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# Dual phosphorus and nitrogen nutrient reduction will be more effective than a phosphorus-only reduction in mitigating diatom and cyanobacterial blooms in Lake Erie, USA–Canada

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## Abstract

Lake Erie, USA–Canada, plays an important ecological and socioeconomic role but has suffered from chronic eutrophication. In particular, western Lake Erie (WLE) is the site of harmful algal blooms (HABs) which are suspected of being driven by excessive nutrient (phosphorus (P) and nitrogen (N)) inputs. During 2022 and 2023, in situ nutrient dilution and addition bioassays were conducted at a WLE bloom-impacted location to investigate whether a nutrient reduction regime would be effective in limiting phytoplankton growth during the June diatom-dominated spring blooms and August cyanobacteria-dominated summer blooms. The primary objectives of this experiment were to (1) Determine if a proposed 40% P-alone reduction would effectively reduce phytoplankton growth and mitigate blooms and (2) assess whether reductions in both P and N are more effective in controlling phytoplankton biomass than exclusive reductions in either N or P. Samples were analyzed for nutrient concentrations and growth rate responses for specific algal groups, utilizing diagnostic (for major algal groups) photopigments. Results indicated that although both 20% and 40% dilutions led to lower phytoplankton biomass and growth rates, 40% reductions were more effective. Our results support the USA–Canada Great Lakes Water Quality Agreement recommendation of a 40% P reduction, but also indicate that a parallel reduction of N input by 40% would be most effective in controlling bloom magnitudes. Overall, our findings underscore the recommendation that a year-round dual N and P 40% reduction is needed for long-term control of eutrophication and algal blooms, including cyanobacteria and diatoms, in Lake Erie.

Lake Erie (USA and Canada), the southernmost and shallowest of the Laurentian Great Lakes, has experienced nearly a century of excessive nutrient loading and eutrophication, culminating in the mid-1900s with dense blooms of nitrogen ($N_2$)-fixing cyanobacteria *Aphanizomenon* and *Dolichospermum* (*Anabaena*) (Davis 1964; Matisoff and Ciborowski 2005). After phosphorus (P) regulations were enacted during the 1970s following the Great Lakes Water Quality Agreement (GLWQA) of 1972, the lake showed signs of recovering from eutrophication and cyanobacterial blooms did not occur in the 1980s and early 1990s (DePinto et al. 1986; Makarewicz 1993). However, since the late 1990s, cyanobacterial blooms dominated by the non-$N_2$-fixing *Microcystis* spp. have become an annual summer occurrence in western Lake Erie (Stumpf et al. 2016). In order to counter the

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Additional Supporting Information may be found in the online version of this article.

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deleterious effects of resurgent eutrophication, the proliferation of cyanobacterial harmful algal blooms (CyanoHABs) and central basin hypoxia, a 40% load reduction in total phosphorus (TP), including dissolved reactive P (DRP), entering the Western and Central Basins of Lake Erie has been enacted by the US and Canadian binational GLWQA to achieve a 6000 metric tons (MT) Central Basin load (GLWQA 2015; US EPA 2015a). Specifically, to address CyanoHABs in the western basin, a 40% P load reduction was recommended from the dominant watershed tributary, the Maumee River, during the March–June time frame rather than annually because there is a better correlation between bloom biomass and the March–June cumulative P load than the cumulative annual P load (Stumpf et al. 2012). The actual loading target is 860 MT of TP and 186 MT of DRP, which equates to a 40% reduction from the 2000–2015 annual average and is also equivalent to the loads observed in 2008. The target 40% P load reduction was the product of an ensemble of empirical data and process-based models (Scavia et al. 2016; Verhamme et al. 2016).

There is a growing consensus among limnologists and aquatic ecologists that the availability of both N and P is key to controlling primary production mediated by microalgae, including eukaryotic taxa (diatoms, chlorophytes, cryptophytes, and various flagellates) and prokaryotic cyanobacteria (Elser et al. 2007; Conley et al. 2009; US EPA 2015a,b); and the percentage of USA lakes co-limited by N and P is significant (McCullough et al. 2024) and appears to have increased (Rock and Collins 2024). The GLWQA (2015) has specifically called for adaptive management that examines how changes in both P and N would affect CyanoHABs in Lake Erie; especially since *Microcystis*, the dominant bloom-former in heavily-impacted western Lake Erie, does not fix $N_2$ (Steffen et al. 2014). Exclusive emphasis on P-only load reduction in the GLWQA (2015) has been in part based on the longstanding paradigm that P plays the major role in constraining phytoplankton biomass (Schindler et al. 2008). However, research specific to *Microcystis* in western Lake Erie has shown that regenerated N plays an important role in supporting blooms (Hoffman et al. 2022). Researchers argue that additional studies at the ecosystem level are required to understand the complex ecological dynamics driving nutrient-limitation and toxicity (Stow et al. 2022); however, a growing body of evidence involving multiyear observations and dynamic molecular models for western Lake Erie *Microcystis* blooms suggest that N-limitation will result in lower microcystin concentrations at the cellular level (Gobler et al. 2016; Hellweger et al. 2022a). These findings point to the potentially important role of N in not only bloom dynamics but, importantly, toxicity, and thus the assumption that most nutrient best management practices will target reductions of both N and P.

In addition to the P loading targets aimed at minimizing cyanoHABs, the GLWQA (2015) has an additional target to minimize central basin hypoxia (dissolved oxygen [DO] $< 2$ mg $L^{-1}$) and anoxia (DO $\cong 0$ mg $L^{-1}$). The GLWQA P loading target to reduce hypoxia is also a 40% P load reduction, but on an annual period (not just a springtime target). The just-deep enough bathymetry of the central basin results in a thin hypolimnion that was likely hypoxic prior to European settlement of the area, based on the absence of burrowing mayfly tusks in sediment cores (Reynoldson and Hamilton 1993). Recent evidence suggests that the hypoxic/anoxic area is becoming larger and forming earlier in the year with eutrophication (Zhou et al. 2013; Zhou et al. 2014). Central basin hypoxia is more associated with springtime diatom blooms than summertime cyanobacterial blooms (Reavie et al. 2016). Therefore, there is a need to also determine how the diatom bloom will respond to P or P and N nutrient reductions to gain insights into how the central basin hypoxic zone will be impacted.

Previous studies addressing causative agents of eutrophication in Lake Erie noted that $N_2$ fixing cyanobacterial genera were also present (e.g., *Anabaena* [now designated *Dolichospermum*], *Aphanizomenon*) (Davis 1964; Matisoff and Ciborowski 2005), and thus capable of supplying N internally. This led some investigators to discount the potential role of externally supplied N in controlling freshwater and coastal eutrophication (Schindler et al. 2008). However, various studies have shown that in most water bodies harboring $N_2$ fixers, this process falls far short of satisfying N demands to support ecosystem-scale primary production, including eutrophic waters where cyanobacteria are often abundant (Howarth et al. 1988; Scott and McCarthy 2010; Paerl et al. 2016). In concert, these studies point to the importance of externally- and internally-supplied N (in addition to P) in controlling freshwater eutrophication and bloom dynamics.

The objective of this study is twofold: (1) Determine if the 40% proposed P reduction will effectively reduce the growth rates of both spring–early summer diatom-dominated blooms and summer cyanobacteria-dominated blooms in the most severely impacted western Lake Erie, and (2) if concurrent reductions of N and P could lower growth rates of both blooms more than P-only reductions. Our study focused on two elevated production and bloom periods in western Lake Erie; the mid-June blooms dominated by diatoms and other eukaryotic phytoplankton taxa, and the mid-August cyanobacteria-dominated bloom periods. We utilized in situ microcosm nutrient enrichment and dilution bioassays that employed both nutrient addition and dilution treatments on natural phytoplankton communities at a location frequented by blooms and routinely monitored for water quality to determine which nutrient(s) were limiting algal production and to what extent nutrient over-enrichment occurred in western Lake Erie during 2022 and 2023. In this work, we did not study an N-only reduction scenario because numerous modeling studies suggested that a P-only approach will lower cyanoHAB biomass in western Lake Erie (Scavia et al. 2016; Hellweger et al. 2022a). However, we addressed the question if reductions of both P and N will slow growth rates more than P only reductions, and we applied this question to both hypoxia-causing diatom blooms and toxin-producing cyanoHABs. Our work complements recent dilution experiment assays that investigated P-only vs. dual nutrient reductions (Barnard et al. 2021; Baer et al. 2023), but we expand upon those studies by (1) adding a focus on diatom-dominated communities which are key bloom formers and hence large contributors to C fixation and hypoxia potentials (cf. Zepernick et al. 2024), (2) quantifying how different phytoplankton groups (diatoms, green algae, cyanobacteria, and cryptophytes) each responds to the P-only vs. P and N dual reductions, and (3) including different reduction percentages (20%, 40%, and 60%, instead of just the GLWQA recommended 40%) to determine what nutrient reduction percentage will be most effective.

## *Methods and materials*

### Bioassay methods

In situ bioassay experiments were conducted at the Ohio State University Stone Laboratory at Put-in Bay, Ohio (eastern edge of the western basin of Lake Erie) to examine the impacts of nutrient enrichment and dilution on phytoplankton production and growth rates (Fig. S1). Bioassays were deployed during mid-June and mid-August, when Lake Erie is typically dominated by diatoms and cyanobacteria, respectively (Chaffin et al. 2018a). This enabled us to evaluate seasonal nutrient limitation and proposed nutrient (P and N) reductions needed to control algal growth in two successive years (2022–2023). Western Lake Erie water (~ 200 liters) was collected from a site 3 km west of the Stone Laboratory, on the south side of Rattlesnake Island (Location: N 41°40.155, W 82°51.193) for all bioassays (Fig. 1). Water was collected at the site in 20 liter prerinsed (with site water) Nalgene carboys. At the laboratory, carboys were emptied into a large prewashed plastic tank that was constantly stirred to maintain uniformity of the bioassay water that was dispensed into triplicated 4-L Cubitainers® for each treatment and controls. Cubitainers® were then transferred to floating "corrals" that were suspended off finger piers adjacent to the Stone Laboratory (Fig. S1). Cubitainers® are chemically-inert, polyethylene, 85% PAR transparent vessels (Paerl et al. 2011).

Nutrient additions in bioassays typically double to triple the ambient concentration (Schelske 1984), therefore we

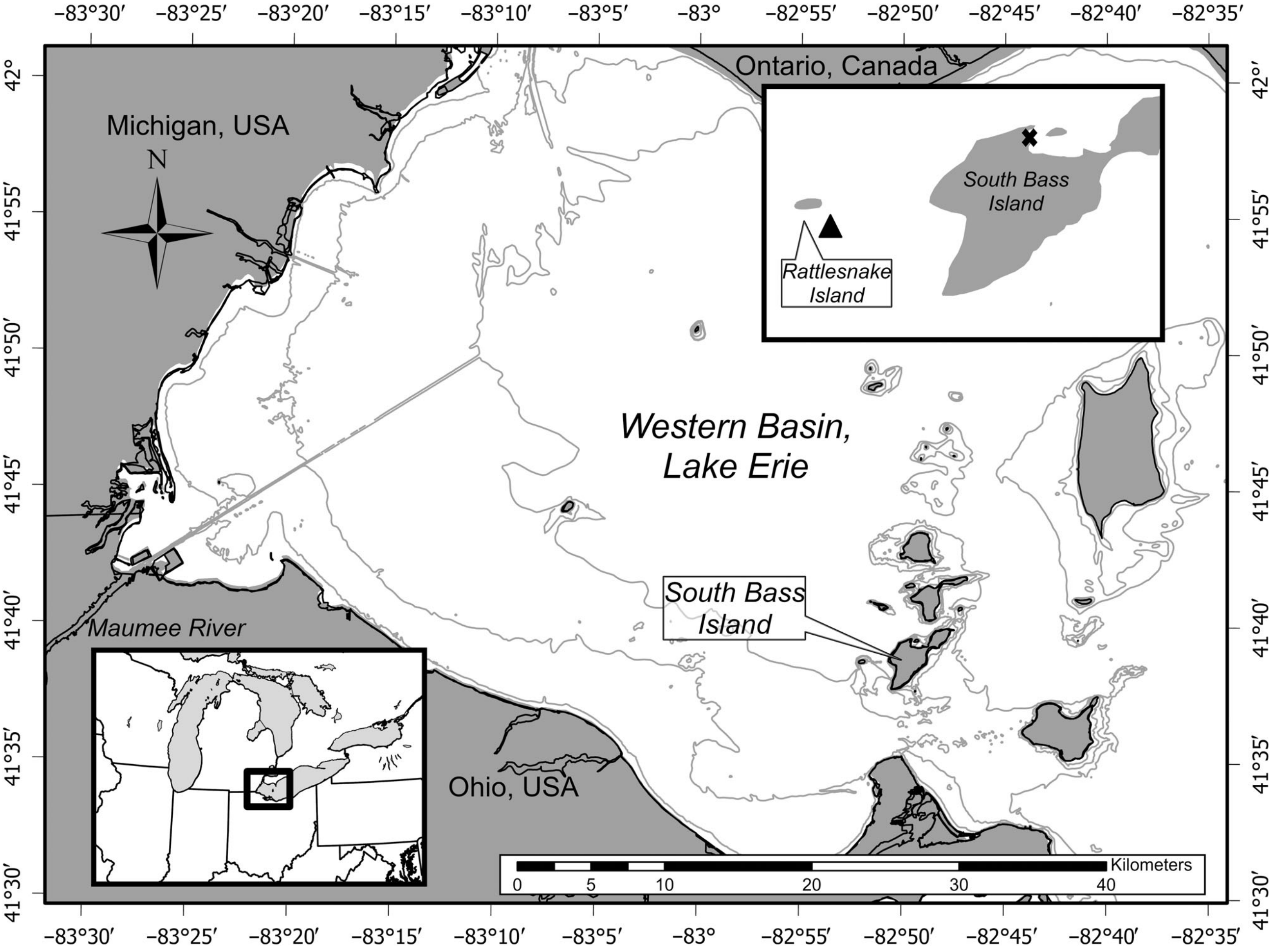

**Fig. 1.** Map of Western Lake Erie, with an insert of bioassay water sampling site (triangle) and location of the Stone Laboratory on South Bass Island (X).

based our N and P additions on inorganic N (ammonium, $NH_4^+$, and nitrate, $NO_3^-$) and P ($PO_4^-$) concentration information gathered from ongoing environmental monitoring and historic data collected near Stone Laboratory (Chaffin et al. 2018a). The nutrient dilution component was designed to test if the 40% decrease in ambient P concentrations, as targeted by the GLWQA, would decrease total phytoplankton (including bloom taxa) biomass and growth rates of dominant phytoplankton taxonomic groups, based on high performance liquid chromatography (HPLC) determination of diagnostic photopigments (chlorophylls and carotenoids) (Pinckney et al. 2001). We also included a 20% dilution treatment to test how the phytoplankton would respond while approaching the 40% load reduction. We included an additional 60% dilution during the 2023 experiments in case the 40% load reduction did not achieve the desired impacts.

We used a slightly different protocol for the dilution experiments in 2022 and 2023. In 2022, we diluted the lake water with a major ion solution (MIS), as in Barnard et al. (2021). The 40% dilution treatment consisted of 2.1 liters of lake water and 1.4 liters of MIS, and the 20% dilution treatment consisted of 2.8 liters of lake water and 0.7 liter of MIS (Table S1). The 0% dilution treatment consisted of just 3.5 liters of lake water. All containers were enriched with 50 $\mu$mol $L^{-1}$ silica as $Na_2SiO_3$ and 10 mg C $L^{-1}$ as $NaHCO_3$ to prevent Si and C limitation. This dilution procedure with added MIS, Si, and C results in a dilution of only P and N of the ambient water, which we termed "N&P co-dilution" treatment. To compare the N&P co-dilution treatment to a P-only dilution treatment, we added 50 $\mu$mol $L^{-1}$ nitrate as $KNO_3$ to a set of Cubitainers® (termed "P dilution, excess $NO_3$") and 50 $\mu$mol $L^{-1}$ ammonium as $NH_4Cl$ to another set of Cubitainers® (termed "P dilution, excess $NH_4$") (to test for the difference between N forms). All dilution percentages and dilution treatments were triplicated.

In the 2023 dilution experiments, we altered the experimental design to maintain a consistent initial amount of algae across all dilutions, and added a 60% dilution level (Table S2). The 60% dilution level consisted of 1 liter of lake water, 0.4 liter of 0.45 $\mu$m-filtered lake water, and 2.1 liters of MIS. The 40% dilution level consisted of 1 liter of lake water, 1.1 liters of 0.45 $\mu$m-filtered lake water, and 1.4 liters of MIS. The 20% dilution level consisted of 1 liter of lake water, 1.8 liters of 0.45 $\mu$m-filtered lake water, and 0.7 liter of MIS. The 0% dilution consisted of 1 liter lake water and 2.5 liters of 0.45 $\mu$m-filtered lake water. Like the 2022 dilutions, 50 $\mu$mol $L^{-1}$ silica and 10 mg C $L^{-1}$ DIC were added. Unlike the 2022 dilutions, we did not include separate N form treatments due to the addition of the 60% dilution. Nitrate and ammonium were jointly added to the "P-only dilution" treatment.

To accompany the dilution experiments, we conducted the more-common nutrient enrichment bioassay to determine if N, P, or both nutrients were limiting phytoplankton growth in natural irradiance and temperature conditions. The enrichment experiments were set up similarly to the 0% dilutions with treatments of added 50 $\mu$mol $L^{-1}$ N (as nitrate (+$NO_3$), ammonium (+$NH_4$), or both nitrate and ammonium (+$NO_3$+$NH_4$)), a P-only enrichment (5 $\mu$mol $L^{-1}$ as $KH_2PO_4$ [+P]), and P and N co-enrichments (+P+N).

### Nutrient concentration determinations

Nutrient samples from the ambient, initial site water were collected in 50 mL Falcon tubes by collecting the filtrate from Chl *a* samples, and were frozen at −20°C until analysis. A continuous segmented flow auto-analyzer (QuAAtro SEAL Analytical, Mequon, WI) was used to quantify nitrate, nitrite, ammonium, dissolved reactive P (DRP), and silicate using standard US EPA methods (Chaffin et al. 2019).

### Phytoplankton biomass determinations

The Cubitainers® were incubated for three days and subsampled daily to quantify chlorophyll *a* (Chl *a*), chlorophyll *b* and carotenoid photopigments diagnostic of major algal groups by high performance liquid chromatography (HPLC). Photopigments were determined on 50 mL water samples collected on GFF filters, which were frozen at −20°C and subsequently extracted using a tissue grinder in 90% acetone (Arar and Collins 1992). Extracts were analyzed by HPLC (Pinckney et al. 2001), calibrated with a purified Chl *a* standard (Turner Designs, Sunnyvale, CA; Sigma Chemicals). Diagnostic photopigments (chlorophylls and carotenoids) representing major phytoplankton classes were calibrated using certified standards (DHI Horsholm, Denmark). The following diagnostic photopigments encoded for the dominant phytoplankton classes: Chlorophyll *b* for chlorophytes, alloxanthin for cryptophytes, fucoxanthin for diatoms, and zeaxanthin for cyanobacteria (Paerl et al. 2003). Parallel samples were collected and preserved with Lugol's iodine solution for subsequent microscopic analyses of phytoplankton taxa.

### Phytoplankton growth rate calculations

Pigment concentrations used to calculate growth rates are presented in the Supporting Information document (Figs. S2–S5). Pigment concentration-derived growth rates were calculated with first-order kinetics by plotting the natural log of pigment concentration vs. time (in days) and the linear regression trend line is the growth rate (Chaffin et al. 2022). This method for calculating growth rate has advantages over the specific growth rate equation (i.e., $\mu = (\ln(C_f) - \ln(C_0))/t_f - t_0$) in that it allows for all concentration data to be used (i.e., $T_0$, $T_1$, $T_2$, $T_3$, as opposed to using just $T_0$ and $T_{final}$ data), and integrates over the entire experiment. The use of linear regression does introduce an error term; however, the error associated with linear regression decreased as growth rate increased (Fig. S6) and indicates that the differences in growth rates among treatments were associated with the dilution and treatments and not the error term. No error term can be estimated for the specific growth rate equation. Furthermore, the first-order kinetic method and the specific growth rate equation gave nearly identical estimates of growth rates (slopes of the methods to estimate growth rates ranged from 0.987 to 1.007 for the different pigments; $R > 0.989$; Fig. S6). The calculated growth rates were then plotted against the percentage of diluted P. In these growth curves, if the P-only dilution and the N and P co-dilution curves were similar, dual nutrient management would not be supported. If the N and P co-dilution growth curve was lower than the P-only dilution growth curve, dual nutrient management would be supported.

### Statistical analyses

We conducted a one-way ANOVA (ANOVA) with a post hoc Tukey test to determine if any significant differences of total Chl *a* existed between the treatments for the nutrient enrichment bioassays. A two-factor (P-dilution percentage X N&P co-dilution) multivariate ANOVA (MANOVA) was conducted on each experiment to determine the main effects on phytoplankton growth rates. When significant ($p < 0.05$, from Pillai's Trace statistic), follow-up two-factor ANOVAs were conducted for each phytoplankton group. IBM SPSS v29 was used for all statistical tests.

## *Results*

### June 2022

Lake water collected for the June 2022 bioassay had high nitrate (31.8 $\mu$mol L$^{-1}$), low ammonium (2.2 $\mu$mol L$^{-1}$), and low DRP concentration (< 0.03 $\mu$mol L$^{-1}$, Table 1). The June 2022 bioassay had the highest initial Chl *a* concentration among the four bioassays and was dominated by diatoms, based on high fucoxanthin concentration (Table 1).

**Table 1.** The initial concentrations of the major phytoplankton groups based on taxa-specific pigments, and nitrate plus nitrite, ammonium, and dissolved reactive phosphorus (DRP) in the ambient, pre-diluted water.

| | June 2022 | August 2022 | June 2023 | August 2023 |
|---|---|---|---|---|
| Fucoxanthin ($\mu$g L$^{-1}$)<br>Diatoms | 4.213 | 0.473 | 0.126 | 0.226 |
| Alloxanthin ($\mu$g L$^{-1}$)<br>Cryptophytes | 0.171 | 0.327 | 0.165 | 0.139 |
| Zeaxanthin ($\mu$g L$^{-1}$)<br>Cyanobacteria | 0.049 | 0.764 | 0.022 | 0.157 |
| Chlorophyll *b* ($\mu$g L$^{-1}$)<br>Chlorophytes | 0.248 | 0.523 | 0.041 | 0.085 |
| Chlorophyll *a* ($\mu$g L$^{-1}$)<br>Total biomass | 12.767 | 8.615 | 1.036 | 2.883 |
| $NO_3 + NO_2$ ($\mu$mol L$^{-1}$) | 31.85 | 2.56 | 56.56 | 4.004 |
| $NH_4$ ($\mu$mol L$^{-1}$) | 2.15 | 2.55 | 2.15 | 1.06 |
| DRP ($\mu$mol L$^{-1}$) | 0 | 0.05 | 0.08 | 0.646 |

The traditional nutrient enrichment bioassay results are shown in Fig. 2. Chlorophyll *a* concentration in the post-incubation control was approximately double that of the initial levels, which indicates that algal growth was sustained for 3 d on ambient nutrients, on intracellular stores of nutrients, or both. There was no significant difference in Chl *a* concentration among the control, +$NO_3$-only, +P-only, and +P+$NO_3$ treatments. The +$NH_4$-only treatment lowered Chl *a*, whereas +P+$NH_4$ resulted in the highest Chl *a* concentration.

In the June 2022 dilution assays, the percentage of P diluted and whether N was in excess or co-diluted yielded significant effects (Table S1). In the June 2022 dilution assays, the ammonium enrichment (the P dilution, excess $NH_4$ treatment) resulted in a significantly ($p < 0.001$, Table S1) lower growth rate for total algae, diatoms, cryptophytes, and green algae, indicating inhibition of growth. In contrast, cyanobacteria had a higher growth rate in the ammonium enrichment (the P dilution, excess $NH_4$ treatment) (Fig. 3). The main effect for percentage of P dilution was significant ($p < 0.001$, Table S1). Total algae ($p = 0.003$) and diatoms ($p = 0.011$) growth rate were lower in the 20% and 40% dilutions than the control. However, cyanobacteria growth rate was higher in the 20% and 40% dilutions than the control ($p = 0.004$). The effect of dilution was nearly significant for the cryptophytes and green algae ($p = 0.068$ and 0.054, respectively). The N&P co-dilution treatment and the P-only-dilution with excess $NO_3$ treatment resulted in a similar growth curve across the dilutions for all types of algae. The similar growth curves for P-dilution and N&P co-dilution suggest that dual nutrient management would not be effective at this time point. However, because the cyanobacteria growth rate in the N&P co-dilution was less than the P-dilution, excess $NH_4$ treatment, dual nutrient management that targeted ammonium would be more effective at controlling cyanobacterial growth than a P-only approach. The interaction between P-dilution percentage and whether N was in excess or co-diluted did not significantly affect growth rates ($p = 0.300$).

### August 2022

Lake water collected for the August 2022 bioassay had low nitrate (2.6 $\mu$mol L$^{-1}$), low ammonium (2.5 $\mu$mol L$^{-1}$), and low DRP concentrations (0.05 $\mu$mol L$^{-1}$, Table 1). The August 2022 bioassay had the second highest initial Chl *a* concentration among the four bioassays and was dominated by cyanobacteria, based on high zeaxanthin concentration (Table 1).

In the traditional nutrient enrichment bioassay, the post-incubation control, +N-only enrichments, and +P-only enrichments resulted in Chl *a* concentrations that were not significantly different among each other and similar to the initial concentration (Fig. 2b). The Chl *a* concentration in the +P +$NO_3$ treatment increased by threefold and the +P+$NH_4$ treatment resulted in a fourfold higher Chl *a* concentration. These results are consistent with P and N co-limitation.

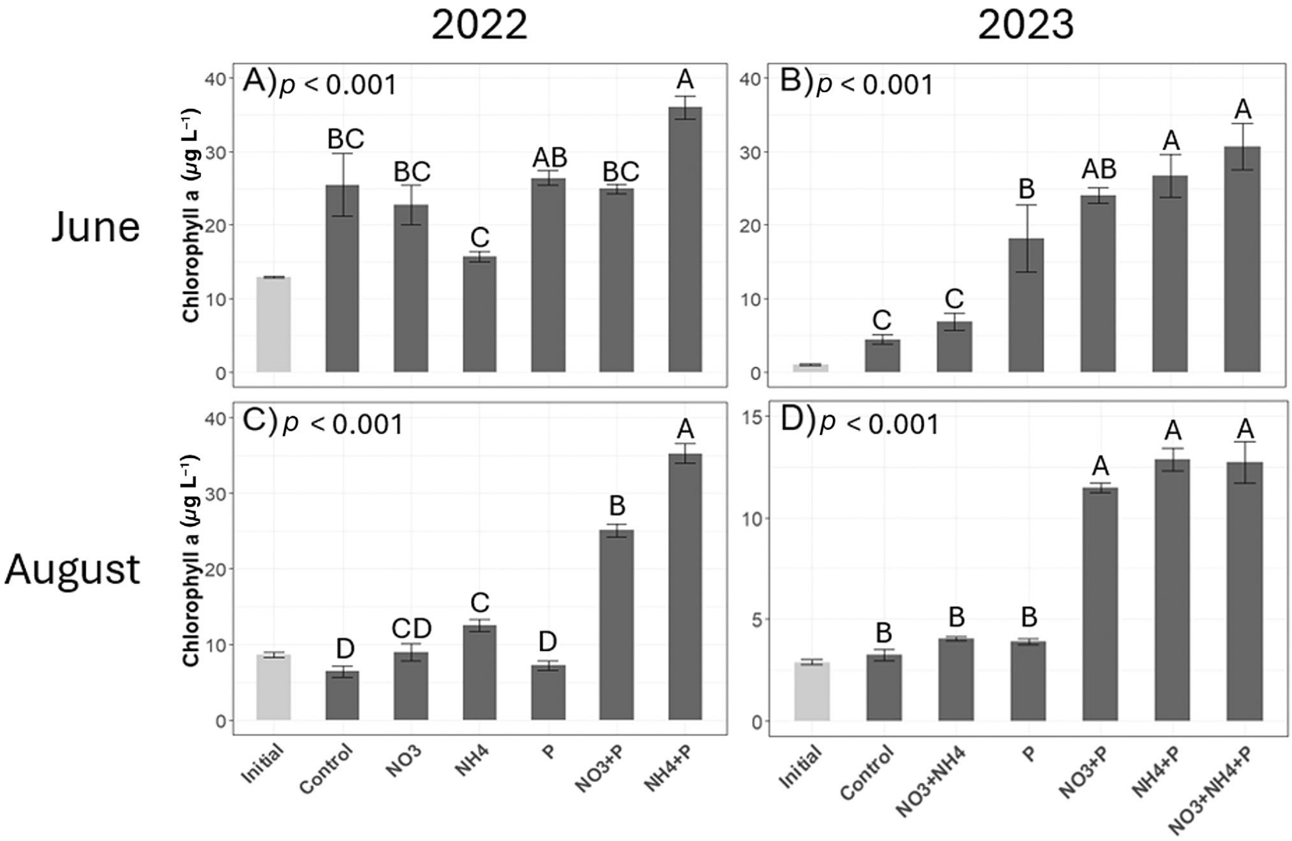

**Fig. 2.** Chlorophyll *a* results from the nutrient enrichment bioassays conducted during June (**a**) and August (**c**) 2022 and June (**b**) and August (**d**) 2023. The initial (light gray) bar represents the preincubation concentrations, and the dark gray bars are the post-incubation treatments. The bars are the mean of three individual replicates ± 1 standard error. The letters above the bars indicate the results of a post hoc Tukey test with the mean of A > the mean of B > the mean of C.

In the August 2022 dilution assays, the percentage of P diluted, if N was in excess or co-diluted, and their interaction had significant main effects on growth rates (Table S2). The P-only dilution, excess ammonium treatment resulted in the highest growth rates for all types of algae (Fig. 4), which suggests that the ammonium inhibition that was observed in June 2022 was not observed in August 2022. Despite the significant effects, the percentage of P dilution did not affect growth in a noticeable pattern like it did in June 2022, and was likely due to the co-limitation of both P and N. The N&P co-dilution treatment resulted in lower growth rates for total algae and all groups of algae than the P-only, excess N treatments (Fig. 4), which suggests that dual nutrient management would lower growth more than a P-only approach.

### June 2023

Lake water collected for the June 2023 bioassay had the highest nitrate concentration among the four experiments (56.6 $\mu$mol L$^{-1}$), low ammonium (2.2 $\mu$mol L$^{-1}$), and low DRP concentration (0.08 $\mu$mol L$^{-1}$, Table 1). The June 2023 bioassay had the lowest initial Chl *a* concentration among the four bioassays and supported a diverse phytoplankton community (Table 1).

In the traditional nutrient enrichment bioassay, the post-incubation control and the +N-only enrichment resulted in Chl *a* concentrations that were not significantly different from each other, whereas the +P-only enrichment and $+NO_3+NH_4+P$ enrichment resulted in an approximately threefold higher Chl *a* concentration (Fig. 2c). These results are consistent with P limitation.

In the June 2023 dilution assays, the percentage of P diluted, if N was in excess or co-diluted, and their interaction had significant effects on growth rates (Table S3). In the June 2023 dilution experiment, there was a clear growth rate reduction with increased dilutions for total algae and all four algal groups (Fig. 5). Additionally, the N&P co-dilution resulted in significantly lower growth rates than the P-only dilution, excess N treatments for total algae and all groups, which supports dual nutrient management. We included a 60% dilution treatment during the 2023 experiments, and at 60% dilution, the P-only and the P&N co-dilution resulted in the same growth rate for all groups except the cyanobacteria, suggesting that for a P-alone approach to be as effective as dual nutrient management, P must be decreased by 60% (Fig. 5).

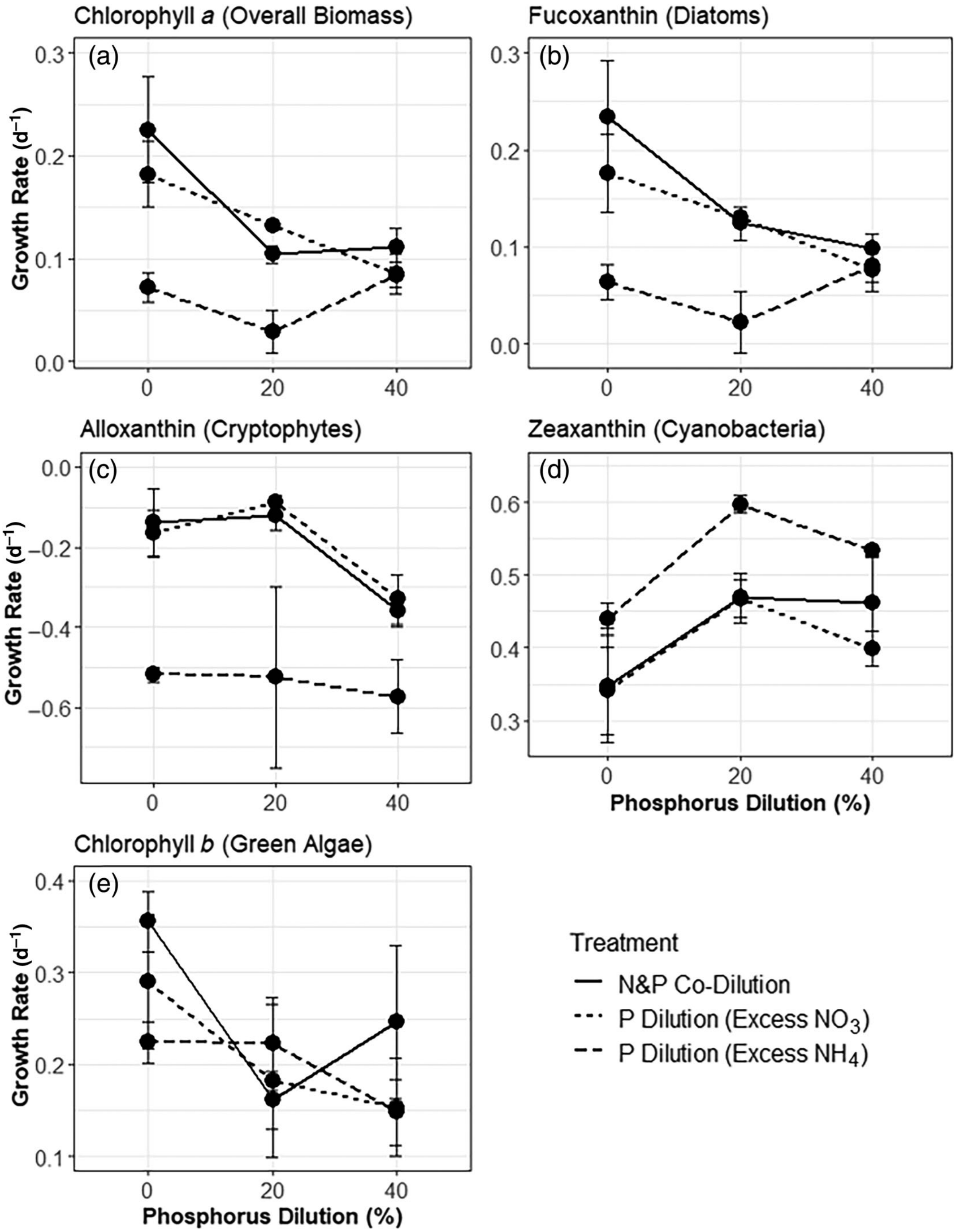

**Fig. 3.** Pigment-based growth rates in the June 2022 dilution experiments as a function of phosphorus (P) concentration dilution. The solid lines are co-dilutions of both nitrogen (N) and P, the dotted lines are the P-only dilution with excess nitrate (50 $\mu$mol L$^{-1}$), and the dashed lines are a P-only dilution with excess ammonium (50 $\mu$mol L$^{-1}$). The values are the mean of three individual replicates ± 1 standard error. Figure designations A through E refer to the different algal groups examined.

### August 2023

Lake water collected for the June 2023 bioassay had low nitrate (4.0 $\mu$mol L$^{-1}$), low ammonium (1.0 $\mu$mol L$^{-1}$), and the highest DRP concentration of the four experiments (0.65 $\mu$mol L$^{-1}$, Table 1). The August 2023 bioassay had the second lowest initial Chl *a* concentration among the four bioassays and was dominated by cyanobacteria, based on high zeaxanthin concentration (Table 1).

In the traditional nutrient enrichment bioassay for August 2023, the Chl *a* concentrations in the control, +N-only enrichment, and the +P-only enrichment did not significantly differ from each other and had Chl *a* concentrations that were

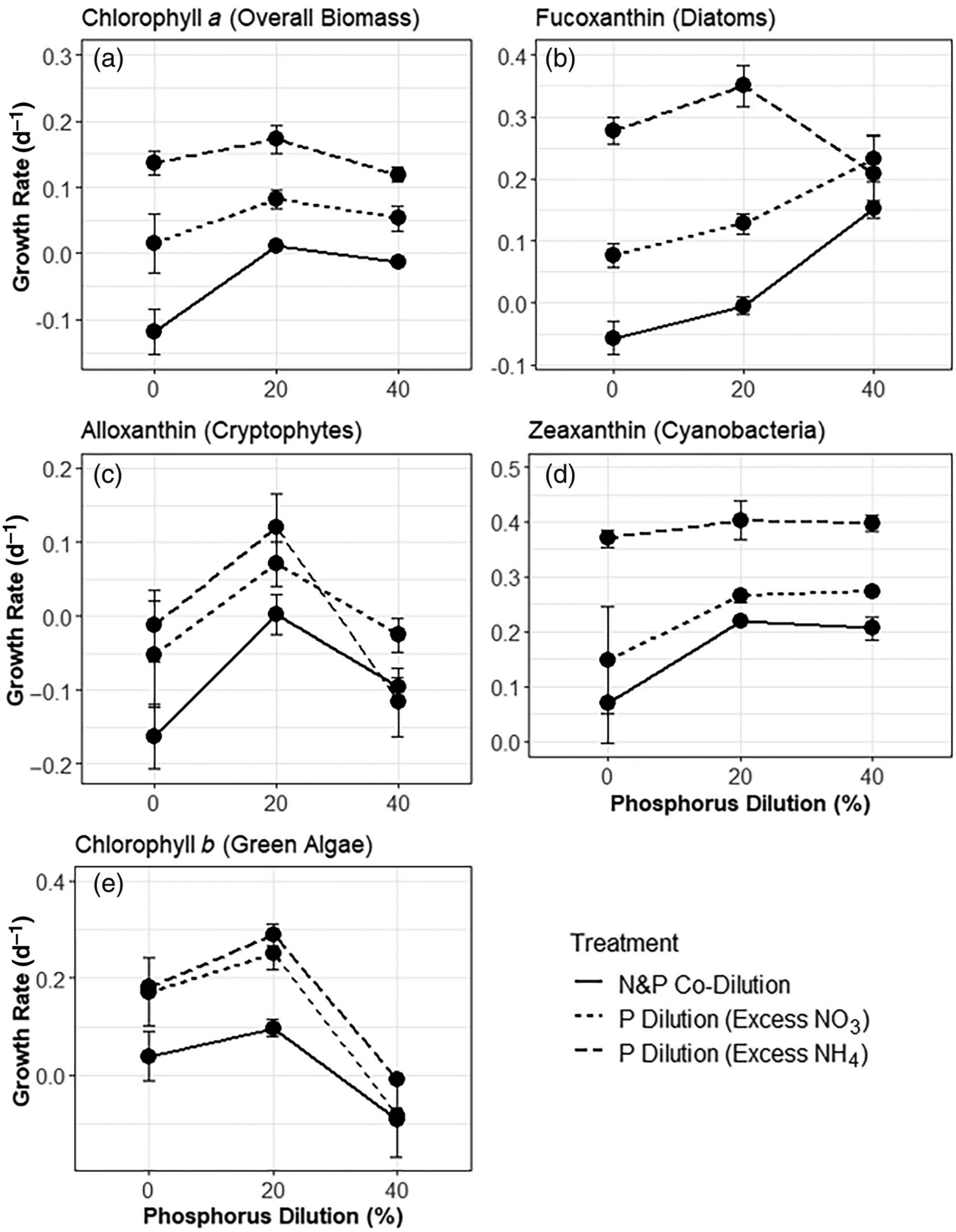

**Fig. 4.** Pigment-based growth rates in the August 2022 dilution experiments as a function of phosphorus (P) concentration dilution. The solid lines are co-dilutions of both nitrogen (N) and P, the dotted lines are the P-only dilution with excess nitrate (50 $\mu$mol L$^{-1}$), and the dashed lines are a P-only dilution with excess ammonium (50 $\mu$mol L$^{-1}$). The values are the mean of three individual replicates ± 1 standard error. Figure designations A through E refer to the different algal groups examined.

similar to the initial level, and the $+NO_3+NH_4+P$ enrichment increased Chl *a* concentration by approximately threefold (Fig. 2d). These results are consistent with N and P co-limitation.

In the August 2023 dilution assays, the main effect of the percentage of P diluted was not significant ($p = 0.284$), whether N was in excess or co-diluted was significant ($p < 0.001$), and their interaction was not significant ($p = 0.149$; Table S4; Fig. 6). The dilution assays in August 2023 did not give clear results for the effects of P-only vs. P&N co-dilution (Fig. 6). The diatoms had significantly higher growth rates in the P&N co-dilution ($p = 0.013$). The

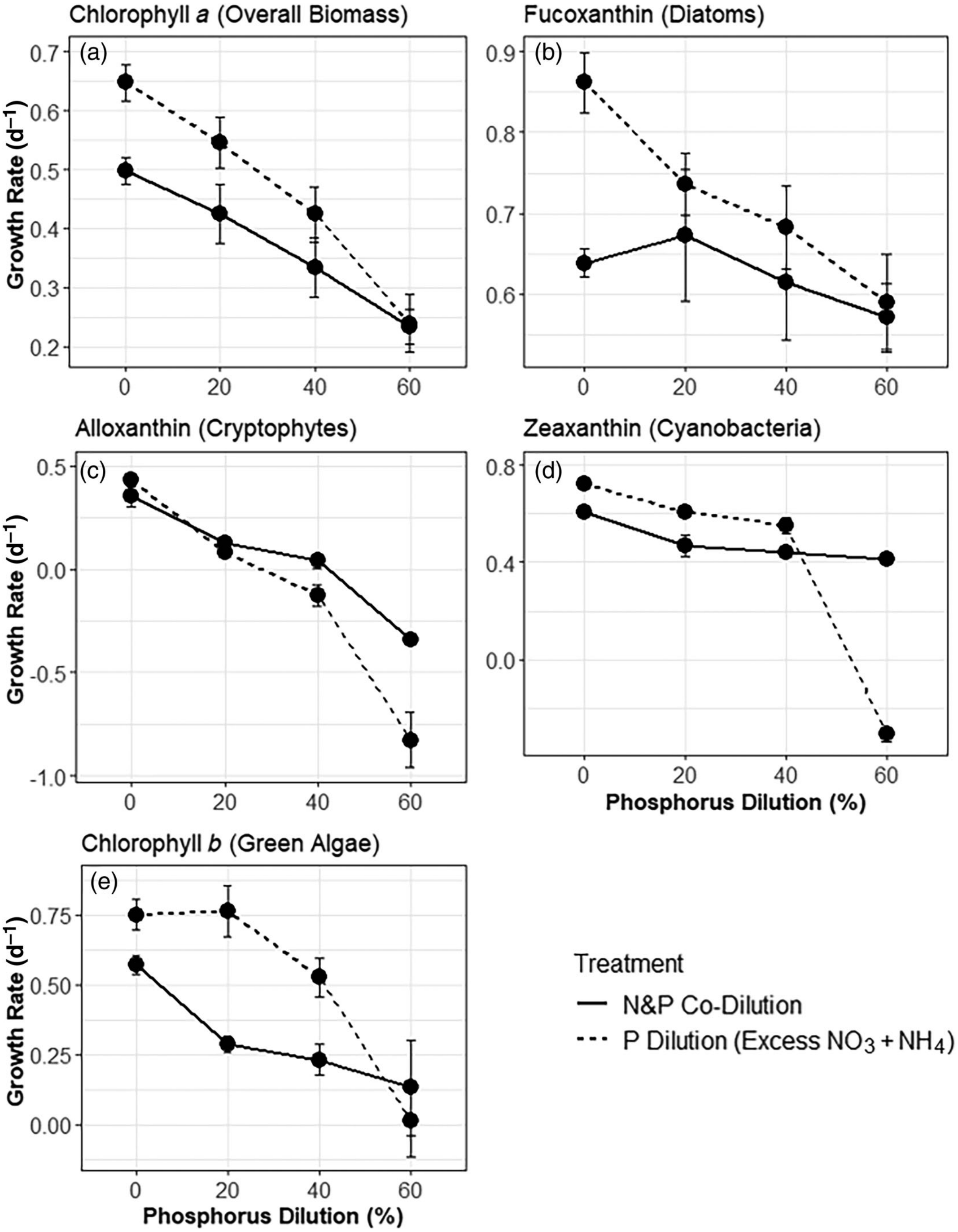

**Fig. 5.** Pigment-based growth rates in the June 2023 dilution experiments as a function of phosphorus (P) concentration dilution. The solid lines are co-dilutions of both nitrogen (N) and P, and the dashed lines are a P-only dilution with excess N (25 $\mu$mol L$^{-1}$ of each ammonium and nitrate). The values are the mean of three individual replicates ± 1 standard error. Figure designations A through E refer to the different algal groups examined.

cyanobacteria and green algae had significantly lower growth rates in the P&N co-dilutions ($p = 0.010$ and 0.003, respectively), but oddly, at 20% P-dilution, the growth rates for both groups were the same, whether or not N was in excess or co-diluted (Fig. 6).

## Discussion

We are the first to show together that co-dilutions of both P and N lowered growth rates during times when N was not limiting (June and dominated by diatoms) and that phytoplankton requires enrichments of both P and N to reach their

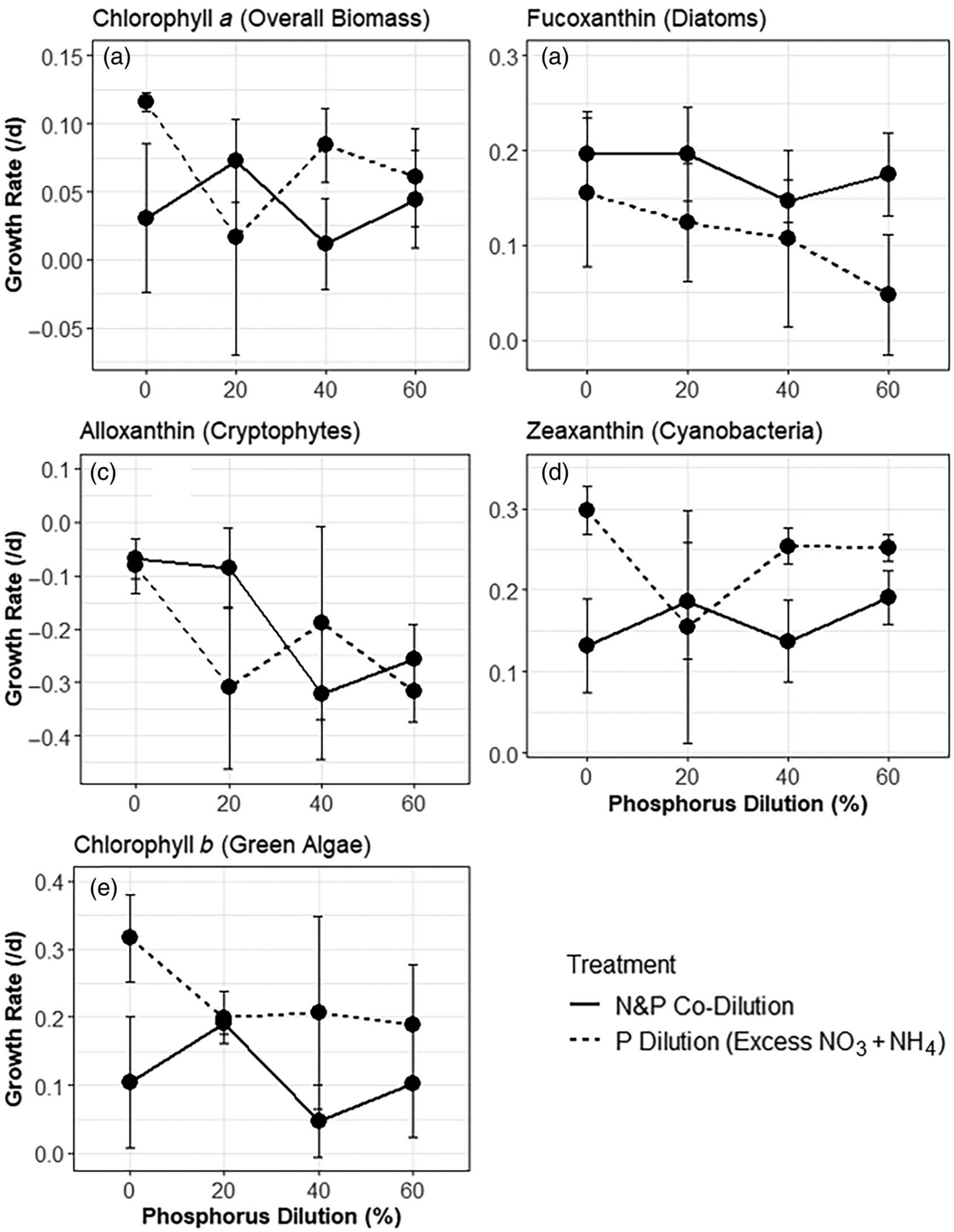

**Fig. 6.** Pigment-based growth rates in the August 2023 dilution experiments as a function of phosphorus (P) concentration dilution. The solid lines are co-dilutions of both nitrogen (N) and P, and the dashed lines are a P-only dilution with excess N (25 $\mu$mol L$^{-1}$ of each ammonium and nitrate). The values are the mean of three individual replicates ± 1 standard error. Figure designations A through E refer to the differnt algal groups examined.

highest biomass during times when cyanobacteria form blooms (August). Cyanobacterial bloom biomass would likely be higher during the late summer and fall if not for N limitation due to low ambient nitrate and ammonium concentrations (Table 1), a small abundance of $N_2$ fixing taxa compared to *Microcystis* (Nauman et al. 2024), and insufficient ammonium regeneration to keep up with N demand (Hoffman et al. 2022). While prior research focus has largely been on summer and fall (July–early October) cyanobacterial blooms (e.g., Chaffin et al. 2018b; Baer et al. 2023), we decided to additionally examine nutrient limitation of spring diatom blooms, as they comprise an important part of overall annual primary production, carbon flux and hypoxia dynamics in the lake (cf. Zepernick et al. 2024; Reavie et al. 2016). Dilution bioassays indicate that both 20% and 40% dilutions are capable of effectively reducing phytoplankton biomass and growth rates, but 40% reductions were more consistently and significantly effective. Overall, results substantiate the GLWQA recommended implementation of a 40% reduction in P inputs (GLWQA 2015); however, our results also point to the need for parallel reductions in N inputs to effectively control algal production and bloom potentials during both diatom and cyanobacterial bloom periods. We suggest that both P and N reductions should be similar in magnitude (40%) as an initial target, with possible adjustments to these reductions once their effectiveness can be gauged by employing long-term (multi-annual) monitoring. Furthermore, our experimental design and results support *dual* P and N management, and we, along with others advocating for dual nutrient management, do not recommend N-only management for Lake Erie or other freshwater bodies experiencing eutrophication and increasing bloom activities.

A complicating factor when expecting short-term (weeks to months) benefits of nutrient reductions on eutrophication and algal blooms is the chronic buildup of legacy nutrients accumulating in both watershed and in-lake sediments over nearly a century of accelerated nutrient loading to the lake. King et al. (2017) showed that DRP concentrations of agricultural runoff within the western Lake Erie are consistently high from storm to storm, whereas nitrate concentrations decrease from storm to storm, which indicates legacy P, but not legacy N, in the watershed. Legacy P from the lake bottom can be released into the water column following wind-driven resuspension, particularly in the spring/early summertime when episodic thunderstorms and bloom initiation coincide (Del Giudice et al. 2021) and from stratification and anoxic hypolimnion (Gibbons and Bridgeman 2020). Legacy nutrients in lake sediments is particularly true for P, which unlike N does not have a way of "escaping" as gaseous forms (Paerl et al. 2016). Recent studies on shallow lakes have emphasized this important distinction in the long-term roles that N and P play once external loading has been reduced (Shatwell and Köhler 2019; Xu et al. 2021; Graeber et al. 2024). These studies emphasize the legacy loading effect of P, where the time scales for "weaning" the system of P tend to be much longer than for N, because N can be lost via denitrification and ammonification, while P largely continues cycling between the sediments and water column and is mainly lost via advective flushing out of the system (Holmroos et al. 2012; Scott et al. 2019). This distinction has served as the rationale for aggressively reducing external loads of N along with P (Lewis and Wurtsbaugh 2008; Paerl et al. 2016; Wurtsbaugh et al. 2019).

It is essential to pursue a nutrient reduction strategy for Lake Erie that includes both N and P expediently, as the benefits will take some time to manifest themselves. The sooner a dual nutrient strategy is implemented, the shorter the delay between action and benefits will be realized. There are examples where dual nutrient reductions have yielded benefits within a matter of months to several years, depending on size, morphology and internal cycling dynamics of N and P (Kronvang et al. 2005; Søndergaard et al. 2017; Shatwell and Köhler 2019). For example, Shatwell and Köhler (2019) showed that N concentrations in the shallow, eutrophic Lake Müggelsee (Germany) responded immediately to loading reduction whereas P concentrations remained elevated for about 20 years; evidence that reducing N in addition to P will lead to more rapid recovery from eutrophication than P reductions alone. Based on these and current studies, the benefits of a long-term goal of a dual nutrient reduction strategy for stemming freshwater eutrophication and HABs are now recognized and beginning to be implemented by environmental agencies globally (US EPA 2015a; Tammeorg et al. 2023; Janse et al. 2024). Given its diverse and dynamic water- and airshed, urban and industrial centers and socioeconomic importance, Lake Erie represents a formidable but necessary challenge in achieving this goal.

The debate over dual nutrient management is centered around whether or not N should be managed in conjunction with P, and is not arguing for N-only management. Arguments against dual nutrient management often reference small-scale experimental results, like bottle assays, in which phytoplankton biomass was stimulated by the *addition of N* and P, whereas the question about dual management is about the *need to decrease N* and P (Schindler 2012; Schindler et al. 2016). Deploying the dilution assay approach counters this argument as it *decreases* both N and P. Dilution assays are less commonly used than the more common nutrient enrichment assays (Schelske 1984), but have been used before to determine the limiting nutrient in hypereutrophic waters when phytoplankton would not respond to nutrient enrichment (Paerl and Bowles 1987; Carrick et al. 1993). Diluting lake water in a stepwise fashion allowed us to generate growth rate curves vs. the dilution percentage. However, because dilution experiments dilute all nutrients, the essential nutrients were amended back into the lake water (i.e., MIS, C, and Si), and amending the diluted water with all but one nutrient (P) allowed us to quantify the effects of that one non-amended nutrient. We built upon this approach to amend with all but P and N to quantify the effects of a dual nutrient management scenario. Collectively, these results support the need for dual nutrient management and that the arguments made by Schindler (2012) are not supported.

Nitrogen availability has been linked to cyanotoxin (microcystin) production by Lake Erie *Microcystis* (Gobler et al. 2016). The dual nutrient vs. P-only management debate was highlighted by a modeling result that claimed a 40% P reduction will make Lake Erie *Microcystis* blooms more-toxic (Hellweger et al. 2022a). Their model suggested that *Microcystis* blooms will have 20% less biomass but microcystin concentrations will increase by 15% to 25% due to clearer water, higher oxidative stress ($H_2O_2$), and a higher availability of nitrate, which favors microcystin-producing strains (Hellweger et al. 2022a). However, others wrote rebuttals claiming that Hellweger et al.'s model excluded too many in-lake processes and included too many simplifications extrapolating cellular-level based model to the ecosystem (Huisman et al. 2022; Stow et al. 2022); but also see the response to comments (Wilhelm et al. 2022; Hellweger et al. 2022b). Regardless of the impacts on cellular toxicity, the impacts on bloom biomass and overall primary production are clear in that both N and P enrichment play a key stimulatory role during bloom succession. Furthermore, the majority of the P-only vs. dual nutrient management debate has focused solely on planktonic blooms. This debate would be more encompassing if benthic blooms were also factored in. For example, benthic cyanobacteria biomass in Lake Erie in situ nutrient diffusing substrata experiments were highest in treatments that elevated both P and ammonium (Nauman et al. 2024), which are also stimulatory for planktonic *Microcystis* blooms and is compelling evidence for dual nutrient management.

Interestingly, we note that in the June 2022 bioassay, only cyanobacteria were stimulated by ammonium enrichment ($50\,\mu\text{mol L}^{-1}$), while this proved inhibitory to eukaryotic algal groups. Diatoms are known to be nitrate specialist (Glibert et al. 2016; Swarbrick et al. 2019) and some marine diatoms can be inhibited by ammonia ($NH_3$) concentrations greater than $100\,\mu\text{mol L}^{-1}$ (Bates et al. 1993). Other studies have reported a range of ammonium concentrations in natural systems that suppress diatom growth—range from as low as $12\,\mu\text{mol L}^{-1}$ (Yoshiyama and Sharp 2006) to high as $124\,\mu\text{mol L}^{-1}$ (Liu et al. 2022). The toxicity of ammonia/ammonium increases with increasing pH as more of it is in the unionized, ammonia form (Azov and Goldman 1982); however, at pH levels in June ($\sim 8.3$), majority should be ionized as nontoxic ammonium. Chlorophytes are known to be more tolerant than diatoms to high levels of ammonia, and cyanobacteria show a preference for reduced forms of N (i.e., ammonium) (Blomquist et al. 1994; Newell et al. 2019), which was supported by our study. However, the ammonium inhibition was somewhat relieved by P additions; most likely because P was the limiting nutrient at that time. Therefore, particular attention should be paid to all forms of N discharged to the lake as efforts to mitigate CyanoHABs are implemented. On the other hand, ammonium had a stimulatory effect on the growth rate of all algae during August 2022, including the diatoms (Fig. 4b). We cannot determine from our data collected why ammonium was inhibitory (toxic or otherwise) during June but stimulatory in August.

The June experiments of both years showed a clear growth rate reduction with increased P dilution and a P and N co-dilution (Figs. 3a, 5a); however, that result was not obtained during the August experiments (Figs. 4a, 6a). The likely explanation for the nonsignificant effect of dilution percent on phytoplankton growth rate in the August experiments was the low concentrations of ambient DRP, nitrate, and ammonium (Table 1), as the enrichment experiment showed co-limitation of N and P (Fig. 2). Collectively, these results suggest that the dilution assay method is better suited for the spring and early summer when nutrient concentrations are high enough to be diluted. While P was the primary limiting nutrient during the June experiment and many other reports suggest primary P limitation in the early summer of previous years in Lake Erie (Chaffin et al. 2013; Chaffin et al. 2018b), the fact that the N&P co-dilution treatment lowered the growth rates more than P-only dilution suggests that N limitation can be induced during the early summer. However, in late summer and fall, once nitrate has been depleted in the water column and ammonium regeneration cannot totally meet the N demand (Hoffman et al. 2022), phytoplankton are constrained by both P and N. Because we showed that N limitation can be induced in the early summer, and it is well known that low N availability constrains growth in late summer and fall, these findings provide strong support that dual nutrient management will be more effective than a P-only approach at minimizing both HAB biomass and central basin hypoxia that is connected to springtime diatom blooms (Reavie et al. 2016).

The goal of the GLWQA (2015) is to reduce P loading into Lake Erie, and there is no target for in-lake P concentration. Therefore, the main caveat of our design is that we tested reduction in concentrations with the assumption that reduced loads will result in reduced concentrations. However, there is only a weak positive correlation between springtime Maumee River P load and in-lake concentration during the summer, and that correlation becomes weaker further into the lake (Rowland et al. 2020). There is no feasible method to experimentally test how a P load reduction will affect algal growth in Lake Erie. While the dilution experiments do not test the load reduction scenario, they offer the only experimental method available to test how less nutrients will affect phytoplankton growth. Numerous modeling exercises have been conducted on load reductions and suggest the P load reductions will lower phytoplankton biomass (Scavia et al. 2016; Verhamme et al. 2016), but no modeling study included a co-nutrient (P and N) scenario. The third option to evaluate P load reduction is to collect new empirical data on P load and bloom biomass annually and see how that relationship changes over time (i.e., Stumpf et al. 2012, 2016). Cost–benefit analysis of a P-only approach and dual nutrient management need to be conducted, but there are cost-effective strategies of N management from agriculture lands (Gu et al. 2023). Making assessments of how phytoplankton respond to P or P and N load reductions should be based on an ensemble of models, empirical data, and experiments and evaluated with an adaptive management mindset.

## Conclusions

Overall, our results agree with previous report (Baer et al. 2023) that a 40% reduction in both P and N will be more effective at reducing cyanobacterial blooms in Lake Erie than a P-only approach. Additionally, our results indicate that a 40% reduction in both P and N will slow diatom bloom growth, and therefore, lessen the carbon load to the central basin and minimize hypoxia. It is stressed that nutrient input reductions should be practiced on a year-round basis to mitigate both spring and summer blooms, which will also lessen central basin hypoxia. While the emphasis should be placed on mitigating problematic summer CyanoHABs, spring diatom blooms contribute significant amounts of organic carbon that will enhance internal loading, especially of P, stored in sediments from many decades of enhanced loading (i.e., legacy P). This constitutes a biologically-available source of P and N supporting summer cyanobacterial blooms. Controlling watershed inputs of both P and N will be beneficial in ultimately reducing the internal legacy pool of these nutrients and achieving the goal of reversing eutrophication and mitigating CyanoHABs and reducing central basin hypoxia.

## Data availability statement

Data used in this manuscript are listed and provided in Supporting Information. Data are available upon request from the senior author.

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## Conflict of Interest

None declared.

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