Title: The Effect of Single Versus Dual Nutrient Decreases on Phytoplankton Growth Rates, Community Composition, and Microcystin Concentration in the Western Basin of Lake Erie

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Highlights

- Dual nutrient decreases resulted in negative growth rates for cyanobacteria during late season experiments, suggesting the possibly to diminish or shorten the bloom

- Dual nutrient decreases may reduce microcystin production during the bloom.
- Decreases in both P and N may be more effective to mitigate HABs in Lake Erie than P reduction management strategies alone.

Abstract

The primary management strategy for minimizing harmful algal blooms (HABs) in Lake Erie has been to reduce springtime loading of phosphorus (P) to the lake. However, some studies have shown that the growth rate and toxin content for the HABs-causing cyanobacterium *Microcystis* also respond to the availability of dissolved inorganic nitrogen (N). This evidence is based on both observational studies that correlate bloom development with changes in N forms and concentrations in the lake, and experiments in which P and/or N are added at concentrations in excess of those present in the lake. The goal of this study was to determine whether a combined decrease in N and P concentrations from ambient levels in Lake Erie could limit the development of HABs more than a reduction in P concentration only. To directly test the impact of P-only versus dual N and P concentration decreases on phytoplankton in the western basin of Lake Erie, we evaluated changes in growth rate, community composition, and microcystin (MC) concentration through eight bioassay experiments performed from June through October 2018, which encompassed the normal Lake Erie *Microcystis*-dominated HAB season. Our results showed that during the first five experiments covering June 25 to August 13, the P-only and the dual N and P decrease treatments had similar effects. However, when ambient N became scarce later in the season, the N and P

decrease treatments resulted in negative growth rates for cyanobacteria, whereas –P only decreases did not. During low ambient N conditions, dual nutrient decreases lowered the prevalence of cyanobacteria among the total phytoplankton community and decreased microcystin concentrations. The results presented here complement previous experimental work on Lake Erie and suggest that dual nutrient control could be an effective management strategy to decrease microcystin production during the bloom and even possibly diminish or shorten the duration of the bloom based on creating nutrient limiting conditions sooner in the HAB growing season.

Keywords: Microcystis, Eutrophication, Lake Erie, Nitrogen, Phosphorus

1. Introduction

Many freshwater and coastal marine ecosystems experience cyanobacterial harmful algal blooms (HABs) due to anthropogenic nutrient pollution, land use practices, and global climate change (Paerl et al., 2016a; Paerl and Huisman, 2009; Smith, 2003). The western basin of Lake Erie is particularly susceptible to cyanobacterial blooms owing to abundant agricultural activity within the Maumee River watershed that results in high nutrient loads and its shallow water depth that result in warm temperatures, relatively low volume to dilute nutrient concentrations from Maumee River input, and relatively high light exposures (Bullerjahn et al., 2016, MacIsaac et al., 1992). Those blooms are dominated by *Microcystis,* which can produce peptide toxins called microcystins (MCs) (Rinta-Kanto et al., 2005; Steffen et al., 2017), that lead to serious threats to public health and wildlife (Bullerjahn et al., 2016; Chorus, 2012; Pouria et al., 1998; Qin et al., 2009).

Beginning in the 1960s, Lake Erie experienced poor water quality and algal blooms owing to nutrient pollution (Steffen et al., 2014). Water quality and HABs in the lake have fluctuated since then in response to management actions and changes in nutrient loading (Watson et al 2016; Makarewicz and Bertram 1991; Stumpf et al 2016). The Great Lakes Water Quality Agreement (GLWQA) of 1972 identified reductions to phosphorus (P) loads as a primary goal for managing eutrophication in the lake (DePinto et al., 1986; Stow et al., 2020). Phosphorus was targeted both because this element is routinely implicated in eutrophication of freshwater lakes (Schindler et al., 2016) and was considered the primary limiting nutrient in Lake Erie at that time (Curl, 1959; Schelske,

1979). Management actions were therefore enacted to decrease P loading and these goals were targeted at point sources related to wastewater and P-based detergents (Dolan, 1993). Those actions led to a decrease in P loads between the late 70's and early 90's, and eutrophic conditions improved until the late 1990s, when HABs began to reemerge as a major water quality concern (Stumpf et al., 2012). In response to this resurgence of HABS, the current water quality management target is to decrease P loading from the Maumee River, a major source of agricultural P runoff, into western Lake Erie by 40% by 2025 as compared to 2008 baseline P loads (Annex 4, 2015; Verhamme et al., 2016). Despite overall reductions in total phosphorus loads to the lake, the forms of P entering the lake have also changed and input of soluble reactive P (Baker at al. 2014; Maccoux et al. 2016). The contemporary P loading targets are based on an ensemble of deterministic models and statistical models based on the relationship between interannual total P load and bloom magnitude (Scavia et al., 2016; Kane et al., 2014; Scavia et al., 2014; Stumpf et al., 2012). Despite these research findings and the past successes in decreasing P inputs, the resurgence of HABs in western Lake Erie over the past decade (Stumpf et al. 2016) warrants investigation into the role of other nutrients or factors besides P.

A number of factors have been proposed for explaining the recent increase in HABs, including changes in the forms of P entering the lake from its watershed (Bertani et al., 2016), invasive mussels that recycle P (Hecky et al., 2004; Vanderploeg et al., 2001), and changes in precipitation patterns that alter the timing and magnitude of P inputs (Paerl et al., 2016a, Michalak et al., 2013; Paerl and Huisman, 2009). While each of these mechanisms are supported to some extent, the role of nitrogen (N) in influencing

bloom dynamics is now receiving renewed emphasis (Newell et al., 2019; Paerl et al., 2016b). While primary P-limitation of algae has been observed in bioassay experiments performed in the lake (Saxton et al., 2012; Moon and Carrick, 2007; Chaffin et al., 2013; Chaffin et al., 2014), recent evidence suggests that P-limitation can shift to co-limitation or limitation by other nutrients later in the summer season (Barnard et al 2021). Although P limitation is common in lakes, N limitation or N and P co-imitation are pervasive across systems (Elser et al., 1990; Elser et al., 2007; Paerl et al., 2016b). There is evidence that N is both a crucial limiting nutrient in freshwater eutrophication (Conley et al., 2009; Lewis and Wurtsbaugh, 2008; Pearl et al., 2009) and may impact cyanobacterial biomass and toxin concentration (Muller and Mitrovic, 2015; Gobler et al., 2016; Newell et al., 2019; Barnard et al., 2021; Paerl and Otten, 2013). Similarly, when P is widely available there is potential for low N concentrations to constrain the growth of cyanobacteria during bloom conditions (Chaffin et al., 2013; Jeppesen et al., 2005; Paerl et al., 2016b; Gobler et al., 2016).

While a decrease in P is expected to have beneficial impacts on mitigating the occurrence and size of HABs in the western basin of Lake Erie, availability of N may become secondarily limiting or affect the composition of algae and production of toxins. In Lake Erie dissolved inorganic nitrogen (N) is at high concentrations from Spring until early August, and the pattern of this seasonal availability of N may play a role in bloom duration (Gobler et al., 2016). Recent studies have indicated that phytoplankton in Lake Erie, *Microcystis* in particular, are responsive to the availability and forms of nitrogen in the lake (Chaffin et al., 2018; Newell et al., 2019). Bloom development over the course

121 of the season is coincident with a decrease in dissolved inorganic N concentration
122 (Chaffin et al., 2013; Jankowiak et al., 2019). Based on these more recent studies and a
123 broader examination of nutrient effects, it is apparent that a decrease of both N and P
124 inputs has the potential to be more effective in constraining HAB biomass than P-only
125 decreases.

126 In addition to acting as a limiting nutrient for growth of HABs, nitrogen also
127 impacts the production of microcystin (MC) by cyanobacteria. Microcystin is rich in N
128 and its availability has been documented to affect regulation of *mcy* genes necessary for
129 the synthesis of the complete peptide (Harke and Gobler, 2013; Harke and Gobler, 2015;
130 Davis et al., 2015, Ouellette et al., 2006). In *Microcystis*, the transcription of N uptake
131 and MC production genes are both impacted by the same mechanism of the NtcA (global
132 nitrogen regulator) transcription factor, suggesting that N metabolism coincides with
133 microcystin synthesis (Pimentel and Giani, 2014, Harke and Gobler, 2013). Under N-
134 depleted conditions, *Microcystis* allocates its N toward cell functions required for
135 survival and growth (Harke and Gobler, 2013). Multiple studies have emphasized the
136 importance of N in controlling microcystin levels and the proportion of potentially toxin-
137 producing cells in Lake Erie HABs (Jankowiak et al., 2019; Gobler et al., 2016; Barnard
138 et al., 2021; Harke et al., 2015; Wagner et al., 2021) and that N addition can lead to a
139 larger increase in microcystin concentration than P additions (Davis et al. 2015; Donald
140 et al. 2011). One of the challenges for clearly understanding the potential benefits of
141 managing both N and P, is that most previous work regarding dual-nutrient control has
142 focused on nutrient addition experiments, versus responses to N or P decreases. More

143 recent work has employed experimental approaches involving decreasing nutrients to
144 below ambient concentrations to evaluate the impacts of decreased availability (Paerl et
145 al., 2011; Xu et al 2015). A recent study in two locations in Western Lake Erie employed
146 experimental decreases in N and P below those expected for river inputs and found that a
147 P decrease alone is insufficient to limit microcystin production (Barnard et al 2021). That
148 paper also indicated that the degree of co-limitation varied between pre-bloom (June) and
149 mid-bloom (August) conditions, suggesting the need for further investigation on the
150 timing of those shifts in relation to ambient lake conditions.

151 The goal for this study was to determine if decreases in concentrations of both
152 inorganic P and N below ambient concentrations in the lake, compared to decreases in P
153 only, has a greater capability to decrease the growth rate, toxin concentrations and
154 prevalence of *Microcystis* among the phytoplankton community in western Lake Erie.
155 Moreover, we hypothesized that these effects would be mediated by ambient N and P
156 concentrations in the lake, which change dramatically over the course of bloom
157 development. To address this goal, we tested three hypotheses: 1) dual nutrient decreases
158 below ambient concentrations are required to reduce growth rates in comparison to single
159 nutrient control when ambient N concentrations are low; 2) dual inorganic nutrient
160 decreases will decrease the abundance of cyanobacteria within the phytoplankton
161 community, particularly when N is low; and 3) dual nutrient decrease will reduce toxin
162 concentrations, the ratio of toxin to biomass, and proportion of mcyE-containing
163 cyanobacteria when compared to single or no nutrient control. Our experiments were
164 performed with natural phytoplankton communities from the western basin of Lake Erie

in order to identify the role of single and dual nutrient decreases below ambient concentrations on cyanobacterial growth rates, community prevalence, and MC production. These experiments complement earlier work using similar methodology in a different part of the western basin (Barnard et al., 2021), but our design repeated the experiment eight times over the course of the boom season to offer new insight on the phenology of phytoplankton response to nutrient availability and particularly nutrient deplete conditions.

2. Material and Methods

2.1 Sample collection and treatment preparation

We executed a series of experiments to determine the impacts of phosphorus-only decreases and dual-nutrient decreases below ambient concentrations in Lake Erie (Paerl et al., 2016b). In order to test our hypotheses during different stages of the bloom and ambient nutrient concentrations (Supplemental Figure S1), we collected water on eight occasions in 2018 from pre-bloom (June) to post-bloom season (October). Our treatments were -N-P (both soluble reactive P and nitrate decreased 40% compared to ambient), -P (only soluble reactive P decreased 40% compared to ambient), ambient, and +N+P (both nitrate and soluble reactive P increased to 40% above ambient). Lake sampling dates were June 25, July 16, July 23, July 30, August 13, August 27, September 11, and October 1 in the year 2018, and herein experiments will be referred to by these dates. Site selection was based on using a location known to be influenced by Maumee River loads and the availability of real-time nutrient data from a continuous monitoring buoy. We collected water from the NOAA Great Lakes Environmental Research Laboratory

187 monitoring site WE2 (41.76217°, -83.33000°) located in the western basin approximately
188 14.5 kilometers northeast of the Maumee River mouth and 15 kilometers west of the
189 municipal water intake for the city of Toledo, Ohio (CIGLR & NOAA GLERL, 2019).
190 For each sampling event 60 L of lake water were collected via a peristaltic pump
191 1 meter below the water surface and stored in dark insulated containers. Once at the
192 laboratory (approximately 4 hours after collection), the carboys were inverted 10 times to
193 ensure water was well mixed prior to any sub-sampling. We estimated ambient nitrate
194 and SRP concentrations using in-situ WE2 buoy data at noon (12:00pm) of the day of
195 collection. The buoy was integrated with a Wet Labs Hydrocycle P instrument (Anderson
196 et al., 2021) that measured soluble reactive P using the same ascorbic acid and
197 molybdenum method as used in the laboratory (CIGLR & NOAA GLERL, 2019). To
198 dilute nutrients to below ambient concentrations, lake water was mixed with Hard Water
199 Mussel Medium (HWMM, 0.2 mM $MgSO_4$, 1.5 mM $NaHCO_3$, and 0.75 mM $CaCl_2$) and
200 then amended with N or P to create the specific nutrient treatments. HWMM is a salt
201 solution that contains no major nutrients and reflects the major ion chemistry of Lake
202 Erie (Chapra et al., 2012). We routinely check the dissolved inorganic N and P in
203 HWMM and it is below 0.5 µgP L^{-1}, 0.02 mgN L^{-1} as nitrate, and 5 µgN L^{-1} as
204 ammonium.
205 The -N-P treatment contained no additional nutrients added following the 40%
206 dilution with HWMM and represents a total 40% decrease of nutrients and biomass. For
207 the -P treatment, N was added back as nitrate to ambient levels and represents a 40%
208 decrease of phosphorus and biomass. For the ambient treatment, N and P were added

back to ambient levels. If phosphorus levels were not detected via buoy sensors, then 1.5 μg L^{-1} of phosphorus was added to represent a typical low-level concentration observed by laboratory analysis of the weekly NOAA-GLERL monitoring. For the +N+P treatment, N and P were added back to match ambient levels, and then additional N and P was added to represent the nutrient conditions under the influence of spring loads (2 mgN L^{-1} and 20 μgP L^{-1}). If soluble reactive P was reported at less than the instrument detection limit (2.3 μgP L^{-1}), then the final concentration of P in the +N+P treatment was brought to 21.5 μg L^{-1}.

Each treatment was replicated three times in each experiment using transparent 4-L polycarbonate bottles. The mesocosm bottles were placed in an insulated outdoor incubation tank in which water was constantly mixed by pumps to gently mix the bottles and reduce *Microcystis* colony settling (Den Uyl et al., 2021). The tanks were temperature controlled within 1°C of the measured Lake Erie water temperatures at station WE2 at noon (12:00pm) of the collection day by a recirculating temperature bath (Cole-Parmer Instrument Company). We used a neutral density filter membrane applied to each bottle to decrease light intensity to 50% of surface irradiance to represent the light intensity that algal cells would experience at approximately 1 meter depth. An RBR Solo temperature sensor was placed in the tank to monitor tank temperature.

Response variables identified include size-fractionated chlorophyll, fluorometry, pH, quantitative polymerase chain reaction of total cyanobacteria and toxin *mcyE*, and particulate microcystins. The first sampling ($T_{initial}$) occurred immediately after filling the bottles on the day of collection. We sampled the entire range of parameters on days 0, 3

(T_{mid}), and 7 (T_{end}), where data was collected for a total of 7 days for each experiment. However, the June 25 experiment ran for 9 days with mid and endpoint data collected on Day 4 and Day 9 respectively. T_{mid} and T_{end} data was collected from each individual bottle around 9 am local time.

2.2 Biomass Concentrations

Size-fractionated chlorophyll was analyzed by filtering two replicate subsamples (approximately 150 mL) at $T_{initial}$, T_{mid}, and T_{final} through either a 53 μm Nitex screen or a Whatman GF/F filter (Bowers, 1980; Vanderploeg et al., 2001). Screens and filters were frozen and later extracted with N,N-dimethylformamide and analyzed fluorometrically (Speziale et al. 1984) using a Turner Designs 10-AU fluorometer. Total chlorophyll was obtained from the sum of the two size fractions. A benchtop spectrofluorometric instrument (Fluoroprobe, BBE Moldaenke, Series 3) was used to identify algal group-specific in vivo fluorescence of intact samples. The phytoplankton group concentrations were allocated from the total fluorescent concentration to a spectral algal class due to its fluorescence spectrum (Catherine et al., 2012, Chaffin et al., 2013). Class-specific fluorometry data were collected at 9 am each day of the experiments. Particulate carbon (C) and N were determined by flash combustion method using a Carlos Erba EA1110. We estimated exponential growth rates during days 0-3 for both class-specific fluorescence and extracted chlorophyll using the following equation:

$$\frac{(ln(biomass_3) - ln(biomass_0))}{t_3 - t_0}$$

2.3 Particulate Microcystins (MCs)

Particulate MCs were determined from filtered mesocosm samples. Samples underwent three freeze/thaw cycles, QuikLyse Cell Lysis (Abraxis #529911QL), and were quantified using a microcystin-specific enzyme-linked immunosorbent assay (ELISA) (Abraxis #520011; Fischer et al. 2001). Toxin concentration is the overall concentration of particulate microcystins and was determined for all experiments except for the October 1 experiment, when routine lake sampling had indicated that microcystin was below detection.

2.4 Nutrient Concentrations

Concentrations of nitrate, ammonium, and dissolved reactive P were quantified with a Seal AA3 continuous segmented flow analyzer (SEAL Analytical Inc., Mequon, WI) using standard U.S. EPA methods (EPA 353.1, 354.1, 350.1, and 365.1, respectively). Samples for particulate P were collected onto polycarbonate membrane filters with a pore size of 0.2 µm and the P content was determined by persulfate digestion adapted from Menzel and Corwin (1965), followed by the ascorbic acid molybdenum blue method (Strickland and Parsons, 1972).

2.5 qPCR (total cyanobacteria and toxin mcyE)

Estimates of total cyanobacterial cell concentrations and the proportion of *mcyE* containing cyanobacteria within the mesocosms were determined using quantitative polymerase chain reaction (qPCR). We collected samples for qPCR on Millipore Isopore membrane filters with a pore size of 3.0 µm. These filters match those used for long-term

272 monitoring of particulate MC by NOAA GLERL and CIGLR (Burtner et al. 2020). For
273 quantification, total cellular nucleic acids were extracted from filtered mesocosm samples
274 using the Qiagen DNeasy Blood and Tissue Kit, adding a lysate homogenization step
275 (QiaShredder spin-column) prior to DNA purification. DNA extract was frozen at -80 °C
276 until analysis. We performed qPCR using Phytoxigene CyanoDTec kits (Phytoxigene,
277 Inc., Akron, Ohio, US) using the manufacturer's primers, cycling parameters, and copy
278 number estimation. Two cyanobacteria-specific genetic targets were used during this
279 study, the 16S-Cyano rRNA gene (16S rDNA) and the mcyE gene. Targeting the 16S
280 rRNA gene allows for the quantification of the abundance of total *Microcystis* population
281 within the mesocosm. The mcyE gene is found within the microcystin synthetase gene
282 cluster and is one of the genes responsible for the production of microcystin (Genuario et
283 al., 2010; Tillet et al., 2000). The *mcyE* target is only found in potentially-toxic strains of
284 *Microcystis* and allows for the quantification of toxin potential in the mesocosm. qPCR
285 was executed using an Applied Biosystems 7500 Fast Instrument using TaqMan labeled
286 probes (Applied Biosystems) and genus-specific mcyE and 16S-Cyanobacteria rDNA
287 primers. For amplification of both the 16S and mcyE gene targets, the cycling conditions
288 were for 95 °C for 2 minutes for initial denaturation, followed by 40 cycles of 95 °C for
289 15 seconds for denaturation and 60 °C for 30 seconds for annealing-extension.
290 The proportion of *mcyE* containing cyanobacteria refers to the number of *mcyE*
291 gene copies normalized to 16S cyanobacteria abundance at T_{final} and was determined for
292 all experiments except for the June 26 and October 1 experiments. Biomass normalized
293 toxicity refers to particulate microcystins normalized to cyanobacterial fluorescence and

294 was determined for all experiments except for the October 1 experiment. Toxin
295 concentration was normalized to cyanobacterial fluorescence in order to get a more
296 accurate prediction of potential toxin production within the cyanobacterial population.

297 *2.6 Statistical Analyses*

298 For each experiment, separate one-way analysis of variance (ANOVA) tests were
299 used to examine the effect of treatment on total fluorescence and cyanobacterial growth
300 rate, particulate toxins, particulate MCs normalized to cyanobacterial fluorescence, and
301 *mcyE* gene abundance normalized to cyanobacteria abundance. Phytoplankton
302 community composition was log transformed prior to analysis. A Tukey post-hoc test
303 was performed among treatment types. All statistical analyses were performed using R
304 version 3.4.3 (R Core Team 2017) and Figures were made using SigmaPlot version 14.0
305 (Systat Software).

306

307 **3. Results**

308 The initial physical and chemical water quality conditions for each experiment are
309 given in Table 1. Across the experimental sampling dates, ambient surface water
310 temperature ranged from 19.3 to 26.7^{o} C, and the concentrations of phytoplankton
311 biomass (estimated by extracted chlorophyll-a and phycocyanin) indicate HAB
312 conditions were reached by the July 30 sampling event, persisted throughout August, and
313 then declined during September and October. Dissolved inorganic nutrient
314 concentrations (NH_4 , NO_3 , and SRP) showed a pattern of seasonal decline related to

315 phytoplankton assimilation, biomass accrual, and dilution of river inputs (Table 1).
316 Elevated concentrations during the June 25 and September 11 experiments reflect a
317 response to rain events and higher discharge from the Maumee River, as noted by water
318 chemistry changes at the monitoring buoy (Table 1). At the time of collection of the June
319 25 experiment, ambient SRP was 24.4 µg-P L^{-1} with a nitrate concentration of 1.376 mg-
320 N L^{-1}, and the ambient SRP was 17.8 µg-P L^{-1} at the September 11 experiment sample
321 collection (Table 1).

322 Figure 2 shows that fluorescence of the phytoplankton increased rapidly over the
323 first 3-4 days of each experiment, then decelerated or decreased. These time series of
324 fluorescence also indicate that the treatments had varying effects based on time of year
325 and initial ambient conditions. In the beginning of the season, all treatments except the
326 +N+P treatment shared similar trends, however; by the end of the season (August 27,
327 September 11, and October 1 experiments) the –N–P treatment resulted in lower biomass
328 yields compared to the other treatments ($p<0.001$ for August 27 and September 11
329 experiments and, $p = 0.01$ for October 1 experiments) (Figure 2). This pattern of lower
330 yields within the -N-P treatment coincides with minimum dissolved inorganic N (NH_4^+
331 and NO_3^-) concentrations in the lake (Figure 1). The +N+P treatment always resulted in
332 higher yields of biomass, except for the October 1 experiment where the +N+P, ambient,
333 and –P treatments trended similarly (Figure 1).

334 Figure 3 shows that the decreased nutrient treatments had variable effects on the
335 initial growth rates of total phytoplankton and cyanobacteria, depending on the time
336 within the season. The -P treatment in comparison to the ambient treatment resulted in

337 decreased growth rates for total phytoplankton at multiple timepoints, but the largest
338 effects occurred on July 23 (0.055 d^{-1} reduction, 32.75% decrease), July 30 (0.042 d^{-1}
339 reduction, 28.89% decrease) and August 13 (0.014 d^{-1} reduction, 18.27% decrease). The -
340 P treatment in comparison to the ambient treatment had a similar effect on the growth
341 rate of cyanobacteria with the largest decreases on July 23 (0.031 d^{-1} reduction, 31.85%
342 decrease) July 30 (0.047 d^{-1} reduction, 67.39% decrease, and August 13 (0.036 d^{-1}
343 reduction, 58.01% decrease). Compared to the ambient treatments, the -N-P treatment
344 had lower growth rates for total phytoplankton and cyanobacteria at multiple timepoints.
345 The largest decrease in growth rates occurred on August 13, August 27, September 11 for
346 both total phytoplankton and cyanobacteria. Growth rates were reduced 0.038 d^{-1}
347 (49.06% decrease), 0.110 (131.76% decrease) and 0.129 d^{-1} (42.74% decrease)
348 respectively in total phytoplankton, and cyanobacteria growth rates were decreased by
349 0.0465 d^{-1}, (75.93% decreased) 0.188 d^{-1}, (928.34% decrease) and 0.135 d^{-1} (176.11%
350 decrease) for the respective dates. During August and September, the –N–P treatment
351 resulted in lower growth rates of cyanobacteria compared to the –P, ambient and elevated
352 nutrient treatments. In fact, dual nutrient decreases resulted in negative growth rates for
353 cyanobacteria in the August 30 ($p<0.001$) and September 14 ($p<0.001$) experiments, even
354 when the other treatments maintained positive growth rates. The largest effects occurred
355 on August 13 (0.024 d^{-1}, 37.66% decrease), August 27 (0.126 d^{-1}, 126.67% decrease) and
356 September 11 (0.110 d^{-1}, 38.93% decrease) and growth rates for cyanobacteria on August
357 27 (0.204 d^{-1}, 567.31% decrease) and September 11 (0.125 d^{-1}, 187.75% decrease). This
358 pattern of reduced and even negative growth rates within the –N–P treatment coincides

359 with low DIN concentration in the lake (Table 1). While the nutrient decrease treatments
360 had variable effects on growth rates, the +N+P nutrient treatment consistently resulted in
361 higher growth rates for both cyanobacteria and the total community ($p<0.05$) for the July
362 23, July 30, August 13, August 27, and September 11 experiments.
363 Table 2 shows that early in the season (from June 25 experiment to the August 13
364 experiment), all the treatments except the +N+P treatment had similar yield responses.
365 Specifically in the July 24 through September 11 experiments, cyanobacteria accounted
366 for the majority (54.9% to 73.5%) of the phytoplankton community at $T_{initial}$. By T_{mid} of
367 those experiments, cyanobacteria accounted for less than half of the community on
368 average (7.40% to 50.91%) and by T_{final} cyanobacteria accounted for approximately
369 28.77% of the community. Differences among treatments on biomass became more
370 obvious by the end of the season and the greatest reduction in cyanobacterial abundance
371 was observed in the –N–P treatment (Table 2). This pattern of reduced cyanobacteria
372 abundance within the –N–P treatment coincides with the lowest DIN concentration in the
373 lake (Figure 1). Dual nutrient control reduced the abundance of cyanobacteria within the
374 entire phytoplankton community compared to the other treatments (Table 2). For
375 example, the August 30 experiment ($p < 0.001$) had an initial cyanobacterial abundance
376 of 8.26 ($\mu g\ L^{-1}$) and dual nutrient control (–N–P) reduced the final cyanobacterial yield to
377 4.84 ($\mu g\ L^{-1}$) compared to a final concentration of 9.127 ($\mu g\ L^{-1}$) in the ambient treatment
378 (Table 2). This trend was also observed in the September 14 experiment ($p < 0.001$),
379 where dual nutrient decreases reduced cyanobacterial abundance to 5.53 $\mu g\ L^{-1}$ in the -N-
380 P treatment compared to 8.66 $\mu g\ L^{-1}$ in the ambient treatment (Table 2).

Figure 3 shows that dual nutrient control decreased toxins (Panel A) in the July 30 ($p<0.001$) and August 27 ($p<0.001$) experiments and decreased the amount of biomass-normalized toxin (Panel B) in the July 26 experiment ($p < 0.001$). Microcystin concentration and potential (mcyE copies / 16S copies) trended with overall biomass concentration. Toxin production declined over the bloom season from mid-July to October. This pattern is seen in Figure 3, where an increase in nutrients increased toxins (panel A) in the July 26 ($p<0.001$), August 3, ($p = .0107$) and August 30 ($p<0.001$) experiments.

4. Discussion

In the western basin of Lake Erie, nitrogen availability is higher during bloom initiation in early July and declines throughout the summer, reaching minimum concentration in September or October (Chaffin et al., 2011, 2013; Gobler et al., 2016). Studies suggest that microcystin concentrations increase when nitrate concentration and other environmental conditions such as water temperature are conducive to cyanobacterial growth, thus higher concentrations of cyanotoxins are expected during the mid-summer (i.e. higher production) during a period of high nitrogen availability and warm water temperatures (Chaffin et al., 2018; Gobler et al., 2016; Horst et al., 2014; Obenour et al., 2014). Decreasing the availability of both nitrogen and phosphorus could lead to a faster decline in the internal pool of nutrients within the phytoplankton cell (Saxton et al., 2012), and limit the amount of intracellular nutrients available to be allocated to processes by which toxins are produced, which may lead to a reduction in toxicity of a bloom. The peak in available N in the lake is preceded by high loading in

403 spring from the watershed (Song et al. 2022), suggesting that targeting N load decreases
404 throughout the spring and summer is an effective strategy.

405 We hypothesized that decreasing phosphorus would reduce growth rates of
406 cyanobacteria and that decreasing N and P would result in lower growth rates than just
407 decreased P alone. The results presented here suggest that dual nutrient control has the
408 capability to decrease toxicity, production, and toxin potential within blooms faster than
409 with single nutrient (P) control alone, this is supported by previous studies (Chaffin et al.,
410 2018; Barnard et al., 2021; Davis et al. 2009; Davis et al. 2015). While that has been
411 reported previously, this study was directly tied to ambient concentrations in lake water
412 and shows the seasonal progression of this co-limitation effect. In June through mid-
413 August of the Lake Erie HAB season, when DIN was elevated in the ambient lake water
414 (0.3 to 1.3 mg N L^{-1}, Table 1), all treatments except the +N+P treatment produced only
415 small impacts on biomass, growth rates, and toxin. This lack of effect in the nutrient
416 decrease treatments can be attributed to availability of nutrients within the lake water
417 (Table 1, Figure 1). The exact timing and magnitude of response to treatment is
418 dependent on the status of the internal pools of N and P and should reflect this seasonal
419 exposure history (Millie et al., 2009; Kane et al., 2014). By the end of the season
420 treatment effects became stronger, with the -N-P treatment yielding less biomass and MC
421 than the -P only or ambient treatments. In our experiments, this response occurred when
422 ambient nitrate concentration was low, reaching 0.06 mg-N L^{-1} (Table 1, Figure 1).

423 We hypothesized that dual nutrient decreases would decrease the abundance of
424 cyanobacteria among the phytoplankton community. Dual nutrient decreases limited the

425 abundance of cyanobacteria among the phytoplankton community. For the majority of the
426 experiments (July 23 through September 11), cyanobacteria accounted for the majority
427 (54.94% to 73.49%) of the initial phytoplankton community. Our biomass data shows
428 that green algae dominated (>95%) in the June 25 and July 16 experiments, and the algal
429 groups were roughly in the same proportions (no obvious dominant group) in the October
430 1 experiment (Supplemental Figure S1) and these compositional differences can help
431 explain the different yield responses between treatments. Overall, cyanobacterial
432 abundance in the –N–P treatment was often lower than in any of the other treatments,
433 with +N+P yielding the greatest abundance. In the August 27 and September 11
434 experiments, dual nutrient control (–N–P) reduced the final cyanobacterial abundance
435 when compared to the initial biomass, while all other treatments saw increased
436 abundance when compared to $T_{initial}$ (initial abundance). These reductions in abundance
437 could potentially be explained by allowing for algal species that are more efficient at
438 nutrient uptake or have higher growth rates, such as green algae, to outcompete
439 cyanobacteria under nutrient replete conditions.

440 Moreover, our results showed that dual nutrient decreases (–N–P) resulted in
441 negative growth rates (i.e. net mortality) for cyanobacteria in multiple experiments, even
442 when the other treatments maintained positive growth rates. This finding suggests that
443 dual nutrient decreases are, in fact, required to reduce growth rates in comparison to
444 single or no nutrient control under given conditions. Excess nutrients seen in short-term
445 pulse events, as depicted by the +N+P treatment, often resulted in high growth rates for
446 both cyanobacteria and other algal groups. Though cyanobacteria demonstrated a greater

447 proportional response in growth rate of the +N+P treatment and supports the capability of
448 cyanobacteria to exploit excess nutrients seen in storm events. Previous experimental
449 studies in Lake Erie have demonstrated the potential for dual nutrient effects (Chaffin et
450 al. 2018; Barnard et al. 2021), although at concentrations of N and P greater than the
451 ambient levels in the lake. Our results support those findings, but go further to show
452 that dual nutrient decreases below ambient concentrations have the capability to decrease
453 toxicity, production, and toxin potential within blooms faster than with single nutrient
454 control alone. Although our results show an effect of nitrogen concentration, it is critical
455 to note that available N cycles rapidly in Lake Erie (Hampel et al. 2019) and so the
456 magnitude of response to changes in concentration is hard to anticipate. However, it is
457 likely that by decreasing both N and P concentrations in the lake when DIN are low in
458 ambient lake conditions (which will require limiting loads earlier in the year),
459 cyanobacterial abundance can be further limited. Eutrophication thresholds in large lakes
460 range from 0.50 to 1.20 mg L^{-1} for total nitrogen and 0.03 to 0.10 mg L^{-1} for total
461 phosphorus (Smith et al, 1999; Xu et al., 2014) and decreased nutrient contractions below
462 these thresholds may explain the negative growth rate associated with the –N–P treatment
463 in the August 27 experiment. This finding indicates that decreasing nutrient
464 concentrations well below these thresholds may decrease the impacts of HABs. The
465 +N+P treatment almost always resulted in higher yields of biomass, toxin concentration,
466 and increased biomass concentration of phytoplankton across all experiments. This
467 finding was expected and provides a meaningful reminder of the negative outcome of

468 having nutrient concentrations present at these high spring-time levels when
469 cyanobacterial species are likely to be dominant.
470 We hypothesized that dual nutrient decreases would reduce toxin concentration,
471 through both a reduction in the proportion of potential toxin producing cells containing
472 the *mcy*E marker when compared to single or no nutrient control and how much toxin
473 was made for a given amount of cyanobacterial biomass. Biomass normalized toxin
474 concentrations (concentration of particulate microcystins normalized to cyanobacterial
475 fluorescence) (Table 2) declined over the course of the season, suggesting that non-toxic
476 strains of *Microcystis* seemed to grow better than toxin-capable strains during low
477 nutrient conditions. A shift from toxic to non-toxic strains is not uncommon and has been
478 seen in previous studies within Lake Erie (Davis et al., 2015) and within other eutrophic
479 bodies of water (Briand et al., 2009, 2008; Davis et al., 2009; Sabart et al., 2010). A
480 previous study by Chaffin et al. (2018) suggests the shift to non-toxic strains of
481 *Microcystis* from toxic strains is based on the ability to efficiently grow with decreasing
482 nutrients, while toxic strains were likely to become nutrient limited. The results of this
483 study indicate that cyanobacterial blooms biomass and toxin concentration might be
484 responding to dual nutrient limitation when ambient levels of N are low.
485 This study suggests that effective management strategies for reducing the
486 intensity, duration, and toxicity of HABs in Lake Erie might include both nitrogen and
487 phosphorus loading goals. Our conclusions suggest that both P and N decreases would be
488 more effective to mitigate HABs in Lake Erie. While current best management practices
489 are focused on P mitigation to control HABs in Lake Erie, managers must be aware of the

short-term implications that N inputs may have for bloom growth, composition, and toxicity.

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825 **Tables**

826 Table 1. Ambient conditions at sampling site WE2 at the time of initial collection.

Experiment		Temperature (°C)	SRP (µg-P L^{-1})	NH_4 (µg-N L^{-1})	NO_3 (mg-N L^{-1})	Extracted PC (µg L^{-1})	Extracted Chl-*a* (µg L^{-1})
1	June 25	22.1	24.4	131.1	1.3760	0.2	4.9
2	July 16	26.7	3.3	25.0	0.6515	4.4	6.9
3	July 23	24.0	3.7	7.6	0.6765	3.1	9.4
4	July 30	24.4	2.1	3.0	0.7030	18.9	25.6
5	Aug 13	26.0	2.1	3.8	0.3715	15.7	19.4
6	Aug 27	24.4	2.2	3.6	0.0565	18.5	25.6
7	Sept 11	21.8	17.8	33.0	0.0645	5.4	17.0
8	Oct 1	19.3	13.6	9.2	0.1850	2.4	13.5

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831 **Table 2.** The yield of cyanobacterial biomass determined by fluorescence (μg L^{-1}) at initial collection following dilution

832 ($T_{initial}$) and T_{mid} averaged by treatment.

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Experiment		Cyanobacterial biomass as chlorophyll (μg/L), ± denotes one standard error									
		Initial (T_0)		Ambient (T_{mid})		-P (T_{mid})		-N-P (T_{mid})		+N+P (T_{mid})	
1	June 25	1.09	± 0.21	4.13	± 0.30	4.22	± 0.28	4.29	± 0.38		
2	July 16	2.32	± 0.06	2.98	± 0.28	3.08	± 0.15	2.81	± 0.10		
3	July 23	3.17	± 0.18	4.54	± 0.35	4.07	± 0.12	4.1	± 0.10	8.43	± 0.18
4	July 30	5.83	± 0.10	7.25	± 0.49	6.67	± 0.42	6.38	± 0.29	16.1	± 2.14
5	Aug 13	6.53	± 0.19	7.97	± 0.17	7.1	± 0.33	7.19	± 0.33	16.25	± 0.85
6	Aug 27	8.26	± 0.18	9.13	± 0.20	9.44	± 0.39	4.84	± 0.11	18.63	± 0.85
7	Sept 11	6.97	± 0.18	8.66	± 0.21	8.87	± 0.26	5.53	± 0.17	9.17	± 0.32
8	Oct 1	1.83	± 0.18	2.95	± 0.01	3.18	± 0.04	2.86	± 0.06	3.06	± 0.23

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Figure Legends

Figure 1. Total fluorescence ($\mu g\ L^{-1}$) on a log scale over the course of the experiments (June – October) separated by treatment type. Each grouping of lines represents a different experiment. Experiments on June 25 (Exp 1) and July 16 (Exp 2) did not have a +N+P treatment.

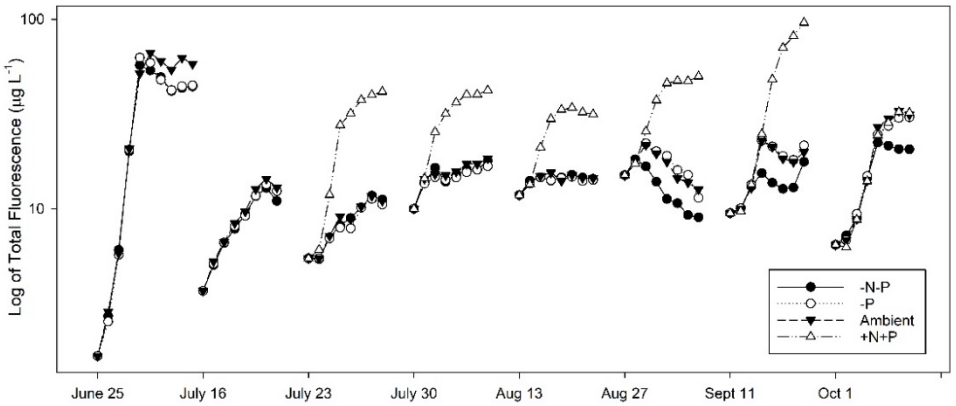
Figure 2. Fluorescence growth rate from day 0 to day 3 (exponential growth phase) for the experiments, separated by cyanobacteria (panel A) and total phytoplankton fluorescence (panel B).

Figure 3. Panel A depicts toxin concentration, as determined by the concentration of particulate microcystins ($\mu g\ L^{-1}$) at T_{mid} for the June 25 through September 11 over the course of the 2018 experimental field season. Panel B depicts biomass normalized toxicity, as determined by the concentration of particulate microcystins ($\mu g\ L^{-1}$) normalized to cyanobacterial fluorescence ($\mu g\ L^{-1}$) at day 3 for each experiment over the course of the 2018 experimental field season. Panel C depicts proportion of *mcyE* bearing cyanobacteria, as determined by the number of *mcyE* gene (copies/mL) normalized to Cyanobacterial-16S gene (copies/mL) at day 7 for each experiment over the course of the 2018 experimental field season. Toxin concentration and biomass normalized toxicity data were not determined for the October 1 experiment. The proportion of *mcyE* bearing cyanobacteria data was not determined for the June 25 or

857 October 1 experiments

858 **Figures**

859 Figure 1.



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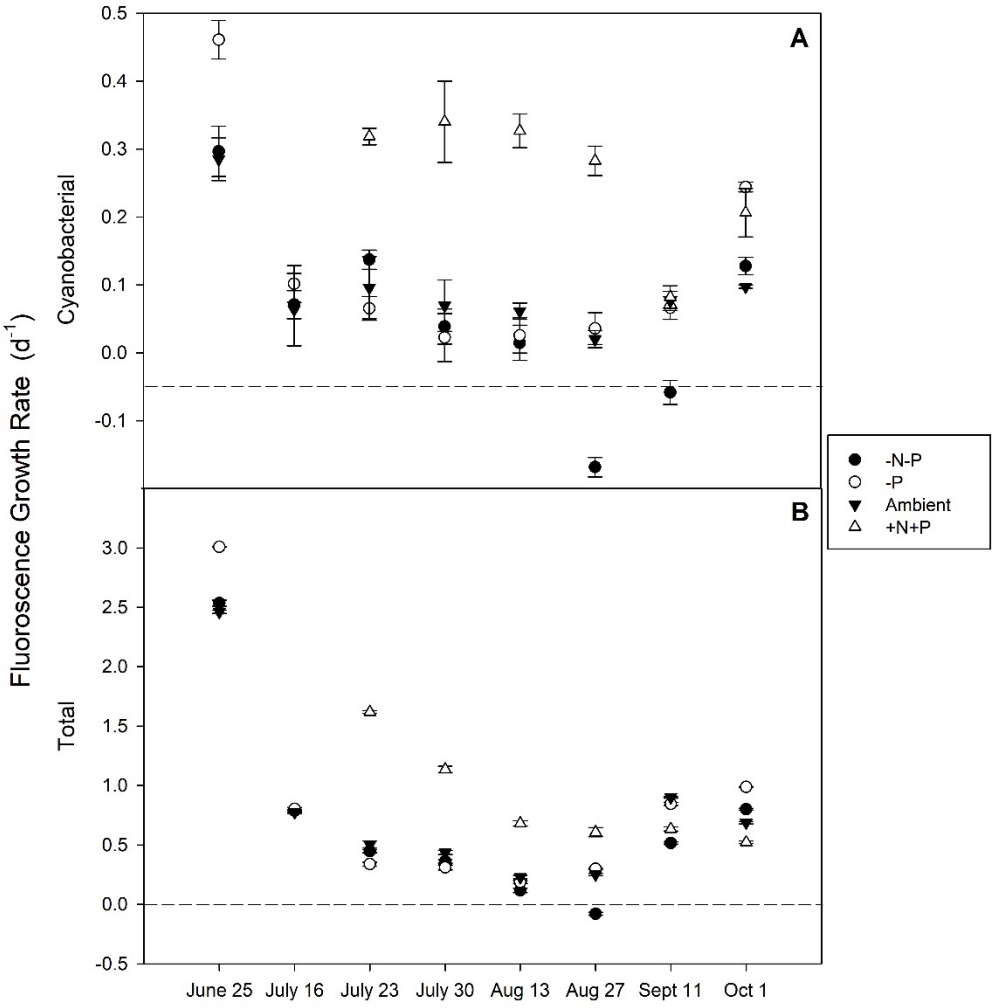
861 Figure 2.



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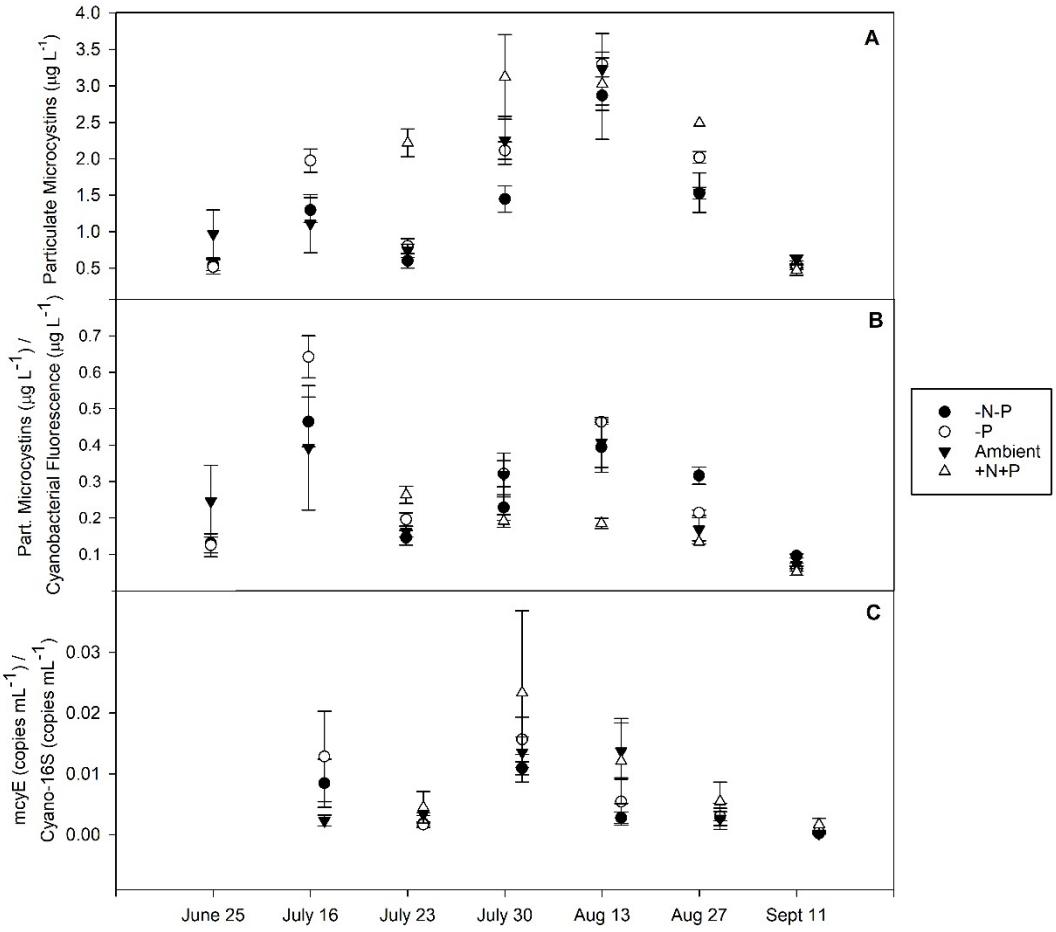

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Data Availability Statement

The full dataset for this work is archived with the University of Michigan Deep Blue Repository and is available at https://hdl.handle.net/2027.42/150644.

Supplementary Figures

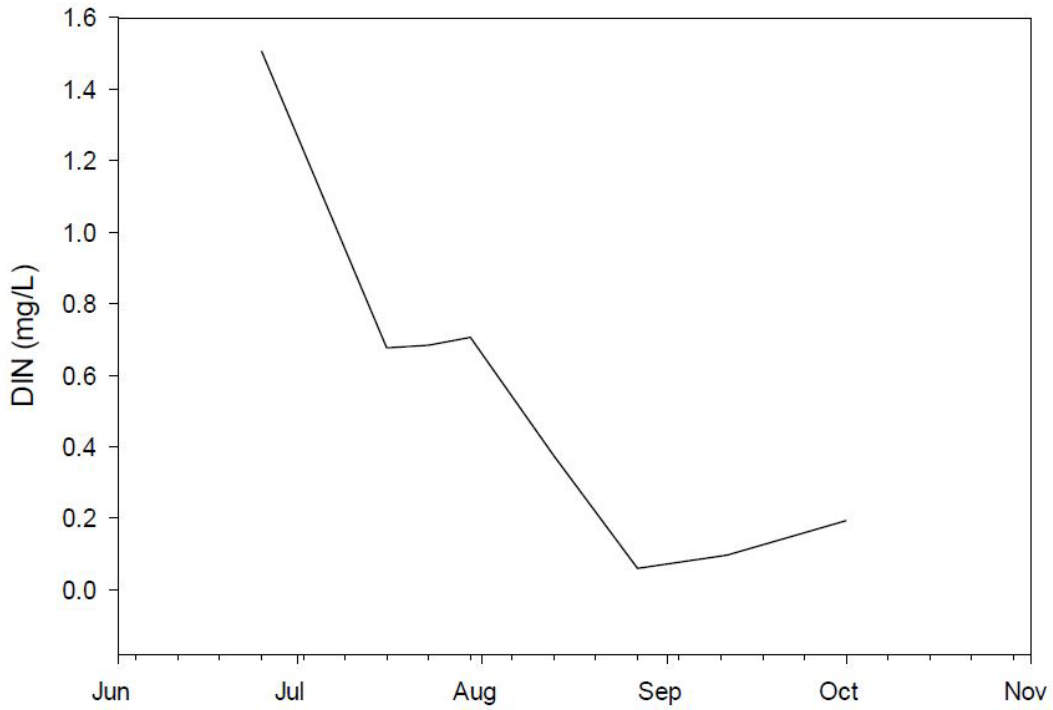

Figure S1. Dissolved inorganic nitrogen (DIN, sum of nitrate, nitrite, and ammonium) during the 2019 bloom season at station WE2 in western Lake Erie.