible for the substantial increase in biomass. Third, our mesocosms lacked herbivores and macrophytes, which can strongly influence periphyton structure and function [58]. Finally, we sacrificed a fully replicated experimental design to exactly mimic the design of a prior benchtop study [24]; although this limited our ability to apply inferential statistics across all treatments, it facilitated US EPA personnel to determine if their previous results could be scaled up from flasks to mesocosms.

## 5. Management Implications

The periphyton community is a vital component in lakes, playing critical roles in nutrient cycling, habitat provision, primary productivity, food for higher trophic levels, and interconnecting not only the benthic and pelagic zones vertically but the littoral and open water zones horizontally [1] [59]. Nonetheless, very little attention has been placed on how this community is affected by management measures taken to control algal blooms in lakes.

The results of our study indicate that periphyton is impacted by hydrogen peroxide and glucose in terms of both biomass and community structure. We recognize that these impacts will not stop the application of algal bloom control measures, which are driven by overriding socio-economic and health factors. However, our data suggest that hydrogen peroxide will reduce if not eliminate the autotrophic component of the periphyton community, which in turn will negatively impact the invertebrate community (and ultimately, the higher trophic food web) that consumes this important source of nutrients [60]. In addition, glucose additions may stimulate heterotrophic bacteria, especially *Asticcacaulis* [61], at the expense of autotrophs, resulting in unknown impacts. More research in this area is recommended before these additions are made in the natural environment.

We suggest that as new approaches are developed to control algal blooms, biological assessments focus not only on the phytoplankton community but also on the attached communities. When feasible, approaches should be devised that limit impacts to the periphyton community (unless of course, toxic taxa are present in the community).

## Funding

This research and A.R. were funded by the Allen and Helen Hunting Research and Innovation Fund held at AWRI. Additional funding was provided by the United States Environmental Protection Agency (US EPA), Office of Research and Development's (ORD's) research programs: Safe and Sustainable Water Resources (SSWR 4.2.2.1).

## Author Contributions

Alison Romanski: data analysis and interpretation, statistical analysis, graphics, and manuscript writing. Charlyn G. Partridge: methodology, data curation, and manuscript writing/review. Alan D. Steinman: conceptualization, acquisition of funds, validation, manuscript review/editing, and on-site project management.

## Acknowledgements

We are grateful to the US EPA team from Cincinnati, including Steve Vesper, David Linz, Ian Struewing, Nathan Sienkiewicz, and Jingrang Lu, for their collaboration and funding for the project. Mike Hassett and Katie Tyrrell assisted with mesocosm maintenance and lab analyses; Renee Tardani assisted with the genomic analysis. Comments from three anonymous reviewers improved the manuscript.

## Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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### Supplemental Information

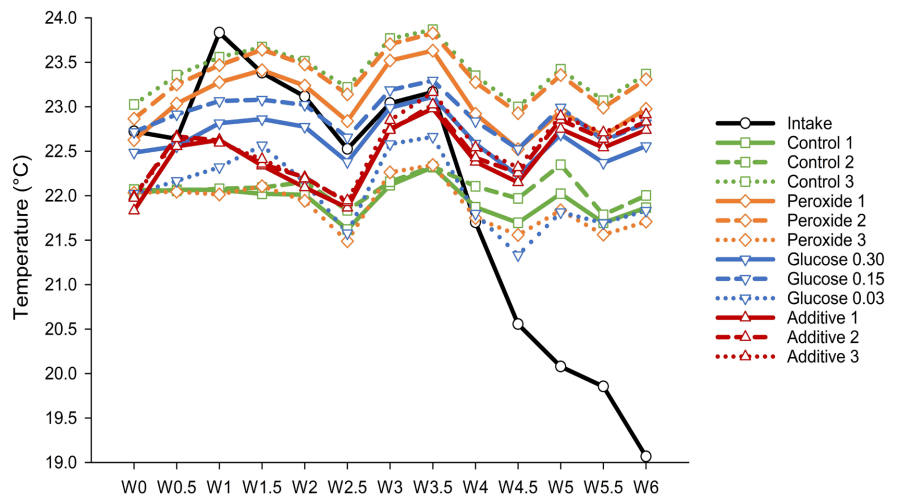


Figure S1. Temperature data from each mesocosm tank over time.

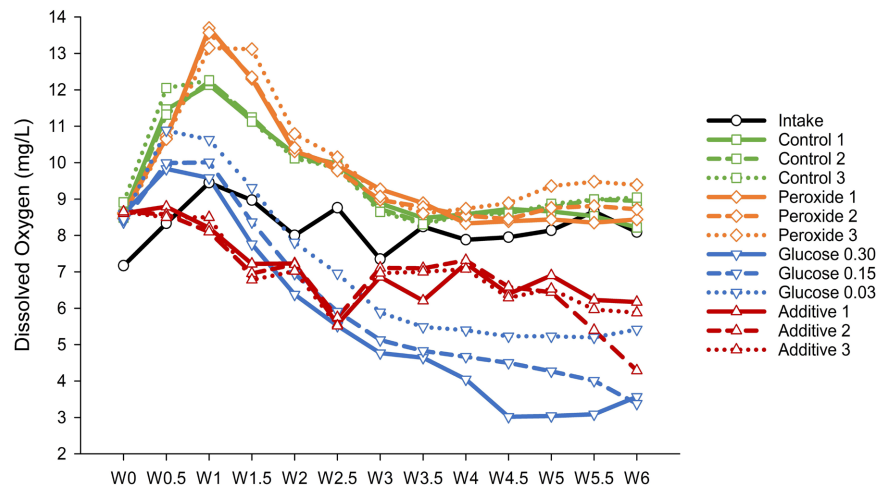


Figure S2. Dissolved oxygen data from each mesocosm tank over time.

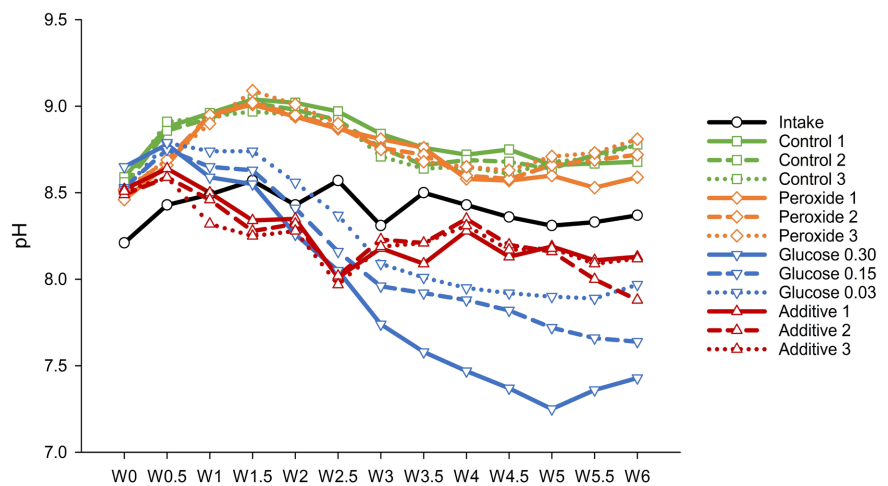
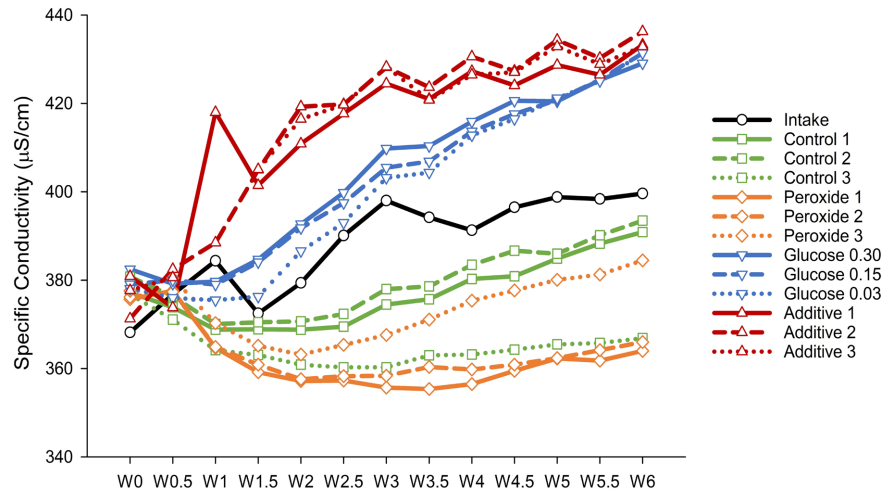
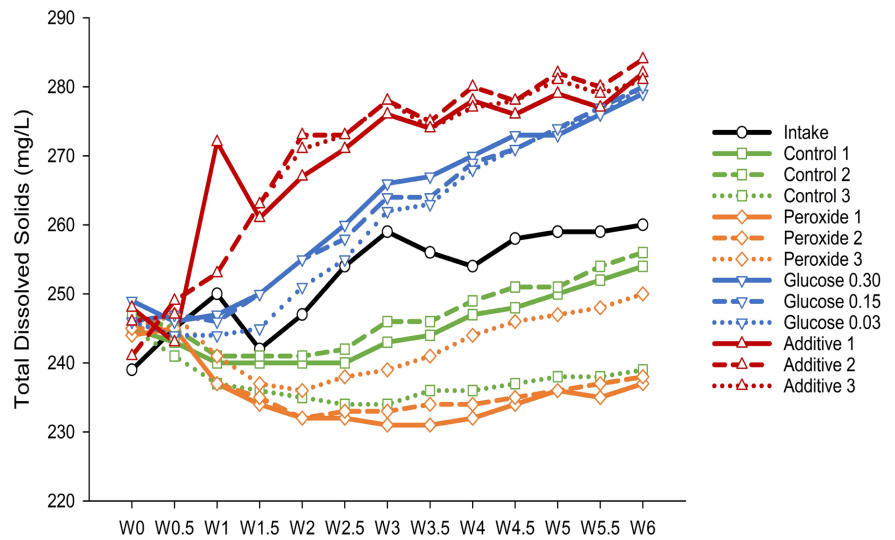


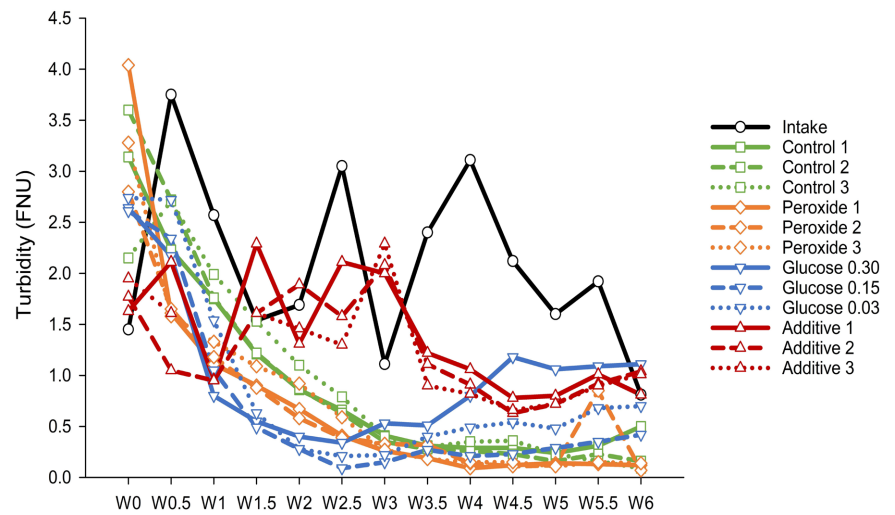
Figure S3. pH from each mesocosm tank over time.



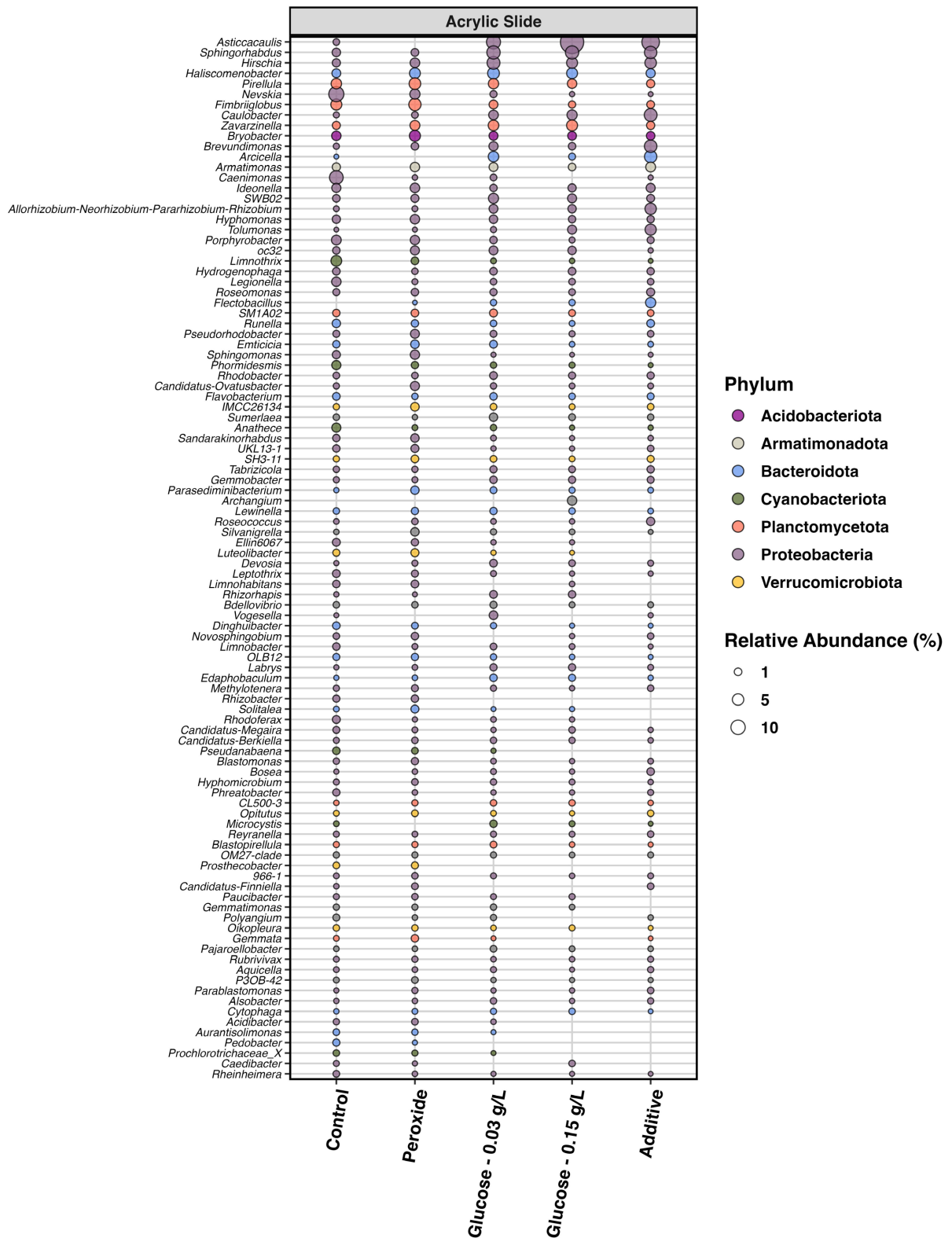
**Figure S4.** Specific conductance data from each mesocosm tank over time.



**Figure S5.** Total dissolved solids data from each mesocosm tank over time.



**Figure S6.** Turbidity data from each mesocosm tank over time.



**Figure S7.** Bubble plot of the 100 taxa with the highest relative abundance listed in order from most abundant at top to least abundant (of the 100) at the bottom. Diameter of bubble refers to abundance and color refers to phylum. Note the top 20 are also shown as **Figure 5** in the main manuscript.