Title

The Role of Host Microbiomes in Modulating Phytoplankton Community Dynamics and Ecosystem Functioning Under Abiotic Stressors

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The Role of Host Microbiomes in Modulating Phytoplankton Community Dynamics and Ecosystem Functioning Under Abiotic Stressors

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Nikki Michaela Mercer

Committee in charge:

Professor Sara Jackrel, Chair
Professor Diana Rennison
Professor Jonathan B Shurin

2022

The Thesis of Nikki Michaela Mercer is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2022

DEDICATION

This thesis is dedicated to everyone who has ever inspired me to pursue a master's degree in the first place and to those who motivated me to complete it in its entirety as it was not an easy journey. Without their support, I wouldn't be where I am today. This thesis is also dedicated to all the species that call this planet home. This work will help me to pursue my dream of helping and protecting animals, as their mere existence in this world is invaluable.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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I would like to express the deepest appreciation for my principal investigator, Professor Sara Jackrel, who has provided me with endless academic support throughout our professional relationship. She brings a wealth of enthusiasm and passion to research, which motivated us to set ambitious research goals. Her scientific expertise is inspiring, and I am forever grateful to have had the opportunity to learn under her guidance.

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Lastly, I would like to give a special thanks to my friends, family, and partner, Jonney Ramos, for their continuous support throughout this whole learning process. Their love and understanding always encourage me to follow my dreams, no matter how big or small they may be.

ABSTRACT OF THE THESIS

The Role of Host Microbiomes in Modulating Phytoplankton Community Dynamics and Ecosystem Functioning Under Abiotic Stressors

by

Nikki Michaela Mercer

Master of Science in Biology

University of California San Diego, 2022

Professor Sara Jackrel, Chair

With increasing anthropogenic stressors affecting aquatic systems, it is becoming crucial to understand what factors promote and maintain phytoplankton populations and communities. Previous research shows that variation within phytoplankton microbiomes affects pairwise species interactions, but the full extent of the impacts that host microbiomes have on species-rich algal communities is not known. Our research aims to test if greater diversity in host-associated microbiomes increases algal community diversity and ecosystem functioning. Furthermore, our research goal is to determine if increased diversity in host-microbiomes will have a stronger

effect on these metrics under more stressful conditions, including nutrient-depleted and warmer environments. This was tested by growing cultures consisting of five species of green eukaryotic algae in varying temperature, phosphorus, and microbiome diversity treatments for six weeks. Overall, our data did not support our hypotheses as cultures inoculated with the highest microbiome diversities did not promote the highest levels of algal community richness or biomass. Additionally, higher bacterial diversity did not mitigate the negative effects of phosphorus or temperature stress for algal communities. Our work empirically concludes that host microbiome composition is a critical factor that influences algal community dynamics and ecosystem functioning, and that it is environmentally dependent. This work develops our understanding of the various biotic and abiotic factors that govern species community composition, which will allow us to better predict how anthropogenic disturbances may affect our natural aquatic systems.

INTRODUCTION

Uncovering the intricacies of Earth's ecological systems is necessary to advance our understanding of how our actions are impacting natural habitats. With more than 71% of our planet's surface covered in water, understanding what factors govern primary productivity in aquatic systems is essential (Sonune & Ghate, 2004). As phytoplankton are the base of the aquatic food web, their community composition, productivity and abundance will ultimately affect all other trophic levels, including humans (Winder & Sommer, 2012). Phytoplankton productivity will ultimately affect the organisms that we rely on for food and our livelihoods.

Understanding what promotes and maintains primary productivity in aquatic systems is becoming of paramount importance as biodiversity loss and anthropogenic climate change are threatening global phytoplankton populations. For instance, the El Niño-Southern Oscillation (ENSO) refers to rising sea surface temperatures in the central and eastern tropical Pacific Ocean (L'Heureux, 2014). This warming event results in reduced coastal upwelling of nutrients in these regions, along with regions that extend further than 50 kilometers from the coast (Benson et al., 2002). This significant decrease in available nutrients in the photic zone results in a bottom-up cascade. The lack of nutrients results in a diminished population of phytoplankton which leads to a decline in abundance of the remaining higher trophic levels (Bunnell et al., 2014). An example of this can be seen in the 2015 ENSO where the reduction of primary producers and increased sea surface temperature resulted in a 52% decline of California sea lion populations and a 61.7% decline in their pup populations, along with a notable drop in their pup body mass (Elorriaga-Verplancken et al., 2016). As climate warming will increase as the global human population rises, the frequency and intensity of events associated with El Niño will also simultaneously increase (A. Timmermann et al., 1999).

Eutrophication is another anthropogenic disturbance that is affecting primary productivity and ecosystem functioning. There are many sources of excess nutrients in aquatic habitats, but much of the excess nitrogen and phosphorus in water bodies originate from fertilizer runoff from agricultural and aquacultural practices, poor sewage disposal, industrial waste discharge, and extreme natural events such as flooding, which bring in excess nutrients from terrestrial to aquatic ecosystems (Díaz, 2010; Nixon, 1995). Eutrophication can ultimately result in diminished primary productivity and the diversity of other trophic levels. Excess limiting nutrients can allow algae to grow at extremely fast rates, resulting in algal blooms. These blooms can become so dense that they inhibit light penetration beneath them, resulting in mortality among aquatic plants and corals (Zohdi & Abbaspour, 2019). In addition, algal blooms sequester much of the existing bioavailable nutrients, leaving low concentrations of nutrients for the phytoplankton themselves and therefore causing algal mortality. The decomposition by bacteria of the highly abundant dead algae takes up all the available oxygen in the water column, creating anoxic conditions called "dead zones," which results in additional mortality among organisms inhabiting this oxygen-depleted environment (Diaz & Rosenberg, 2008). This highlights how eutrophication poses negative impacts on entire aquatic ecosystems. Researchers have also found that eutrophication reduced the beta diversity of phytoplankton communities, due to intensified stress and declining environmental variation, resulting in negative food web effects (Y. Li et al., 2022).

Nutrient limitation can also result from anthropogenic disturbance, such as via the introduction of dreissenid mussels. These mussels have been introduced by humans through ballast water discharge and the movement of mussel-tainted recreational ships. Dreissenid mussels affect the ecosystem they inhabit through both top-down and bottom-up controls. As a top-down control, mussels feed on organic material suspended in the water column, including phytoplankton. These mussels also employ bottom-up control by immobilizing phosphorus in the

nearshore benthic zone, thus competing with offshore phytoplankton communities for this limiting nutrient (J. Li et al., 2021). These top-down and bottom-up processes alter phytoplankton and nutrient levels, resulting in oligotrophic conditions (Bunnell et al., 2014; Engevold et al., 2015). Human activities can therefore exacerbate nutrient limitation, which can cause declines in phytoplankton productivity and diversity (Falkowski et al., 1992; Ogawa & Ichimura, 1984). Therefore, both nutrient-poor and nutrient-rich conditions can limit the productivity and diversity of algal communities, resulting in negative effects in the aquatic food web.

Anthropogenic disturbances are causing declines in global biodiversity, including on a bacterial scale. For example, climate warming has been found to decrease soil microbial diversity by reducing the taxonomic richness of bacteria, fungi, and protists (Wu et al., 2022). Eutrophication has also been found to reduce soil bacterial species richness and diversity (Wang et al., 2018). Similar trends of bacterial diversity significantly declining with eutrophication has been found in aquatic systems (Tang et al., 2021). Conversely, it has also been found that bacterial diversity is positively correlated with trophic status, up until hypereutrophic conditions (Kiersztyn et al., 2019). This offers insight into the complex linkage between bacterial diversity and nutrient levels, but it highlights that peak aquatic bacterial diversity may emerge at intermediate nutrient levels (Kassen et al., 2000). Overall, both nutrient-poor and nutrient-rich conditions can limit the diversity of bacterial communities, which may reduce ecosystem functioning.

Declines in bacterial diversity caused by anthropogenic disturbances may have further cascading implications on the function and community dynamics of their host organisms. For example, anthropogenic sea surface warming in the Mediterranean has elevated mortality rates in marine invertebrates, including the sponge *Ircinia fasciculata*. Elevated water temperatures have

been found to induce unstable shifts in the symbiotic host-associated bacterial communities, resulting in health declines and mortality among marine sponges (Blanquer et al., 2016). This study highlights that microbiome composition affects the fitness of the host species, as the host species undergoes a health decline without its stable symbiotic relationship with the bacteria. Our study will expand on this concept as we aim to understand the role of host microbiome composition and diversity in modulating phytoplankton community composition and biomass. Phytoplankton are a valuable model system for testing this idea because they have been the focal organisms for many ecological theories, regulate key ecological functions, and house bacterial communities that can be manipulated under controlled conditions (Jackrel et al., 2021).

Microbes and eukaryotic hosts have a large array of interactions that range from mutualism and commensalism to predation, competition, and parasitism. These relationships take place in the microscale region encompassing a phytoplankton cell called the phycosphere. The phytoplankton cell itself can change the oxygen and pH values, as well as the concentrations of organic materials in this region. This modified environment enables microbes to migrate into this zone through chemotaxis and live alongside their algal host. These factors all contribute to an environment that attracts bacteria and allows them to thrive (Seymour et al., 2017).

Our work aims to understand how declining diversity within host microbiomes will affect host health, and in turn, algal community dynamics. Further, we aim to discover whether diversity of the host microbiome is particularly important when phytoplankton are experiencing abiotic stress, including elevated temperature and/or phosphorus limitation. Prior work in a different host-microbiome system suggests that the presence of diverse host microbiomes does in fact mitigate the negative effects of abiotic stressors on host health. Specifically, the presence of a microbiome has been found to aid the development of their dung beetle (*Onthophagus gazella*)

hosts, particularly when paired with abiotic stressors. This study found that beetles with high desiccation stress had decreased survival with 34.6% of the larvae with normal microbial communities making it to adulthood. However, those larvae under the same desiccation stress, but with a lessened microbial community, experienced a 100% death rate (Schwab et al., 2016).

Specific to our phytoplankton system, prior studies have found that microbiomes promote fitness among their algal hosts. For example, algal monocultures reach greater carrying capacities when grown with, versus without, their host microbiomes (Jackrel et al., 2021). Further, bacteria residing in the phycosphere can affect pairwise species interaction strength in phytoplankton. Specifically, presence of host microbiomes tended to decrease the host's susceptibility to algal competitors in biculture, suggesting that host microbiomes may promote species coexistence (Jackrel et al., 2020). Here we expand on this work by testing how microbiome diversity affects host interactions in more complex communities.

Evaluating how host microbiomes affect multispecies assemblages will advance our understanding of what factors limit or facilitate phytoplankton growth. This will shed light on the broader implications of declining microbial diversity and anthropogenic disturbances, such as nutrient limitation, eutrophication, and climate warming. Advancing our understanding of the independent and interactive effects of microbiome diversity and abiotic stressors on aquatic primary productivity may contribute towards the development of mitigation efforts to protect our aquatic systems from human impacts. Therefore, our objectives are to (1) test whether variation in host-associated microbiome diversity alters phytoplankton community composition and biomass and (2) test whether variation in host-microbiome diversity has a stronger effect on these metrics in stressful environments. We hypothesize that increased diversity in host-associated microbiomes will alter algal community richness, evenness, density, biomass, nutrient cycling,

nitrogen cell uptake, and carbon and nitrogen stable isotopic ratios. In addition, we hypothesize that increased diversity in host-microbiomes will have a stronger effect on these metrics in more phosphorus-depleted and warmer environments. To test these hypotheses, we carried out a 3 x 4 x 2 experimental design in which algal communities were exposed to three microbiome diversity treatments, four phosphorus treatments, and two temperature treatments.

This research advances the field with empirical evidence of the impacts of microbiome composition and diversity on phytoplankton community dynamics. These results will clarify how shifting microbiome composition and diversity will affect species-rich algal communities with and without abiotic stressors. More broadly, this study uses phytoplankton as a model to advance our understanding of how host microbiomes modulate host health and ecology in complex species-rich environments.

MATERIALS AND METHODS

Species Pool

Algal cultures were obtained from the University of Texas Culture Collection of Algae in 2011. Cultures were maintained as laboratory slants under 30 mol/m^2/s lighting at 15°C on COMBO medium. We used five species of eukaryotic green algae: *Chlorella sorokiniana*, *Monoraphidium minutum*, *Scenedesmus acuminatus*, *Selenastrum capricornutum*, and *Coelastrum microporum*. Each species was rendered axenic using ultrasonication and single-cell sorting onto solid media, as described in a previous study (Jackrel et al., 2021). Axenic cultures were then allowed to recruit bacterial communities from an experimental pond facility at the University of Michigan's E. S. George Reserve (ESGR) in Pinckney, MI, USA as described by the same study (Jackrel et al., 2021). We used algal cultures that were inoculated with bacterial communities originating from two of the naturalized experimental ponds that were previously shown to have significantly different bacterial communities based on 16S rRNA sequencing. These two ponds were referred to as Pond 2 and Pond 3 (Jackrel et al., 2021).

Experimental Design and Set Up

To determine media recipes that would reflect the natural range of nutrient conditions, from oligotrophic to hypereutrophic, that are typically found in freshwater lakes, we analyzed total dissolved phosphorus and nitrogen of five types of media through the Marine Chemistry Lab at the University of Washington. We made our five media types by modifying the standard COMBO media recipe to reflect a nutrient gradient as described in prior work (Danger et al., 2007; Kilham et al., 1998; O'Donnell et al., 2013). Based off our total phosphorus and nitrogen

preliminary measurements, we made our medias using the standard COMBO protocol with the exceptions of the following: oligotrophic media - 10% of the $NaNO_3$ and 0% of the K_2HPO_4 stock solutions; mesotrophic media: 10% of the $NaNO_3$ and 1% of the K_2HPO_4 stock solutions; eutrophic media: 10% of the $NaNO_3$ and 2% of the K_2HPO_4 stock solutions; and hypereutrophic media: 10% of the $NaNO_3$ and 10% of the K_2HPO_4 stock solutions.

We grew 15 algal stock cultures in full strength COMBO and incubated them at 18°C at 80 RPM under 81 μE lighting set to a 16:8 h light-dark cycle. These stocks included the axenic strain of each of the five algal species, and a xenic strain of each species originating from Pond 2, and a xenic strain of each species originating from Pond 3.

We used these stocks to implement a 4 x 3 x 2 experimental design to test for the independent and interactive effects of phosphorus, microbiome diversity and temperature on algal community composition and function. Our experimental design consisted of 4 phosphorus treatments (oligotrophic, mesotrophic, eutrophic, hypereutrophic), 3 microbiome diversity treatments (low, medium, high), and 2 temperature treatments (ambient and elevated) for a total of 120 experimental flasks. Experimental flasks were filled with 100 mL of COMBO media of the corresponding phosphorus level and inoculated with the same five-species axenic algal community at a total density of 11,798 cells/mL. The intention was to have the five-species axenic algal community inoculated at a total density of 10,000 cells/mL, with 2,000 cells/mL of each species in each flask. However, due to an error, *Chlorella sorokiniana*, *Monoraphidium minutum*, *Selenastrum capricornutum*, and *Coelastrum microporum* were all inoculated at 2,000 cells/mL, but *Scenedesmus acuminatus* was inoculated at 3,798 cells/mL. This initial inoculation error did not halt the progression of the experiment as this error was consistent for all the treatments. Inoculation volumes were determined by measuring cell densities of the five axenic

algal stock cultures using a hemocytometer and microscope. To create our microbiome diversity treatment, we next created bacterial filtrates from each of the ten xenic algal stocks. We also created presumably axenic 'bacterial filtrates' from the five axenic stocks as a control for our low microbiome diversity treatment. We created these filtrates using sonication and a separation-by-centrifugation process. To execute this, we processed 100 mL from each of the ten xenic algal stocks and each of the five axenic stocks. These stocks were sonicated on ice at 20 W for 30 seconds. This was repeated three times with 1-minute rests between sonications. Then the tubes were centrifuged at 900 g for 15 minutes. The supernatant was then filtered through a 25 mm 3.0 μm filter to remove any remaining algal cells into media bottles. The filtrates from each algal species were combined by pond, so all 5 bacterial filtrates from Pond 2 were combined into one media bottle, all 5 bacterial filtrates from Pond 3 were combined into a second media bottle, and all 5 "bacterial filtrates" from the axenic cultures were combined into a third media bottle.

To create our microbiome diversity treatment, we spiked 30 μl of a bacterial filtrate into our 120 flasks of five-species axenic algal communities. To generate our low microbiome diversity treatment, we spiked our flasks with 30 μl of the axenic filtrate. To create our medium microbiome diversity treatment, we spiked our flasks with 30 μl of the Pond 3 filtrate. To create our high microbiome diversity treatment, we spiked our flasks with 15 μl of the Pond 2 filtrate and 15 μl of the Pond 3 filtrate. We then collected the remainder of each of our bacterial filtrates onto 47 mm 0.22 μm nitrocellulose filters, snap froze them in liquid nitrogen, and stored them at -80°C for microbial sequencing.

To measure the initial algal cell stoichiometry of the axenic stocks, the axenic cell pellets were cleaned of residual bacterial cells and COMBO media by a series of separation-by-centrifugation steps. First, each pellet was resuspended into 75 mL of a sterile NaCl saline and

vortexed for 30 seconds. Next, the resuspended cultures were pelleted and the supernatant was discarded. This resuspension and pelleting process was repeated again for each axenic algal cell pellet. A final 75 mL of sterile NaCl saline was added to each pellet and the resuspended pellet was then collected onto a pre-weighed and pre-combusted (500°C for 4 hours in a muffle furnace) Whatman GF/F glass fiber filter. Filters were dried for at least 48 hours at 60°C in a drying oven, weighed to determine algal biomass, and stored for isotopic analysis. To determine the isotopic ratios of these axenic stocks, the dried axenic cell pellets were processed for their C:N ratios, $\delta^{15}N$, and $\delta^{13}C$ at the UC Davis Stable Isotope Facility.

Each flask was incubated in an 18°C room in their corresponding temperature location (either ambient or elevated) on a shaker at 80 RPM and under 81 μE lighting set to a 16:8 h light-dark cycle, with the spatial location of the flasks being randomized by phosphorus and microbiome treatment. The ambient temperature treatment consisted of no heat being added to the shaker table resulting in an average temperature of 23.25°C ± 1.667°C for the duration of the experiment. The elevated temperature treatment was created by placing heating mats on the shaker table to warm the flasks, which resulted in an average temperature of 28.36°C ± 2.328°C for the duration of the experiment **(Fig. S1.1)**.

To determine how algal community composition varied by treatment over the duration of the study, we preserved 1 mL from each flask with 5 μl of 25% glutaraldehyde. This sampling was repeated weekly for six weeks, storing samples at 4°C. After this sampling, we also refreshed nutrient conditions of each flask by removing 10 mL and adding 10 mL of sterile COMBO media, abiding by the correct phosphorus-level media being added to each flask.

At the end of the six-week study, we aimed to determine the total dissolved phosphorus and total dissolved nitrogen remaining in the spent algal media of each of the 120 flasks. To do

this, we pelleted 50 mL of each culture and transferred 40 mL of the supernatant to a pre-rinsed 60 mL nalgene polypropylene bottle. These 120 bottles were then stored at -20°C and processed for total dissolved nitrogen and phosphorus at the Marine Chemistry Lab of University of Washington.

We then aimed to determine algal cell stoichiometry from each flask. We used cell pellets remaining from the previous step and cleaned the cell pellet of residual bacterial cells and COMBO media by a series of separation-by-centrifugation and sonication steps. First, cell pellets were resuspended in 50 mL of sterile NaCl saline and vortexed for 30 seconds. Each resuspended culture was then sonicated on ice at 20 W for 30 seconds. This was repeated three times with 1-minute rests between sonications. Cultures were then pelleted and the supernatant was discarded. This resuspension and pelleting process was repeated again. A final 50 mL of sterile NaCl saline was added to each pellet and the resuspended pellet was then collected onto a pre-weighed and pre-combusted (500°C for 4 hours in a muffle furnace) Whatman GF/F glass fiber filter. Filters were dried for at least 48 hours at 60°C in a drying oven, weighed to determine algal biomass, and stored for isotopic analysis.

Total Dissolved Nitrogen and Dissolved Phosphorus Analysis

We wanted to look at how the concentrations of the total dissolved nitrogen and total dissolved phosphorus in the algal media varied across samples at Week 6, so we fit a three-way Analysis of Variance Model for these two metrics with the temperature, phosphorus, and microbiome diversity treatments as fixed effects (Chambers et al., 1992). Data for these two metrics passed the model assumption for homogeneity of variance (Fligner-Killeen Test, **Table**

S1.1), and deviated only moderately from normality as illustrated in Q-Q plots located in the Appendix **(Figure S2.1 (a-b); Tables S2.1-S2.2)**.

Isotopic Analysis

We wanted to assess how the C:N ratios, $\delta^{15}N$, and $\delta^{13}C$ in the dried algal biomass varied across samples at Week 6, so we fit a three-way Analysis of Variance Model for these three metrics with the temperature, phosphorus, and microbiome diversity treatments as fixed effects (Chambers et al., 1992). Data for these three metrics passed the model assumption for homogeneity of variance (Fligner-Killeen Test, **Table S1.1**), and deviated only moderately from normality as illustrated in Q-Q plots located in the Appendix **(Figure S2.1 (c-e); Tables S2.3-S2.5)**.

Algal Community Dynamics Analysis

In order to measure richness, evenness, and density across all samples at Week 6, either 400 cells or four full hemocytometer grids (3.6 μl), whichever came first, of each species in each sample were counted (Narwani et al., 2016). We wanted to look at how total density, Shannon's Diversity, species abundances, and weight of the final dried algal biomass varied across samples at Week 6, so we used the "vegan" package in RStudio for analysis (Oksanen et al., 2022; R Core Team, 2022; RStudio Team, 2022). We fit an Analysis of Variance Model for all of these metrics with the temperature, phosphorus, and microbiome diversity treatments as fixed effects (Chambers et al., 1992). Data for these metrics passed the model assumption for homogeneity of variance with the exception of the log mean abundance for *M. minutum* (Fligner-Killeen Test,

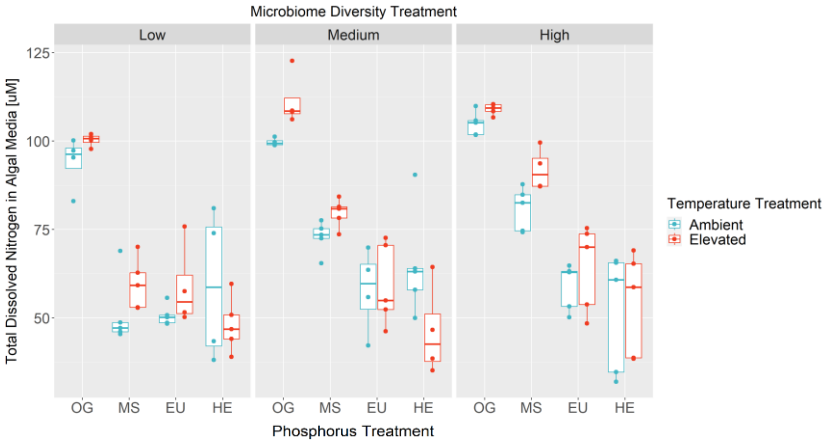
Table S1.1), and deviated only moderately from normality as illustrated in Q-Q plots located in the Appendix **(Figure S2.1 (f-l); Tables S2.6-S2.12)**.

All figures were generated using the "ggplot2" package in RStudio (R Core Team, 2022; RStudio Team, 2022; Wickham, 2016).

RESULTS

Nutrient Cycling

1a)



1b)

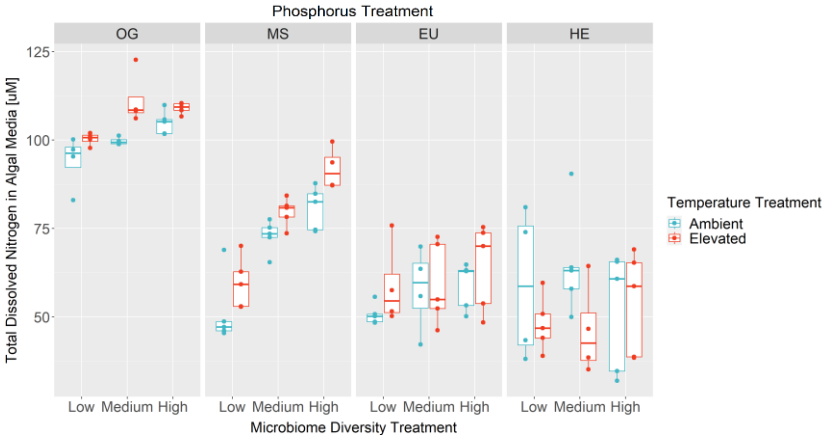
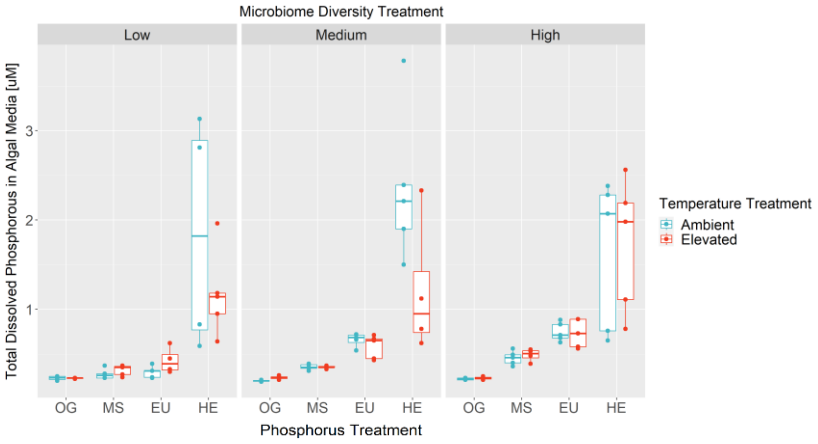


Figure 1 (a,b): Mean total dissolved nitrogen ([µM]) in the algal media of all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. Microbiome diversity treatments represent the level of bacterial diversity that was inoculated into each experimental culture at the beginning of the experiment. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,87} = 14.8$, $p < 0.0001$, Phosphorus-Level: $F_{3,87} = 132.9$, $p < 0.0001$, Temperature: $F_{1,87} = 2.1$, $p = 0.15$. See Table S2.1 for the complete analysis of variance table, including interactions.

2a)



2b)

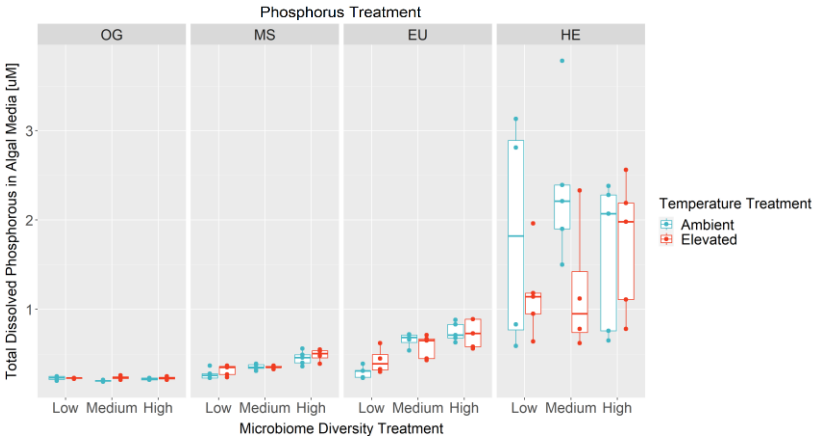


Figure 2 (a,b): Mean total dissolved phosphorus ([µM]) in the algal media of all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. Microbiome diversity treatments represent the level of bacterial diversity that was inoculated into each experimental culture at the beginning of the experiment. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,87} = 31.3$, $p < 0.0001$, Phosphorus-Level: $F_{3,87} = 335.2$, $p < 0.0001$, Temperature: $F_{1,87} = 3.4$, $p = 0.069$. See Table S2.2 for the complete analysis of variance table, including interactions.

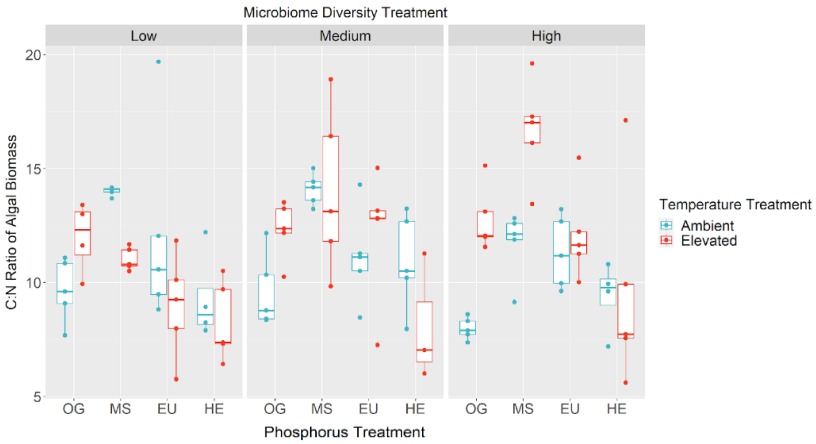
Total dissolved nutrients in the algal media at the six-week time point varied significantly by phosphorus level and microbiome diversity treatments **(Fig. 1)**. Dissolved nitrogen concentrations were negatively correlated with phosphorus level despite being added at a constant concentration for each of our four media types. Specifically, the oligotrophic treatment resulted in having nitrogen concentrations that were nearly two times greater than the concentrations found in the hypereutrophic treatments (Phosphorus-Level: $F_{3,87} = 132.9$, $p < 0.0001$). On the contrary, dissolved phosphorus concentrations were positively correlated with phosphorus level. This positive correlation was expected as phosphorus concentrations varied by trophic level in the original media types (i.e. dissolved P in our hypereutrophic media was about 32 times greater than that of our oligotrophic media). Over the course of the six-week experiment, this value decreased such that the hypereutrophic treatment exhibited dissolved phosphorus concentrations that were only about 7.5 times greater than those in the oligotrophic treatment (Phosphorus-Level: $F_{3,87} = 335.2$, $p < 0.0001$). This indicates that there was a 67.8% drawdown in the available phosphorus over the course of the six-week experiment in the hypereutrophic treatment. The proportion of this phosphorus drawdown decreases with each successive phosphorus level, except for the oligotrophic treatment, which actually experienced a 39.9% increase in the available phosphorus over the course of the six-week experiment. However, this may have been due to phosphorus contamination occurring when the oligotrophic flasks were inoculated with the algal stocks, which all originated from full-strength COMBO media.

Furthermore, the microbiome diversity treatment had a significant effect on total dissolved nitrogen and phosphorus. Specifically, high microbiome diversity caused about a 20% increase in the dissolved nitrogen concentrations when compared to the low microbiome diversity treatment (Microbiome Diversity: $F_{2,87} = 14.8$, $p < 0.0001$). Similarly, high microbiome diversity resulted in about a 33% increase in dissolved phosphorus concentrations when compared to the

low microbiome diversity treatment (Microbiome Diversity: $F_{2,87} = 31.3$, $p < 0.0001$). Lastly, there was a significant pairwise interaction between the temperature and phosphorus treatment for the total dissolved nitrogen in which nitrogen concentrations increased with elevated temperatures for all phosphorus treatments except for the hypereutrophic treatment (Temperature*Phosphorus-Level: $F_{3,87} = 4.0$, $p < 0.01$). These results suggest that varying abiotic stressors in conjunction with differences in phytoplankton microbiome diversity alter levels of total dissolved nitrogen and phosphorus found in the water column of species-rich algal communities.

Cell Stoichiometry

3a)



3b)

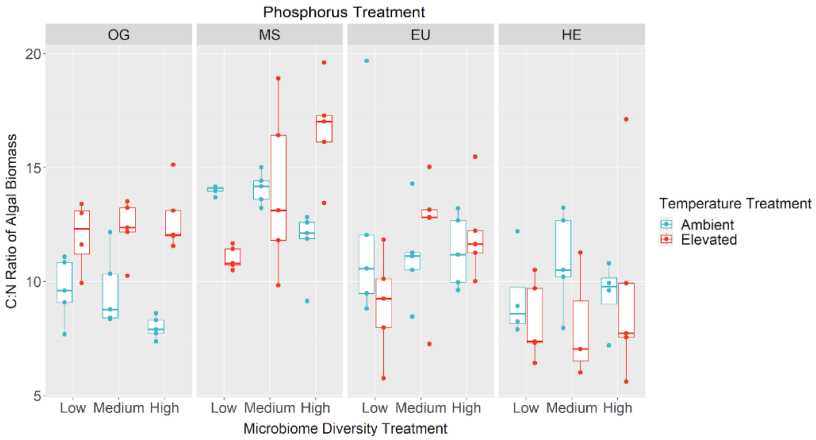
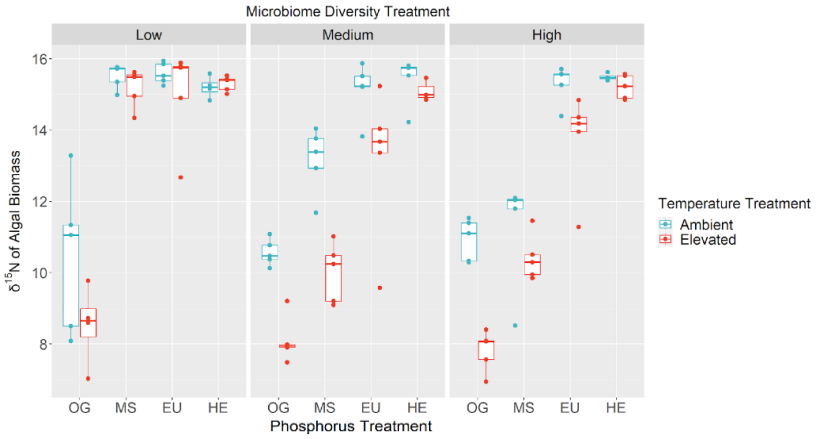


Figure 3 (a,b): Mean Carbon:Nitrogen of dried algal biomass from all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,91} = 2.3$, $p = 0.10$, Phosphorus-Level: $F_{3,91} = 18.2$, $p < 0.0001$, Temperature: $F_{1,91} = 2.3$, $p = 0.13$. See Table S2.3 for the complete analysis of variance table, including interactions.

Algal C:N was significantly affected by phosphorus level, with the mesotrophic conditions resulting in the highest C:N, which were about 20% and 45% greater than algal C:N in the eutrophic and hypereutrophic treatments, respectively (**Fig. 3**, Phosphorus-Level: $F_{3,91} = 18.2$, $p < 0.0001$). The microbiome treatment by itself was not significant, but it had a significant interaction with temperature (Microbiome Diversity*Temperature: $F_{2,91} = 8.1$, $p < 0.001$). On average, the magnitude that algal C:N increased from the low to high microbiome diversity treatment was amplified in elevated temperature conditions, particularly among mesotrophic and eutrophic conditions. Furthermore, the pairwise interaction between temperature and phosphorus treatments was significant (Temperature*Phosphorus-Level: $F_{3,91} = 5.3$, $p < 0.005$). Under oligotrophic conditions, C:N was positively correlated with temperature, but in hypereutrophic conditions, C:N was negatively correlated with temperature.

4a)



4b)

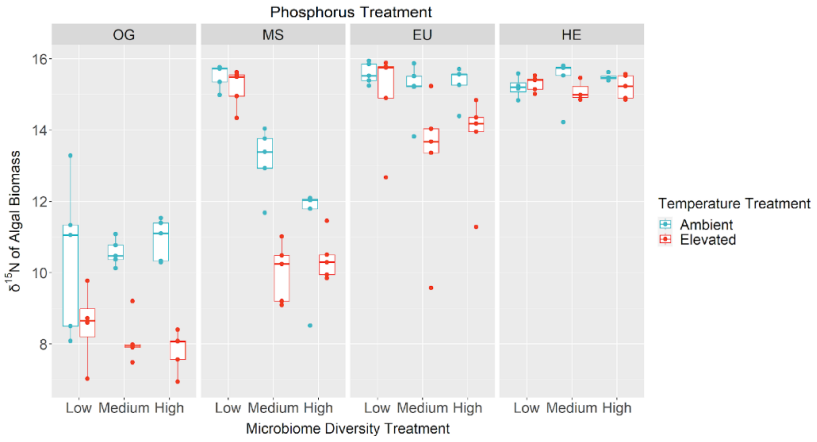


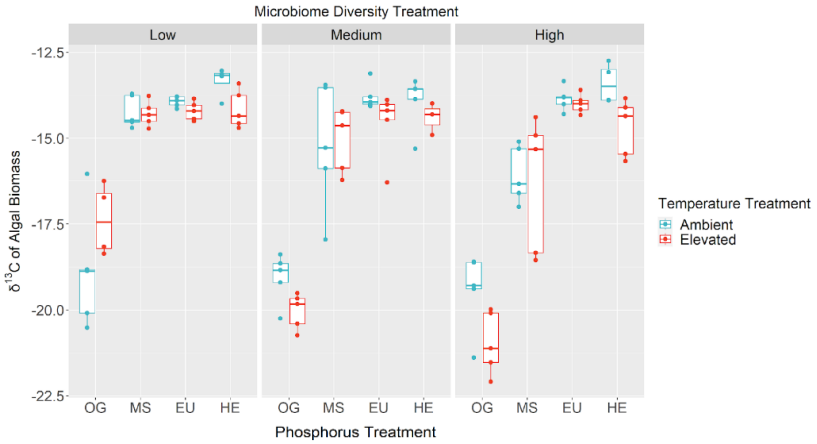
Figure 4 (a,b): Mean $\delta^{15}N$ of dried algal biomass from all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,91} = 25.8$, $p < 0.0001$, Phosphorus-Level: $F_{3,91} = 208.8$, $p < 0.0001$, Temperature: $F_{1,91} = 54.6$, $p < 0.0001$. See Table S2.4 for the complete analysis of variance table, including interactions.

Algal $\delta^{15}N$ was also affected by each of our three experimental treatments **(Fig. 4)**. On average, ambient temperatures had nearly 11% greater $\delta^{15}N$ values than elevated temperature

treatments (Temperature: $F_{1,91} = 54.6$, $p < 0.0001$). Algal $\delta^{15}N$ was also positively correlated with phosphorus level. Specifically, the hypereutrophic treatment had about 62% greater $\delta^{15}N$ values compared to the oligotrophic treatment (Phosphorus-Level: $F_{3,91} = 208.8$, $p < 0.0001$). Lastly, low microbiome diversity resulted in about 12% greater $\delta^{15}N$ values when compared to the medium and high microbiome diversity treatments (Microbiome Diversity: $F_{2,91} = 25.8$, $p < 0.0001$).

Additionally, there were significant interactions among these treatment effects on algal $\delta^{15}N$. For example, algal $\delta^{15}N$ was negatively correlated with temperature for all microbiome treatments, however the degree that $\delta^{15}N$ declined from the low to high microbiome diversity treatment was heightened in elevated temperature conditions (Temperature*Microbiome Diversity: $F_{2,91} = 4.7$, $p = 0.012$). Additionally, algal $\delta^{15}N$ declined with elevated temperatures for all phosphorus treatments, but the magnitude that $\delta^{15}N$ decreased from ambient to elevated temperatures was greater in the oligotrophic and mesotrophic treatments than the eutrophic and hypereutrophic treatments (Temperature*Phosphorus-Level: $F_{3,91} = 5.9$, $p < 0.005$). Algal $\delta^{15}N$ shifts across trophic levels were also highly context dependent on the microbiome diversity treatment (Phosphorus-Level*Microbiome Diversity: $F_{6,91} = 13.3$, $p < 0.0001$).

5a)



5b)

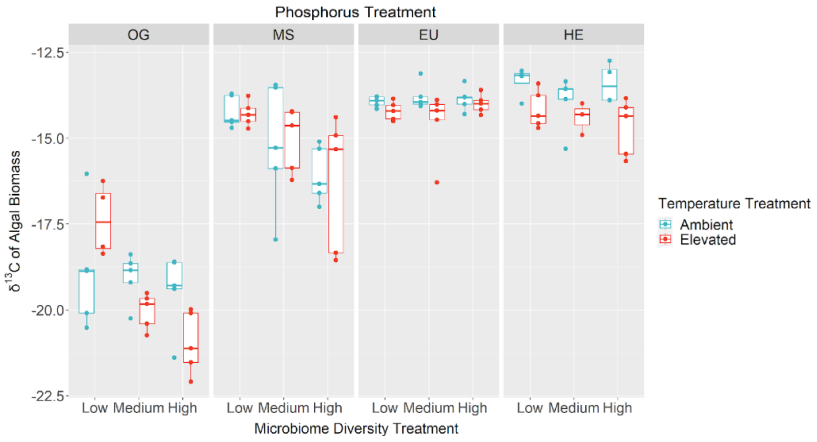


Figure 5 (a,b): Mean $\delta^{13}C$ of dried algal biomass from all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,91} = 12.0$, $p < 0.0001$, Phosphorus-Level: $F_{3,91} = 213.3$, $p < 0.0001$, Temperature: $F_{1,91} = 4.3$, $p = 0.042$. See Table S2.5 for the complete analysis of variance table, including interactions.

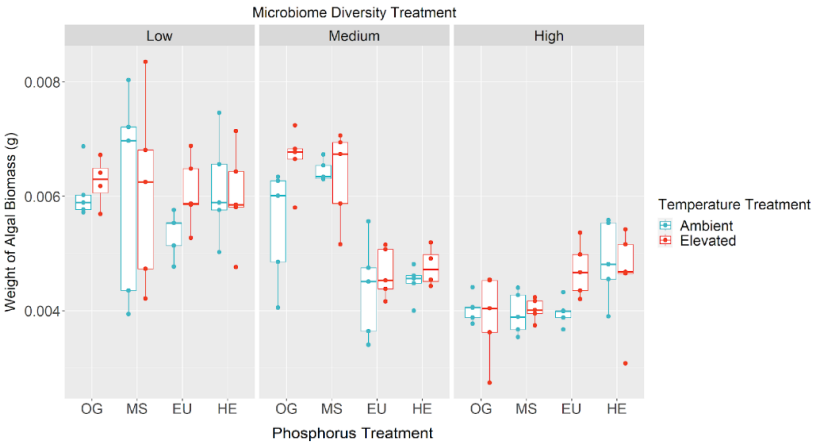
Algal $\delta^{13}C$ was also significantly affected by each of the three experimental treatments **(Fig. 5)**. These results parallel the trends found for $\delta^{15}N$. On average, ambient temperatures had

about 2.3% greater $\delta^{13}C$ values than elevated temperature treatments (Temperature: $F_{1,91} = 4.3$, $p = 0.042$). Algal $\delta^{13}C$ was also positively correlated with phosphorus level. Specifically, the hypereutrophic treatment had approximately 28% greater $\delta^{13}C$ values than the oligotrophic treatment (Phosphorus-Level: $F_{3,91} = 213.3$, $p < 0.0001$). Lastly, low microbiome diversity resulted in the largest $\delta^{13}C$ values, which were about 5% and 7% greater than the $\delta^{13}C$ values in the medium and high microbiome diversity treatments, respectively (Microbiome Diversity: $F_{2,91} = 11.96$, $p < 0.0001$).

The phosphorus and microbiome diversity treatments also had a significant interactive effect on algal $\delta^{13}C$ (Phosphorus-Level*Microbiome Diversity: $F_{6,91} = 3.6$, $p < 0.005$). $\delta^{13}C$ is higher with low microbiome diversity than intermediate and high microbiome diversities in oligotrophic and mesotrophic conditions, whereas this trend does not occur in eutrophic and hypereutrophic conditions. On average, the extent that $\delta^{13}C$ declines from the low to high microbiome diversity treatment is intensified in oligotrophic conditions. These results suggests abiotic stress and host microbiome composition drives variation in algal stoichiometry in species-rich communities.

Algal Community Dynamics

6a)



6b)

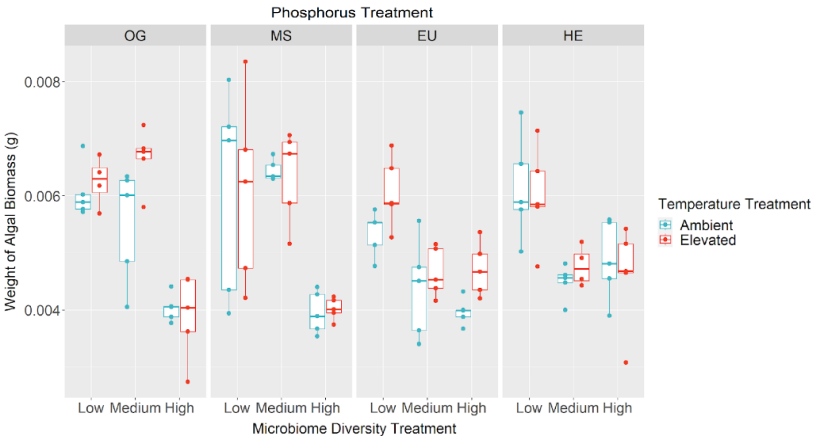
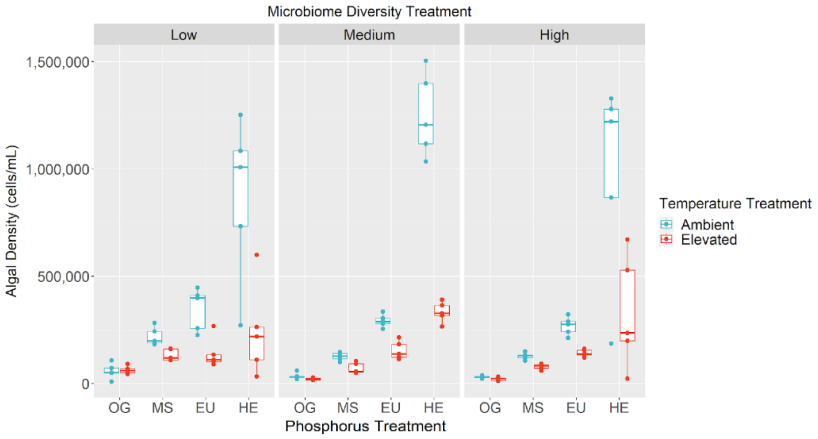


Figure 6 (a,b): Mean mass of the dried algal biomass (g) from all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,94} = 52.0$, $p < 0.0001$, Phosphorus-Level: $F_{3,94} = 3.9$, $p = 0.012$, Temperature: $F_{1,94} = 2.5$, $p = 0.12$. See Table S2.6 for the complete analysis of variance table, including interactions.

We found that total dried algal biomass was negatively correlated with microbiome diversity. Specifically, low microbiome diversity resulted in a nearly 41% increase in algal

biomass when compared to the high microbiome diversity treatment (Microbiome Diversity: $F_{2,94} = 51.98$, $p < 0.0001$). We also found a significant pairwise interaction between the phosphorus and microbiome diversity treatments affecting total dried algal biomass (Phosphorus-Level*Microbiome Diversity: $F_{6,94} = 7.1$, $p < 0.0001$). In eutrophic and hypereutrophic conditions, algal biomass declined an average of 22.5% with the transition from low to medium microbiome diversity. For oligotrophic and mesotrophic conditions, we found only a marginal difference in the final biomass between the low and medium microbiome diversity treatments, but there was an average 36.3% decrease in biomass with the transition from medium to high microbiome diversity. On average, the magnitude of algal biomass declines from the low to high microbiome diversity treatment was amplified in oligotrophic conditions.

7a)



7b)

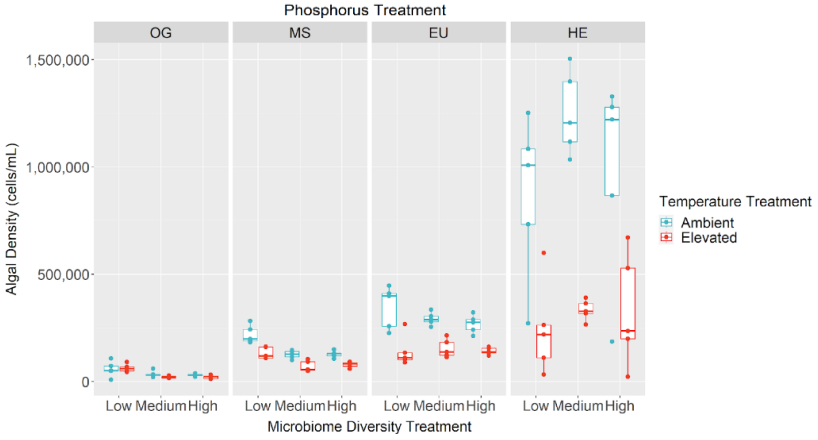
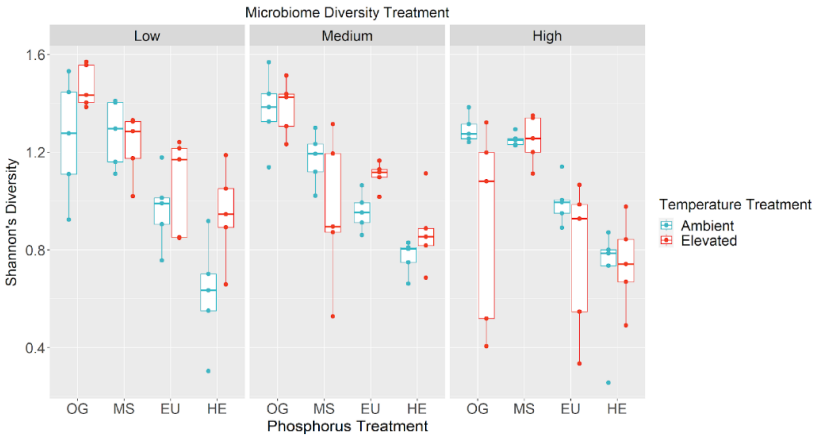


Figure 7 (a,b): Mean total algal density (cells/mL) of all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,96} = 3.7$, $p = 0.030$, Phosphorus-Level: $F_{3,96} = 144.1$, $p < 0.0001$, Temperature: $F_{1,96} = 59.6$, $p < 0.0001$. See Table S2.7 for the complete analysis of variance table, including interactions.

Total algal density (cells/mL) was also affected by each of the three experimental treatments **(Fig. 7)**. On average, ambient temperatures resulted in algal densities that were about

2.7 times greater than the densities found in the elevated temperature treatments (Temperature: $F_{1,96} = 59.6$, $p < 0.0001$). Algal densities were positively correlated with phosphorus level. Specifically, the hypereutrophic treatments resulted in densities that were about 17.6 times greater than the densities found in the oligotrophic treatments (Phosphorus-Level: $F_{3,96} = 144.1$, $p < 0.0001$). Furthermore, low microbiome diversity produced about a 5% increase in algal densities when compared to the high microbiome diversity treatment (Microbiome Diversity: $F_{2,96} = 3.65$, $p = 0.030$). The microbiome diversity treatment was also significant, but context dependent on phosphorus level (Microbiome Diversity*Phosphorus-Level: $F_{6,96} = 3.7$, $p < 0.005$). Densities were greatest with low microbiome diversity in oligotrophic and mesotrophic conditions, whereas densities in hypereutrophic conditions were much higher with intermediate and high microbiome diversity. In addition, the pairwise interaction between temperature and phosphorus treatments significantly affected total algal cell density (Temperature*Phosphorus-Level: $F_{3,96} = 7.7$, $p < 0.0005$). Algal density declined with elevated temperatures for all phosphorus conditions. In addition, the trend of densities being positively correlated with phosphorus level remains true for both ambient and elevated temperatures. However, the algal densities of oligotrophic, mesotrophic, and eutrophic phosphorus levels did not decline as drastically with elevated temperatures as observed in the hypereutrophic treatment. The density of the hypereutrophic treatment at ambient temperature is exceptionally higher than the densities of the other phosphorus conditions at ambient temperature, but it drops to a more similar density value as the other phosphorus treatments at an elevated temperature.

8a)



8b)

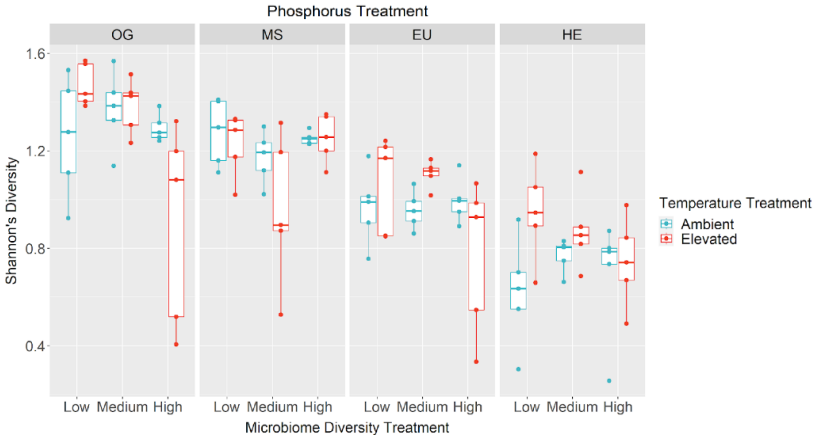


Figure 8 (a,b): Mean Shannon Diversity index of all experimental cultures consisting of 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. *(a) and (b) figures are complementary; they illustrate the exact same data but with different axes.* ANOVA-- Microbiome Diversity: $F_{2,96} = 4.2$, $p = 0.018$, Phosphorus-Level: $F_{3,96} = 44.6$, $p < 0.0001$, Temperature: $F_{1,96} = 0.04$, $p = 0.85$. See Table S2.8 for the complete analysis of variance table, including interactions.

Shannon Diversity of the algal communities was significantly affected by the phosphorus and microbiome diversity treatments (**Fig. 8**). In contrast to the positive correlation between

phosphorus and algal cell density, Shannon Diversity was instead negatively correlated with phosphorus level. Specifically, oligotrophic treatments resulted in about a 65% increase in the diversity metric when compared to the hypereutrophic treatment (Phosphorus-Level: $F_{3,96} = 44.6$, $p < 0.0001$). In addition, low microbiome diversity caused about an 11.7% increase in the diversity metric when compared to the high microbiome diversity treatment (Microbiome Diversity: $F_{2,96} = 4.2$, $p = 0.018$). The microbiome diversity treatment was also significant, but context dependent on temperature and phosphorus level. For example, the Shannon Diversity of the low and medium microbiome diversity treatments were positively correlated with temperature whereas the Shannon Diversity of the high microbiome diversity treatment decreased with elevated temperature. On average, the magnitude that the Shannon Diversity decreases from the low to high microbiome diversity treatment is heightened in elevated temperature conditions (Microbiome Diversity*Temperature: $F_{2,96} = 5.9$, $p < 0.005$). Shannon Diversity was also lower in oligotrophic and mesotrophic conditions with intermediate and high microbiome diversity, than with low microbiome diversity. On average, the magnitude that the Shannon Diversity declined from the low to high microbiome diversity treatment is intensified in oligotrophic conditions (Microbiome Diversity*Phosphorus-Level: $F_{6,96} = 3.0$, $p = 0.011$).

9)

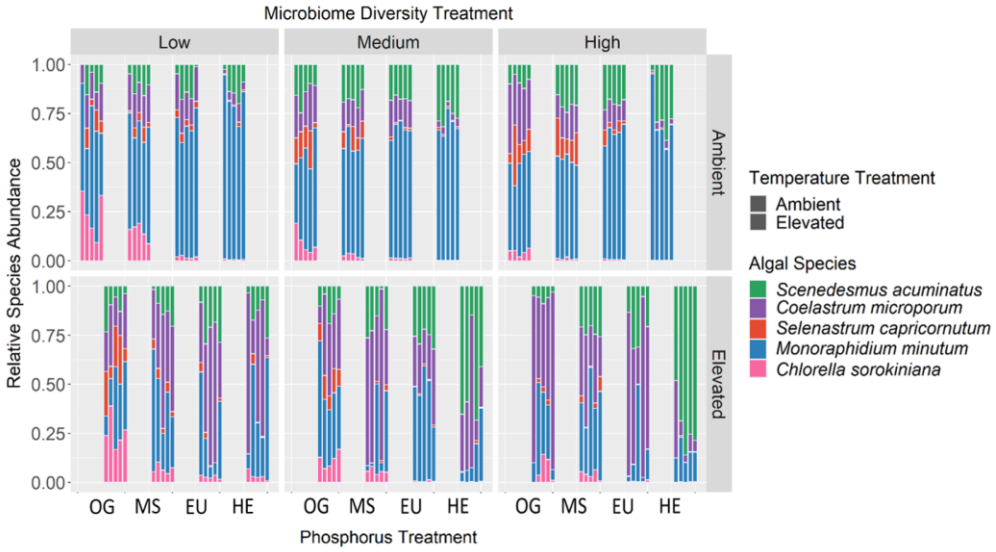


Figure 9: Relative species abundance of all experimental cultures inoculated with 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. See Tables S2.9-S2.12 for the complete analysis of variance tables, including interactions, for the abundances of the individual algal species.

10)

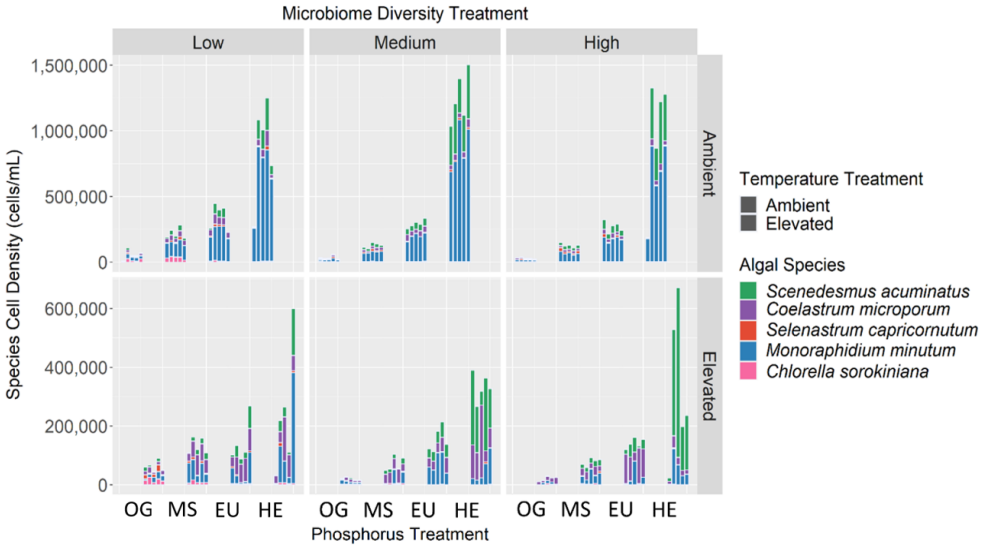


Figure 10: Species cell density (cells/mL) of all experimental cultures inoculated with 5 species of green eukaryotic algae that underwent the combination of three different treatments (Temperature, Phosphorus, and Microbiome Diversity). These results were taken from Week 6, the last time point of the experiment. For phosphorus treatments: OG = oligotrophic conditions, MS = mesotrophic conditions, EU = eutrophic conditions, and HE = hyper-eutrophic conditions. See Tables S2.9-S2.12 for the complete analysis of variance tables, including interactions, for the abundances of the individual algal species.

To explore shifts in algal community composition by treatment, relative species abundances and species cell densities were analyzed **(Fig. 9,10)**. Our treatments had several significant effects on species abundances. For instance, high microbiome diversity tended to lower the abundances of *C. sorokiniana, C. microporum,* and *S. capricornutum* compared to the low microbiome diversity treatment (*C. sorokiniana*: Microbiome Diversity: $F_{2,94} = 105.5$, $p < 0.0001$; *C. microporum*: Microbiome Diversity: $F_{2,96} = 4.2$, $p = 0.018$; *S. capricornutum*: Microbiome Diversity: $F_{2,85} = 11.3$, $p < 0.0001$). In addition, *C. microporum* and *S. acuminatus* abundances were positively correlated with phosphorus level, whereas *C. sorokiniana* abundances were negatively correlated (*C. microporum*: Phosphorus-Level: $F_{3,96} = 56.7$, $p < 0.0001$; *S.*

acuminatus: Phosphorus-Level: $F_{3,95} = 73.0$, $p < 0.0001$; *C. sorokiniana*: Phosphorus-Level: $F_{3,94} = 18.8$, $p < 0.0001$).

Furthermore, the pairwise phosphorus and microbiome diversity treatment interaction played a significant role on the abundances of a few of the algal species. *S. acuminatus* abundances increased with phosphorus level for all microbiome treatments; however, high microbiome diversity only had a considerable effect on increasing abundances of this species in the hypereutrophic condition (*S. acuminatus*: Microbiome Diversity*Phosphorus-Level: $F_{6,95} = 4.7$, $p < 0.0005$). Similarly, *C. microporum* abundances were positively correlated with phosphorus level with the exception that peak abundances occurred in the eutrophic treatment with low and high microbiome diversity. Low microbiome diversity resulted in greater *C. microporum* abundances than high microbiome diversity for all phosphorus conditions (*C. microporum*: Microbiome Diversity*Phosphorus-Level: $F_{6,96} = 4.2$, $p < 0.001$). Lastly, *S. capricornutum* abundances were also larger with low microbiome diversity than in high microbiome diversity for all phosphorus treatments except for the mesotrophic condition (*S. capricornutum*: Microbiome Diversity*Phosphorus-Level: $F_{6,85} = 2.5$, $p = 0.031$).

DISCUSSION

Our results illustrate that host microbiome diversity affects phytoplankton community composition and standing biomass, both independently and in interaction with abiotic stressors. Microbiome diversity was found to increase nutrient bioavailability, as measured by quantifying the total dissolved nitrogen and phosphorus in the water column, which supports the idea that phycosphere bacteria enhance nutrient recycling. Prior research has demonstrated this positive relationship between bacterial diversity and nutrient recycling in both terrestrial and aquatic systems. In an observational study, it has been shown that soil microbial diversity is positively correlated with multi-nutrient cycling (Jiao et al., 2021). For aquatic ecosystems, a correlative study found that greater prokaryote diversity was a hallmark of higher functioning coastal lagoons. These lagoons were characterized by increased prokaryote carbon conversion efficiency (Danovaro & Pusceddu, 2007). Our work provides empirical evidence corroborating the positive relationship between microbial diversity and the bioavailability of nutrients from a controlled manipulative experiment. The mechanism underlying this relationship is unknown, but may be due to higher diversity enabling use of a greater breadth of resources because greater microbial species richness typically correlates with a greater range of functional traits (Loreau, 2001). Another potential mechanism is that communities inhabited by a greater bacterial diversity may adjust more quickly to shifting environmental conditions. This ability of diverse microbial communities to adjust to new conditions may allow for increased ecosystem functioning, such as nutrient recycling (Danovaro & Pusceddu, 2007).

Synthesizing our dissolved nutrient and cell stoichiometry data, we find that as phosphorus concentrations increased, algal communities shifted from being phosphorus-limited to nitrogen-limited. The law of the minimum states that productivity cannot increase if one of the

essential nutrients is insufficient (de Baar, 1994). Supporting this idea, we see that with increased phosphorus concentrations, dissolved nitrogen becomes more limiting as algal cells are able to sequester more of the bioavailable nitrogen into their cells with increasing availability of phosphorus. Despite all experimental flasks containing the identical amount of nitrogen at the start of the study, total dissolved nitrogen was greatest in the oligotrophic treatment, successively going down to the least amount found in hypereutrophic treatments. Considering that the lowest algal C:N was observed in the hypereutrophic treatment, we can infer that this shift in bioavailable nutrients is in part due to assimilation of nitrogen into algal cells.

Unexpectedly, we found that the lowest microbial diversity promoted the highest algal community biomass, algal cell density, and Shannon Diversity of the algal community. This result does not support our hypothesis that increased diversity in host-associated microbiomes will increase algal community biomass, density, richness and evenness. We had based this hypothesis on our prior work, where we had found that the presence of a microbiome typically facilitated pairwise algal species coexistence and reduced competitive exclusion (Jackrel et al., 2020). Our current results highlight that there are more dynamics at play in species-rich algal communities that may alter the typically mutualistic role of host microbiomes that has been observed in algal monocultures and bicultures. Further, unlike the Jackrel et al. 2020 study of pairwise interactions, our research adds abiotic stressors, which may alter the strength and type of these algal-bacterial interactions. While both our prior and current studies sourced the same phycosphere communities, it is possible that these communities shifted over time in our long-term lab stocks. Such changes over time may have caused our high bacterial diversity treatment to include more competitive rather than facilitative phycosphere bacteria. This could explain why the low diversity treatment resulted in higher algal community biomass, cell densities, and

diversity, as the absence of competitive bacterial taxa may have aided the growth and diversity of their algal hosts.

We also found that algal assimilation of nitrogen, as determined through our algal C:N data, and algal density was highly context dependent on our temperature and phosphorus treatments. In oligotrophic conditions, algal cells assimilated more nitrogen at ambient temperatures, whereas in hypereutrophic conditions, the algal cells assimilated more nitrogen with elevated temperatures. This same trend has been observed by researchers studying aquatic plants in which in nutrient-saturated conditions, plants assimilated more nitrogen with elevated temperatures because it allowed decomposition and nutrient cycling rates to accelerate, resulting in more bioavailable nutrients for the plants, meaning decreased C:N ratios. They found that plant growth rates responded comparably to this nutrient-temperature interaction. In contrast, in conditions where nutrient concentrations were very limiting, plants were unable to assimilate more nitrogen with elevated temperatures as simply there were not enough nutrients available to facilitate any further growth (Zhang et al., 2020). This highlights that heightened temperatures can pose opposite effects on plant C:N ratios and thus plant growth, depending on what stressful nutrient condition they reside in. Further, our results demonstrated that elevated temperatures can either promote or hinder algal standing biomass, depending on what other abiotic stressors are present. It is also important to note that total algal densities decreased with elevated temperatures for nearly all experimental treatments, highlighting that elevated temperatures mainly served as a stressor to the algae. Algae tend to have higher growth rates in response to warmer temperatures up until a thermal optimum. After this point, algal growth can significantly decline (Larsdotter, 2006). Prior studies have found that maximum algal growth rates are achieved at 23°C, before increasing temperatures start to have a negative effect on algal growth (Pawlita-Posmyk et al., 2018). This could explain why our elevated temperature treatments are reducing algal densities.

Our ambient temperature treatments were maintained at an average of 23.25°C, whereas our elevated temperature treatments had an average of 28.36°C. Our ambient temperature treatment was close to the optimal growth temperature for most algae, whereas our elevated temperature treatment was warmer than the thermal optimum. Even though warmer temperatures tend to increase algal growth, our elevated temperature treatment was above the optimum, thus resulting in lower algal densities when compared to our ambient temperature treatment.

Our stable isotope ratio data further advance our understanding of how our treatments resulted in nutrient limitation. The degree of fractionation of stable nitrogen isotopes is tightly correlated with the bioavailability of nutrients in the water column. In environments with an abundance of dissolved inorganic nitrogen, algae preferentially assimilate ^{14}N over ^{15}N, which leads to decreased algal $\delta^{15}N$ values. Similarly, nitrogen-limited conditions lead to increased algal $\delta^{15}N$ values (Ho et al., 2021). Our stable nitrogen isotope data confirms trends observed in our total dissolved nitrogen data: dissolved nitrogen was negatively correlated with phosphorus level, because with excess phosphorus, algal cells were able to assimilate more nitrogen, while algal $\delta^{15}N$ was positively correlated with phosphorus levels. In addition, high microbiome diversity resulted in the greatest levels of dissolved nitrogen concentrations and the lowest levels of algal $\delta^{15}N$. These observations highlight that the bioavailability of dissolved nitrogen significantly affects the stable nitrogen isotopes present in the phytoplankton.

Our isotopic data also sheds light on our algal cell density results. Since algae tend to prefer lighter isotopes, when populations are growing slowly and not close to carrying capacity, cells can preferentially assimilate the lighter isotopes (Phelps et al., 2021). However, at high algal densities, these preferred lighter isotopes become depleted, resulting in heavier isotopic signatures at higher densities. Indeed, we found that when algal densities were lower, lighter

nitrogen and carbon isotopes were more prevalent in the algal biomass. In addition, in treatments where algal densities increased, the algae tend to assimilate the heavier isotopes of both nitrogen and carbon. As expected, heavier stable isotopes were more prevalent in our treatments that experienced the highest algal densities, particularly phosphorus-rich conditions and the low microbiome diversity treatment.

Overall, we found that the magnitude of negative effects of high microbiome diversity intensified in temperature-stressed conditions. For example, the lowest algal nitrogen uptake and $\delta^{15}N$ values corresponding with the highest microbiome diversity, particularly at elevated temperatures. In addition, the lowest levels of Shannon Diversity of algal communities corresponded with the highest microbiome diversity at elevated temperature conditions. These findings do not support our hypothesis that increased diversity in host microbiomes will have the most beneficial effects on algal communities in the most abiotically stressful environments, as higher prokaryote diversity did not alleviate the adverse effects of temperature stress on algal nitrogen assimilation, nitrogen stable isotopes, or on community diversity. These results of the pairwise interactions between microbiome diversity and abiotic stressors are inconsistent with previous research where it was found that host microbiome diversity aided host health when in response to abiotic stress (Rolli et al., 2015; Schwab et al., 2016).

A potential explanation for the deleterious effects of our high microbiome diversity treatment could be algal pathogens, as algal standing biomass significantly declined in the presence of microbial diversity. This may elucidate why we are finding contradictory results in comparison with prior studies. Our high bacterial diversity treatment is likely resulting in more competitive algal-bacterial interactions, as opposed to mutualistic ones. This indicates that the presence of competitive bacterial taxa paired with an abiotic stressor may trigger declines in algal

biomass and cell production. Some prior research has found that antagonistic interactions occur more frequently in microbial communities than mutualisms (Kramer et al., 2020), and perhaps this may relate to algal-bacterial interactions. A great number of microbial taxa are known to have adverse effects on phytoplankton. Bacterial parasitism often occurs through competition for bioavailable resources, thus taking away necessary nutrients from the phytoplankton hosts. Such parasitic bacteria and fungi can cause phytoplankton mortality (Ramanan et al., 2016). For example, *Phlyctidium scenedesmi* is a known algal-antagonist chytrid as its competitive abilities have resulted in significant declines in *Scenedesmus* abundances in open pond systems. Furthermore, it was discovered that there were multiple coexisting chytrids that were associated with diminished algal productivity in the family *Scenedesmaceae* (Carney & Lane, 2014). As two of our five algal species belong to the family *Scenedesmaceae* (*Coelastrum microporum* and *Scenedesmus acuminatus*), our algal community could be predisposed to such antagonistic bacteria. We may have had an increased occurrence of algal-bacterial competitive or pathogenic interactions in the higher microbiome diversity treatment due to the lack of co-evolution. Our algae came from lab stocks from the University of Texas Culture Collection of Algae in 2011, whereas our bacterial communities originated from the naturalized ponds in University of Michigan's E. S. George Reserve. Since they did not originate from the same source, there was a lack of an opportunity for the bacterial communities to coevolve alongside their algal hosts to become effective symbionts. Future studies could address this limitation by using algae and bacteria recently isolated from the same or similar natural environments.

Generally, prior research has found that the occurrence versus complete absence of a host microbiome can improve algal health and carrying capacity. However, our prior work has also found that relatively lower phycosphere diversity (but not axenic) was correlated with an increase in algal fitness among algal monocultures (Jackrel et al., 2021). Rather than an intentional

manipulation, this decline in diversity occurred over a four-week time period of culture maintenance in the lab. In this prior work, our sequencing data suggested that this result may have been caused not necessarily by declining diversity itself, but instead an increase in the relative abundance of nitrogen fixers that would facilitate algal growth combined with a decline in the relative abundance of antagonistic Gammaproteobacteria. This study only focused on phytoplankton monocultures, yet this still aligns with our current results as we comparably found that our low microbiome diversity treatment resulted in the highest algal community standing biomass. It is important to note that our work used the same five algal species and the same source of bacterial communities as Jackrel et al., 2021. It may be possible to glean further insights into the relationship between microbiome diversity and host fitness by assessing higher temporal resolution of our current study. Our data from our week-six timepoint, corresponded to 420 - 1,120 host generations, considering average doubling times of 9 – 24 hours for the algal species used in this study (Jackrel et al., 2021). If algal cell densities and bacterial diversity were assessed at each of our six weekly time points, we would have more conclusive data on whether declining microbial diversity is a causative driver of increased algal fitness.

Our study had several limitations. First, our algal media analyzed for dissolved nutrients may have contained a subset of the bacterial community in addition to the COMBO medium of experimental cultures because we relied solely on centrifugation to remove algal and bacterial cells from the supernatant rather than filtration. Without passing the supernatant through a 0.22 μm filter, it remains possible that our elevated dissolved nutrient concentrations in the high microbiome diversity treatment was in part due to bacterial cells remaining in the supernatant. Greater nutrient cycling efficiency is a widespread attribute of communities with higher productivity (Danovaro & Pusceddu, 2007). Given that high bacterial diversity decreased algal community biomass, densities, diversities, and stable isotope ratios, it is possible that bacterial

diversity also decreased dissolved nutrients via reduced nutrient cycling rates. However, this result remains inconclusive given that our algal media measurements may have housed part of the bacterial communities. As a separate limitation from whether all bacteria were removed prior to measuring dissolved nutrients, it should also be noted that our data cannot disentangle the mechanism driving the relationship between microbiome diversity and dissolved nutrients. The positive correlation between microbiome diversity and concentrations of dissolved nutrients in the water column may either indicate recycling of nutrients by bacteria, or that bacterial interactions with algae reduces the uptake of nutrients by algae. Lastly, it is important to note that our microbiome diversity treatment is inherently confounded with bacterial composition, which is a limitation for the majority of empirical tests of the relationship between bacterial diversity and ecosystem function. Effects of our microbiome diversity treatment may have been due to the total number of bacterial taxa present, or may have also been caused by the types of functions these bacteria can perform. Further evaluation of the bacterial communities by 16S rRNA sequencing could give insights into the relationship between bacterial taxonomic diversity and functional diversity in our study, disentangling how shifts in bacterial composition versus diversity are affecting phytoplankton community dynamics.

With global human populations rising, anthropogenic disturbances are posing serious threats to the world's biodiversity, both terrestrial and aquatic. Phytoplankton are the photosynthetic primary producers in aquatic systems, in which their fitness either directly or indirectly affects all other trophic levels. This is due to them being the base of the aquatic food web and owing to the fact that they play a major role in regulating Earth's biological processes as they produce about 50-80% of the world's oxygen (NOAA, 2021). Climate warming along with nutrient limitation and eutrophication due to human activities are further harming the health of global phytoplankton populations, along with their associated bacteria. In order for effective

restorative measures to be taken, all factors that regulate these ecological communities must be fully understood. Our work contributes to the field of microbial ecology as we empirically find that host microbiome diversity is a significant factor in determining algal community dynamics and ecosystem functioning. Additionally, this is one of the first studies to investigate how the effect of microbiome diversity on eukaryotic interactions in species-rich communities shifts with the introduction of abiotic stressors. Future research should aim to discover which genera of bacteria contribute the most in promoting host community fitness and diversity. This could be done by isolating specific genera and essentially repeating our experiment but instead of inoculating the algal flasks with different levels of microbial diversities, they could be inoculated with single bacterial genera. By quantifying the diversity and fitness metrics of the host communities, it could be determined which bacterial genera have the highest disposition to facilitate mutualistic interactions with their host species. This could help reveal which bacterial genera are more likely to promote the standing biomass and diversity of the host species. Perhaps it is not overall bacterial taxonomic richness that is directly affecting these metrics, but instead the presence of specific functionally-important bacterial genera. If these can be identified, along with their effects being successfully extrapolated and observed in natural settings, then aquatic conservation efforts could be strengthened if these genera are also protected. In addition, it would be useful to test to what degree our abiotic stressors affect the microbiome in terms of its composition and diversity. We test the extent that the combination of phosphorus and temperature stress and microbiome diversity has on algal community standing biomass and composition, but we did not specifically test how these abiotic factors influence the host microbiome itself. This would offer us more insight into what degree abiotic conditions affect the microbiome, based on its standing original biodiversity. This data could be obtained through 16S rRNA sequencing data, which would allow us to see if the bacterial diversity in each experimental treatment is

different over time from their original values (low, intermediate, and high diversity). If they are, this would indicate that our abiotic treatments are shifting their compositions as well, highlighting that there may be a confounding variable at play.

On a broader scale, future work should aim to discover all the elements that influence prokaryote and eukaryote diversity, along with uncovering how stressors brought upon by climate change may impact the strength and type of bacteria-eukaryote interactions. Addressing these areas of research still largely unknown will pave the way to better understanding how microbes and their host species interact with each other, along with determining how anthropogenic disturbances may impact their relationships, and consequently whole ecosystems.

APPENDIX

S1.1)

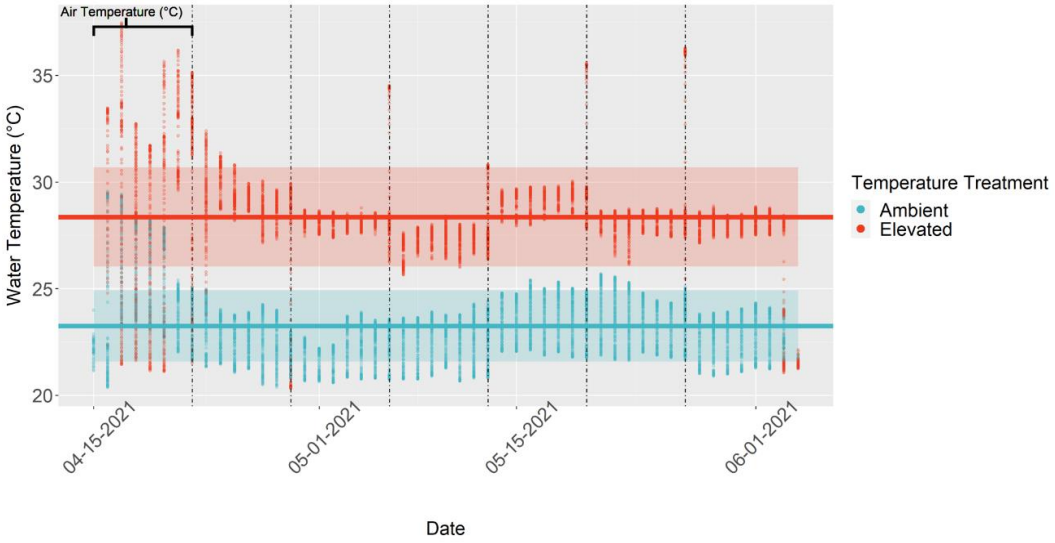


Figure S1.1: Water temperature (°C) of the Ambient and Elevated temperature treatments measured by a HOBO® temperature logger. The ambient temperature treatment resulted in an average temperature of 23.25°C ± 1.67°C, with the elevated temperature treatment having an average temperature of 28.36°C ± 2.33°C. The reason for the discrepancies from 04-15-2021 to 04-22-2021, is due to the HOBO® data logger not being submerged in water, so the temperatures for the first week of the experiment display air temperature values. The vertical dashed lines denote the sampling days.

Table S1.1: Fligner-Killeen Test of Homogeneity of Variances for the various metrics measured, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

metric	med chi-squared	df	p-value
Mean total dissolved nitrogen	34.309	23	0.06082
Reciprocal mean total dissolved phosphorus	29.367	23	0.1685
Mean C:N of algal biomass	17.376	23	0.7904
Mean $\delta^{15}N$ of algal biomass	15.635	23	0.8704
Mean $\delta^{13}C$ of algal biomass	28.75	23	0.1888
Mean algal biomass	27.519	23	0.2345
Log mean total algal density	22.365	23	0.4983
Mean Shannon Diversity	26.024	23	0.2997
Log mean abundance of *Scenedesmus acuminatus*	20.133	23	0.6339
Log mean abundance of *Coelastrum microporum*	27.034	23	0.2545
Log mean abundance of *Selenastrum capricornutum*	14.496	23	0.9118
Log mean abundance of *Chlorella sorokiniana*	28.788	23	0.1874
Log mean abundance of *Monoraphidium minutum*	35.233	23	0.04931

S2.1 (a-l))

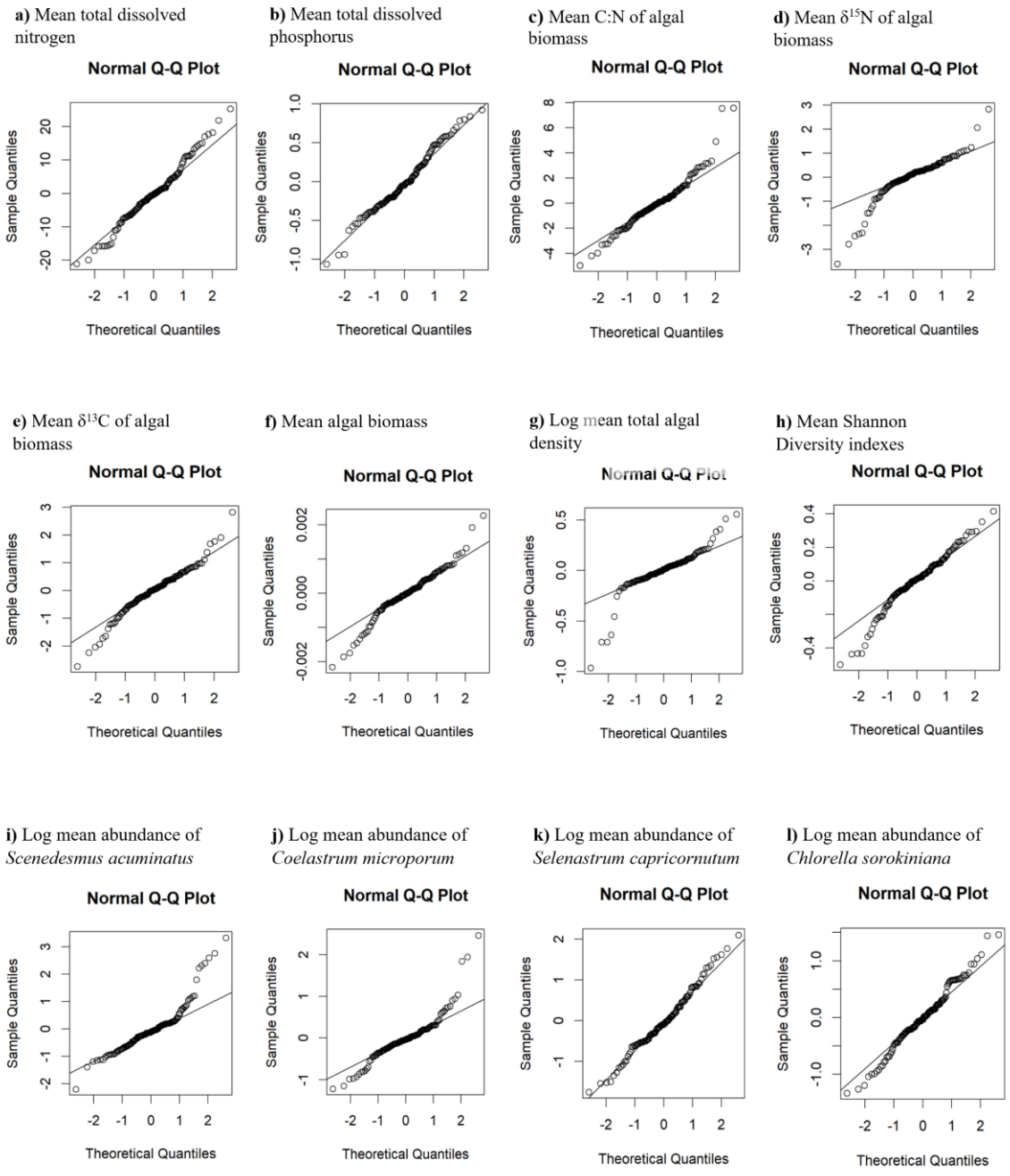


Figure S2.1 (a-l): Normality plots of measured metrics, with temperature, phosphorus, and microbiome diversity treatments as fixed effects.

Table S2.1: Three-way Analysis of Variance for mean total dissolved nitrogen in the algal media, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	208	208	2.074	0.153451
Phosphorus Treatment	3	39964	13321	132.936	**< 2 x 10^{-16}**
Microbiome Treatment	2	2969	1484	14.814	**2.91 x 10^{-6}**
Temperature*Phosphorus	3	1209	403	4.021	**0.009923**
Temperature*Microbiome	2	128	64	0.636	0.531598
Phosphorus*Microbiome	6	2669	445	4.440	**0.000579**
Temperature*Phosphorus *Microbiome	6	532	89	0.884	0.509906
Residuals	87	8718	100		

Table S2.2: Three-way Analysis of Variance for reciprocal mean total dissolved phosphorus in the algal media, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	0.65	0.65	3.385	0.0692
Phosphorus Treatment	3	193.37	64.46	335.219	**< 2 x 10^{-16}**
Microbiome Treatment	2	12.05	6.02	31.327	**5.66 x 10^{-11}**
Temperature*Phosphorus	3	0.84	0.28	1.454	0.2327
Temperature*Microbiome	2	0.96	0.48	2.492	0.0886
Phosphorus*Microbiome	6	11.58	1.93	10.038	**2.08 x 10^{-8}**
Temperature*Phosphorus *Microbiome	6	2.72	0.45	2.356	**0.0372**
Residuals	87	16.73	0.19		

Table S2.3: Three-way Analysis of Variance for mean Carbon:Nitrogen of dried algal biomass, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	11.2	11.23	2.281	0.134409
Phosphorus Treatment	3	268.1	89.38	18.149	**2.59×10^{-9}**
Microbiome Treatment	2	23.1	11.53	2.342	0.101910
Temperature*Phosphorus	3	78.3	26.11	5.303	**0.002053**
Temperature*Microbiome	2	79.1	39.54	8.029	**0.000615**
Phosphorus*Microbiome	6	11.9	1.98	0.402	0.876010
Temperature*Phosphorus *Microbiome	6	48.9	8.14	1.653	0.141608
Residuals	91	448.1	4.92		

Table S2.4: Three-way Analysis of Variance for the mean $\delta^{15}N$ of dried algal biomass, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	52.2	52.22	54.637	**6.83×10^{-11}**
Phosphorus Treatment	3	598.6	199.52	208.767	**$< 2 \times 10^{-16}$**
Microbiome Treatment	2	49.2	24.61	25.751	**1.37×10^{-9}**
Temperature*Phosphorus	3	16.8	5.60	5.861	**0.00105**
Temperature*Microbiome	2	9.0	4.48	4.688	**0.01155**
Phosphorus*Microbiome	6	76.5	12.74	13.335	**8.68×10^{-11}**
Temperature*Phosphorus *Microbiome	6	7.2	1.19	1.249	0.28906
Residuals	91	87.0	0.96		

Table S2.5: Three-way Analysis of Variance for mean $\delta^{13}C$ of dried algal biomass, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	3.7	3.68	4.252	**0.04205**
Phosphorus Treatment	3	553.0	184.35	213.252	**$< 2 \times 10^{-16}$**
Microbiome Treatment	2	20.7	10.34	11.958	**2.45×10^{-5}**
Temperature*Phosphorus	3	2.3	0.78	0.898	0.44562
Temperature*Microbiome	2	3.1	1.54	1.783	0.17402
Phosphorus*Microbiome	6	18.8	3.13	3.615	**0.00292**
Temperature*Phosphorus *Microbiome	6	9.6	1.60	1.850	0.09802
Residuals	91	78.7	0.86		

Table S2.6: Three-way Analysis of Variance for mean dried algal biomass, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	1.490×10^{-6}	1.487×10^{-6}	2.467	0.1196
Phosphorus Treatment	3	6.970×10^{-6}	2.324×10^{-6}	3.855	**0.0119**
Microbiome Treatment	2	6.268×10^{-5}	3.134×10^{-5}	51.982	**6.27×10^{-16}**
Temperature*Phosphorus	3	2.040×10^{-6}	6.810×10^{-7}	1.129	0.3413
Temperature*Microbiome	2	6.200×10^{-7}	3.090×10^{-7}	0.512	0.6009
Phosphorus*Microbiome	6	2.579×10^{-5}	4.298×10^{-6}	7.129	**2.79×10^{-6}**
Temperature*Phosphorus *Microbiome	6	2.510×10^{-6}	4.180×10^{-7}	0.694	0.6549
Residuals	94	5.667×10^{-5}	6.030×10^{-7}		

Table S2.7: Three-way Analysis of Variance for the log mean total algal density, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	2.920	2.920	59.613	**1.1×10^{-11}**
Phosphorus Treatment	3	21.178	7.059	144.134	**$< 2 \times 10^{-16}$**
Microbiome Treatment	2	0.358	0.179	3.650	**0.029657**
Temperature*Phosphorus	3	1.132	0.377	7.704	**0.000115**
Temperature*Microbiome	2	0.009	0.004	0.087	0.916949
Phosphorus*Microbiome	6	1.074	0.179	3.655	**0.002601**
Temperature*Phosphorus *Microbiome	6	0.213	0.035	0.724	0.631394
Residuals	96	4.702	0.049		

Table S2.8: Three-way Analysis of Variance for the mean Shannon Diversity, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	0.001	0.0012	0.035	0.85136
Phosphorus Treatment	3	4.612	1.5373	44.596	**$< 2 \times 10^{-16}$**
Microbiome Treatment	2	0.290	0.1449	4.204	**0.01778**
Temperature*Phosphorus	3	0.271	0.0904	2.623	0.05497
Temperature*Microbiome	2	0.410	0.2048	5.941	**0.00369**
Phosphorus*Microbiome	6	0.614	0.1023	2.967	**0.01061**
Temperature*Phosphorus *Microbiome	6	0.429	0.0716	2.077	0.06300
Residuals	96	3.309	0.0345		

Table S2.9: Three-way Analysis of Variance for the log mean abundance of *Scenedesmus acuminatus*, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	11.59	11.59	12.962	**0.000508**
Phosphorus Treatment	3	195.64	65.21	72.960	**< 2 x 10^{-16}**
Microbiome Treatment	2	3.03	1.51	1.692	0.189592
Temperature*Phosphorus	3	0.72	0.24	0.270	0.846723
Temperature*Microbiome	2	0.26	0.13	0.144	0.865845
Phosphorus*Microbiome	6	25.29	4.21	4.715	**0.000304**
Temperature*Phosphorus *Microbiome	6	4.18	0.70	0.780	0.587681
Residuals	95	84.91	0.89		

Table S2.10: Three-way Analysis of Variance for the log mean abundance of *Coelastrum microporum*, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	3.70	3.702	10.620	**0.001548**
Phosphorus Treatment	3	59.25	19.749	56.653	**< 2 x 10^{-16}**
Microbiome Treatment	2	2.92	1.459	4.184	**0.018101**
Temperature*Phosphorus	3	0.01	0.003	0.008	0.999040
Temperature*Microbiome	2	0.04	0.018	0.052	0.949126
Phosphorus*Microbiome	6	8.87	1.478	4.240	**0.000789**
Temperature*Phosphorus *Microbiome	6	3.98	0.664	1.904	0.087993
Residuals	96	33.47	0.349		

Table S2.11: Three-way Analysis of Variance for the log mean abundance of *Selenastrum capricornutum*, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	45.98	45.98	62.954	**7.67×10^{-12}**
Phosphorus Treatment	3	4.63	1.54	2.112	0.104666
Microbiome Treatment	2	16.44	8.22	11.252	**4.62×10^{-5}**
Temperature*Phosphorus	3	7.85	2.62	3.584	**0.017069**
Temperature*Microbiome	2	13.56	6.78	9.281	**0.000226**
Phosphorus*Microbiome	6	10.74	1.79	2.451	**0.031071**
Temperature*Phosphorus *Microbiome	6	4.24	0.71	0.967	0.452805
Residuals	85	62.08	0.73		

Table S2.12: Three-way Analysis of Variance for the log mean abundance of *Chlorella sorokiniana*, with the temperature, phosphorus, and microbiome diversity treatments as fixed effects.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Temperature Treatment	1	2.24	2.24	5.918	**0.01688**
Phosphorus Treatment	3	21.41	7.14	18.829	**1.21×10^{-9}**
Microbiome Treatment	2	79.92	39.96	105.451	**$< 2 \times 10^{-16}$**
Temperature*Phosphorus	3	5.15	1.72	4.529	**0.00519**
Temperature*Microbiome	2	0.42	0.21	0.548	0.58012
Phosphorus*Microbiome	6	3.66	0.61	1.609	0.15312
Temperature*Phosphorus *Microbiome	6	7.57	1.26	3.329	**0.00512**
Residuals	94	35.62	0.38		

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