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Effect of hydrogen peroxide on natural phytoplankton and bacterioplankton in a drinking water reservoir: Mesocosm-scale study

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ABSTRACT

Cyanobacterial blooms are increasingly reported worldwide, presenting a challenge to water treatment plants and concerning risks to human health and aquatic ecosystems. Advanced oxidative processes comprise efficient and safe methods for water treatment. Hydrogen peroxide (H_2O_2) has been proposed as a sustainable solution to mitigate bloom-forming cyanobacteria since this group presents a higher sensitivity compared to other phytoplankton, with no major risks to the environment at low concentrations. Here, we evaluated the effects of a single H_2O_2 addition (10 mg L^{-1}) over 120 h in mesocosms introduced in a reservoir located in a semi-arid region presenting a *Planktothrix*-dominated cyanobacterial bloom. We followed changes in physical and chemical parameters and in the bacterioplankton composition. H_2O_2 efficiently suppressed cyanobacteria, green algae, and diatoms over 72 h, leading to an increase in transparency and dissolved organic carbon, and a decrease in dissolved oxygen and pH, while nutrient concentrations were not affected. After 120 h, cyanobacterial abundance remained low and green algae became dominant. 16S rRNA sequencing revealed that the original cyanobacterial bloom was composed by *Planktothrix, Cyanobium* and *Microcystis*. Only *Cyanobium* increased in relative abundance at 120 h, suggesting regrowth. A prominent change in the composition of heterotrophic bacteria was observed with *Exiguobacterium, Paracoccus* and *Deinococcus* becoming the most abundant genera after the H_2O_2 treatment. Our results indicate that this approach is efficient in suppressing cyanobacterial blooms and improving water quality in tropical environments. Monitoring changes in abiotic parameters and the relative abundance of specific bacterial taxa could be used to anticipate the regrowth of cyanobacteria after H_2O_2 degradation and to indicate where in the reservoir H_2O_2 should be applied so the effects are still felt in the water treatment plant intake.

1. Introduction

Cyanobacterial blooms in freshwater are directly related to anthropogenic eutrophication and represent a major concern for drinking water supply (Pearl and Otten, 2013; Codd et al., 2016; Huisman et al., 2018). The ideal solution to this situation is an effective control of nutrient inputs from watersheds (Paerl, 2017). However, this is a challenging and long-term measure. As a short-term alternative, Advanced Oxidative Processes (AOPs) have been proposed to control cyanobacterial blooms due to the speed, efficiency and harmless by-products generated (Matthijs et al., 2012; Pestana et al., 2015; Weenink et al., 2015; Menezes et al., 2020).

Studies in temperate regions have demonstrated the effect of hydrogen peroxide (H_2O_2) on phytoplankton community, promoting eukaryotic organisms over cyanobacteria (Matthijs et al., 2012; Lurling et al., 2014; Weenink et al., 2015; Yang et al., 2018). The use of H_2O_2 either in laboratory-scale experiments (Weenink et al., 2015), or in situ application in a eutrophic lake (Matthijs et al., 2012) resulted in the selective control of cyanobac-

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teria in *Planktothrix*-dominated phytoplankton communities, with no major effects on eukaryotic phytoplankton or zooplankton. The higher sensitivity of cyanobacteria to H_2O_2 compared to eukaryotic phototrophs can be explained by the absence of compartmentalized organelles protected by membranes, as well as the lack of Mehler reaction as found in higher plants and algae (Latifi et al., 2009; Allahverdiyeva et al., 2013; Passardi et al., 2007). This reaction consists of O_2 reduction to superoxide anion (O_2^-) by electrons from photosystem I (PSI). Superoxide is then converted to H_2O_2 by superoxide dismutase and finally to water by peroxidase. In cyanobacteria, flavoproteins suppress O_2^- generation and the electrons from PSI are donated to O_2 producing H_2O without the formation of reactive oxygen species (ROS) (Latifi et al., 2009; Allahverdiyeva et al., 2013). Thus, cyanobacteria do not possess an anti-ROS system as efficient as that of green algae and higher plants (Passardi et al., 2007).

In tropical regions, the composition and biomass of cyanobacterial blooms can differ from those in temperate regions and tend to last longer due to extended warm periods and higher light intensity (Kosten et al., 2011; Pearl and Paul, 2012; Paerl et al., 2017; Huisman et al., 2018; Vanderley et al., 2021). The efficiency of H_2O_2 is influenced by light intensity and nutrient availability (Piel et al., 2019; Sandrini et al., 2020). It is also dependent on H_2O_2 decomposition and its residence time in water (Matthijs et al., 2012; Weenink et al., 2015), which can be affected either by biotic factors, such as phytoplankton and bacterioplankton composition, or abiotic factors as water reductive power (affected by dissolved solutes or organic matter) (Arvin and Pedersen, 2015; Weenink et al., 2015). In general, all these factors might be considerably different comparing temperate to tropical zones (Sobek et al., 2007; Kosten et al., 2011; Carrasco Navas-Parejo et al., 2020; Toming et al., 2020; Vanderley et al., 2021) but investigations on the efficiency of H_2O_2 in suppressing cyanobacteria in tropical aquatic environments are scarce.

Although the effect of H_2O_2 against phytoplankton is well established, the treatment outcome for the bacterioplankton is less investigated but this community can contribute to H_2O_2 decomposition and affect cyanobacterial control. Recently, Lusty and Gobler (2020) evaluated the effect of H_2O_2 (4 mg L^{-1}) on cyanobacteria and the overall microbial community in laboratory scale experiments using environmental samples from four eutrophic lakes in the United States. They observed a variable reduction in the relative abundance of cyanobacteria (29%-85%) accompanied by a decrease on Actinobacteria and an increase in Bacteroidetes and Proteobacteria. Bacteria vary in their susceptibility to ROS, depending on both enzymatic and non- defense mechanisms (Daly, 2009). Thus, changes in bacterioplankton composition according to their antioxidant defenses are expected as a result of H_2O_2 application, which can influence its residence time and its efficiency in mitigating cyanobacteria.

Due to eutrophication, water quality in artificial reservoirs in the semi-arid region is deteriorating to a point where conventional water treatment plants (WTP) are not technically or economically able to process raw water into the Brazilian drinking standards (Barros et al., 2020). High amounts of particulate or dissolved organic matter produced mostly by phytoplankton (Pestana et al., 2019), overload the treatment steps, increasing the amount of chemicals needed, frequency of settling tank discharge and filter wash, and ultimately decreasing WTP efficiency and finished water quality (Barros et al., 2020). Thus, H_2O_2 could be used as a cheap and easy pre-treatment method for cyanobacteria control before water treatment. Despite the widespread occurrence of cyanobacterial blooms in tropical regions, the effectiveness of H_2O_2 to suppress that in natural phytoplankton communities has still not been accessed. The effect of H_2O_2 on co-occurring heterotrophic bacteria must also be considered. Besides their role on H_2O_2 degradation, changes in the composition of microbial communities can potentially affect biogeochemical cycles, nutrient availability, water quality and the aquatic ecosystem balance. In a previous study, using H_2O_2 (10 mg L^{-1}) we observed the suppression of a *Planktothrix*-dominated cyanobacterial community in laboratory-scale experiments with no effects on eukaryotic phototrophs using raw water from a reservoir in the Brazilian semi-arid region (Rocha, 2020). To further characterize the effects of H_2O_2 over phytoplankton communities and to simulate the treatment in a natural environment, we tested 10 mg L^{-1} H_2O_2 in a mesocosm system installed into a reservoir used for drinking water supply in the Brazilian semi-arid region. We assessed physical and chemical parameters for freshwater quality and also the effect of H_2O_2 on the bacterioplankton community composition over time. We hypothesized that the treatment would be effective in suppressing cyanobacteria but would also affect the associated heterotrophic bacterioplankton, altering the proportion of susceptible/resistant bacterial species.

2. Methodology

2.1. Study area

This study was performed in Gavião reservoir (3°59'03"S/38°37'13"W), altitude of 65 m relative to the sea level. This is a semi-arid region with high solar radiation reaching approximately 5 kWh m^{-2} day^{-1}, 8 h per day, annual average atmospheric temperature of 32°C and irregular and low rainfall periods from January to May (FUNCEME, 2017; Barros et al., 2019). The reservoir has a water storage capacity of 33 million m^3 (Cogerh, 2019) and is used exclusively for public water supply to Fortaleza, capital of Ceará state, Brazil, serving about 3 million people (Fig. 1). The water treatment plant (WTP) adjacent to the reservoir operates by conventional treatment set (coagulation, direct filtration, and disinfection) and is managed by the Water and Wastewater Company of the State of Ceará.

2.2. Mesocosms experimental set-up and water sampling

Mesocosm experiments were carried out from 15 February to 20 February 2019 in the Gavião reservoir. Six impermeable and semi-transparent plastic bags were built in a cylindrical shape with 2 m length and 1.5 m diameter (3.5 m^3). Mesocosms were filled with about 3000 L of water. The whole structure was located in the lacustrine zone of the Gavião reservoir with an average depth of 10 m, near to the water treatment plant-intake point. Mesocosms and full-scale structures in the water can be seen in Supplementary Figure 1. The water column depth (1.5 m) in the mesocosms was previously established to ensure that the experiment took place in the euphotic zone. The extent of the euphotic zone was calculated by multiplying the value of Secchi's disk depth by the K factor of 3, frequently used for tropical waters in Brazil (Esteves, 2011). Mesocosms were set as control with no H_2O_2 (n=3) and as treatment condition (n=3) consisting of a single addition of H_2O_2 (10 mg L^{-1}). The experiment was maintained for 120 h (5 days) and water samples were taken immediately before the application of H_2O_2 (T0) and at times 24, 72, and 120 h. For each sampling time biotic (section 2.3) and abiotic parameters were evaluated (Table 1).

Stocked H_2O_2 (60% by weight – Sigma Aldrich®) was diluted to achieve the initial concentration that was determined by iodometric titration (Skellon and Wills, 1948). The degradation of H_2O_2 was estimated at each sampling time using a semi-quantitative method with colorimetric strips (MQuant™ Peroxide-Test, Merck®) as a pragmatic approach, focusing on the impact on all tested parameters before and after H_2O_2 consumed rather than on its kinetics of degradation. All samples were collected around 9:00 am, the

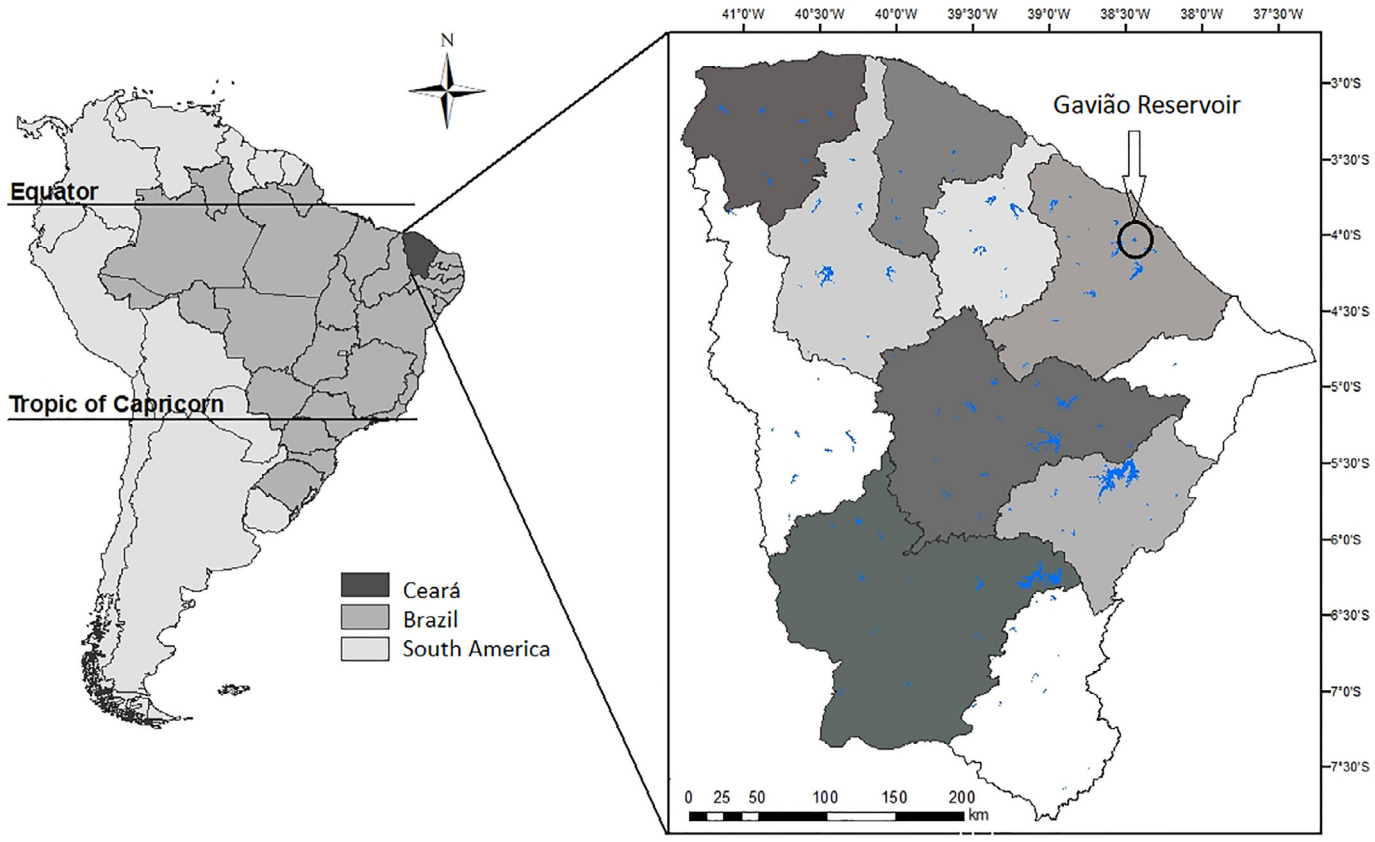

Fig. 1. Gavião reservoir location, Ceará state, Brazil. Source: Adapted from Barros *et al.* (2019).

Table 1
Physical or chemical parameters, methods and location of analysis.

Parameter	Method/Equipment	Where
Transparency	Secchi disk	*in situ*
Temperature and pH	YSI probe model 60 - Yellow Springs Instruments, EUA	*in situ*
Dissolved oxygen	YSI probe model 55 - Yellow Springs Instruments, EUA	*in situ*
Conductivity	Conductivity meter 105A+, Orion Research, EUA	*in situ*
Turbidity	Hach model 2100P, EUA	Laboratory
True color	Genesys spectrophotometer 10S UV-Vis - Thermo Scientific, EUA	Laboratory
Total organic carbon (TOC) and dissolved carbon (DOC)	Sievers InnovOx Laboratory TOC Analyzer (General Electrics, USA)	Laboratory
Ammonia	4500-NH_3 C - APHA (2005)	Laboratory
Nitrite, nitrate, orthophosphate, sulfate, fluoride, and chloride.	Samples were filtered through a glass fiber 0.45-μm membrane before analyses by Ion Chromatographic using Dionex ICS-1100 (Thermo Scientific, EUA)	Laboratory

same time of H_2O_2 application at the beginning of the experiment. We used a Van Dorn bottle to collect water from the center of the mesocosm in a depth of 75 cm. Each sample was placed in a 1 L amber glass flask, refrigerated, and transported to the laboratory.

2.3. Biological parameters

2.3.1. Chlorophyll-a concentration

The chlorophyll-a concentration was estimated using a PHYTO-PAM II Phytoplankton Analyzer (Walz, Germany) with different wavelengths: blue (470 nm), green (520) and red (645 and 665 nm). It is possible to distinguish phytoplanktonic groups based on the different types of light-harvesting pigment antennae and estimate chlorophyll-a concentrations (µg L^{-1}) of cyanobacteria, green algae, and diatoms (Walz, 2003). Chlorophyll-*a* estimation was used to evaluate the biomass of the natural phytoplankton community and to evaluate the respective effects of H_2O_2 treatment on those separate groups.

2.3.2. Bacterioplankton composition

2.3.2.1. Sample processing and DNA extraction. Water samples were taken from the mesocosms at times 0, 24, 72, and 120 h in aliquots of 300 mL and refrigerated until reaching the laboratory. Samples were then filtered through 0.22 μm SteritopTM filter units (Merck

Millipore®, Massachusetts, US) and the filters were kept at -80 °C until DNA extraction. DNA was extracted from the cells collected in the filters using a DNA Fecal/Soil Microbe Miniprep kit (Zymo® Research, California, US), following the standard protocol from manufacturer. DNA purification, amplification of the v4 region of the bacterial 16S rDNA gene (16S rRNA) and the preparation of DNA libraries were performed as described in Santos et al. (2020).

2.3.2.2. Sequencing and bioinformatics analysis. Sequencing procedures (Illumina) and initial bioinformatics steps such as quality trimming and alignment of resulting sequences were performed according to Santos *et al.* (2020). Chimeras were detected using VSEARCH and then excluded (Rognes et al., 2016). Taxonomic classification was carried out using the SILVA database (Quast et al. 2013) with a confidence threshold of 80%. Sequences not assigned as Bacteria or classified as chloroplast, mitochondria, Archaea or unknown were discharged. Taxa with only one or two sequences were removed. The total number of sequences in each sample was randomly normalized to equal that of the sample with the lower number of sequences. Then, the sequences were clustered into operational taxonomic units (OTUs) using a sequence similarity cut-off of 97%. The taxonomic assignment of OTUs was performed according to SILVA database v138 (release date December 16, 2019). The composition of microbial communities was evaluated according to the relative abundance of taxa (Phylum and Order levels), considering only those OTUs that contributed more than 1% to total sequences. A non-metric multidimensional scaling (nMDS) and clustering dendrogram were applied to ordinate the 22 samples based on OTU composition using a dissimilarity matrix based on Bray Curtis distance. Rarefaction curves as well as Shannon diversity index, Sobs richness and evenness were calculated for each sample using Mothur v1.43 software (Schloss et al. 2009). Sequence information for this project is available at the NCBI database (BioProject accession number PRJNA659532).

2.4. Statistical analysis

2.4.1. Limnological parameters

Multivariate analysis by PERMANOVA was applied to verify the effects of H_2O_2 over time (0, 24, 72, and 120 h) on all limnological parameters evaluated (abiotic parameters and chlorophyll), considering α=0.05. D'Agostino & Pearson normality test was applied to verify the distribution of data considering $p<0.05$. To support more specifically the difference within each parameter as an interaction consequence between control and treatment over time, two-way ANOVA was used considering α=0.05 and uncorrected Fisher's LSD post hoc to detect the difference at the respective time points. Statistical analysis was performed by R software version 1.3.959 and Graphpad Prism 8.0

2.4.2. Composition of bacterial communities

OTU frequencies were used as input to perform a permutational multivariate analysis of variance using two factors (two-way PERMANOVA, $p<0.05$) as experimental condition (with or without H_2O_2) and time (0, 24, 72 and 120 h) that were used to evaluate the effects of the treatment on bacterioplankton. The null hypothesis was rejected if the p-value was <0.05, assuming the alternative hypothesis that there was a significant effect of H_2O_{22} on bacterial community structure over time. A mixed-effects model of ANOVA was used to evaluate differences on diversity and richness indexes considering $p<0.05$ and Sidak's multiple comparison tests. These analyses and charts were performed in Graphpad Prism 8.0 (GraphPad Software, LaJolla California, US). We performed a linear discriminant analysis (LDA) and effect Size (LEfSe) (Segata et al., 2011) following the Hutlab Galaxy web framework (available on http://huttenhower.sph.harvard.edu/galaxy/) using LDA score of 3.0 as threshold (log 10 transformed) to select bacterial taxa with a significant contribution ($p<0.05$) to the differentiation between treatment and control over time. From this, the statistical analysis consisted of a two-tailed nonparametric Kruskal-Wallis test and an unpaired Wilcoxon test to reveal significant differences in most abundant OTUs comparing treatment and control (considering all replicates) over time. We also used a similarity percentage analysis (SIMPER) (Clarke, 1993) to select the main OTUs that contributed to the differentiation between control and treatment analyzing at each sampling time, separately (24, 72 and 120 h).

2.4.3. Association between OTUs and limnological parameters

Canonical correspondence analysis (CCA) was used to associate limnological parameters with the major OTUs, corresponding to those selected by SIMPER analysis and presenting a relative contribution higher than 1% or cumulative contribution of 50% or more at each sampling time (24, 72 or 120 h). All three phytoplankton groups (cyanobacteria, green algae, and diatoms) and abiotic parameters that presented statistical differences comparing treatment and control were used. The CCA chart was represented using the PAST3 software (Hammer et al., 2001). A correlation matrix using Spearman ($p<0.01$ and a threshold for r value=$\pm$0.6) was constructed to calculate significant associations among abiotic and biotic parameters included in the CCA.

3. Results

3.1. Abiotic parameters

In a multivariate analysis including all parameters tested here, the H_2O_2 treatment influenced abiotic parameters and chlorophyll from phytoplankton (commonly known as limnological parameters) used as water quality indicators as supported by PERMANOVA analysis (p=0.02; F=25.20) with a great interaction between time and condition (p=0.024; F=4.0793). H_2O_2 was no longer detected in the mesocosms after 72 h (data not shown). Both transparency ($p<0.0001$, F=69.67) and turbidity ($p<0.0001$, F=50.73) were affected by the treatment at all sampling times (24, 72 and 120 h). The initial transparency value was 47 cm and increased to 64 cm in the control at 24 h, without further modification. In the treated mesocosms, transparency continued to increase to 82, 115 and 105 cm at 24, 72 and 120h, respectively (Table 2). Turbidity was initially 11 NTU (Nephelometric Turbidity Units) and remained 11-14 in the control. In the treated water, it decreased to 8.3, 5.5 and 5.5 NTU at 24, 72 and 120 h, respectively. Other abiotic parameters were significantly different in the treatment compared to the control at certain sampling times (Table 2). Conductivity showed a significant variation over the treatment (p=0.005, F=7.2) presenting an increase at 120 h (p=0.0007), reaching an average of 585 $\mu S\ cm^{-1}$ compared to 575 $\mu S\ cm^{-1}$ from control. Nonetheless, no difference was observed between the treatment and control conditions for conductivity-related salt anions such as chloride (p=0.27), sulphate (p=0.57) and fluoride (p=0.57). The pH significantly decreased in the H_2O_2 treatment compared to control ($p<0.0001$, F=42.16), from 9.2 to 8.1 at 72 h and from 9.0 to 8.3 at 120 h (Table 2). No significant change in TOC was observed, whereas DOC decreased from 22 to 18.5 mg L^{-1} comparing control and treatment at 72 h (p=0.003, F=7.71). DO also decreased significantly at 72 h, 11.4 in control and 6.4 mg L^{-1} in the treatment ($p<0.0001$, F=21.26). No significant difference was observed for nutrients comparing treatment and control, considering ammonia (p=0.59), nitrite (p=0.73), nitrate (p=0.50) or orthophosphate (p=0.12). Nutrient concentrations were affected by enclosure over time, both in the treatment and in the control condition. Temperature or true color did not change in response to the treatment over time (p=0.53).

Table 2
Influence on abiotic parameters of 10 mg L^{-1} H_2O_2 in raw drinking water over 120 h. The parameters that presented a significant difference ($p<0.05$) between control and experimental group at each sampling time are labeled in bold. The values represent average and standard deviation (n=3). The abbreviations: DOC – dissolved organic carbon; TOC – total organic carbon; DO – dissolved oxygen; Chl – chlorophyll; NTU – nephelometric turbidity unit.

Parameters	Initial time (T0)	24 h		72 h		120 h	
		Control	Treatment	Control	Treatment	Control	Treatment
Transparency (cm)	47.3.2	**64.3±5.5**	**81.7±3.2**	**62.7±4.5**	**115.3±3.5**	**68.7±2.9**	**105.0±3.0**
Turbidity (NTU)	11.2±0.2	**11.5±0.1**	**8.3±0.3**	**14.4±0.3**	**5.5±0.9**	**13.1±0.8**	**5.5±1.7**
Conductivity (μS cm^{-1})	568±3.1	565±1.15	567.3±1.5	573±1.1	580.3±3.5	**574±1**	**582±0.6**
pH	8.8±0.2	9.1±0.1	8.9±0.1	**9.1±0.07**	**8.1±0.1**	**9.0±0.05**	**8.3±0.1**
DOC (mg L^{-1})	16.1±0.5	16.5±0.8	16.0±0.7	**22.1±0.2**	**18.5±1.1**	21.8±2	17.8±0.6
DO (mg L^{-1})	8.0±0.3	10.3±0.4	9.9±0.8	**11.4±1.1**	**6.4±0.2**	9.1±0.5	8.0±0.7
True Color (mg L^{-1})	22.6±2.5	19.7±2.5	24.1±2.5	21.2±0	18.3±2.5	21.2±0	16.8±0
Temperature (°C)	31.3±0.4	30.8±0.2	30.4±0.2	31.5±0.1	31.3±0	31.1±0.7	31.3±0.2
TOC (mg L^{-1})	20.9±2.5	20.4±1.2	19.0±1.2	26.3±1.8	25.7±2.7	29.7±3.2	23.2±1.2
Ammonia (mg L^{-1})	0.05±0.8	0.06±0.1	0.1±0.1	0.2±0.05	0.2±0.03	0.1±0.05	0.07±0.06
Nitrite (mg L^{-1})	0.7±0.06	0.8±0.06	0.8±0.1	1.0±0.1	1.0±0.1	0.8±0.03	0.9±0.1
Nitrate (mg L^{-1})	0.4±0.1	0.5±0.2	0.6±0.2	0.1±0.05	0.2±0.1	0.4±0.2	0.2±0.3
Orthophosphate (mg L^{-1})	0.7±0.2	0.9±0.5	1.4±0.2	1.6±0.2	1.4±0.4	1.4±0.1	1.4±0.08
Sulphate (mg L^{-1})	7.0±0.3	7.8±0.3	7.7±0.7	7.5±0.4	7.7±0.4	8.0±0.1	8.3±1.2
Chloride (mg L^{-1})	82.2±3.2	84.9±3.7	88.8±6.0	86.7±5.4	83.1±0.5	83.3±2.2	85.0±3.2
Fluoride (mg L^{-1})	0.2±0.02	0.3±0.2	0.6±0.06	0.6±0.02	0.8±0.1	1.1±0.6	1.5±0.3

3.2. Effect of H_2O_2 on phytoplankton

According to the estimated chlorophyll concentration, biomass of all three phytoplankton groups was affected by H_2O_2 over the time: cyanobacteria ($p<0.0001$, $F=21.45$), green algae ($p<0.0001$, $F=58.38$), and diatoms ($p<0.0001$, $F=24.97$). Cyanobacteria showed the highest chlorophyll value at T0 (~ 35 µg L^{-1}) and decreased consistently over time compared to the control condition. At 72 h, cyanobacteria could not be detected (Fig. 2A). Green algae chlorophyll also decreased significantly at 72 h ($p=0.0003$ for both), reduced by 50%. However, at 120 h a substantial increase in green algal biomass by chlorophyll estimation was observed ($p=0.03$) reaching about 25 µg L^{-1} while in the control it remained at 13 µg L^{-1} (Fig. 2B). The initial chlorophyll concentration of diatoms was 1 µg L^{-1} and this group could not be detected at 24 h in the treatment condition ($p=0.04$), but subsequently increased at 120 h, reaching a biomass three times higher than the control (Fig. 2C). In the treated water, green algae biomass was higher than in the control at 120 h, while the inverse was observed for cyanobacteria, suggesting a substitution in dominance.

3.3. Effect of H_2O_2 on bacterioplankton

3.3.1. Bacterial diversity and ordination

After normalization, 32221 sequences were considered in each sample to describe the bacterial communities (Supplementary Table 1). The sample T3M5 (no H_2O_2 at 72 h) was lost during DNA processing and could not be analyzed. Rarefaction curves approached saturation with coverage of bacterial diversity reaching 97-98% for all samples (Supplementary Table 1 and Supplementary Figure 2). Clustering of samples revealed an average similarity of 60% within the control and treatment groups and a dissimilarity of 70-80% between them (Supplementary Figure 3). Considering Shannon diversity index and sobs richness estimation, H_2O_2 did not affect richness at any time ($p=0.07$, $F=5.58$) (Supplementary Figure 4A) but decreased community diversity ($p=0.001$, $F=63.51$) at 24 and 72 h compared to the control condition (Supplementary Figure 4B). Thus, compositional changes occurred in bacterial communities after H_2O_2 addition without a decrease in the number of species. Ordination of samples corresponding to treatment and control conditions over time was represented in non-metric multidimensional scaling (nMDS). Samples corresponding to the two conditions distributed distantly and those corresponding to the treatment at different sampling times were also separated, with each replicate grouped within its respective time (Supplementary Figure 5). Samples collected at 24 and 72 h after H_2O_2 addition were more similar than that recovered at 120 h. These differences were confirmed by two-way PERMANOVA for treatment vs. control ($p<0.0001$, $F = 26.562$) and treatment over the time, 24, 72, 120 h ($p<0.0001$, $F=4.516$) with the interaction between these factors ($p<0.03$) (Supplementary Table 2).

3.3.2. Bacterioplankton composition

The composition of bacterial communities changed over time after H_2O_2 treatment (Fig. 3, Supplementary Table 3). The original community was mainly composed of Actinobacteria (21%), Cyanobacteria (20%), Plantomycetes (15%) and Proteobacteria (11%), followed by Verrucomicrobia (9%), Chloroflexi (8%) and Bacteroidetes (7%). Very few changes were observed in the control over time. An increase of Cyanobacteria relative abundance occurred at 24 h (25%) and 72 h (27%), and then returning at 120 h to a value similar to the initial abundance. Actinobacteria relative abundance continuously decreased reaching 11% at 120 h, while Verrucomicrobia increased to 21% at the final time. Conversely, the composition of bacterioplankton in the treatment was completely modified. Samples collected at 24 and 72 h were more similar, showing a notable expansion of Firmicutes from 0.2% at time 0 to 40-50%. Other phyla that increased in abundance in these samples were Deinococcus (from 0.01% at time 0 to 7-11%) and Proteobacteria (from 11% at time 0 to 17-23%). In parallel, Actinobacteria, Verrucomicrobia, Planctomycetes and Chloroflexi decreased in abundance. At 120 h, bacterioplankton profile in H_2O_2 samples was dissimilar from time 0 and from the control at this time, including dominant phyla such as Bacteroidetes (19%), Verrucomicrobia (24%) as well as Proteobacteria (26%) (Fig. 3).

3.3.3. Effect of H_2O_2 on Cyanobacteria

The H_2O_2 treatment decreased the relative abundance of Cyanobacteria from 20% at T0 to about 1% at 24 and 72h and 7% at 120 h. The OTU composition within the cyanobacterial community was also affected by the treatment (Fig. 4). *Cyanobium, Planktothrix* and *Microcystis* were the dominant genera in the original cyanobacterial community accounting for 30, 27 and 16%, respectively (Fig. 4). In the control, they remained as the main genera until the end of the experiment. In the treated water, in which

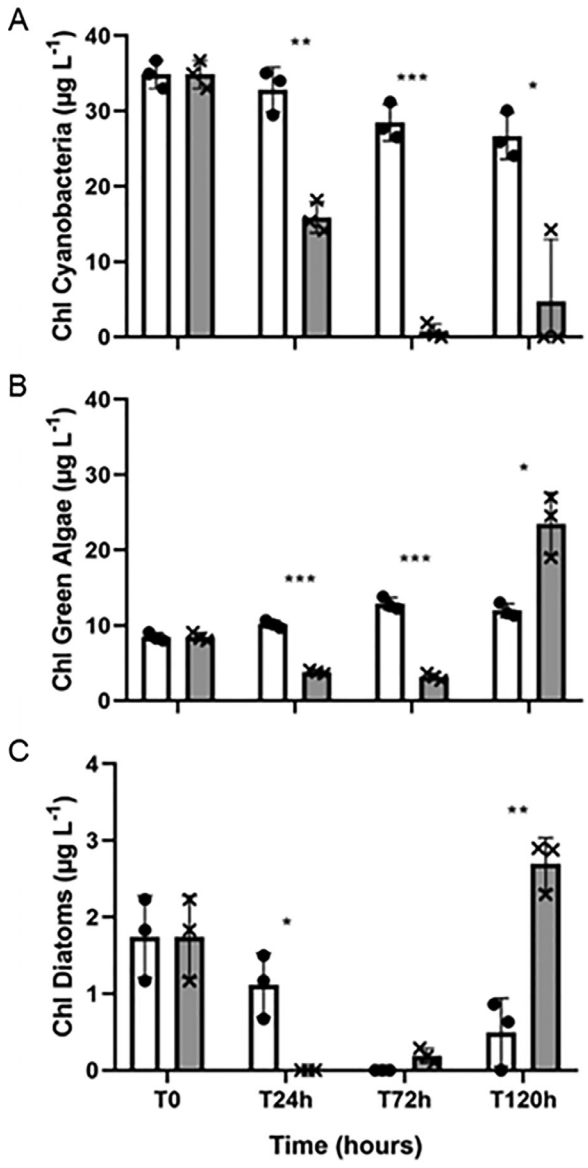

Fig. 2. Effects of 10 mg L^{-1} H_2O_2 on phytoplankton biomass by chlorophyll estimation for the groups (A) cyanobacteria, (B) green algae, and (C) diatom over 120 h. Results are expressed as the average ± SD. Grey and white bars represent treatment with H_2O_2 and control with no H_2O_2, respectively. Individual values of each replicate are represented by x for treatment and • for control (n=3). The asterisks represent statistical difference between treatment and control, with * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$).

a drastic decline in cyanobacterial abundance was observed, *Microcystis* was dominant accounting for 60% of all cyanobacterial OTUs at 24 h, while *Cyanobium* corresponded to 20%. From 72 h to 120 h, *Microcystis* abundance decreased (10 and 5%, respectively) while *Cyanobium* abundance increased (53 and 64%, respectively). *Planktothrix* abundance remained low after the treatment (4-8%). Thus, at the end of the experiment, when cyanobacterial abundance tended to increase, the community composition differed from the original one (Fig. 4).

3.3.4. Bacterial taxa distinguishing control and H2O2 treatment

To highlight the main bacterioplankton taxa associated with the differentiation between control and treatment, regardless of time, we performed LDA and LEfSe analysis using the relative abundances of OTUs as input. Considering the LDA score (log10) with a cut-off of 3.0, we selected 67 OTUs that contributed to the discrimination of treatment and control (Supplementary Figure 6). Among them, the most representative taxa in the control condition were an unclassified genus from *Chthoniobacteraceae* (4%) and *Terrimicrobium* sp. (3%) and the cyanobacterial genera *Planktothrix* (6-10%), *Microcystis* (3%), *Cyanobium* (3%) and *Raphidiopsis* (*Cylindrospermopsis*) (1%) (Fig. 5A-F). The dominant genera in the treatment condition were *Exiguobacterium, Deinococcus, Paracoccus* and *Rhizobium* (Fig. 5G-I). At 24 and 72 h, the relative abundance of *Exiguobacterium* reached 40-50%. *Paracoccus* corresponded to 4-9% and *Deinococcus* had an abundance of 10%, while their abundance was close to zero in the control condition or at time 0 (T0) (Fig. 5G-I). Other taxa predominant in the control condition were *Mycobacterium* and unclassified genera of *Solirubrobacterales, Caldilineaceae, Chloroflexaceae* and *Pirellulaceae* (Supplementary Figure 6). We also selected the main OTUs represented in the H_2O_2 treatment condition after 120 h to characterize the bacterioplankton composition after H_2O_2 degradation. According to the LEfSe analysis, the five most relevant were *Luteolibacter* sp. (~15%), *Exiguobacterium* sp. (~5%), *Comamonadaceae* unclassified (~3%), *Spirosomaceae* sp. (~3%) and *Prosthecobacter* sp. (~3%) (Supplementary Figure 7).

3.3.5. Correlation between bacterial groups and physical-chemical parameters

We selected the main OTUs that contributed to the difference between control and treatment at each sampling time according to SIMPER analysis (Supplementary table 4) and tested their correlation with limnological parameters (chlorophyll groups and abiotic factors) affected by the H_2O_2 treatment (Table 2). CCA ordination (Fig. 6) showed that all sampling times in the control condition grouped, including T0, with turbidity and chlorophyll of cyanobacteria as the main limnological parameters associated with this group. The high turbidity was positively correlated with four different cyanobacterial OTUs, *Cyanobium* (OTU16) (r=0.84), *Planktothrix* (OTU03) (r=0.81), *Microcystis* (OTU14) (r=0.79) and *Raphidiopsis* (*Cylindrospermopsis*) (OTU21) (r=0.76), and a heterotrophic uncultured *Chthoniobacteraceae* (OTU02) (r=0.75). Turbidity and chlorophyll of cyanobacteria had a positive correlation with some heterotrophic bacteria such as *Mycobacterium* (OTU06), *Terrimicrobium* (OTU09), a *Solirubrobacterales* member (OTU10) and a *Caldilineaceae* member (OTU28); and a negative correlation with transparency (Supplementary Table 5). As an early effect of H_2O_2 at 24 h, transparency increased and cyanobacterial chlorophyll decreased, and there was a significant positive correlation between transparency and *Exiguobacterium* (OTU01) (r=0.77), *Paracoccus* (OTU05) (r=0.8) and *Deinococcus* (OTU08) (r=0.79) (Fig. 6). A significant negative correlation was estimated between these three OTUs and chlorophyll of cyanobacteria (r= -0.71, -0.74 and -0.75, respectively) (Fig. 6 and Supplementary Table 5). At 72 h, transparency and turbidity were still significantly different between experimental conditions and pH, DOC and DO decreased in H_2O_2 samples compared to control (Table 2). In the treatment, *Exiguobacterium* (OTU01), *Paracoccus* (OTU05) and *Deinococcus* (OTU08) were still positively correlated with transparency (r=0.77, 0.80 and 0.79 respectively), and also an unclassified *Sphingobacteriaceae* (OTU25) (r=0.66) (Fig. 6). *Rhizobium* (OTU39) and *Flavobacterium* (OTU42) were positively correlated with transparency (r=0.66, 0.73 and 0.6, respectively) and negatively correlated to the turbidity (r= -0.65 and -0.74, respectively) and pH (r= -0.62 and -0.80, respectively). *Flavobacterium* (OTU42) (p=0.0001) also showed a negative correlation with DO (r= -0.75). No significant correlation was observed between any OTU and DOC (Fig. 6, Supplementary Table 5). At 120 h, transparency, turbidity, pH, conductivity and cyanobacterial chlorophyll differentiated control and treatment. A positive correlation was established between many OTUs and the higher conductivity and transparency in the treat-

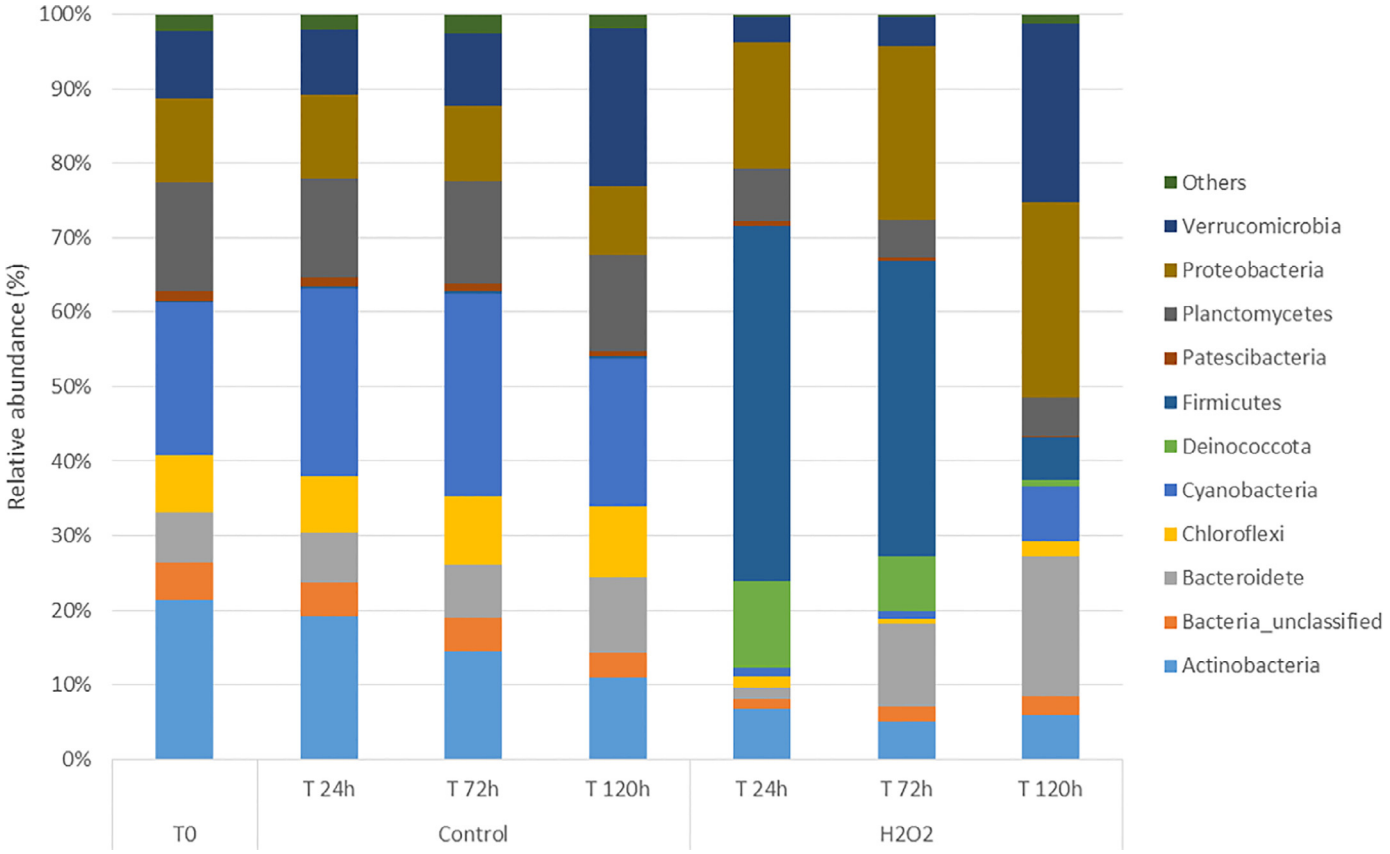

Fig. 3. Relative abundance of bacterial OTUs at phylum level at time 0 and in control or treatment (H_2O_2 addition) after 24, 72 and 120 h. Each bar plot represents the phyla average from triplicates samples (n=3) in each sampling time and condition.

ment: *Blastomonas* (OTU65) (conductivity: r=0.84; transparency: r=0.68), *Rhodobacter* (OTU81) (conductivity: r=0.75; transparency: r=0.80), *Lacibacter* (OTU160) (conductivity: r=0.80; transparency: r=0.75) and an unclassified *Spirosomaceae* (OTU37) (conductivity: r=0.82; transparency: r=0.72). The abundance of an unclassified *Sphingobacteriaceae* (OTU25) was related only to conductivity (r=0.77). OTUs negatively related to pH and associated with the treatment were *Spirosomaceae* (OTU37) (r= -0.74), *Rhizobium* (OTU39) (r= -0.62), *Flavobacterium* (OTU42) (r= -0.80), *Blastomonas* (OTU65) (r= -0.71), *Rhodobacter* (OTU81) (r= -0.63) and *Lacibacter* (OTU160) (r= -0.71) (Fig. 6, Supplementary Table 5). Although *Luteolibacter* (OTU17) (p=0.2), *Comamonadaceae* unclassified (OTU29) (p=0.6) and *Prosthecobacter* (OTU88) (p=0.2) were related to the treatment condition at 120 h, they did not present a significant correlation with abiotic factors (Supplementary Table 5). Moreover, green algae chlorophyll was positively correlated to *Cyanobium* sp. (OTU16) (r=0.62) that maintained its presence in the control condition and a slight increase in the treatment samples. *Prosthecobacter* sp. (OTU88) was positively related to diatom chlorophyll (r=0.74), whereas a *Comamonadaceae* unclassified (OTU29) was positively related to both factors (r=0.6 for green algae and 0.73 for diatom group).

4. Discussion

The studied reservoir is used for drinking water supply with a recent history of cyanobacteria proliferation, with dominance of *Planktothrix agardhii* and *Raphidiopsis raciborskii* (Barros et al., 2019). In this study, H_2O_2 was applied in mesocosm scale to mitigate a cyanobacterial bloom and to improve water quality parameters. Simultaneously, we investigated the effect of this treatment on the composition of the bacterioplankton community. A single concentration of 10 mg L^{-1} H_2O_2 was added and biotic and abiotic parameters were monitored over 120 h (five days). Studies reported that 5 mg L^{-1} of H_2O_2 would be enough to control cyanobacteria in a freshwater environment (Matthijs et al., 2012; Weenink et al., 2015). The concentration used here (10 mg L^{-1}) was based on a previous experiment performed in microcosm scale using water from Gavião reservoir, considering its hypereutrophic state and high organic carbon content. This concentration suppressed cyanobacteria with less effect on eukaryotic phytoplankton (Rocha, 2020). Although cyanobacteria is selectively affected by H_2O_2 compared to other phytoplankton, attention must be paid not to harm other organisms such as zooplankton (Reichwaldt et al., 2012; Spoof et al., 2020). Spoof *et al.* showed the vulnerability of different classes of zooplankton from Lake Köyliönjärvi (Finland) after exposure to 10 and 20 mg L^{-1} H_2O_2. Zooplankton sensitivity can vary depending on the species and this issue should be evaluated in case of in situ application of H_2O_2 for cyanobacterial control.

The efficiency of H_2O_2 for cyanobacterial suppression *in situ* depends on its degradation time, which is affected by organic carbon concentration and bacterioplankton density and composition (Weenink et al., 2015). Fan et al. (2019) using water samples from Lake Taihu found that 10 mg L^{-1} effectively inhibited the growth of cyanobacteria over 15 days leading to the dominance of eukaryotic green algae but with no information regarding H_2O_2 degradation time. Curiously, in Lake Taihu DOC concentrations vary from 2 to 8 mg L^{-1} according to site or season, two or three times less than DOC in Gavião reservoir (Ye et al., 2011; 2015; Huang et al., 2017). Even so, in Gavião reservoir, 10 mg L^{-1} of H_2O_2 suppressed cyanobacteria over 120 h, a reduced time compared with those

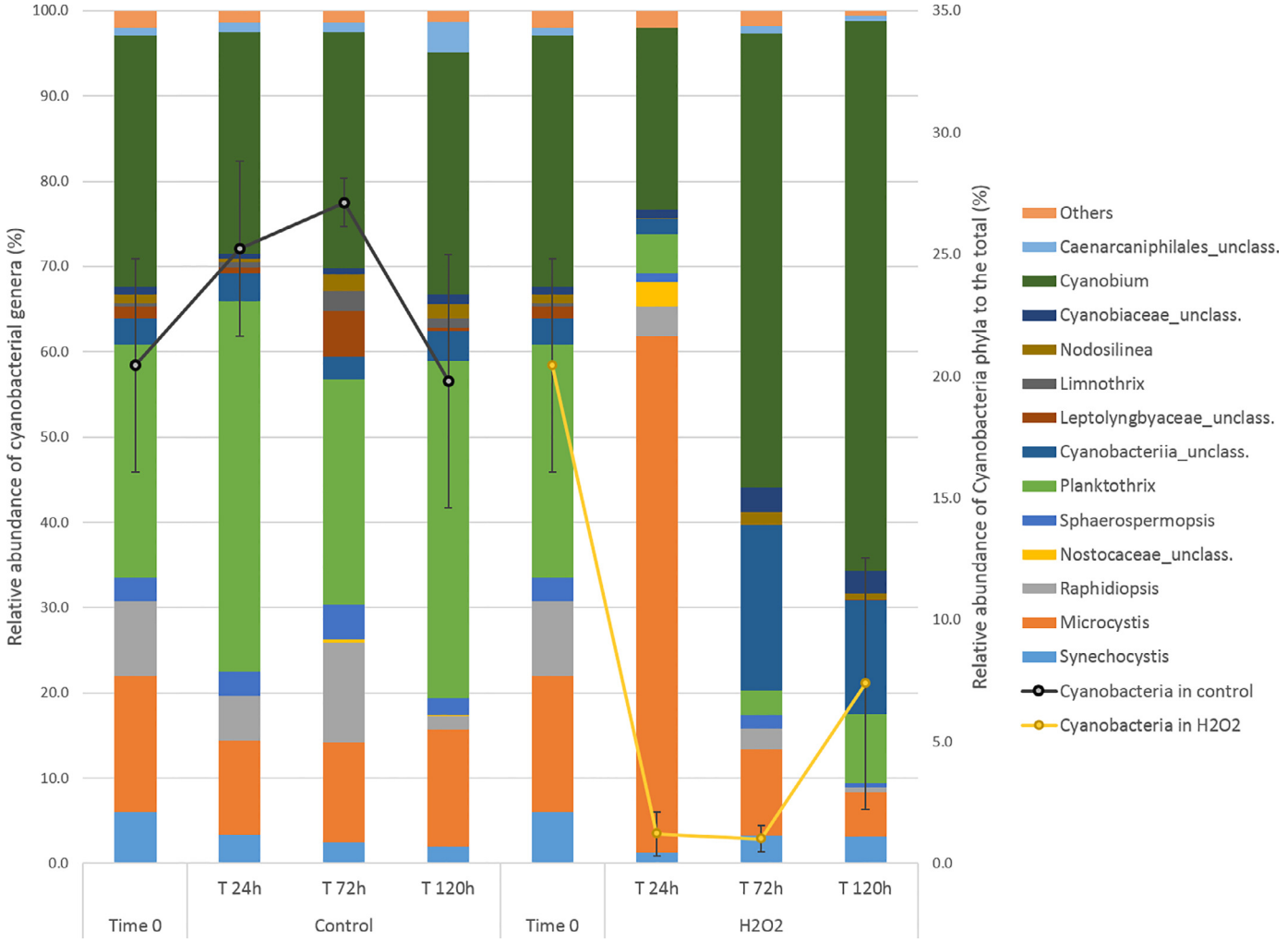

Fig. 4. Relative abundance on average for OTU-based Cyanobacteria genera (bar chart) and Cyanobacteria phyla (lines) (n=3). Black line means control condition and yellow line means treatment condition with respective standard deviation

studies that applied 10 mg L^{-1} or less in lower organic carbon concentration environments (Matthijs et al., 2012; Weenink et al., 2015; Fan et al., 2019). In our experiment H_2O_2 was not detected from 72 h on. After that, cyanobacteria remained less abundant in the treatment than the control, but a slight increase was observed at 120 h, according to chlorophyll and 16S rRNA sequencing analysis. DOC concentration decreased at 72 h in the treatment condition compared to the control, which could be associated to photodegradation or microbial activity (Ye et al., 2015; Huang et al., 2017), although no bacterial OTU was correlated to this decrease.

Apart from cyanobacteria, eukaryotic phytoplankton was also initially suppressed by H_2O_2 treatment, but green algae dominated after H_2O_2 degradation. This evidence a higher sensitivity of cyanobacteria to the treatment or a higher recovery/adaptability of green algae (Barrington et al., 2013; Yang et al., 2018; Lusty and Gobler, 2020). Sinhá et al. (2018) observed that a decrease of cyanobacteria biomass was followed by an increase in the abundance of eukaryotic diatom *Synedra* sp. and green algae *Cladophora* sp., suggesting that these species benefited from the collapse of cyanobacteria and utilized the available nutrients. In another study where a *Microcystis aeruginosa* bloom was suppressed with H_2O_2 application, growth of Chlorophyte (*Chlamydomonas* spp.) was promoted (Wang et al., 2019). Although the composition of the eukaryotic phytoplankton was not evaluated in our experiment, *Aulacoseira* sp. and *Fragilaria* sp. were identified as the main diatom species in the raw water and the cyanobacteria *Planktothrix* and *Raphidiopsis* were present at the time of the study (Cagece Internal Reports, February 2019).

Along with phytoplankton suppression, transparency increased after H_2O_2 application and was correlated to cyanobacterial chlorophyll over time, differently from other studies (Matthijs et al., 2012; Sinhá et al., 2018). Thus, in our case, the use of high H_2O_2 concentrations improved water quality parameters. DO concentrations decreased at 72 h, which could be related to the collapse of cyanobacteria and reduced photosynthetic activity leading to lower oxygen production (Crafton et al., 2019). Other studies in freshwater environments suggested that increases in pH and DO can be attributed to cyanobacterial growth in massive blooms and high nutrient levels (Paerl et al., 2001; Gao et al., 2012).

H_2O_2-based treatment has been described to trigger re-oligotrophication, leading to an alternative equilibrium in the environment (Matthijs et al., 2012), but in our case, no changes were observed in nutrient concentration as a result of H_2O_2 addition. Many studies have observed an increase in nutrient availability after H_2O_2 treatment with an increase in phosphate and ammonia that could be related to microbial cell lysis (Matthijs et al., 2012; Bauzá et al., 2014; Sinhá et al., 2018). The maintenance or increase in nutrient availability would favor cyanobacterial regrowth over time (Paerl and Otten, 2013; Huisman et al., 2018). Furthermore, changes in nutrient concentrations could be associated with the

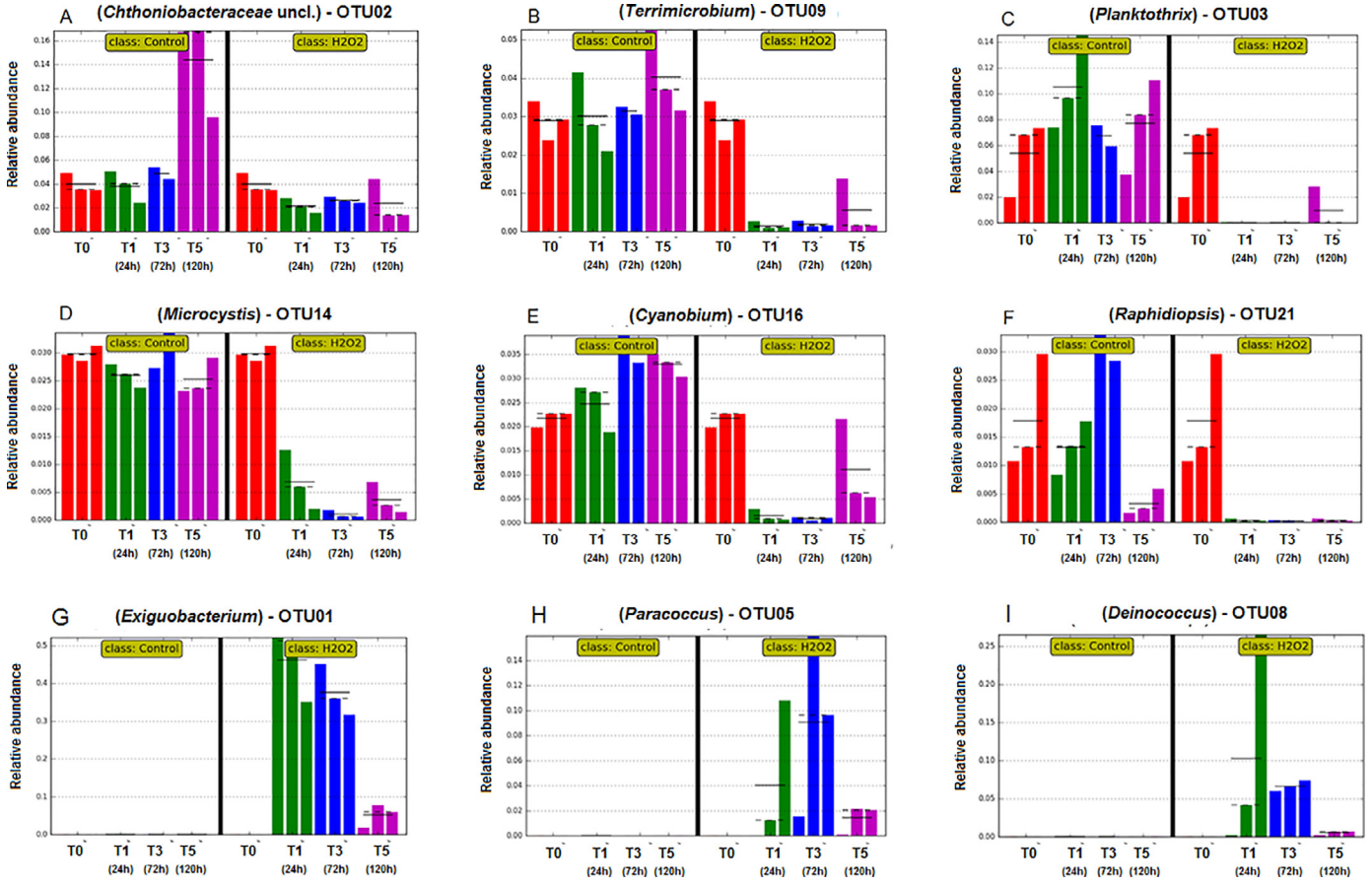

Fig. 5. Relative abundance of the main genera of heterotrophic bacteria and cyanobacteria in the control or treatment condition over time according to the LefSE analysis (p value <0.05 and a LDA score (log10) >3.0). In the control condition, the main OTUs corresponded to the heterotrophic bacteria *Chthoniobacteraceae unclassified* (A) and *Terrimicrobium* (B) and to the cyanobacteria *Planktothrix* (C), *Microcystis* (D), *Cyanobium* (E) and *Raphidiopsis* (*Cylindrospermopsis*) (F). In the treatment condition, the main OTUs corresponded to *Exiguobacterium* (G), *Paracoccus* (H) and *Deinococcus* (I).

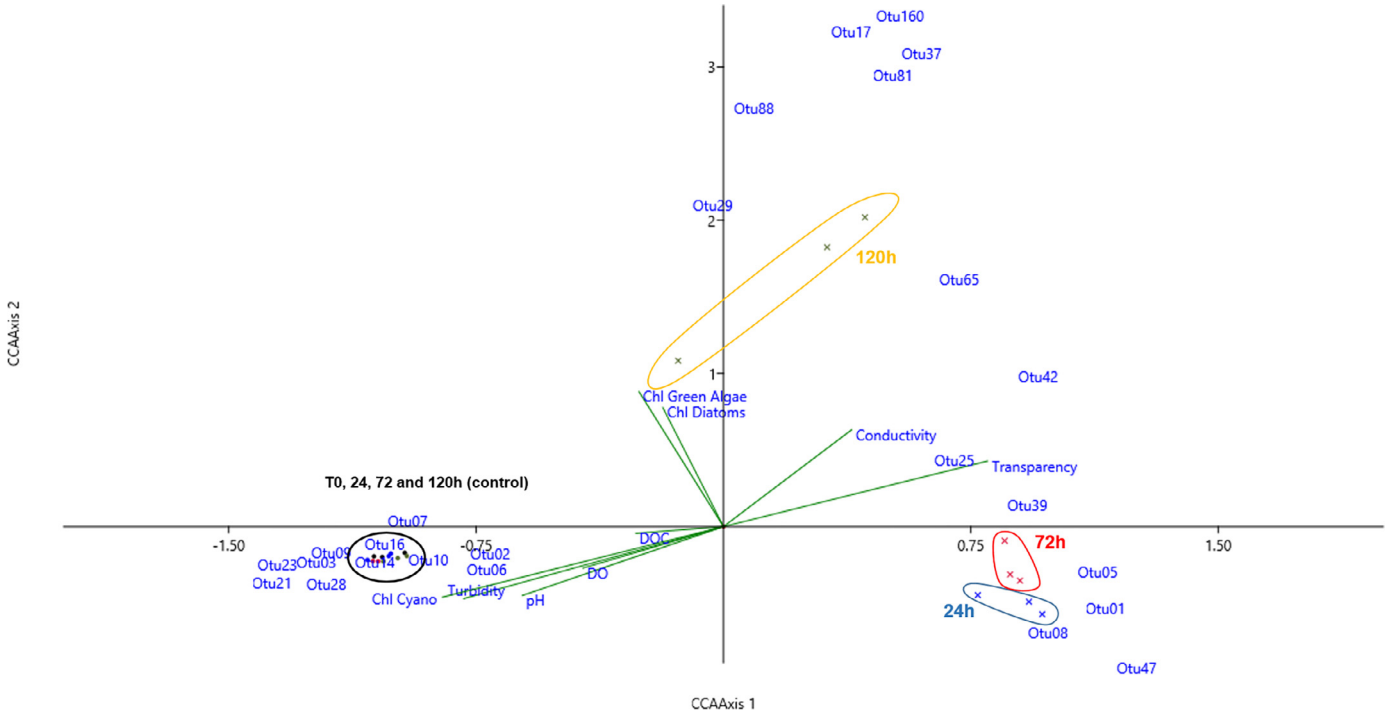

Fig. 6. Canonical correspondence analysis (CCA) considering the main OTUs that contributed to the differentiation between control and treatment at each sampling time (by SIMPER) and physical chemical parameters significantly different at each time.

species composition of the cyanobacterial bloom, the redox potential of the water body, and the growth of the associated microbial community. After H_2O_2 degradation, an increase in cyanobacterial biomass and in the relative abundance of sequences was observed at 120 h, suggesting regrowth with a different community composition from the original one. *Cyanobium* was the main genus dominating the cyanobacterial community in the treated mesocosms but sequences from *Planktothrix* and *Microcystis* were also detected. Picoplankton cyanobacteria like *Cyanobium* (0.2 – 2 µm) are abundant but underestimated in freshwater systems either in oligo-mesotrophic or hypereutrophic conditions (Callieri, 2008; Gaysina et al., 2019, Cabello-Yeves et al., 2017; Guedes et al., 2018). Representative species of picocyanobacteria can reach a growth rate of 0.5 d^{-1} in oligotrophic marine environments (Cruz and Neuer, 2019). In our experiment, as the cyanobacterial bloom was suppressed by H_2O_2, *Cyanobium* with its relatively faster growth rate may have benefited from the alleviation of competition and the availability of nutrients (Callieri, 2010; Leblanc et al., 2018). Thus, long-term microscopy analysis, chlorophyll measurements and molecular tools would be important to monitor the development of the remaining cyanobacterial community and optimize mitigation strategies.

Besides Cyanobacteria, the abundance of other phyla decreased after the H_2O_2 treatment. This was the case of Actinobacteria, Verrucomicrobia, Planctomycetes and Chloroflexi, indicating that members of these phyla were also sensitive to the oxidative process. Conversely, some bacterial taxa were resistant to the H_2O_2 treatment including Firmicutes, Proteobacteria and Deinococci members. *Paracoccus* and *Deinococcus* contributed in similar proportions to the bacterial communities of treated samples (24 and 72 h). Members from both genera are known to produce enzymes involved in bacterial defense against the deleterious effects of oxidative stress (Wang and Schellhorn, 1995; Baker et al., 1998). *Deinococcus* is known for its capacity to survive in diverse extreme environmental conditions such as ionization, UV radiation and desiccation (Daly, 2009). Ionization and UV radiation can mediate and intensify the production of ROS. *Deinococcus radiodurans* cells have high catalase activity and increase it when exposed to H_2O_2. When pre-treated with sub-lethal doses of H_2O_2 cells became resistant to higher concentrations of H_2O_2 and also more resistant to UV light and γ rays, showing the potential to enhance survival to diverse stress factors (Wang and Schellhorn, 1995; Slade and Radman, 2011).

The main genus in the treatment condition was *Exiguobacterium* and its cosmopolitan distribution may be related to an adaptability to extreme environmental conditions including high UV intensity (Strahsburger et al., 2018). An inherent characteristic of this genus is the intense activity of catalase (Yumoto et al., 2004; Takebe et al., 2007). Strains from this genus present the complete pathway for carotenoid C30 synthesis (White et al., 2019), which might contribute to ROS quenching as well as protect against photo-damage. *Exiguobacterium* showed a positive correlation with transparency, a consequence of the collapse of cyanobacteria. Tian et al. (2012) showed that an extract of *Exiguobacterium* sp. had an intense algicidal activity against cyanobacteria inhibiting the growth of *Planktothrix, Synechococcus* and *Chroococcus* in 2 days, without activity against green algae. After H_2O_2 degradation, *Exiguobacterium* abundance decreased and *Cyanobium* dominated the cyanobacterial community. A major contributor at this later time was *Luteolibacter* that increased in abundance compared to the control condition. A correlation between this genus and picocyanobacteria has been previously reported during a *Synechococcus*-dominated cyanobacterial bloom (Guedes et al., 2018). Additionally, Cardman et al. (2014) suggested that members of Verrucomicrobia, including *Luteolibacter* sp. could be involved in algal polysaccharide degradation in marine environments. These results indicate that further investigation using metagenomics approaches can confirm the prevalence of specific bacterial taxa after the oxidizing process and subsequent compositional changes that precede cyanobacterial regrowth. In this scenario, it is important to know resistant bacteria that dominate the bacterioplankton after an oxidizing treatment for water treatment purposes since they could imply risks for water consumption. Additionally, understanding the dynamics of microbial communities after H_2O_2 treatment could reveal bacterial taxa potentially useful in biomonitoring conditions before the return of cyanobacterial blooms.

We did not evaluate the potential effects of H_2O_2 on cyanobacterial toxins since none of the routinely monitored cyanotoxins (microcystin, saxitoxin, and cylindrospermopsin) were detected before and throughout the experimental procedure (Cagece Internal Reports, 2019, Document no.5, annex 20 Health Ministry-Brazil – Brazil, 2017). Although the efficiency of H_2O_2 to eliminate microcystin has been observed both *in situ* (Matthijs et al., 2012) and in cyanobacterial cultures (Huo et al., 2015; Menezes et al., 2020), other studies reported toxin remaining (Lürling et al., 2014; Spoof et al., 2020). In case the toxins released over H_2O_2 process and not chemically or biologically degraded are still present at the WTP intake, then other steps could be adopted at WTP for complete removal such as activated carbon adsorption or chemical oxidation. The advantage of the H_2O_2 treatment is that it does not need new infrastructure added to the WTP and can be applied to a small part of the reservoir (close to the intake) only when raw water quality worsens to a certain threshold.

5. Conclusion

We evaluated the effects of H_2O_2 on phytoplankton, bacterioplankton and abiotic parameters using a mesocosm system into a hypereutrophic reservoir located in a semi-arid region, subjected to high irradiance, high temperature and presenting a high carbon content. Along with improving water quality, a single application of 10 mg L^{-1} H_2O_2 suppressed the phytoplankton community over 72h, with a subsequent dominance of green algae after H_2O_2 degradation. The original *Planktothrix*-dominated cyanobacterial bloom was suppressed and an increase of *Cyanobium* sp. was apparent. This suggests regrowth of cyanobacteria, linked to the availability of nutrients. The heterotrophic bacterial community composition changed over time and was dominated by taxa that presented resistance to H_2O_2, particularly *Exiguobacterium*. Thus, H_2O_2 imposes a stress condition upon the bacterioplankton with strong compositional changes in the relative abundances of diverse phyla. Longer observation periods are needed to determine the time of the return of a cyanobacterial bloom after H_2O_2 degradation. Our results suggest that monitoring abiotic parameters and the relative abundance of specific taxa could be used to anticipate the regrowth of cyanobacteria. An important aspect to observe for the success of this process is where to apply H_2O_2 so that its effects are still felt in the WTP intake. For that, in addition to the knowledge of for how long the water quality improvement lasts, a study of the reservoir hydrodynamics (residence time, preferential pathways) is needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117069.

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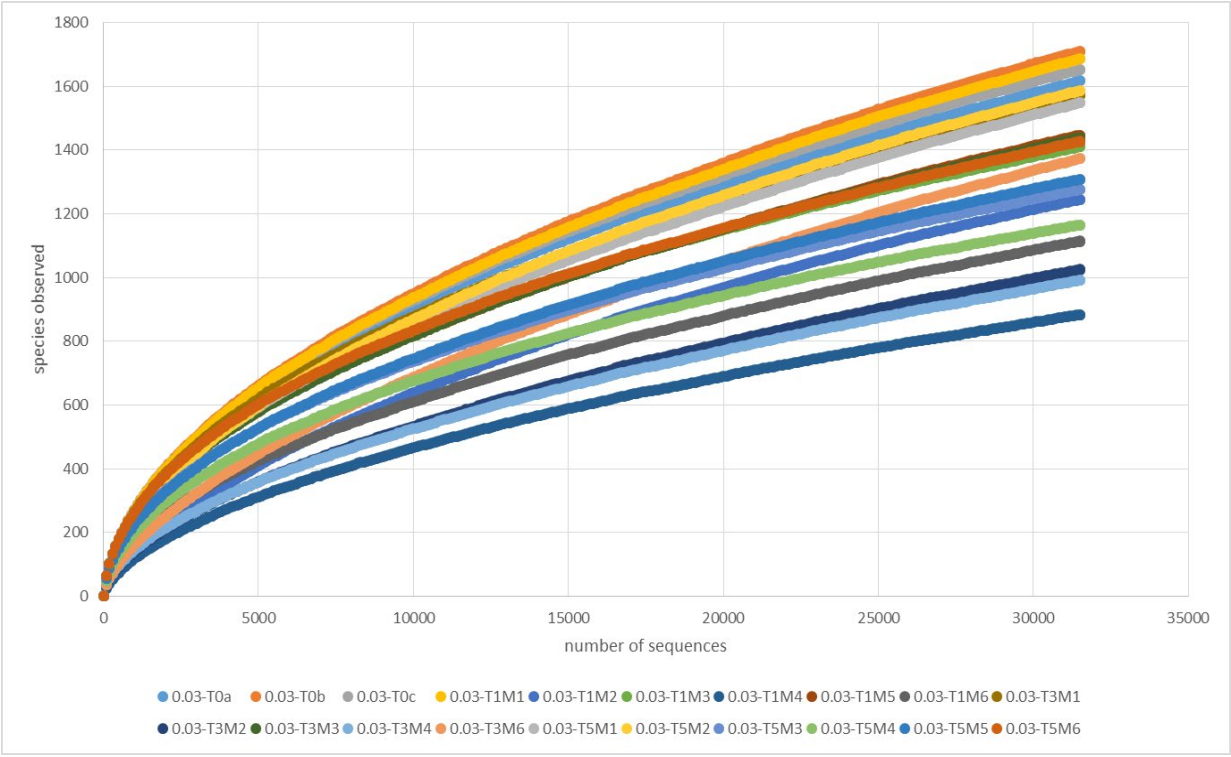
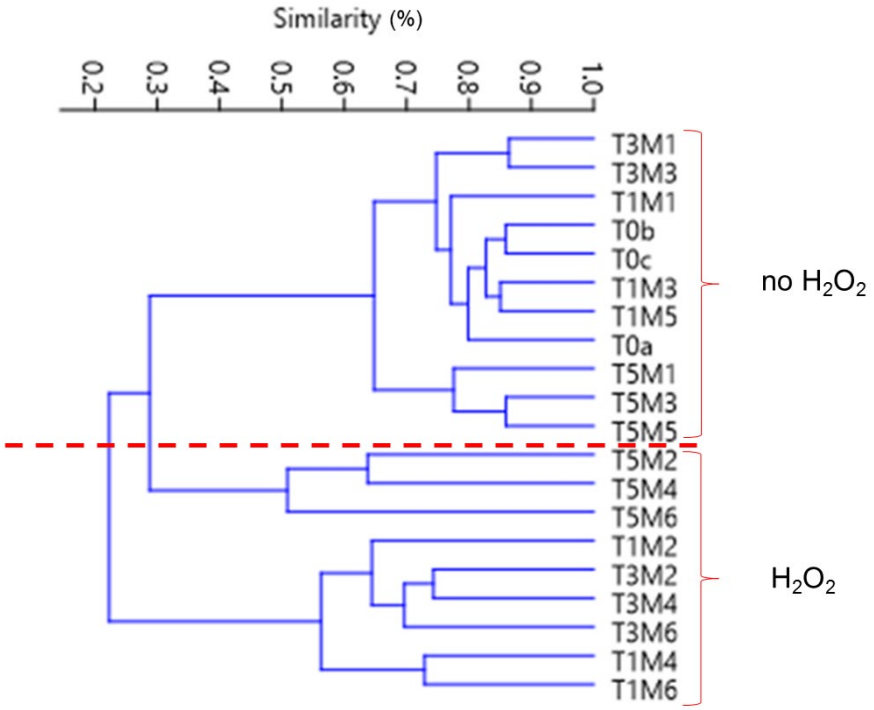
Supplementary Material

Supplementary Figures

Supplementary Figure 1: Design of experimental platform, using illustration of individual mesocosms (A), the floating platform in the top view (B) and real photographs of structure assembled in the Gavião reservoir (C, D and E). The mesocosms were kept floating by PVC-pipes attached to a floating platform made of a wood deck supported by six empty plastic barrels to ensure stability and allow water sampling.

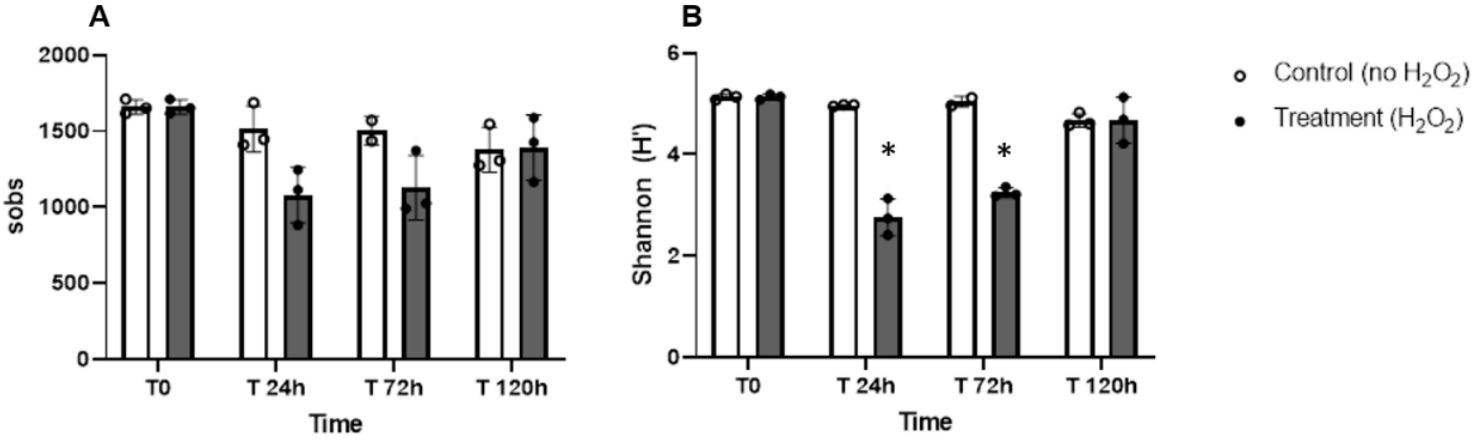


Supplementary Figure 2: Rarefaction curves relating observed species with the number of sequences for all samples after normalization.



15

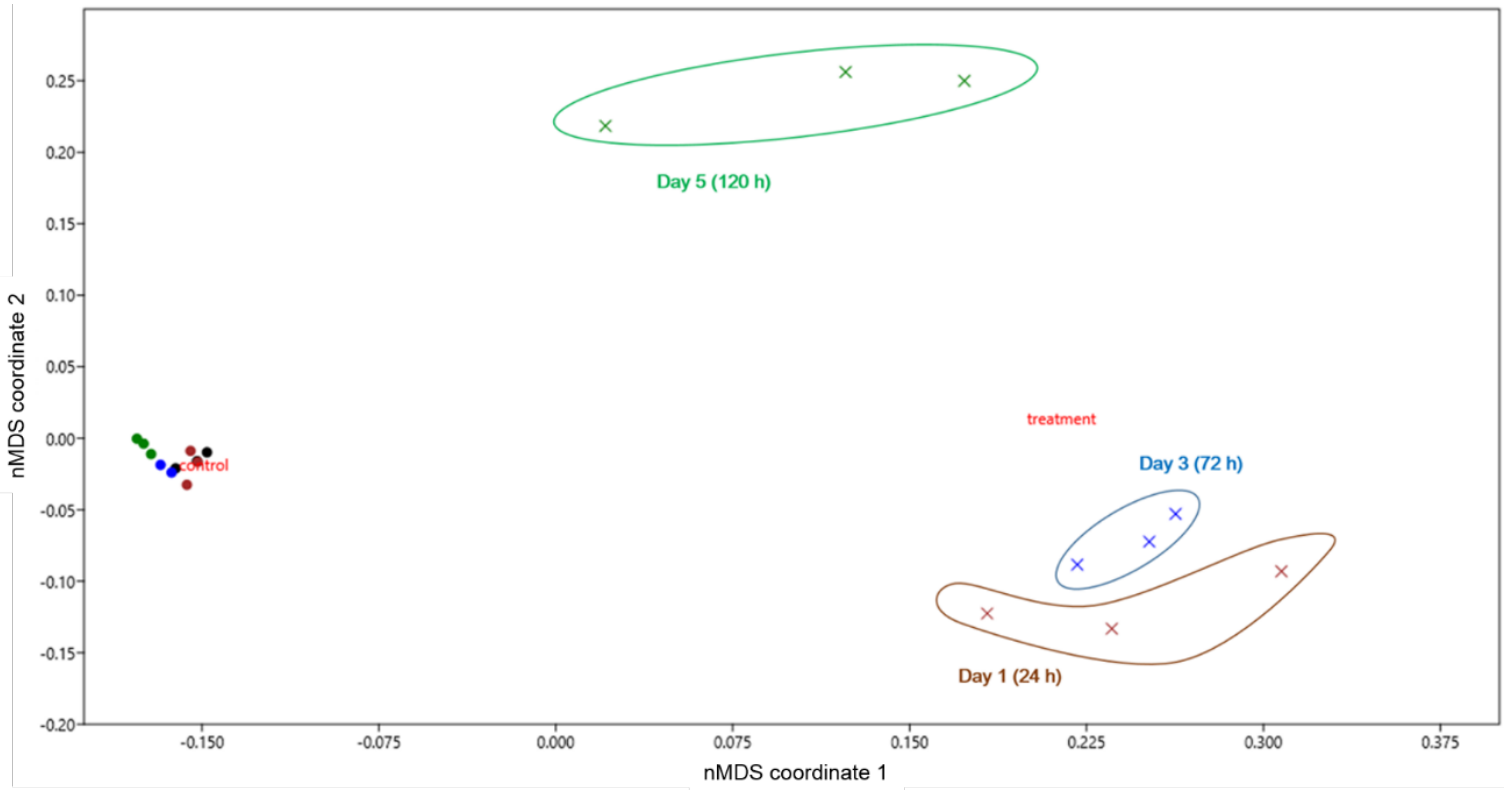
16 Supplementary Figure 3: Hierarchical clustering of samples considering a matrix using
17 Bray-Curtis distance. The dashed red line separates treatment and control groups.



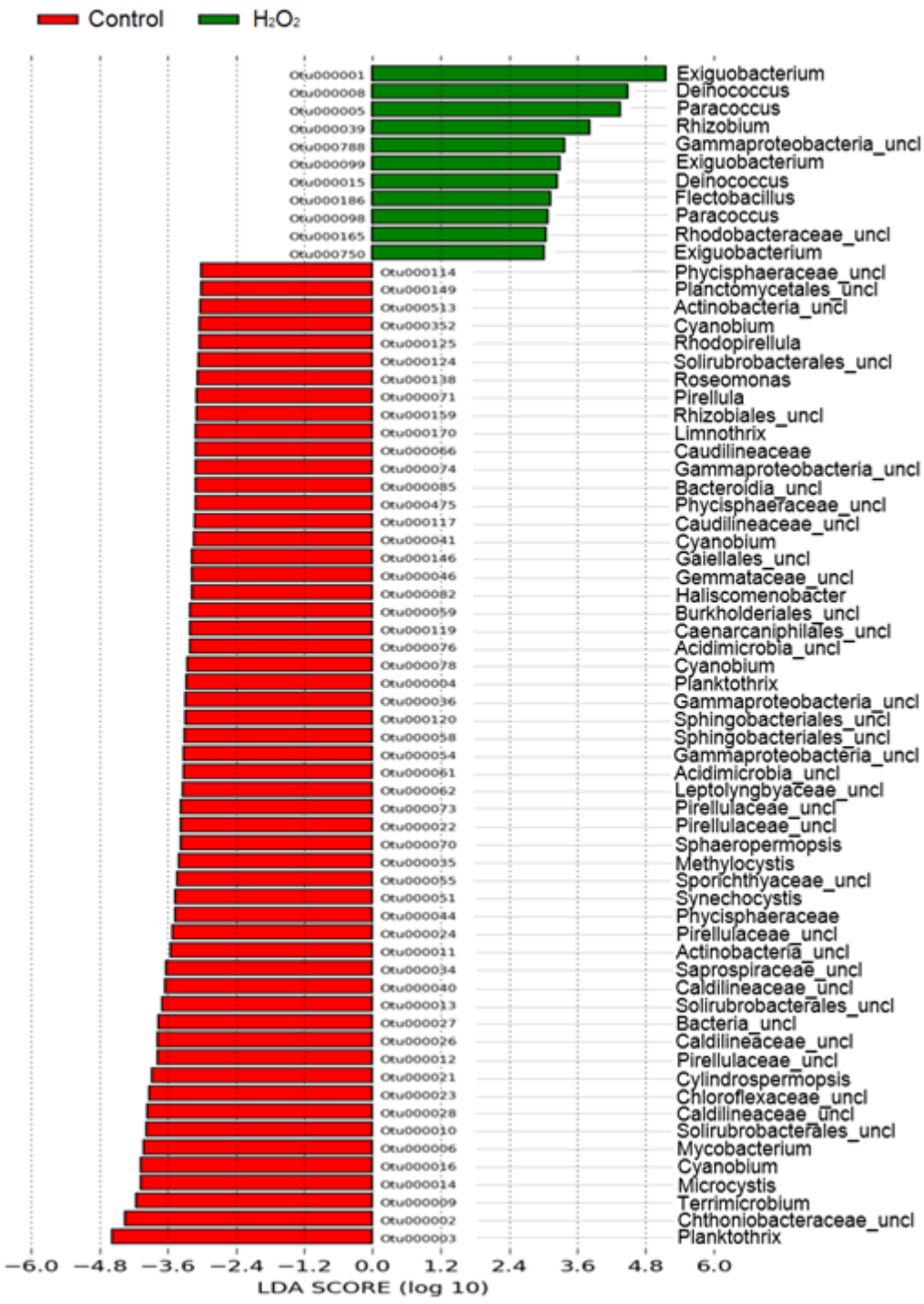
18 Supplementary Figure 4: Alpha diversity considering sobs richness (A) and Shannon
19 diversity (B) of samples from each condition (treatment x control) over the time. Grey
20 and white bars represent the treatment and control conditions, respectively. Asterisks
21 mean significant differences between control and treatment at each sampling time
22 ($p<0.05$) considering mixed-effects model ANOVA ($p<0.05$) using Sidak's as *post hoc*
23 test.

24 Supplementary Figure 5: Ordination of samples according to the bacterial community
25 composition by non-metric multidimensional scaling (nMDS) using Bray-Curtis

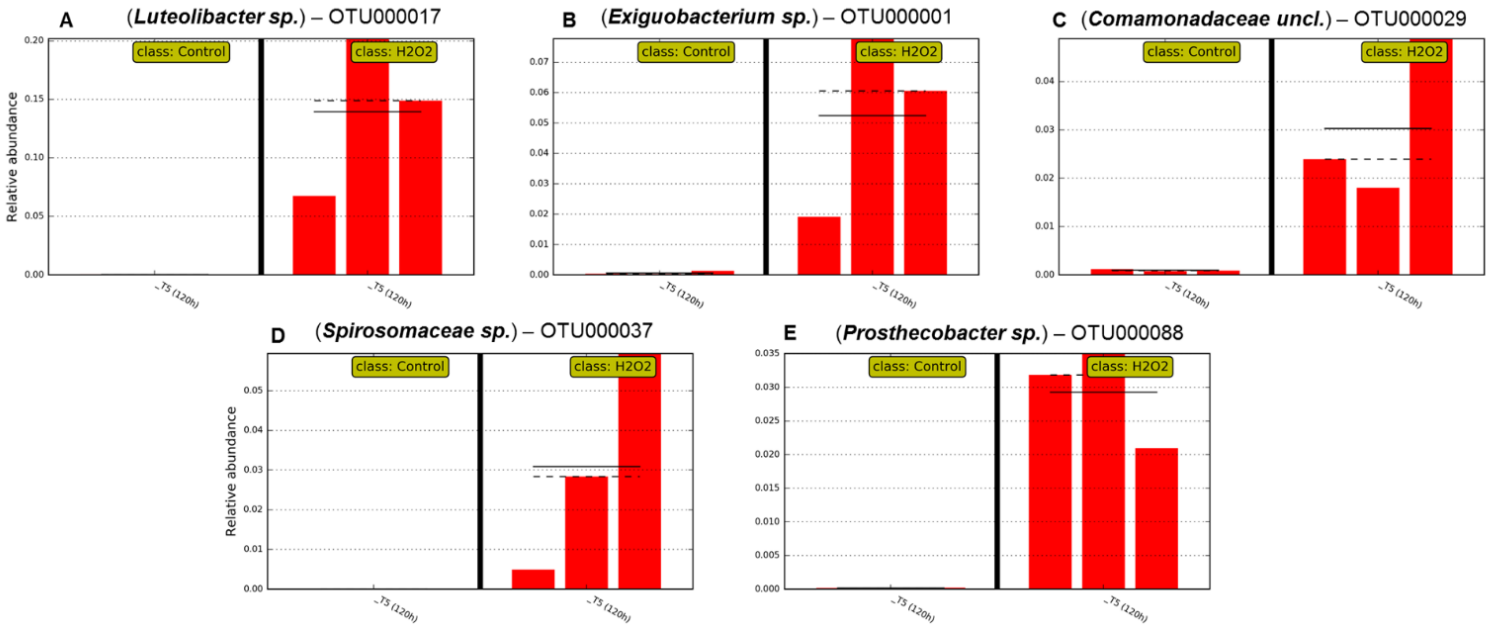
distance. Samples correspond to control or H_2O_2 treatment collected over the time, 24,



72 and 120 h (n=3).



Supplementary Figure 6: Linear discriminant analysis coupled to effect size (LEfSe) of bacterial abundance in treatment and control. Taxa with significant difference distribution between treatment (H2O2) and control (no H2O2) groups were selected using a p value <0.05 and a LDA score (log10) >3.0.



Supplementary Figure 7: Relative abundance of the top five bacteria in treatment and control condition at 120h sampling time according to LEfSe analysis, where A) *Luteolibacter* sp., B) *Exiguobacterium* sp., C) *Comamonadaceae* unclassified., D) *Spirosomaceae* sp. and E) *Prosthecobacter* sp.

52 **Supplementary Tables**

53 Supplementary Table 1: Sample identification, sequencing data and diversity indexes

Sample	Description	DNA concentration (ng/uL)	Number of sequences		Coverage	Richness	Diversity	Evenness
			Before trim qual	After trim qual		Sobs	Shannon (H')	ShannonEven
T0a	Time 0	10.8	103444	32221	0.975	1617	5.08	0.688
T0b	Time 0	13.106	50162	32221	0.973	1711	5.14	0.691
T0c	Time 0	7.762	84658	32221	0.974	1653	5.19	0.700
T1M1	No H_2O_2 (T 24h)	24.855	220482	32221	0.973	1687	4.98	0.671
T1M2	H_2O_2 (T 24h)	21.904	65561	32221	0.979	1245	2.74	0.385
T1M3	No H_2O_2 (T 24h)	15.593	96268	32221	0.980	1410	4.98	0.687
T1M4	H_2O_2 (T 24h)	17.373	89357	32221	0.985	883	2.41	0.356
T1M5	No H_2O_2 (T 24h)	12.076	266828	32221	0.977	1447	4.95	0.681
T1M6	H_2O_2 (T 24h)	12.487	134237	32221	0.982	1114	3.13	0.446
T3M1	No H_2O_2 (T 72h)	26.633	100340	32221	0.975	1571	5.12	0.696
T3M2	H_2O_2 (T 72h)	8.088	149253	32221	0.982	1025	3.36	0.485
T3M3	No H_2O_2 (T 72h)	23.73	38774	32221	0.978	1438	4.97	0.683
T3M4	H_2O_2 (T 72h)	5.693	363589	32221	0.982	991	3.21	0.466
T3M6	H_2O_2 (T 72h)	8.584	98866	32221	0.975	1373	3.18	0.440
T5M1	No H_2O_2 (T 120h)	20.208	86240	32221	0.974	1548	4.83	0.658
T5M2	H_2O_2 (T 120h)	23.88	155713	32221	0.975	1587	4.69	0.636

T5M3	No H_2O_2 (T 120h)	22.109	102664	32221	0.981	1276	4.58	0.641
T5M4	H_2O_2 (T 120h)	17.982	84591	32221	0.983	1165	4.22	0.598
T5M5	No H_2O_2 (T 120h)	26.747	100176	32221	0.980	1308	4.61	0.642
T5M6	H_2O_2 (T 120h)	18.224	165810	32221	0.978	1427	5.13	0.707

Supplementary Table 2: Analysis of two way PERMANOVA considering condition (control or treatment) and time (0, 24, 72 and 120h) as groups and using Bray-Curtis matrix distance of similarity and $p<0.05$ for hypothesis test.

Two-way PERMANOVA

Permutation N: 9999					
Source	Sum of square roots	df	Mean square	F	P
Condition	1.9493	1	1.9493	26.562	0.0001
Time	0.99428	3	0.33143	4.5162	0.0001
Interaction	-0.036183	3	-0.012061	-0.16435	0.037
Residual	0.88064	12	0.073386		
Total	3.788	19			

Supplementary Table 3: Relative abundance (%) of bacterial phyla in control and treatment samples over the time 0 (A), 24h (B), 72 (C) and 120h (D). Average values of replicates are also shown. (n=3).

(A)

	Time 0a	Time 0b	Time 0c	**average**
Actinobacteria	20.3	21.2	22.2	21.2
Bacteria_unclassified	4.4	5.3	5.6	5.1
Bacteroidetes	7.0	6.5	6.8	6.8
Chloroflexi	7.6	7.5	7.9	7.7
Cyanobacteria	24.2	21.6	15.6	20.4
Deinococcota	0.0	0.0	0.0	0.015

Firmicutes	0.1	0.2	0.3	0.2
Patescibacteria	1.0	1.5	1.5	1.3
Planctomycetes	13.7	14.8	15.2	14.6
Proteobacteria	10.7	11.3	11.6	11.2
Verrucomicrobia	8.5	7.9	10.6	9.0
Others	2.4	2.2	2.4	2.3

61

62 (B)

	No H2O2 (M1)	No H2O2 (M3)	No H2O2 (M5)	H2O2 (M2)	H2O2 (M4)	H2O2 (M6)	**avg no H2O2**	**avg H2O2**
Actinobacteria	17.2	20.0	20.1	4.0	5.4	10.8	19.1	6.7
Bacteria_unclassified	4.9	4.2	4.6	0.8	0.9	2.3	4.6	1.3
Bacteroidete	6.4	7.0	6.8	1.2	1.0	2.5	6.7	1.6
Chloroflexi	9.6	6.9	5.9	0.9	1.1	2.4	7.5	1.5
Cyanobacteria	29.1	24.5	22.0	0.5	0.9	2.2	25.2	1.2
Deinococcota	0.0	0.1	0.0	29.7	4.5	0.2	0.1	11.5
Firmicutes	0.2	0.5	0.1	37.1	52.5	53.7	0.3	47.7
Patescibacteria	1.0	1.2	1.3	0.3	0.7	1.1	1.2	0.7
Planctomycetes	12.1	13.2	14.5	4.0	5.7	11.5	13.3	7.1
Proteobacteria	10.8	11.4	11.6	19.0	24.0	7.6	11.3	16.9
Verrucomicrobia	6.3	8.6	11.0	2.3	3.0	4.6	8.6	3.3
Others	2.3	2.1	2.0	0.2	0.2	0.9	2.1	0.5

63

64 (C)

	No H2O2 (M1)	No H2O2 (M3)	H2O2 (M2)	H2O2 (M4)	H2O2 (M6)	**avg no H2O2**	**avg H2O2**
Actinobacteria	13.9	15.1	4.8	5.2	5.3	14.5	5.1
Bacteria_unclassified	4.8	4.1	1.6	2.1	2.0	4.4	1.9
Bacteroidete	7.7	6.6	14.8	6.5	11.9	7.2	11.1
Chloroflexi	9.0	9.2	0.7	0.7	0.7	9.1	0.7
Cyanobacteria	26.4	27.8	0.7	0.7	1.6	27.1	1.0

Deinococcota	0.1	0.0	8.0	7.6	7.0	0.0	7.5
Firmicutes	0.2	0.4	32.4	37.6	48.9	0.3	39.6
Patescibacteria	1.1	0.9	0.3	0.4	0.4	1.0	0.4
Planctomycetes	14.3	13.4	4.5	5.3	5.7	13.8	5.2
Proteobacteria	10.5	9.5	28.4	29.9	11.4	10.0	23.2
Verrucomicrobia	9.2	10.2	3.6	3.8	4.6	9.7	4.0
Others	2.6	2.6	0.2	0.3	0.4	2.6	0.3

D)

	No H2O2 (M1)	No H2O2 (M3)	No H2O2 (M5)	H2O2 (M2)	H2O2 (M4)	H2O2 (M6)	**avg no H2O2**	**avg H2O2**
Actinobacteria	12.0	10.6	10.2	6.4	3.5	8.0	10.9	6.
Bacteria_unclassified	3.5	3.5	3.2	2.5	1.9	2.9	3.4	2.
Bacteroidetes	8.1	9.2	12.6	15.5	23.5	17.4	10.0	18.
Chloroflexi	9.6	9.2	10.0	2.6	1.1	2.4	9.6	2.
Cyanobacteria	24.8	20.1	14.4	4.6	4.2	13.3	19.8	7.
Deinococcota	0.1	0.0	0.0	0.8	1.4	0.2	0.0	0.
Firmicutes	0.4	0.2	0.1	6.8	8.3	2.2	0.3	5.
Patescibacteria	0.8	0.8	0.4	0.1	0.1	0.2	0.7	0.
Planctomycetes	13.5	11.8	13.4	4.3	2.9	8.3	12.9	5.
Proteobacteria	9.9	9.5	8.7	30.9	24.0	23.6	9.4	26.
Verrucomicrobia	15.0	23.2	24.9	23.7	28.5	20.1	21.0	24.
Others	2.2	1.7	1.9	1.7	0.5	1.4	1.9	1.

Supplementary Table 4: Main OTUs that contributed to the difference between control and treatment at each time: 24h (a), 72h (b) and 120 (c) according to SIMPER analysis. OTUs were selected considering a contribution above 1% or a cumulative contribution of 50%

(A)

24h (treatment vs control)
Overall dissimilarity: 75.04

Taxon	Av. dissim	Contrib. %	Cumulative %	Mean control	Mean h2o2
Otu01	23.02	30.67	30.67	40	1.45E+04
Otu03	5.239	6.982	37.66	3,32E+03	14
Otu08	5.121	6.824	44.48	14,3	3.24E+03
Otu47	3.191	4.252	48.73	47	2.04E+03
Otu05	2.006	2.673	51.4	6	1.27E+03
Otu09	1.428	1.903	53.31	950	49.7
Otu07	1.168	1.557	54.86	881	145
Otu16	1.159	1.544	56.41	779	48.7
Otu10	0.9891	1.318	57.73	946	323
Otu06	0.9642	1.285	59.01	1,08E+03	469
Otu14	0.9547	1.272	60.28	818	216
Otu28	0.9193	1.225	61.51	633	53.3
Otu02	0.8747	1.166	62.68	1.20E+03	680

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74 (B)

72h (treatment vs control)					
Overall dissimilarity: 81.62					
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean control	Mean h2o2
Otu01	18.75	22.98	22.98	2.00E+01	1.18E+04
Otu05	4.522	5.541	28.52	3.50E+00	2.85E+03
Otu03	3.355	4.111	3.26E+01	2.12E+03	7.33
Otu08	3.317	4.064	36.69	1.25E+01	2.10E+03
Otu25	2.263	2.773	39.47	1.50E+00	1.43E+03
Otu16	1.757	2.152	41.62	1.14E+03	30
Otu21	1.523	1.866	4.35E+01	967	6.67
Otu09	1.478	1.81	45.29	993	61.3
Otu14	1.47	1,801	47.09	959	32

Otu23	1.368	1.675	48.77	863	0.667
Otu42	1.236	1.514	50.28	1.5	780
Otu39	1.158	1.418	51.7	1.5	731
Otu02	1.115	1.366	53.07	1.54E+03	835
Otu28	0.8843	1.083	54.15	587	29.7
Otu06	0.8404	1.03	5.52E+01	875	345

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76 (C)

120h (treatment vs control)					
Overall dissimilarity: 72.79					
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean control	Mean h2o2
Otu17	6.955	9.554	9.554	8.33	4.39E+03
Otu02	5.984	8.221	17.78	4.53E+03	756
Otu03	3.373	4.634	22.41	2.43E+03	303
Otu01	2.592	3.561	25.97	19.7	1.65E+03
Otu09	1.737	2.386	28.36	1.27E+03	178
Otu37	1.542	2.118	30.48	0.667	972
Otu29	1.466	2.013	32.49	30	954
Otu88	1.456	2	34.49	5	922
Otu23	1.292	1.774	36.26	846	31.7
Otu06	1.13	1.552	37.81	934	222
Otu81	1.113	1.529	39.34	2.33	704
Otu16	1.089	1.495	40.84	1.04E+03	351
Otu14	1.082	1.486	42.33	797	116
Otu160	0.9758	1.341	43.67	1.33	616
Otu25	0.9362	1.286	44.95	1.67	592
Otu28	0.8992	1.235	46.19	608	41.3
Otu65	0.7712	1.059	47.25	4	490
Otu42	0.7236	0.994	48.24	1.67	458

Otu05	0.7151	0.9824	49.22	4	455
Otu10	0.6141	0.8436	50.07	576	189

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Supplementary Table 5: Spearman correlation between the main OTUs, which contributed to the difference between control and treatment over the time, with physical chemical parameters that showed difference over the treatment according to Guedes et al., 2020. Spearman r values are labeled considering a negative (in red) or positive (in green) correlation, from a significant p-value ($p<0.01$).

(r) Sperman	Transparency	Turbidity	pH	Conductivity	DO	DOC	Chl Cyano	Chl Green Algae	Chl Diatoms
Otu01	0.775	-0.658	-0.407	0.264	-0.206	-0.139	-0.712	-0.399	-0.252
Otu02	-0.472	0.760	0.467	-0.173	0.344	0.406	0.497	0.280	-0.150
Otu03	-0.702	0.810	0.710	-0.485	0.592	0.171	0.772	0.410	0.138
Otu05	0.803	-0.722	-0.577	0.530	-0.384	0.022	-0.747	-0.240	-0.042
Otu06	-0.774	0.708	0.646	-0.628	0.358	-0.119	0.821	0.035	0.027
Otu07	-0.719	0.356	0.465	-0.492	0.287	-0.278	0.730	0.389	0.531
Otu08	0.797	-0.687	-0.520	0.447	-0.318	-0.045	-0.755	-0.350	-0.239
Otu09	-0.622	0.764	0.559	-0.242	0.317	0.250	0.622	0.378	0.017
Otu10	-0.823	0.664	0.596	-0.679	0.325	-0.153	0.893	-0.032	0.085
Otu14	-0.915	0.791	0.669	-0.531	0.459	-0.029	0.887	0.308	0.096
Otu16	-0.622	0.846	0.588	-0.158	0.481	0.445	0.551	0.624	0.013
Otu17	0.265	-0.477	-0.393	0.473	-0.549	-0.015	-0.306	0.323	0.567
Otu21	-0.881	0.767	0.643	-0.492	0.481	0.059	0.885	0.296	0.103
Otu23	-0.735	0.839	0.538	-0.133	0.390	0.489	0.661	0.587	0.091
Otu25	0.666	-0.551	-0.683	0.777	-0.530	0.337	-0.595	-0.002	0.221

Otu28	-0.803	0.752	0.706	-0.541	0.462	-0.034	0.783	0.200	-0.027
Otu29	-0.107	-0.128	0.081	0.074	-0.042	-0.163	0.090	0.600	0.736
Otu37	0.683	-0.717	-0.748	0.821	-0.729	0.190	-0.714	0.102	0.338
Otu39	0.734	-0.656	-0.626	0.601	-0.475	0.122	-0.708	-0.245	-0.026
Otu42	0.604	-0.746	-0.806	0.550	-0.757	-0.104	-0.593	-0.223	0.255
Otu47	0.310	-0.286	-0.092	0.051	0.017	-0.054	-0.215	-0.369	-0.254
Otu65	0.804	-0.564	-0.711	0.848	-0.527	0.410	-0.829	0.148	0.090
Otu81	0.760	-0.581	-0.638	0.759	-0.536	0.409	-0.771	0.230	0.310
Otu88	-0.255	-0.088	0.187	-0.162	0.105	-0.299	0.205	0.569	0.742
Otu160	0.723	-0.626	-0.713	0.806	-0.620	0.368	-0.757	0.199	0.298