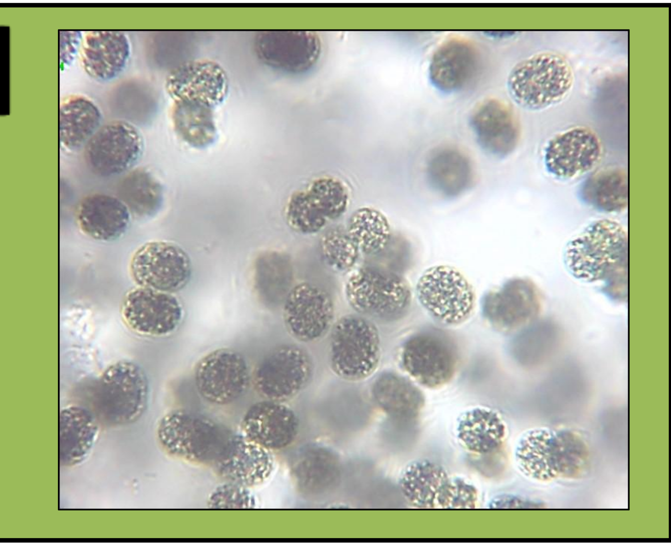
Microcystis Growth Suppression- a Laboratory Study of Three Chemical Treatment Methods



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Abstract

Harmful algal blooms of the toxin-producing cyanobacterium *Microcystis aeruginosa* have become a growing problem for southwest Florida freshwater bodies. Recently a 2018 bloom in the Caloosahatchee River and a 2016 bloom in Lake Okeechobee both led to the declaration of a state of emergency. Current long-term mitigation efforts such as nutrient reduction do not protect against the immediate risk these blooms pose for residents and wildlife, many short-term chemical spraying methods can cause harm through biomagnification of toxic chemicals and increased toxin release upon application. Hydrogen peroxide and L-lysine have shown promising results in selectively inhibiting the growth of *M. aeruginosa* and are more ecologically friendly due to fast degradation in water or biological enhancement of non-target organisms, respectively. We further explored three chemical treatment methods for the rapid suppression of *M. aeruginosa,* the use of amino acid L-lysine, hydrogen peroxide and combined treatments of both chemicals, which have never been tested before. Two *M. aeruginosa* strains, NIES-90 and FD4, were initially examined. The two L-lysine treatments 3 mg/L and 8 mg/L yielded significant concentration-dependent growth inhibition ranging from 43-94% when compared to controls. The two hydrogen peroxide treatments 16.7 mg/L and 33.3mg/L resulted in similar growth inhibition ranging from 40-89%. The combined treatments 3 mg/L L-lysine: 16.7 mg/L hydrogen peroxide and 8 mg/L L-lysine: 33.3 mg/L HP resulted in 82-99% decline of growth. Five more *M. aeruginosa* strains which vary in ecological adaptions such as toxin-production and colony formation were also used to assess variability in treatment sensitivity. Other abundant algae found in southwest Florida water bodies were also tested and found largely to be less sensitive compared to *M. aeruginosa*. Results of toxicity trials were then used to determine treatment concentrations for application in a mesocosm study. Water and natural phytoplankton communities from the Caloosahatchee River were pumped into mesocosms for a 7-day treatment observation. All three treatment applications led to reduced cyanobacterial populations when compared to controls.

Introduction

- Nutrient reduction is considered the best strategy to reduce occurrences of cyanobacteria HABs, however, these strategies are long-term solutions (Matthijs et al., 2012).
- Short-term alleviation methods like copper sulfate spraying can harm unintended organisms and persist in sediments (Hanson and Stefan, 1984).
- Hydrogen peroxide (HP), is a promising algaecide alternative due fast degradation rates in water and selective targeting of cyanobacteria (Matthijs et al., 2012).
- L-lysine, an essential amino acid, is also a promising alternative in selectively inhibiting the growth of *M. aeruginosa* where oxidative stress and antagonism between related amino acids may be the mode of action (Takamura et al., 2004).
- The goal of this research was to explore more ecologically friendly rapid-bloom suppression methods for the toxic cyanobacterium *M. aeruginosa.*

Methods

- Cyanobacteria and other algae were cultured in BG-11 to exponential phase before beginning treatment experiments. Cultures were incubated at 25⁰ C in a Precision Plant Growth Chamber.
- A preliminary experiment was conducted examining two concentrations for each treatment using FD4. A second experiment examining growth inhibition was done for multiple *M. aeruginosa* strains and other abundant cyanobacteria and eukaryotic algae found in Southwest Florida.
- Growth of algae was examined spectrophotometrically at an optical density of 750 nm.
- A mesocosm experiment was also completed. 250 L of river water was pumped from the Caloosahatchee River Franklin Lock and Dam (S-79) into 100 gal tubs (n=12) and 10 mL of concentrated *M. aeruginosa* biomass was added to each mesocosm. Three replicate mesocosms were done for each treatment and control. L-lysine treatments were examined at a concentration of 8 mg/L, HP concentration was 33.3 mg/L and mixed treatment concentrations of 8 mg/L L-lysine: 33.3 mg/L HP.

Discussion

- Found concentrations of L-lysine, HP, and mixed treatments that successfully inhibited the growth of multiple strains of *M. aeruginosa.*
- Non-axenic cultures of *M. aeruginosa,* many of which form colonies (FD4, AL2, HC1), were found to be largely more susceptible to L-lysine compared to HP. Possible result of bacterial degradation (Kim et al., 2019).
- Axenic cultures of *M. aeruginosa* were more sensitive to HP, genome analysis by Kim et al. (2019) found that NIES-843 and several other *M. aeruginosa* strains do not have a gene for producing catalase and that bacteria having the gene helped the growth of *M. aeruginosa* treated with HP by providing this catalase function.
- Mixed treatments tended to be the most successful at inhibiting the growth of *M. aeruginosa*. In water bodies where HP decomposition rates are high paired treatments may be suitable over using higher doses of HP. Such methods should be explored further.
- L-lysine was not inhibitory to the growth of most other Cyanophyceae and Chlorophyceae strains, which is in agreement with Hehmann et al. (2002), who found that these two phyla along with Bacillariophyceae were not sensitive to L-lysine. The mode of action of L-lysine and its specificity to *M. aeruginosa* should be explored further through genomic studies.
- HP is increasingly regarded as an ecologically friendly algaecide alternative due to its selectivity on cyanobacteria (Drabkova et al., 2007a; Matthijs et al., 2012). Our findings with *Chromochloris zoginginesis* support this claim.
- Success of these experiments allowed us to further examine treatment mitigation in mesocosm experiments at the Franklin Lock and Dam (S-79), along the Caloosahatchee River (Fig. 4). Mixed treatments were found to be most successful at reducing the abundance of *M. aeruginosa.*

Results

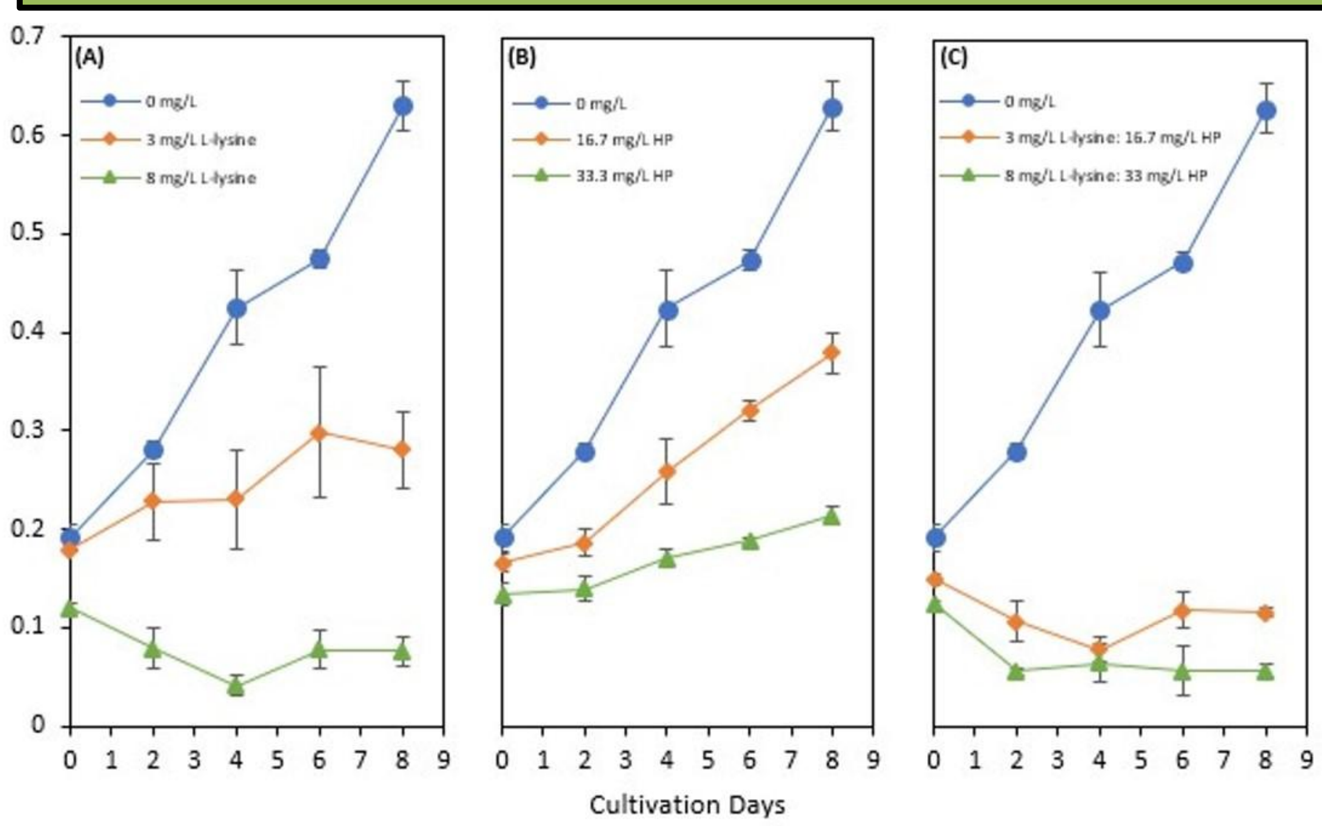

Figure 1. Susceptibility of *M. aeruginosa* strain FD4 to varying concentrations of L-lysine (A), hydrogen peroxide (B) and mixed treatments of L-lysine and hydrogen peroxide (C). Data presented are expressed as ± standard deviation.

Table 1. *Microcystis aeruginosa* strains used. The Caloosahatchee River is denoted by CR*.

Strain	MC-Toxin Production	Axenic	Isolation location
NIES-90	X		Lake Kawaguchi, Japan
NIES-88	X		Lake Kawaguchi, Japan
NIES-102	X	X	Lake Kasumigaura, Japan
NIES-843	X	X	Lake Kasumigaura, Japan
NIES-4325		X	Lake Abashiri, Japan
FD4			CR*, Fort Denaud, FL
HC1	X		CR*, Alva, FL
AL2	X		CR*, Alva, FL

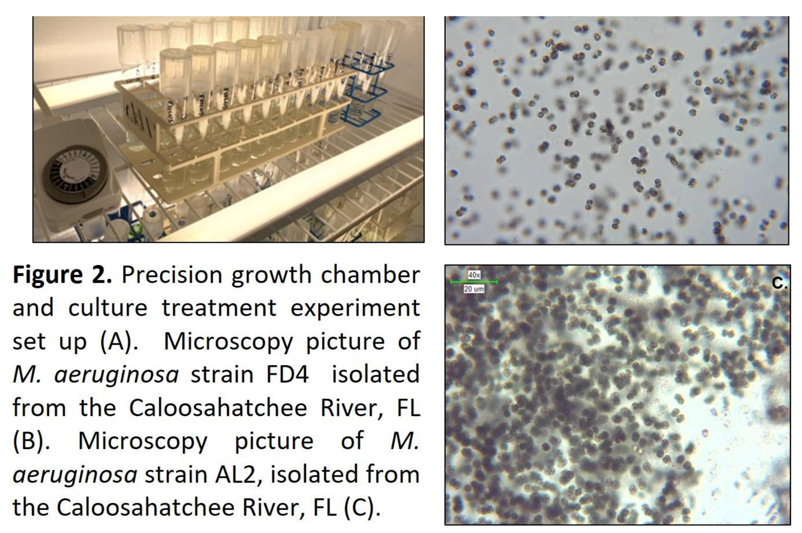

Figure 2. Precision growth chamber and culture treatment experiment set up (A). Microscopy picture of *M. aeruginosa* strain FD4 isolated from the Caloosahatchee River, FL (B). Microscopy picture of *M. aeruginosa* strain AL2, isolated from the Caloosahatchee River, FL (C).

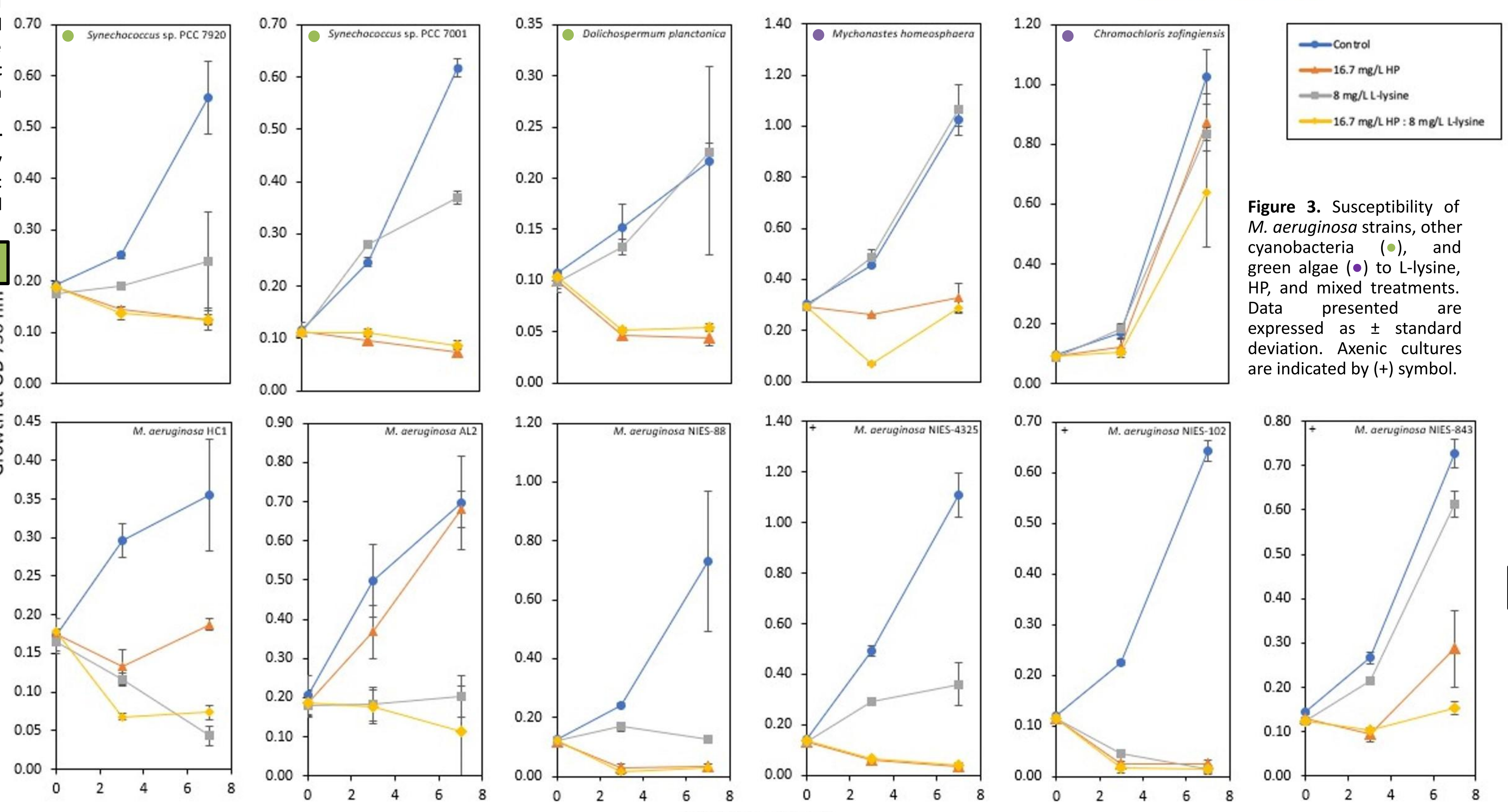

Figure 3. Susceptibility of *M. aeruginosa* strains, other cyanobacteria (●), and green algae (●) to L-lysine, HP, and mixed treatments. Data presented are expressed as ± standard deviation. Axenic cultures are indicated by (+) symbol.

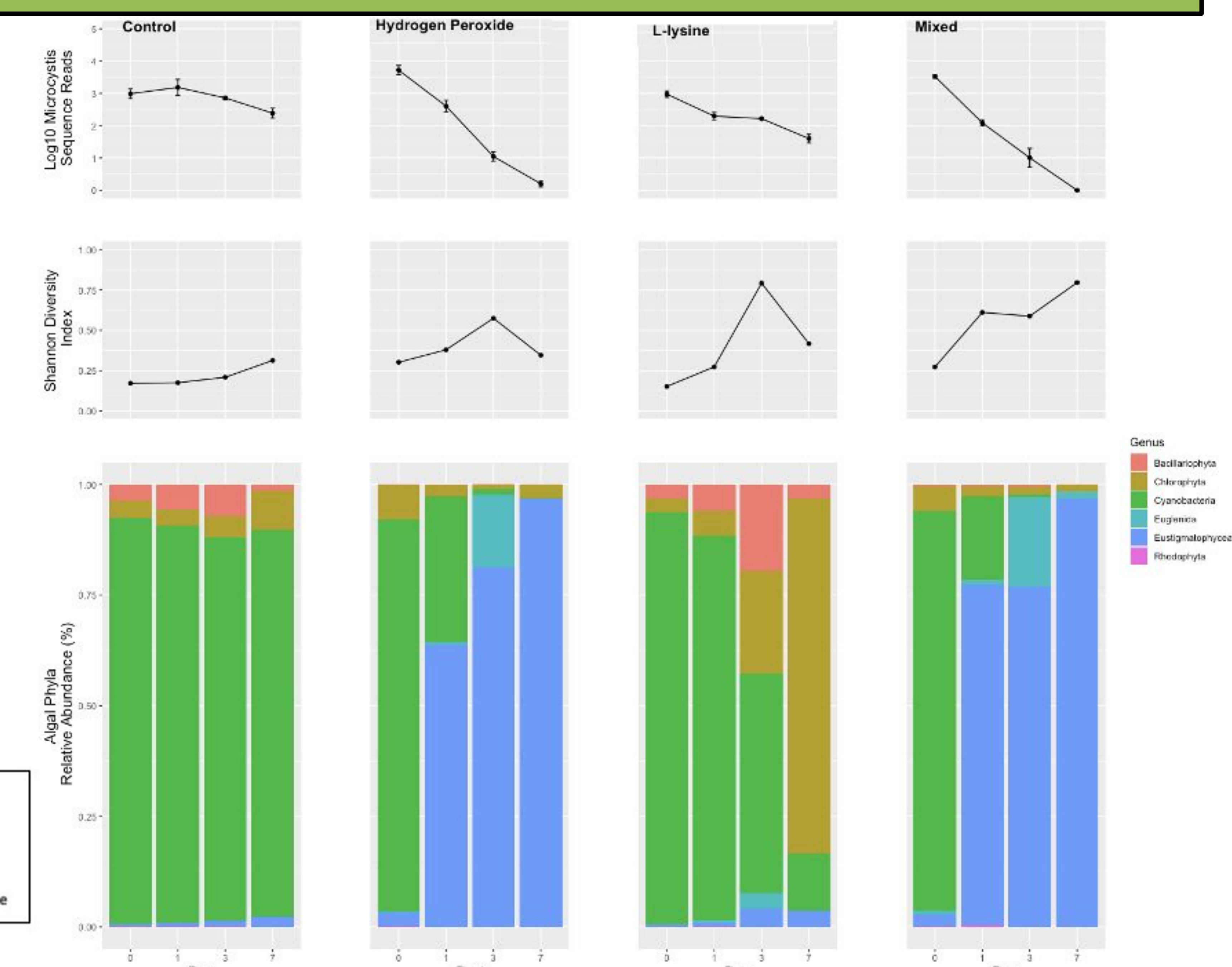

Fig.4 Preliminary results of 7-day mesocosm treatment experiment. Analysis based off average sequence read abundance of 16S rRNA sequence reads from surface water samples on days 0, 1, 3, and 7. Effects of treatments on *Microcystis* relative abundance (±SD)(top). Shannon diversity index for algal communities (middle). Shifts in relative abundance of algal communities during treatment experiment (bottom). Experimental set up at CR*(left).

Acknowledgments

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