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Biochar as a Lake Management Option for Harmful Algal Blooms: Lab Experiments and Bioassays

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Biochar as a Lake Management Option for Harmful Algal Blooms: Lab Experiments and Bioassays

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by

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Abstract

Harmful Algal Blooms (HABs) are an increasing global concern for water management due to their increased frequency, distribution, and toxin production. In freshwaters the growth of cyanobacteria (commonly known as blue-green algae), due to anthropogenic nutrient enrichment is the primary driver of HABS; these are often referred to as cyanoHABs. The management of cyanoHABs should be focused on in the watershed through best management and conservation practices or the physical, chemical and biomanipulation of the lake or reservoir that is experiencing these blooms. In this study, we examine the use of biochar as an option for the management or treatment of cyanoHABs.

Biochar is organic carbon produced from the pyrolysis of wood or other organic material. Biochar has been previously used in wastewater treatment and environmental remediation, and this project will test the ability of biochar to remove nutrients such as phosphate, nitrate and ammonia from freshwaters, and how biochar affects algal growth and microcystin concentrations.

A series of adsorption experiments were completed in the lab to evaluate how Biochar Now, a commercially available product, removed dissolved nutrients from water, and several bioassays were completed to evaluate its effect on cyanoHABs and microcystin concentrations. The main objectives of this study will be to 1) to determine the capacity of biochar to remove dissolved nutrients from aqueous solution, 2) to evaluate the effect of biochar on algal growth and cyanoHABs using bioassays, and 3) to evaluate the effect of biochar on free and total microcystin concentrations in cyanoHABs using bioassays. Results showed that biochar did not readily absorb nutrients or metals from aqueous solutions, and biochar did not have a relationship with microcystin production in the bioassays. However, while results were variable, bioassays showed that biochar might influence chlorophyll-a concentrations.

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Table of Contents

Introduction

Harmful algal blooms (HABs) are an increasing global concern for water management due to their increased frequency, distribution, and toxin production, as increased eutrophication continues to change freshwater ecosystems. The term harmful algal bloom was first used when applied to marine algae, that produced toxins or had other adverse effects (Davidson et al., 2011). In freshwaters the growth of cyanobacteria (commonly known as blue-green algae), are the primary driver of HABs (hereafter, cyanoHABS). Numerous cyanobacterial genera have optimal growth rates at higher water temperatures, where global warming and increased surface water temperatures play an integral role in their persistence and expansion across the globe (Paerl and Otten, 2013).

Some of the most common genera of cyanobacterial HABs that produce toxins are *Microcystis, Anabaena, Aphanizomenon,* and *Dolichospermum* (Carmichael, 1994). CyanoHABs, have adverse environmental and organismal effects, and they are also harmful to human health (Paerl et al., 2013). When these blooms produce toxins, they reduce water clarity and prevent the growth of aquatic plants. These toxins also have severe health effects on both humans and animals, such as acute, chronic, and sub-chronic poisoning, organ damage and failure, and in extreme cases death (Carmichael and Boyer, 2016). Some commonly known cyanotoxins are microcystin, cylindrospermopsin, anatoxin, guanitoxin, saxitoxin, nodularin and lyngbyatoxins which can have acute and chronic effects on human and animal health (Blaha et al., 2009). Cyanotoxin concentrations and frequency can vary between bloom events during a single bloom, therefore there is a significant need for monitoring each event. Microcystin, one of the most commonly produced toxins by cyanobacteria, is the most often detected toxin in most freshwater systems across the world (Wood et al., 2015).

There are several drivers of HABs, such as increased CO_2 concentrations which leads to global warming, increases in acidity and dissolved oxygen levels, and the enhancement of light intensity (Loreau et al., 2018). However, it is widely known that increased water temperatures, in concert with anthropogenic nutrient enrichment are the primary drivers for HABs in freshwaters (Wang et al. 2019b). The reduction of point and non-point source pollution is necessary to control phosphorus and nitrogen inputs into freshwaters, however, in scenarios where this cannot be achieved, treatment must be considered for HABs and associated toxins (Xu et al., 2022).

Some conventional methods used in treating cyanoHABs in drinking water, involve both physical and chemical processes (Xuexiang et al., 2016). These processes include sedimentation, coagulation, disinfection, flocculation, filtration, and adsorption. Emerging in the 1900s sedimentation, coagulation, and flocculation were commonly used for the removal of suspended solids in water, and when combined with chlorination, was successful in improving water quality and decreasing the occurrence of water-borne diseases (Xuexiang et al., 2016). However, cyanobacterial cells may be more challenging to remove due to their morphological properties and low densities (Ghernaout et al., 2010) when using these traditional methods. Past research shows that coagulation techniques were not as efficient in the removal of cyanobacterial cells and toxins (e.g., Chow et al., 1999).

In freshwaters, some common techniques used to treat cyanoHABs are the addition of chemicals such as copper sulfate and hydrogen peroxide. Studies show that in eutrophic lakes, where cyanobacteria dominated the phytoplankton communities in prairie lakes, copper sulfate was successful in decreasing algal biomass in freshwater bodies (Hanson et al., 1984). However, copper sulfate concentrations took about 8 to 10 days to be completely removed from the lakes (Whitaker et al., 1978). Copper sulfate has also been known to contribute to fish kills while being

used in treating cyanoHABs (Korosi et al., 2012). Similarly, hydrogen peroxide treatments, reduce HABs in freshwaters (Matthijs et al., 2012), but may have adverse effects on fish life stages and other non-target aquatic organisms. Additionally, hydrogen peroxide needs to be significantly diluted when being used as a treatment before application in lakes and other freshwater systems for. (Rach et al., 2011).

Adsorption methods using activated carbon have also been explored as an option for treating drinking water, wastewater, and even natural waters (Thompson et al., 2016). Biochar, the solid product of a thermochemical process known as biomass pyrolysis (Weber et al., 2018), has been considered a potential substitute for activated carbon in environmental remediation and wastewater treatment due to its low cost, relative abundance, and comparative sorptive abilities (Kearns et al., 2014). While several studies have examined the use of biochar for the treatment of wastewater (Shyam et al., 2022, Takaya et al., 2016), there is still some uncertainty in understanding how biochar in its unactivated state can be used as a possible management option for cyanoHABs in lakes.

To determine if biochar can be used as a suitable treatment for HABs we will conduct adsorption experiments and eight bioassays at Lake Fayetteville. The main objectives of this study will be to 1) to determine the capacity of biochar to remove dissolved nutrients from aqueous solution, 2) to evaluate the effect of biochar on algal growth and cyanoHABs using bioassays, and 3) to evaluate the effect of biochar on free and total microcystin concentrations in cyanoHABs using bioassays. Biochar as a potential treatment can create possible benefits for freshwater and lakewater management, as it may present an opportunity for eradication of the long-standing issue of cyanoHABs and decreased water quality in freshwaters.

Literature Review

Biochar is a carbon-based product produced from the pyrolysis of organic substances or biomass. These substances include plant material and organic waste such as manure and agricultural by-products. Pyrolysis is the process by which the biomass is heated in the absence of oxygen, which results in a stable highly porous material, called biochar (Lehmann and Joseph, 2015). This product is known to have a large surface area and is commonly used in the agriculture space for soil fertility improvement. Recently, biochar has gained significant attention as a potential solution for soil remediation, water quality improvement and the enhancement of agricultural productivity.

Biochar has several physical and chemical properties that makes it a suitable material for soil and water quality improvement. Biochar is known for its high porosity, which is one of its most important physical properties. The porosity of biochar is created during the pyrolysis process when the biomass breaks down into a permeable structure. The high porosity of biochar also enhances its ability to increase soil water holding capacity (Mohamed et al., 2016), promote growth of beneficial microorganisms (Cui et al., 2021), adsorb and retain contaminants such as metals and other compounds such as nutrients, from soil and aqueous solutions (Luo et al., 2022, Brewer et al,2014). The high porosity and surface area are the reasons this product has been extensively studied (Luo et al., 2022).

The potential applications of biochar have also been studied extensively. Biochar has been used as a soil amendment to improve soil fertility and increase crop yields (Lehmann et al., 2015). It has also been used as a carbon sequestration tool to store carbon for hundreds of years, reducing the amount of carbon dioxide in the atmosphere and mitigating climate change (Lehmann et al.,

2015). In addition, biochar has been studied as a source of renewable energy, with potential uses as a fuel for heating and cooking (Jeffery et al., 2011).

Biochar also has numerous chemical properties that are useful. Generally, it has a neutral to slightly alkaline pH due to the presence of calcium, magnesium, and potassium ions. This property helps to buffer acidic soils and improve nutrient availability (Lehmann and Joseph, 2015). Biochar has a high cation exchange capacity (CEC) due to the presence of functional groups such as carboxyl and hydroxyl groups. This property enhances nutrient retention and availability in the soil (Brewer et al., 2014). Its CEC enhances soil fertility and promotes plant growth, while its low electrical conductivity and pH buffering capacity help to maintain soil health. However, the properties of biochar can vary depending on the base material used and pyrolysis conditions, and further research is needed to optimize its use in different soils and environments (Crombie et al., 2013).

Some research found that biochar application significantly increased crop yields, with a 55% increase in maize yield and a 92% increase in soybean yield (Steiner et al., 2007). Other findings showed that biochar application significantly increased rice yield, with a 50% increase in rice yield (Schulz et al.,2012). Biochar has also been shown to improve soil health and reduce the negative impacts of heavy metals on soil, its application has been known to reduce the bioavailability of heavy metals in soil, leading to decreased toxicity and improved soil health (Xu et al., 2016).

Biochar also has the potential to be used as a filtration medium for removing heavy metals from contaminated water (Reddy et al., 2014). Other studies found that biochar was effective at removing heavy metals such as cadmium, copper, and zinc from contaminated water, and that it has also been proven to be useful in removing organic contaminants (Lehmann et al., 2015). Some

research found that biochar was effective at removing organic contaminants such as phenol, 4-chlorophenol, and 2,4-dichlorophenol from contaminated water (Ahmad et al. ,2014).

Biochar can also be used in constructed wetlands for removing contaminants from wastewater. Zhang et al. (2022) found that biochar-amended wetlands were effective at removing contaminants such as nitrogen, phosphorus, and organic matter from wastewater. The study also found that biochar-amended wetlands were more effective at removing contaminants than wetlands without biochar. In addition to its use in water filtration systems, biochar can also be used in agriculture to reduce nutrient runoff into waterways. When biochar is added to soil, it can help to retain nutrients, reducing nutrient concentrations in surface runoff (Lehmann et al., 2015). Doydora et al. (2011) found that biochar reduced the amount of nitrate and phosphorus in runoff from agricultural fields.

Some studies have also evaluated biochar particle size, and how this characteristic can influence its ability to be effective in water treatment. For example, a study by Houben et al. (2013) compared the effectiveness of biochar produced from wood, rice husk, and wheat straw for removing heavy metals from contaminated water. The study found that biochar produced from wood was the most effective at removing heavy metals, followed by biochar from rice husk and wheat straw. Liu et al. (2013) evaluated the effectiveness of biochar produced from different feedstocks (e.g. wood, corn stover, peanut shells) for removing organic pollutants from contaminated water. Results showed that biochar produced from wood was the most effective at removing organic pollutants, with removal rates ranging from 75% to 97%.

Ahmad et al. (2014) also examined the effectiveness of biochar produced from different feedstocks (e.g. rice husk, corn cob, wheat straw) for removing phosphorus from wastewater was

evaluated. The study found that biochar produced from rice husk was the most effective at removing phosphorus, with removal rates ranging from 90% to 99%.

In recent years, biochar has been promoted as a way of removing nutrients from aqueous solutions, including wastewater, lakes (Xu et al., 2019, Takaya al., 2016) and ponds (Lui et al., 2016). Studies have shown the ability of biochar to readily uptake nutrients and other pollutants from aqueous solution. (Lui et al., 2013, Mohan et al., 2014, and Zhang et al., 2020). Research showed that biochar made from corn cobs had a maximum capacity to remove nitrogen from wastewater of 0.08 mg/g at an initial concentration of 5 mg/L, and biochar made from garden wood had a maximum capacity to remove phosphorus from wastewater of 0.036 mg/g at an initial concentration of 5 mg/L (Lui et al., 2013).

Biochar has not been widely used as a lake treatment option, however few studies have examined the possibility of biochar for the reduction of algae and cyanobacteria in freshwaters (Kiani et al., 2023 & An et al., 2019). Most of its uses in water quality and lake remediation are centered around sorption and removal of nutrients, metals, contaminants, and toxins (Table 1).

Reference	C.A Takaya et al.	Zhanghong Wang et al.	Hafshejani et al.	Sivaprasad Shyam et al.	Kyung-Won Jung et al.	Ying Yao et al.
Biochar base material	Oak wood	Oakwood Ash	Sugarcane Bagasse	Feedstock	Brown marine macroalgae	Sugar beet tailings
Mass of Biochar (g)	0.1	0.1	0.05	-	0.05	0.1
Volume of solution	100 mL	50mL	Not Specified	-	50 mL	50 mL
Time period	2.5 hrs, 5hrs, 7.5 hrs, 10hrs 24hrs	0.25 hrs to 48 hrs	24hrs	-	48 hrs	24 hrs
Concentration Range	0 to 30 mg/g (PO4) and 105.8 - 146.4 mg/g(NH4)	1–1500 mg/ L1 for NH4 1–2000 mg/ L for NO3 3–3000 mg /L for PO4	1-50 mg/L NO3	1 to 6000 ppm	5 to 200 mg/L	15 to 640 mg/L
Nutrients/ Elements Measured	PO4 and NH4	NH4, NO3 and PO4	NO3	PO4	PO4	PO4
Temperature of sample	Room Temperature	Room Temperature	Room Temperature		Room Temperature	Room Temperature
Filtration method/ size of the filter	0.45um	0.22 um	0.45um		0.45 um	0.22 um

Table 1: Summary of methods used in studies where biochar was used for the removal of nutrients and other inorganic substances from aqueous

Study Site

Lake Fayetteville is a 0.6 km^2 recreational lake with a 24 km^2 catchment area in northwest Arkansas, United States. The lake has a maximum depth of 15 m and an average surface area of 1km^2. In 1949, Lake Fayetteville was constructed as a drinking water source, however, it has been used mainly for recreational purposes including the city park that surrounds it. The lake has been known to be a hypertrophic reservoir (Grantz et al., 2014) and cyanobacteria has dominated the phytoplankton community since 1968 (Meyer, 1971). Cyanobacterial HABs (cyanoHABs) are an annual issue at this lake (Wagner et al., 2021, Haggard et al., 2023), where water samples, especially surface scum, had total microcystin concentrations exceeding recreational guidelines (8 ug/L EPA, 2019).

Clear Creek and Brush Creek are the primary tributaries supplying flow to Lake Fayetteville. Clear Creeks watershed's land cover is dominated by agriculture, where over 50% of the watershed is attributed to agricultural activity such as livestock and crop production. Urbanized spaces account for almost 40% of the watershed, and the remaining areas mainly consist of forestry (8.4%) and wetlands. The lake's watershed as a whole is mostly forested, where forests account for 60% of the land cover, followed by 20% open water, 20%, urbanized spaces, 19% pasture, and 1% wetlands (Stroud Water Research Center, 2021).

Figure 1: Map of Lake Fayetteville, in Fayetteville, Arkansas with sampling location indicated.

Methods

Sorption Experiments

Forty 125 ml flasks were used in sorption experiments to measure the uptake of dissolved nutrient concentrations. The nutrients measured in this sorption experiment were NO_3-N, Soluble Reactive Phosphorus (SRP), and NH_4-N.The initial concentration for each nutrient in aqueous solution was 0, 0.01, 0.025, 0.05, 0.1, 0.5 1.0, 5.0, and 10.0 mg/L, and each flask was filled with a volume of 100 ml. After each flask was filled with the nutrient solution, 0.2 g of biochar was measured and placed into each flask. A biochar mass-to-volume ratio of 0.2 g to 100 ml of the aqueous solution was used in these experiments, which is within the range observed in the literature, seen in (Table 1). The forty flasks were capped with parafilm, shaken and incubated at room temperature for 24 hours. pH measurements were taken throughout all experiments.

After the incubation period, the flasks were removed from the incubator and mixed thoroughly for five (5) minutes. After mixing, a 10 ml sample was taken from each flask and placed into a labeled centrifuge tube, and then centrifuged at 3175 rpm for 20 minutes. The samples were then filtered (0.45 µm) and placed into 20 ml plastic vials, and analyzed for dissolved nutrients. Dissolved nutrient analysis was performed using a Skalar San++ System Wet Chemistry Autoanalyzer, including SRP via EPA Method 365.1, NH_4-N via EPA Method 351.2 and NO_3N plus NO_2-N via EPA Method 353.2 (https://awrc.uada.edu/). This experiment was repeated three times, to determine the capacity of biochar to remove nutrients from aqueous solution.

Field Sampling and Water Quality Analysis

Routine monitoring of lake Fayetteville was conducted weekly. Three sites were sampled, the dam, inlet, and Clear Creek. At each site, an alphasampler was used to collect three water samples. Water samples were collected in 1L bottles, stored in a cooler, and then delivered to the water quality lab.

A total of eight bioassays were conducted in the spring and summer months on March 27, April 29, May 18, May 25 June 18, June 22, July 6 and August 3 of 2022. This was done by collecting water off of the marina dock in Lake Fayetteville. A 20 L plastic container was filled with water at the lake, along with 3 additional 1 L bottles to evaluate the physiochemical properties of the lake prior to incubation. These samples were analyzed in the field for dissolved oxygen (DO), conductivity, and temperature, using a YSI ProSolo Digital Water Quality Meter, and for pH using the Oakton pH Testr 30+ Waterproof Pocket Tester. The initial samples were also analyzed for Chlorophyll-a (CHL-a) pigments, total and free microcystin (MC), dissolved nutrients including NH_4-N, SRP, and (NO_3-N plus NO_2-N) and chlorophyll raw fluorescence (CHL RFUs), phycocyanin raw fluorescence (PC RFUs) and the phycocyanin to chlorophyll a ratio (PC:CHL).

Chlorophyll-a pigment was measured using APHA Method 10200 H3, where 100 ml of sample was filtered through a 0.7 µm membrane filter, the filter was then placed in a 15 ml centrifuge plastic tube and 10 ml of aqueous 90% acetone was added to the tube. The samples were then stored in a freezer at -18 °C, and later analyzed using the Turner Designs Fluorometer.

Total MC was analyzed using EPA Method 546, where 20 ml of unfiltered water was placed in a 40 ml glass amber vial (Haggard & Austin, 2023) These samples were put through

three (3) freeze-thaw cycles, to lyse the cells. These samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) Kit on an Abraxis Plate Reader.

Dissolved nutrient analysis was performed using a Skalar San++ System Wet Chemistry Autoanalyzer, as previously described.

Finally, Chlorophyll (CHL) RFUs and phycocyanin (PC) RFUs and the PC:CHL ratios, were measured using a Turner Designs CyanoFluor Handheld HAB Indicator.

Lake Fayetteville HAB and Biochar Bioassays

The plastic container filled with lake water was brought back to the water quality lab. The container was placed on a magnetic stirrer, and continuously mixed, while simultaneously pouring 700 ml of lake water into 1 L glass bottles. Bioassays had both nutrient and biochar treatments. The nutrient treatments were 1). control (no nutrient addition), 2). control with biochar, 3). NO_3-N, N (1.0 mg/L) and PO_4-P, (0.1 mg/L) PN, and 4) PN with biochar. The biochar treatments were, 1). free biochar, 2). single bagged biochar, 3). double bagged biochar and 4). rinsed double bagged biochar. Biochar treatments were changed as we conducted the bioassays. Each treatment was done in replicates of four, and there was a total of 16 or 24 experimental units per bioassay, depending on the treatments used.

The bottles were placed in an incubator (VWR, model VRI6P) in a randomized block design. The units were incubated at the water temperature recorded at the time of collection, for seven days. There was a LED panel lamp (Werker Lamps, model FIX12539) inside of the incubator, with a set light intensity of 140 µmol m2 /s. The light was set on a 10 h light and 14 h dark cycle throughout the entire incubation period. After incubation, CHL-a, CHL RFUs, PC RFUs, PC:CHL, dissolved nutrient concentrations and MC concentrations were measured using the previously described methods.

Statistical Analysis

The effect of biochar on nutrient treatments was analyzed using analysis of variance (ANOVA), and treatment means were separated using the least significant difference (LSD) with a significance level of 0.05 ($\alpha = 0.05$) in R. The data was log-transformed prior to statistical analysis.

Results

Sorption Experiments

Generally, biochar did not readily adsorb nutrients and metals from aqueous solutions. Final dissolved NH_4-N concentrations in the aqueous solution were slightly less than the initial concentration (Fig 2 C). NH_4-N was only slightly adsorbed by biochar across the concentration range of 0.25 to 10mg/L. However, at all concentrations, desorption of ammonia into solution was also observed. Lower concentrations of 0.25 mg/L, showed higher rates of desorption (1%) of NH_4-N.

Like NH_4-N, SRP was not adsorbed by biochar or showed little change from initial concentrations (Fig 2B). Even at the high end of the concentration range, (5 to 10 mg/L), there was only slight (0.2%) uptake by biochar. Typically, at the low end of the concentration range, desorption occurred from biochar, or small particles of biochar interfered with SRP analysis. For example, at the lowest end of the range, 0.01 mg/L up to 7% desorption was observed. Desorption percentages gradually decreased as SRP concentrations increased throughout the experiments. Only at the 1.0 mg/L initial concentration, we were able to see some sorption taking place, which was still less than 1% of the initial SRP concentrations.

The nitrate-N experiments followed a similar trend to the SRP experiments. At the lower initial concentrations, (0.01 and 0.1mg/L), we typically saw desorption ranging from

0.2% to 5.7%. However, as the concentration range increased, from 0.25 mg/L to 10 mg/L no adsorption was observed. Overall, the sorption taking place in the NO_3 experiments was minuscule, with a high of 0.6% and there were still some cases of desorption on the high end of the concentration range.

In the Zn experiments (fig D), we examined the capacity of biochar to adsorb zinc from solution. Here, we saw adsorption in the low end of the concentration range, where initial concentrations of 0.25 showed up to 0.9 % adsorption. However, as initial concentrations increased, sorption capacity decreased and progressed to desorption. For example, at an initial concentration of 1 mg/L we saw up to 0.12% sorption, but at 5 mg/L there was only desorption of 0.2%. At the highest initial concentration of 10mg/L desorption increased up to 0.63%.

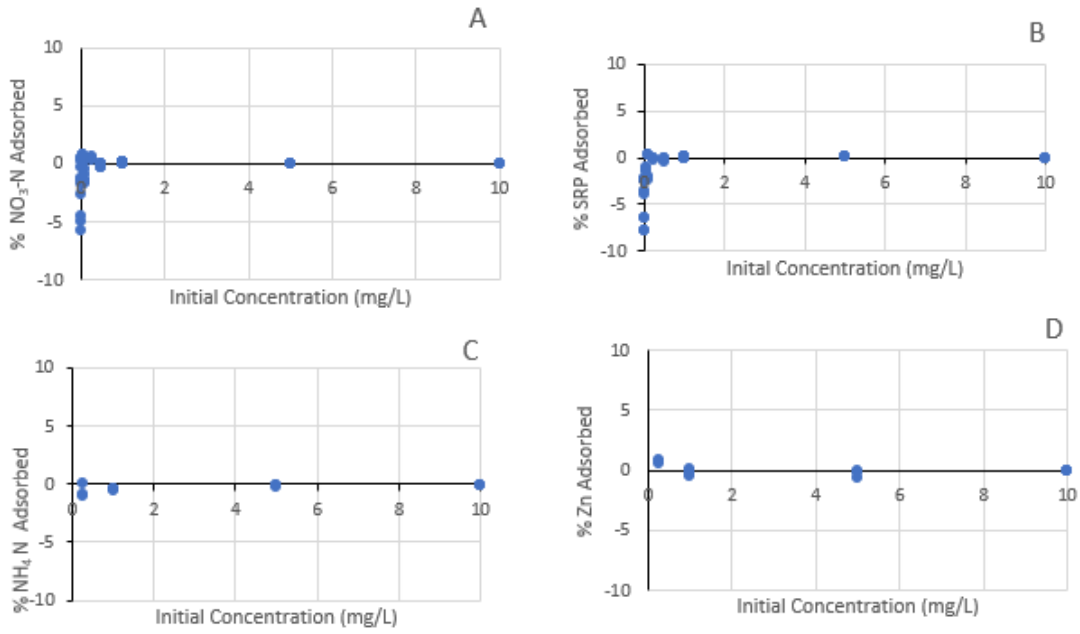

Figure 2. NO_3-N (A), SRP (B), NH_4-N (C), and Zn (D) absorbed by biochar (0.2 g) added to aqueous solutions (100ml) with defined initial concentrations.

Biochar and CyanHabs

Bioassay 1

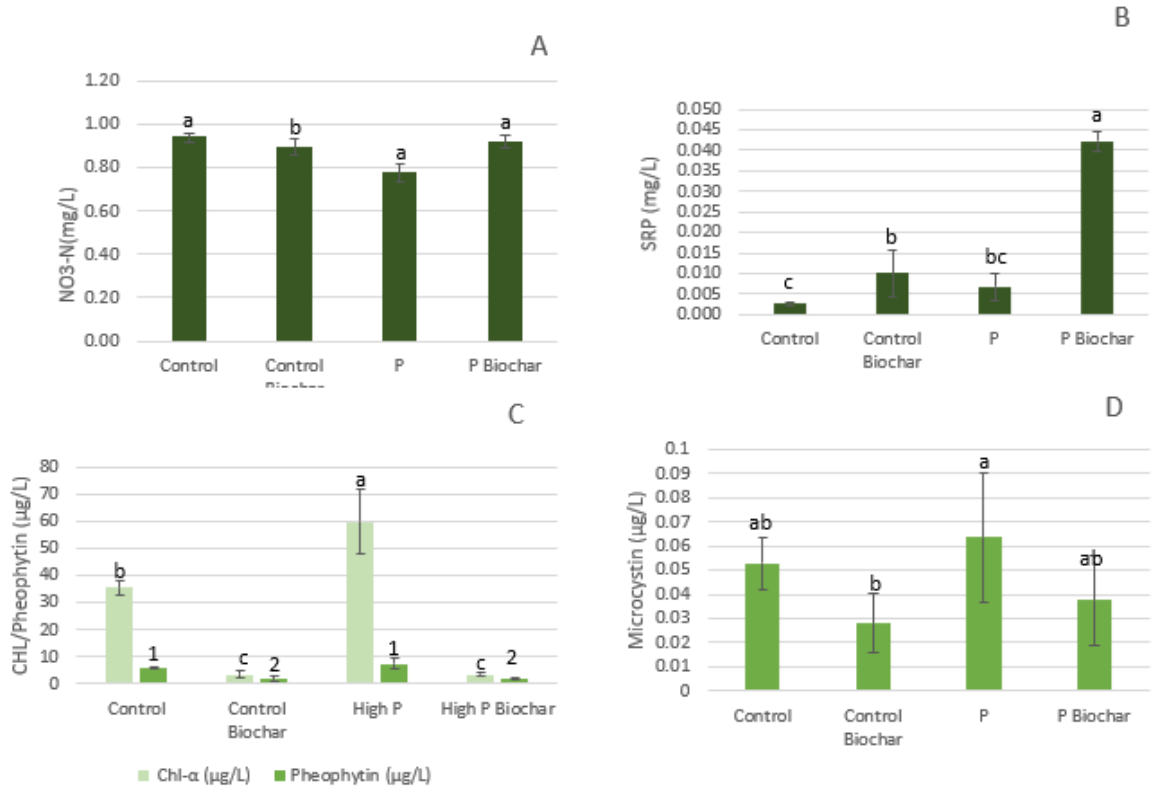

Figure 3. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C) an Microcystin (D) in lake water after 7 days of incubation at 27.1 °C on March 27, 2022.

Biochar was applied as a powder (1 g/L) in the first bioassay. Biochar dispersed along the surface of the water, and then after seven days most of the biochar settled to the bottom of the bottles. The light source was beneath the bottles, so we thought this might inhibit light from reaching the cyanobacteria and algae within the water in the bottles.

After seven days, mean nitrate-N concentrations across the treatments ranged from 0.77 to 0.94 mg/L. There was no significant difference between the control (0.94 mg/L) and the control with biochar (0.89 mg/L) nor were the controls different than the P addition with

biochar. However, mean nitrate concentrations (0.77 mg/L) were least in the bottles with only P added to stimulate cyanobacterial and algal growth.

After the incubation, SRP concentrations were highly variable across the treatments, ranging from 0.003 to 0.042 mg/L. Not surprisingly, the greatest SRP concentration (0.04 mg/L) was found in the bottles with added P and free biochar. However, the bottles with only P added had final SRP concentrations (0.007 mg/L) that were not different than the control (0.003 mg/L) or the control with free biochar (0.01 mg/L). The control had lower mean SRP concentrations than what was observed in the control with biochar, which suggested no adsorption but actual elevated SRP.

Chlorophyll-a concentrations proved to be much less in biochar treatments after seven days and greater in companion non-biochar treatments. Both the control with biochar and the P added with free biochar treatments had mean chlorophyll-a concentrations that were not different (3.4 µg/L). In the control and P treatments without free biochar, we saw significantly greater chlorophyll-a concentrations, averaging up to 60 µg/L with P addition.

Total microcystin concentrations were consistently low in this experiment, with a range of 0.01 to 0.09 µg/L, and these concentrations were less than the reported method detection limits for the lab (MDL=0.1 µg/L). Mean total microcystins in bottles with biochar were not different from the control without biochar nor the treatment with P added and free biochar. Generally, there was no real pattern in the total microcystin concentrations across the treatments.

Bioassay 2

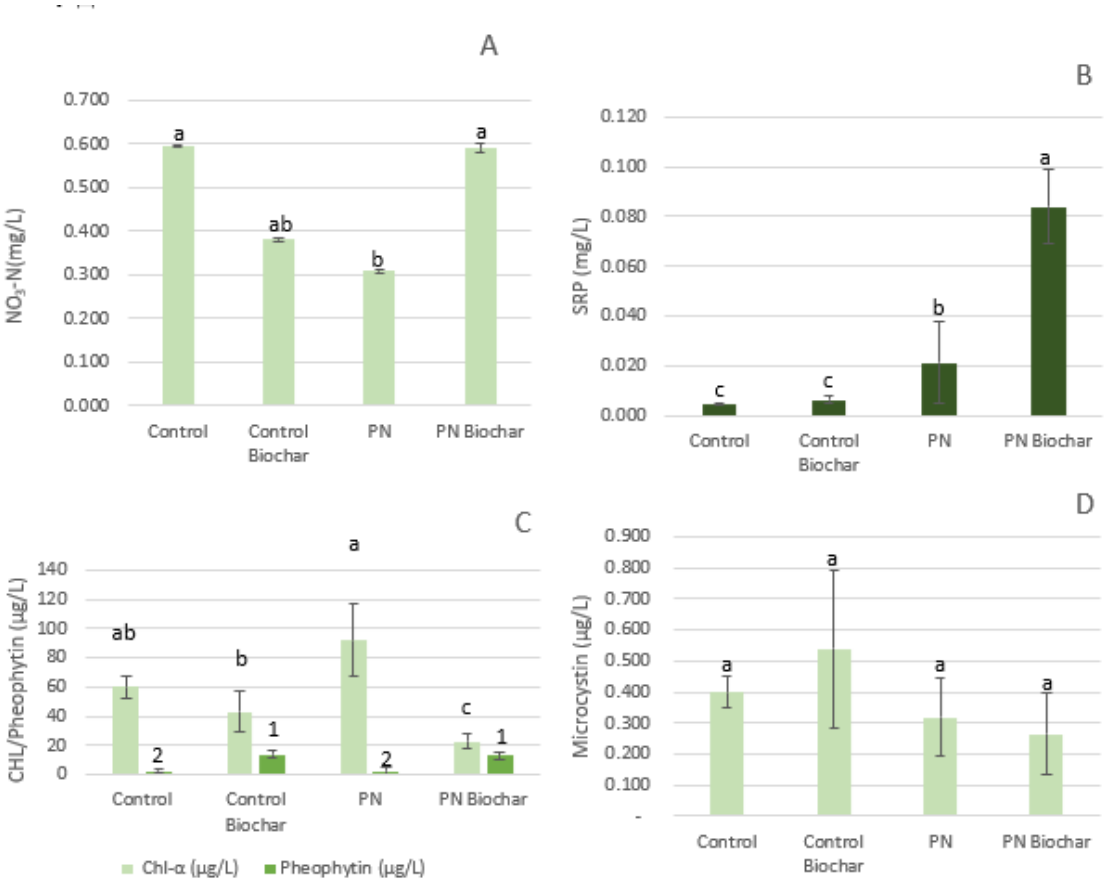

Figure 4. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C) Microcystin (D) in lake water after 7 days of incubation at 19.4 °C on April 29, 2022.

Biochar was single bagged in a permeable cloth mesh at 1g/L in the second bioassay. The cloth mesh was suspended in the bottles for the incubation period. In this instance, there were fewer particles settling to the bottom of the bottles when compared with the free biochar treatments. However, there were still some residual particles settling on the bottom of the flask.

After seven days of incubation, mean nitrate-N concentrations in all the treatments ranged from 0.31 to 0.59 mg/L. There was no significant difference between the control (0.59 mg/L) and the control biochar (0.38 mg/L) treatments. The control with biochar also had no

significant differences, from the P and N addition treatments. Biochar treatments with PN addition proved to have the highest N concentrations.

SRP concentrations were extremely variable across the different treatments. After incubation, we saw elevated levels of SRP (0.084 mg/L) in the biochar treatments with P and N additions. The mean concentration in the control (0.005 mg/L) and the control with biochar (0.006 mg/L) were not significantly different.

Like the first bioassay, chlorophyll-a concentrations proved to be significantly less in the biochar treatments, when compared to the treatments without biochar added in bags. Mean concentrations in the control without biochar (60.1 µg/L) and the P and N addition treatments without biochar (91.9 µg/L) were not significantly different. However, the biochar treatments were significantly different from each other. The control with biochar had an average concentration of 42.8 µg/L which was greater than the PN biochar treatments with a mean of 22.7 µg/L.

Total microcystin concentrations were generally higher in this bioassay than in the first. With mean concentrations ranging between 0.27 and 0.54 µg/L. However, there was no significant difference across all treatments in total microcystins. Similar to the first bioassay, no trend was observed in mean total microcystin concentrations.

Bioassay 3

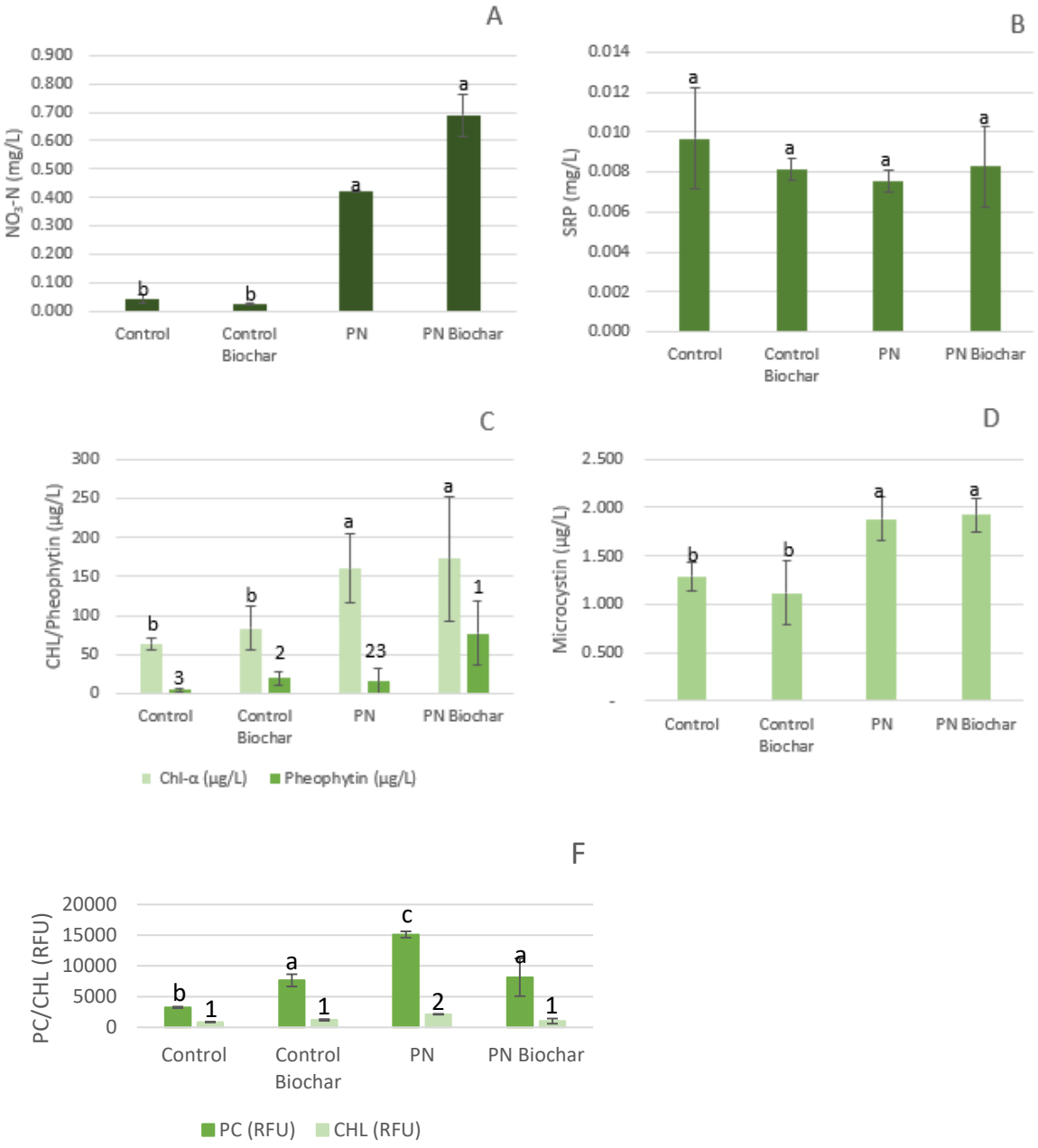

Figure 4. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C) and Microcystin (D) and Phycocyanin and Chlorophyll-a RFUs (F) in lake water after 7 days of incubation at 29.6 °C on May 18, 2022.

In the third bioassay, the mesh cloth sacks were doubled, and 1g/L of biochar was placed in each sack and suspended inside the bottles. There was visibly less biochar powder

seeping out of the sacks and settling to the base of the bottles inside the incubator after seven days.

After incubation, mean nitrate-N concentrations in all treatments ranged from 0.03 to 0.69 mg/L. There was no significant difference between the control (0.05 mg/L) and the control with biochar (0.03 mg/L) treatments. As expected, the control treatments were significantly less than the nutrient addition treatments. Biochar with nutrient additions (0.69 mg/L) and biochar without nutrient additions (0.42 mg/L) was not significantly different from each other.

Mean SRP concentrations in this bioassay ranged from 0.008 to 0.01 mg/L. Average SRP concentrations in the control (0.01 mg/L) were not significantly different from the control with biochar (0.008 mg/L). This relationship was also observed in the nutrient addition treatments, which reflected no significant difference in SRP concentrations in treatments with biochar (0.008 mg/L) and without biochar (0.008 mg/L). Generally, SRP concentrations remained consistent across all treatments in this experiment.

Chlorophyll-a concentrations ranged between 63.7 to 172.7 µg/L in this bioassay. Chlorophyll-a in this bioassay, followed a different trend from the first two bioassays. In this case, the mean concentration in controls without biochar (63.7 µg/L), and the controls with biochar (83.5 µg/L) were not significantly different. The nutrient addition treatments were also not statistically different but these were both greater than either controls.

Mean total microcystin concentrations ranged between 1.12 to 1.92 µg/L. Average concentrations between the control (1.29 µg/L) and the control with biochar (1.15 µg/L) were not significantly different. Both controls were significantly different from the nutrient addition treatments, as expected. However, average concentrations in nutrient treatments without biochar (1.88 µg/L) were not significantly different from nutrient biochar treatments (1.92 µg/L)

Mean phycocyanin (PC) RFUs in this bioassay ranged between 3247 and 15,121 RFUs across treatments. The controls without biochar (3247 RFUs) were significantly less than controls with biochar (7651 RFUs). The opposite was observed in the nutrient addition treatments, where PN units averaged 15,121 RFUs without biochar and 8150 RFUs with biochar. Chlorophyll-a (CHL-a) RFUs were generally low in this bioassay ranging from 859 to 2131 RFUs. These samples were clearly cyanobacteria dominant with phycocyanin to chlorophyll-a (PC: CHL) ratio of 9.8302, prior to incubation.

Bioassay 4

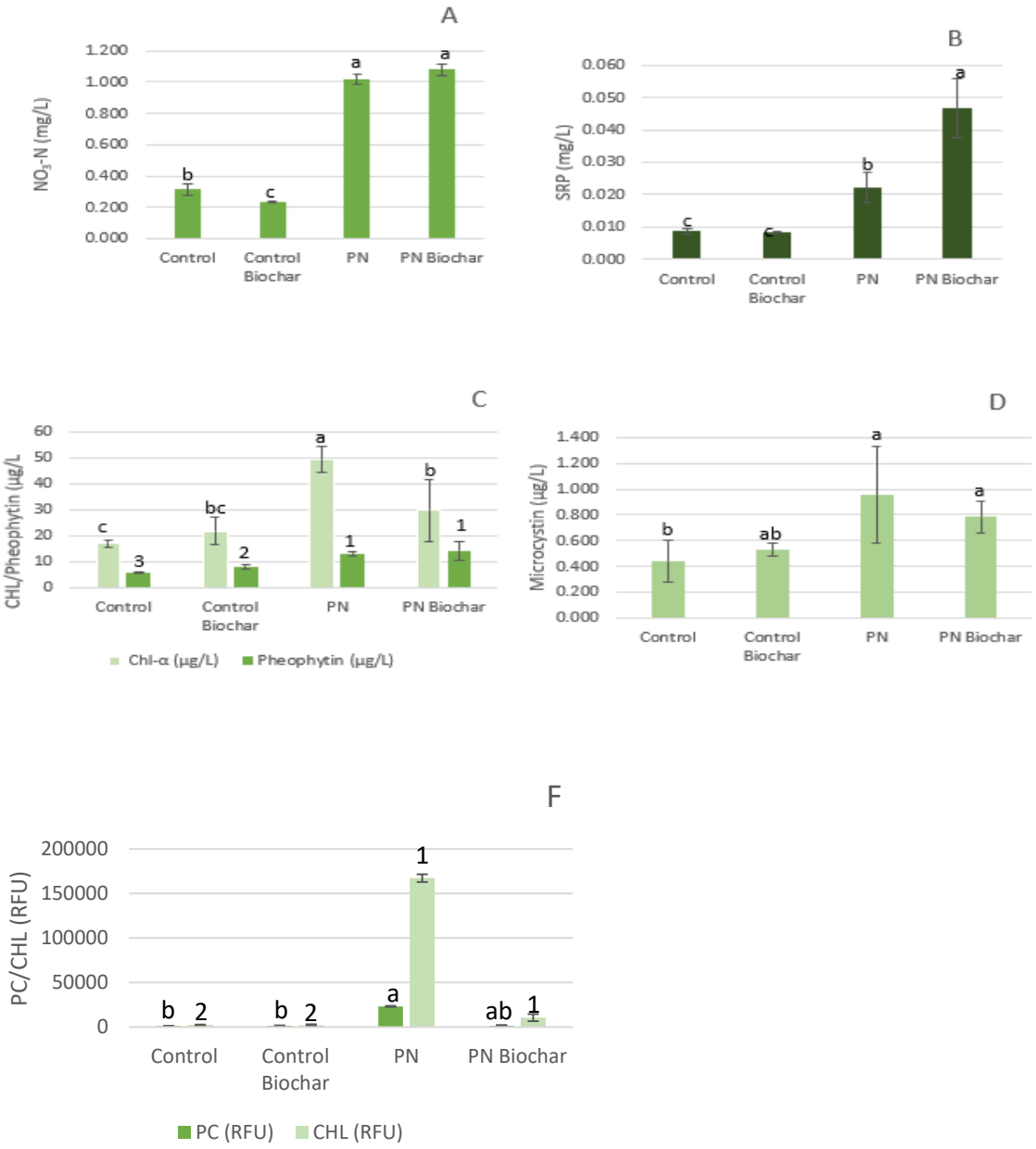

Figure 5. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C), Microcystin (D) and Phycocyanin and Chlorophyll-a RFUs (F) in lake water after 7 days of incubation at 26.1 °C on May 25, 2022.

In bioassay four, 1g/L of biochar powder was placed in the cloth mesh sacks and these sacks were suspended inside the bottles. In this instance, the bags were removed from the bottles before mixing and processing the samples. There was visibly less biochar in the bottles

than in the previous treatments, however, the solution was still relatively cloudy, with some fine biochar particles settling out of the bags to the bottom of the bottles.

After the seven-day incubation period, mean nitrate-N concentrations ranged between 0.24 mg/L and 1.08 mg/L. Mean nitrate-N concentrations were significantly different in the control (0.31 mg/L) and the control with biochar (0.24 mg/L) which were both significantly less than the nutrient addition treatments. However, there was no significant difference in mean nitrate-N concentrations between the nutrient addition treatments with biochar (1.02 mg/L) and without biochar (1.08 mg/L).

SRP concentrations followed a similar trend to the other bioassays after the incubation period. The controls with biochar and without biochar were not significantly different, mean SRP concentrations in both treatments were 0.009 mg/L. Mean SRP concentration in nutrient-addition treatments without biochar (0.02 mg/L) and with biochar (0.05 mg/L) were significantly different, and both were greater than mean SRP concentrations when compared to the controls.

Mean chlorophyll-a concentrations across all treatments ranged between 16.9 to 49.1 µg/L after incubation. The control (16.9 µg/L) and the control without biochar (21.7 µg/L) were not significantly different. Mean chlorophyll-a concentrations in the biochar treatment were not significantly different, where mean chlorophyll-a in the nutrient addition with biochar was 29.6 µg/L. The greatest mean chlorophyll-a concentration was 49.1µg/L in nutrient treatments without biochar.

Total microcystin concentrations ranged between 0.43 to 0.95 µg/L in this bioassay. As anticipated, control and nutrient treatments were significantly different, as nutrients were added to stimulate cyanobacterial and algal growth. However mean microcystin concentrations in

control treatments were not significantly different, nor were the nutrient addition treatments different from each other.

Mean phycocyanin RFUs were generally low across control and biochar treatments (1171-1651 RFUs) and were not significantly different from each other. However, PC concentrations in nutrient addition treatments without biochar (23,187 RFUs) were significantly greater than all other treatments. Chlorophyll-a followed a similar trend where all control and biochar treatments (2274 – 2361 RFUs) were significantly lower than the nutrient addition treatments without biochar (167,257 RFUs). These experiments did not show cyanobacteria dominance, as phycocyanin RFUs were consistently lower than chlorophyll-a RFUs across all treatments.

Bioassay 5

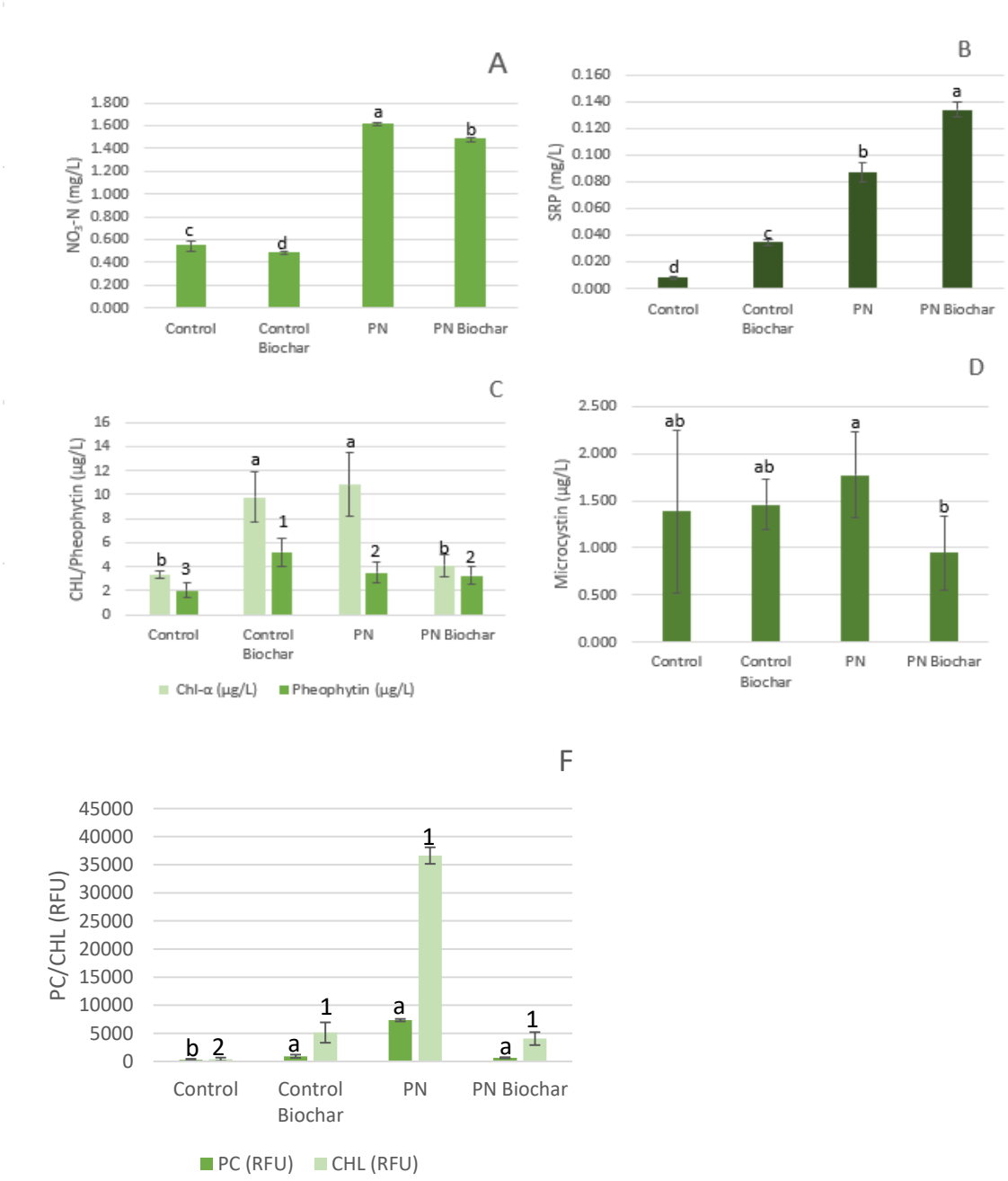

Figure 6. NO_3-N (A), SRP (B), Chlorophyll-a and Pheophytin (C), Microcystin (D) and Phycocyanin and Chlorophyll-a RFUs (F) in lake water after 7 days of incubation at 24.2 °C on June 18, 2022.

In this experiment, 1 g/L of biochar was placed into cloth sacks and rinsed thoroughly before being suspended inside the bottles during incubation. The sacks were removed from

each bottle before mixing on a bidiurnal cycle. The bottles were relatively clear, and there were little amounts of biochar particles settling to the base of the bottles.

Nitrate-N concentrations were highly variable in this bioassay. Mean concentrations ranged between 0.48 and 1.62 mg/L. All treatments were significantly different from each other, and there was no real pattern across treatments.

SRP concentrations were also variable. Mean concentrations ranged between 0.008 and 0.31 mg/L. While results were variable, there was still a trend observed. Mean SRP concentrations in biochar treatments were greater than their companion non biochar treatments.

Chlorophyll-a had average concentrations ranging from 3.3 µg/L to 10.9 µg/L. There was a significant difference between the control (3.3 µg/L) and the control with biochar (9.8 µg/L), and between the PN addition treatments without biochar (10.9 µg/L) and with biochar (4.1 µg/L). In PN treatments, we observed that in the biochar treatments there was significantly less chlorophyll-a. However, the control treatments did not follow that same pattern. Instead, we saw high chlorophyll-a concentrations in the biochar treatments as opposed to those without biochar.

Total microcystins averaged between 0.94 and 1.77 µg/L in this bioassay. There was no significant difference between the controls with biochar (1.46 µg/L) and the controls without biochar (1.39 µg/L). However, in the PN treatments, we saw that treatments without biochar had higher concentrations (1.77 µg/L) than treatments with biochar (0.94 µg/L).

Mean phycocyanin RFUs ranged between 295 and 7338 RFUs across all treatments. Controls without biochar had the lowest PC RFUs (295 RFUs), which was significantly less than the control with biochar (921 RFUs). However, in nutrient addition treatments, PN with biochar (92 RFUs) had significantly less phycocyanin concentrations than PN treatments without biochar (217 RFUs). CHL-a RFUs ranged consistently higher than PC concentrations

in this bioassay (377- 36,633 RFUs). Mean Chlorophyll-a RFUs in controls without biochar (377 RFUs) was significantly less than controls with biochar (5132 RFUs). However, the opposite was seen in our nutrient addition treatments, where PN without biochar (36,633 RFUs) was significantly greater than PN with biochar (4055 RFUs). Like bioassay 4, this experiment did not show cyanobacteria dominance, as phycocyanin RFUs were consistently lower than chlorophyll-a RFUs across all treatments.

Bioassay 6

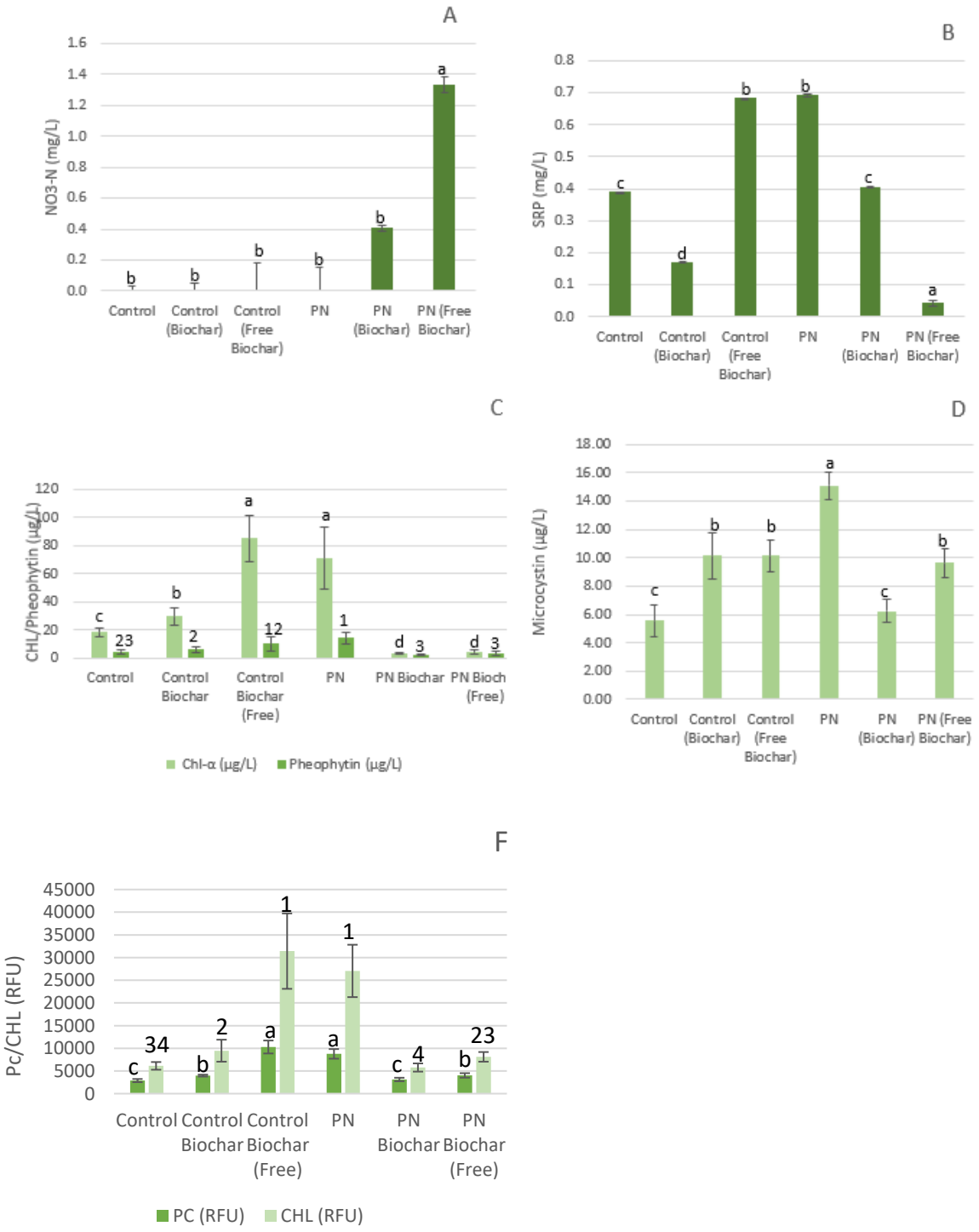

Figure 7. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C), Microcystin (D) and Phycocyanin and Chlorophyll-a RFUs (F) in lake water after 7 days of incubation at 29.7°C on June 22, 2022.

In bioassay 6 we moved from having 16 experimental units to 24 units. This was to compare how free biochar treatments compared with double bagged biochar treatments after a 7-day incubation period. In this instance, 1g/L of biochar was administered freely into the free biochar treatments, and the cloth mesh sack with biochar were suspended inside of the bagged treatment bottles. In the free biochar treatments, the biochar spread across the surface of the water and then sank to the base of the bottles. The bagged biochar treatments were relatively clear with some fine particles settling out of the bags.

Mean nitrate-N concentrations ranged between 0 and 1.3 mg/L. All treatments were not significantly different apart from the free biochar treatments (1.3 mg/L). This is congruent with the sorption experiments where we saw desorption of N in solution.

Mean SRP concentrations ranged between 0.04 to 0.69 mg/L. In this bioassay the N concentrations were highly variable and did not show any sort of trend. Overall, there was no relationship observed between biochar treatments and nutrient concentrations.

Chlorophyll-a concentrations ranged between 2.2 µg/L and 84.9 µg/L. Controls with free biochar (84.9 µg/L) and bagged biochar (29.5 µg/L) were significantly greater that the control without biochar. However, among the PN treatments, we saw biochar treatments being significantly less than PN treatments without biochar added to the bottles.

Total microcystin concentrations in this bioassay were relatively high, with means ranging between 5.6 µg/L and 15.1 µg/L. Controls with free and bagged biochar (10.17 µg/L) had significantly greater microcystin concentrations than the control without biochar (3.6 µg/L). However, in the nutrient addition treatments, PN treatments with free biochar (9.67 µg/L) and bagged biochar (6.23 µg/L) were significantly less than the PN treatments without biochar (15.1 µg/L).

Mean PC RFUs in this bioassay ranged between 2882 to 10,287 RFUs. The controls were all significantly different from each other, with controls with free biochar having the highest PC concentrations (10,287 RFUs) followed by the controls with bagged biochar (3975 RFUs) and the control without biochar (2882 RFUs). Nutrient addition treatments without biochar (8767 RFUs) were not significantly different from the control with free biochar, nor were the PN treatments with bagged biochar (3123 RFUs) and with free biochar (4018 RFUs) significantly different from the companion control treatments. Mean CHL-a RFUs ranged more than two times greater than PC concentrations across all treatments indicating that these experiments were not dominated by cyanobacteria.

Bioassay 7

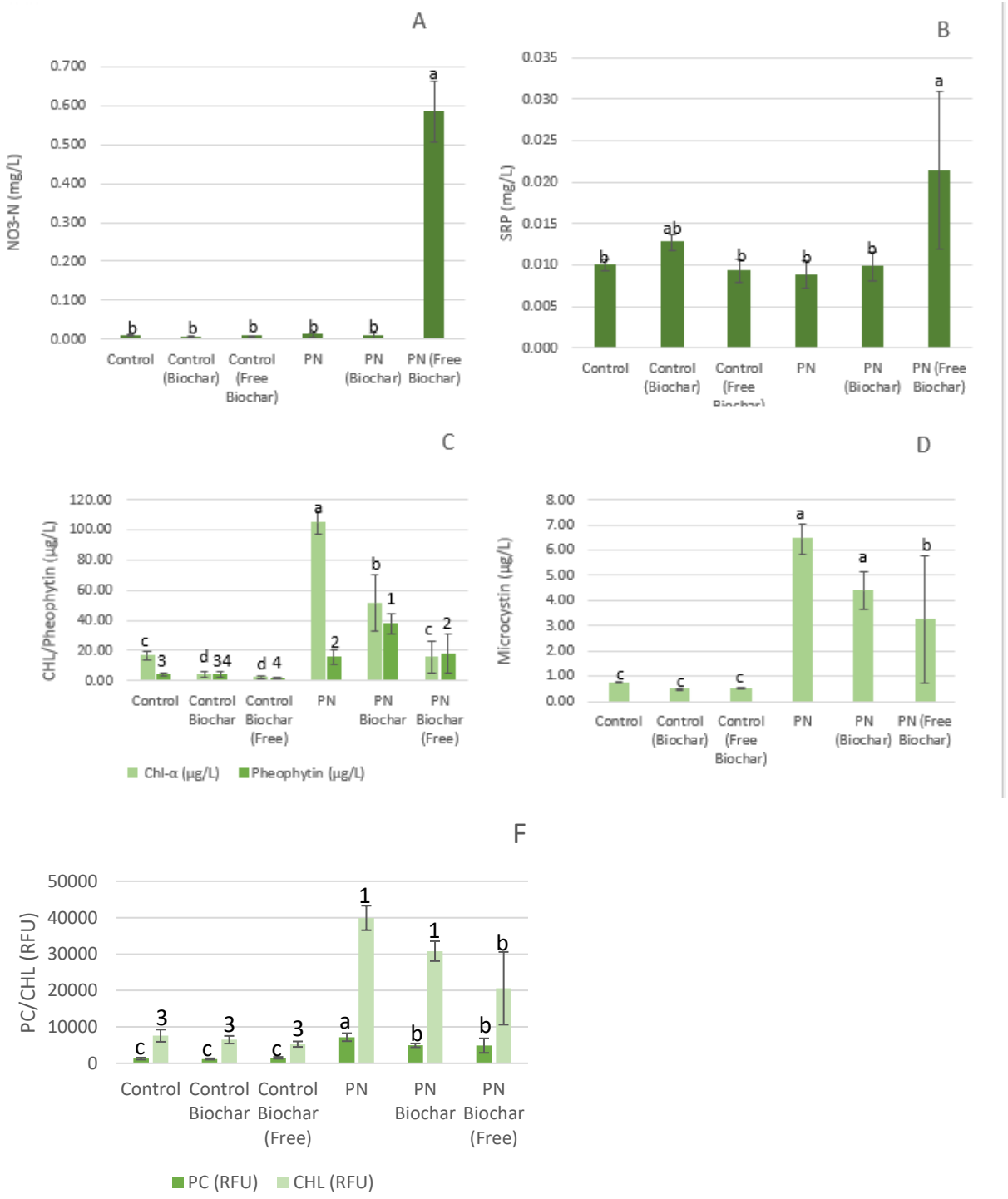

Figure 8. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C), Microcystin (D) and Phycocyanin and Chlorophyll-a RFUs (F) in lake water after 7 days of incubation at 30.3 °C on July 6, 2022.

Like bioassay 6 both double bagged and free biochar treatments were administered in this experiment creating a total of 24 experimental units. In the free biochar treatments, 1g/L of biochar was emptied into each of the bottles, and in the bagged treatments, 1g/L of biochar was placed inside of doubled permeable sacks and suspended inside of the bottles. In this experiment, free biochar treatments were cloudy and settled to the base of the bottles, preventing any light from passing through from the source below. The double bagged treatments remained relatively clear in comparison with the free biochar treatments, however there were still some fine particles settling out of the sacks to the bottom of the bottles.

Mean nitrate-N concentrations ranged between 0.006 to 0.585 mg/L. None of the treatments were significantly different, apart from the free biochar treatment which was significantly higher than all other treatments at 0.58 mg/L. These results were not consistent with previous bioassays.

After incubation SRP concentrations were generally not significantly different across all treatments, apart from free biochar experiments, where controls all ranged between (0.009 – 0.013 mg/L) and nutrient addition treatments with bagged biochar and without biochar ranged between (0.009- 0.021 mg/L). Mean concentrations ranged between 0.009 and 0.02 mg/L across all treatments.

Chlorophyll-a concentrations proved to be significantly less in biochar treatments after seven days and greater in companion treatments without biochar added. Controls with free biochar (2.06 µg/L) and bagged biochar (3.93 µg/L) were not significantly different. However, both biochar controls were less than the controls without biochar (16.21 µg/L). The nutrient addition treatments followed a similar trend. With PN treatments without biochar had highest concentrations of chlorophyll-a pigment (104.78 µg/L) followed by the bagged biochar treatments (51.52 µg/L) and finally the free biochar treatments (15.54 µg/L).

Mean total microcystins across all controls were 0.48 to 6.45µg/L. PN addition treatments had the highest MC concentrations as expected. However, among the PN treatments, there were significant differences between free biochar treatments (3.26 µg/L) and non-biochar PN treatments (6.45 µg/L). There was no significant difference between bagged biochar treatments (4.339 µg/L) and PN treatments without biochar.

Mean PC concentrations ranged between 1185 RFUs and 7171 RFUs across all treatments. All PC controls with bagged and free biochar (1185, 1502 RFUs) were not significantly different from controls without biochar (1283 RFUs). Nutrient addition treatments were significantly greater than the controls. PN treatments without biochar had significantly the highest PC concentrations (7171 RFUs) followed by bagged biochar (4938 RFUs) and free biochar treatments (4861 RFUs). Mean CHL-a concentrations ranged between 5259 and 39,925 RFUs. CHL-a concentrations were at least six times greater than PC concentrations in each respective treatment, indicating that these experiments were not dominated by cyanobacteria.

Bioassay 8

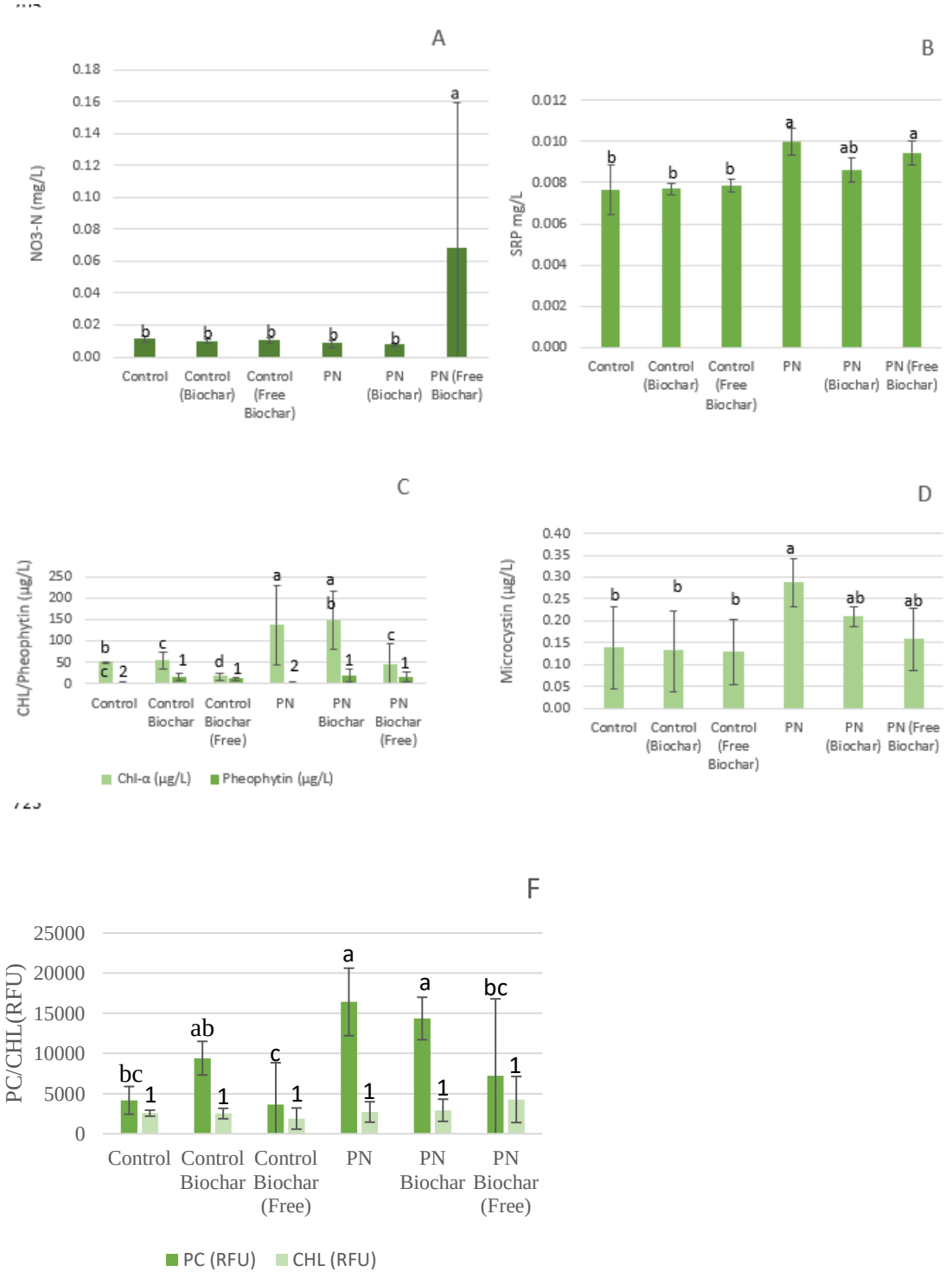

Figure 9. NO_3-N (A), SRP (B) Chlorophyll-a and Pheophytin (C), Microcystin (D) and Phycocyanin and Chlorophyll-a RFUs (F) in lake water after 7 days of incubation at 30.4°C on August 3, 2022.

Biochar was applied as a powder (1g/L) in the free biochar treatments and placed in single bagged mesh sacks in the bagged treatments. Free biochar treatments were expectedly cloudy, and single bagged treatments were visibly less cloudy with some particles sinking to the bottom of the bottles. This could have possibly inhibited the amount of light reaching the cyanobacteria and algae in the bottles, as the light source was below the bottles.

After seven days, mean nitrate-N concentrations across all treatments ranged from 0.008 to 0.068 mg/L. There was no significant difference across all treatments apart from the free biochar treatments (0.068 mg/L) as expected. Free biochar treatments showed higher concentrations of N than all other treatments.

Mean SRP concentrations ranged between 0.008 and 0.01 mg/L. All controls with and without biochar were not significantly different and had mean SRP concentrations close to 0.008 mg/L. While the controls were different from the nutrient addition treatments, those PN treatments were also not significantly different from each other with means ranging between 0.009 and 0.010 mg/L. Concentrations across this experiment were generally low.

Mean chlorophyll-a concentrations in the final bioassay ranged between 1.2 µg/L and 91.7 µg/L. Average concentrations across the controls were highly variable and did not show any true pattern. However, in the PN addition treatments, free biochar treatments (51.4 µg/L) and bagged biochar treatments (67. µg/L) were significantly less than PN treatments without biochar (91.74 µg/L). Not surprisingly, there was also significantly less chlorophyll-a in the free biochar treatments than the bagged treatments.

Total microcystins ranged between 0.13 and 0.29 µg/L in this experiment. There was no significant difference between controls or between the controls and the nutrient addition biochar treatments. PN treatments without biochar had the highest MC concentrations in this experiment, which may have been due to more cyanobacteria present in those treatments.

Mean PC concentrations ranged between 3656 and 16,410 RFUs. Controls with free biochar (3656 RFUs and controls without biochar (4159 RFUs) were not significantly different. However, controls with bagged biochar had the highest PC (9410 RFUs) concentrations among the controls, which was significantly different from free biochar treatments, but not different from controls without biochar. Nutrient addition treatments without biochar (16,410RFUs) and with bagged biochar (14,354 RFUs) were not significantly different from each other but were significantly greater than PN treatments with free biochar (7219 RFUs). CHL-a were not significantly different across all treatments, with mean concentrations ranging from 1896 and 4266 RFUs. These experiments had an initial PC: CHL ratio of 1.6196, and consistently higher concentrations of PC than CHL-a suggesting that these samples were cyanobacteria dominant.

Discussion

Biochar and Nutrient Uptake from Aqueous Solution

Biochar has many beneficial agronomic uses. These include the ability to adsorb and retain contaminants such as metals and other compounds such as nutrients from soil and aqueous solutions (Luo et al., 2022, Brewer et al,2014), maintain soil fertility and health through nutrient retention (Crombie et al., 2013) and has uses as a soil amendment to improve soil fertility and increase crop yields (Lehmann et al., 2015). Recently biochar has been promoted as a means to adsorb nutrients from aqueous solutions such as ponds (Lui et al., 2016), lakes (Xu et al., 2019) and even wastewater (Takaya et al., 2016).

However, we were not able to show that biochar, in its untreated or non-activated state, readily removed nutrients and metals (NH_4, NO_3, SRP and Zn) from aqueous solution. Neither the sorption experiments, nor the bioassays suggested that nutrients were readily adsorbed by biochar. In our experiments, we saw desorption of nutrients into solution, or little sorption. Sorption across all experiments ranged between 0.03% and 8.91 %. Bioassays showed that dissolved nutrient concentrations were often greater in bottles with biochar added relative to the bottles without biochar. This was likely due to fine biochar particles interfering with the colorimetric analysis, desorption of nutrients from the biochar and, or algal and cyanobacterial uptake of nutrients in the bioassay.

Several other studies have also shown that untreated biochar readily removes nutrients and other contaminants from aqueous solutions (Lui et al., 2013, Mohan et al., 2014 & Zhang et al., 2020). A study showed that biochar derived from scorn cobs had maximum nitrogen removal capacity of 0.08mg/g at an initial concentration of 5 mg/L and biochar derived from garden wood had maximum removal of phosphorus of 0.036 mg/g in wastewater at an initial concentration of 5mg/L (Lui et al., 2013).

However, there are some studies that have shown biochar to be effective in nutrient adsorption when activated (Wang et al., 2019). Research showed that biochar that had been modified with different combinations of acids (Wang et al., 2019 & Zhang et al., 2019) had sorption of NH_4 and SRP up to 90.7 and 22.8% respectively. Other studies showed that biochar that had been activated using ethanol had even higher removal efficiency (Xu et al., 2016), with cadmium removal of up to 96%. While our experiments did not reflect that biochar might have removed nutrients and metals from aqueous solution, biochar when activated might be able to remove nutrients from aqueous solutions. However, we wanted to test biochar as marketed for lake management (i.e, untreated or not activated). Biochar not activated by chemical treatment was not effective at nutrient and Zn removal from water.

Biochar and Lake Water Quality

Biochar has not been widely used as a lake treatment option, however few studies have examined the possibility of biochar for the reduction of algae and cyanobacteria in freshwaters (Kiani et al., 2023 & An et al., 2019). Most of its uses in water quality and lake remediation are centered around sorption and removal of nutrients, metals, contaminants, and toxins(Table 1).

However, in our bioassays, while sorption was not generally observed, results showed reduced chlorophyll-a in biochar treatments. During the incubation period the samples were placed under a 10 h light and 14 h dark cycle, and the light source was fixed to the bottom of the incubator. In our free biochar treatments, most of the biochar settled to the bottom of the bottles. We suspected that this may have inhibited light from reaching the cyanobacteria and algae within the water in the bottles. However, in our bagged biochar treatments we still observed reduced chlorophyll-a concentrations, particularly in our nutrient addition treatments with

biochar. While we do not believe the reduced chlorophyll-a concentrations were due to nutrient adsorption, it may have been due to organic amendment effects.

Conclusion

While biochar has been marketed as an option for nutrient and contaminant sorption, our results did not show that biochar readily absorbed nutrients and metals from aqueous solutions. In some cases, we saw increased concentrations of nutrients in both the sorption experiments and the bioassays.

In the bioassays we also did not observe any trend or relationship between total microcystins and biochar, as microcystin concentrations were highly variable across all treatments. There was some relationship between algal biomass and biochar, but we generally saw decreased chlorophyll-a across biochar treatments in the bioassays. However, results were too variable to conclude that biochar did affect algal growth.

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