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Summer phytoplankton nutrient limitation in Maumee Bay of Lake Erie during high-flow and low-flow years

Justin D. Chaffin [a,*,1], Thomas B. Bridgeman [a,1], Darren L. Bade [b,2], Courtney N. Mobilian [a,1]

[a] Department of Environmental Sciences and Lake Erie Center, University of Toledo, 6200 Bayshore Dr., Oregon, OH 43616, United States
[b] Department of Biological Sciences, Kent State University, 256 Cunningham Hall, Kent, OH 44242, United States

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ABSTRACT

Algal production in Maumee Bay in western Lake Erie is highly affected by inputs of nitrogen (N) and phosphorus (P) from the Maumee River, which drains predominantly agricultural lands, leading to the formation of cyanobacterial blooms. In a 3-year study, precipitation and discharge ranged from relatively low (2012) to relatively high (2011) with corresponding changes in the size of the cyanobacterial bloom. This study aimed to quantify the relation between river discharge and algal nutrient limitation in Maumee Bay. During the summer growing seasons, 20 nutrient enrichment bioassays were performed to determine which nutrient (P or N) might limit phytoplankton growth; and ambient N and P concentrations were monitored. The bioassays suggested that phytoplankton growth shifted from P-limited to N-limited during summer of the low and intermediate discharge years (2012 and 2010, respectively), whereas during the high discharge year (2011) phytoplankton were nutrient-replete before becoming N-limited. Phosphorus-replete growth during the high discharge year likely was due to high P loads from the river and dissolved P concentrations greater than 1 μmol/L. Symptoms of N-limited growth occurred during August and September in all three years and during July of 2012 when NO_3^- plus NH_4^+ concentration was less than 7.29 μmol/L suggesting low or no correspondence between N-limitation and size of the cyanobacterial bloom. Occurrence of a relatively small cyanobacterial bloom in 2012 following the record-breaking bloom in 2011 suggests the possibility of fast-reversal of eutrophication in Maumee Bay if P loading from the watershed could be decreased.

Introduction

Land use is a major factor in determining nutrient export from watersheds to lakes (Carpenter et al., 1998). Nutrient export from agricultural watersheds can degrade water quality of lakes by increasing concentrations of potentially limiting nutrients (Tilman et al., 2001), and the rates of export are highly dependent on weather patterns because increases of rainfall accelerate nutrient loading (Haygarth et al., 1999). Phosphorus (P) has long been recognized as the main limiting nutrient in freshwater ecosystems (Reynolds, 2006; Schindler, 1977) and excessive P loading often results in symptoms of eutrophication including cyanobacterial blooms (Downing et al., 2001).

During the mid-1900s Lake Erie (North America) was eutrophic, with dense cyanobacterial blooms, due to excessive P loading (Davis, 1964; Matisoff and Ciborowski, 2005). Regulations set by the United States and Canada in the 1970s restricted P loads into the lake, and water quality quickly improved (DePinto et al., 1986). Cyanobacterial blooms were absent during the 1980s and early 1990s (Makarewicz, 1993). However, following the brief (~20 years) period of recovery and despite the ongoing P regulations, western Lake Erie has returned to eutrophic conditions (Conroy et al., 2005b), and harmful cyanobacterial blooms have been an annual occurrence since the mid-1990s (Millie et al., 2009). The return of cyanobacterial blooms has corresponded to a substantial increase in dissolved reactive P (DRP) loading from the Maumee River (Joosse and Baker, 2011). Agricultural non-point sources are considered to be the main contributor to re-eutrophication of Lake Erie (Richards et al., 2012).

Phytoplankton primary production in Lake Erie water generally has been considered to be P-limited throughout the improving P conditions of the 1980s (Hartig and Wallen, 1984) and into the early 2000s (Wilhelm et al., 2003). However, given the increasing rate of DRP loading over the past 15 years (Joosse and Baker, 2011), P-limitation may be decreasing and the importance of N may be increasing. Furthermore,

* Corresponding author at: F.T. Stone Laboratory, Ohio State University and Ohio Sea Grant, P.O. Box 119, 878 Bayview Ave., Put-In-Bay, OH 43456, United States. Tel.: +1 419 285 1845.
E-mail addresses: chaffin.46@osu.edu (J.D. Chaffin), thomas.bridgeman@utoledo.edu (T.B. Bridgeman), dbade@kent.edu (D.L. Bade), courtney.mobilian@rockets.utoledo.edu (C.N. Mobilian).
[1] Tel.: +1 419 530 8384.
[2] Tel.: +1 330 672 3613.

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high densities of exotic *Dreissena* mussels, which excrete ammonium and dissolved P at low N-to-P ratios, may also be driving a shift to N-limitation (Conroy et al., 2005a). Finally, nitrate concentration and the total N-to-total P (TN:TP) decline throughout summer (Chaffin et al., 2011) suggesting that N-limitation may become important in late summer. Conversely, *Microcystis aeruginosa* dominates the current cyanobacterial blooms in the western basin (Millie et al., 2009). *Microcystis* is not a N-fixer, and so its dominance would not necessarily suggest N-limitation. Because the above evidence indicates the possibility of both P and N limitation, a reassessment of Maumee Bay phytoplankton nutrient status is in order.

The Maumee River watershed is the largest watershed in the Great Lakes basin and is 87.8% agricultural (Han et al., 2011). The Maumee River loads high amounts of suspended sediments (Richards et al., 2008) and nutrients (Baker and Richards, 2002) into Maumee Bay in the southwest end of Lake Erie. Most of the nutrient export from the Maumee River occurs during large rainstorms (Richards et al., 2010), and large rainstorms that occur during the spring months can result in summer cyanobacterial blooms in Lake Erie (Stumpf et al., 2012).

The Maumee River loaded high amounts of P and N into Lake Erie during 2011 which resulted in a record-breaking cyanobacterial bloom (Bridgeman et al., 2013; Stumpf et al., 2012). In contrast, 2012 was a very dry year. There were two goals for this study, 1) compare nutrient limitation of phytoplankton in Maumee Bay during low-flow and high-flow years, and 2) determine the concentrations of N, P, and TN:TP ratios that will induce N or P limitation of phytoplankton growth. To test the hypothesis that increased nutrient loading via river discharge results in increased size of the cyanobacterial bloom in Maumee Bay, algal nutrient limitation was assessed over a 3-year period (2010–2012). Fortuitously, 2011 was one of the wettest and 2012 one of the driest on record (Stumpf et al., 2012). During the summers of 2010, 2011, and 2012 we monitored N and P concentrations, chlorophyll *a* levels, and light climate, and also conducted 20 nutrient enrichment bioassays with Maumee Bay water to determine nutrient limitation of phytoplankton growth.

Methods

Maumee River discharge and nutrient loading

The Maumee River cumulative discharge, cumulative total N load, and cumulative total P load were calculated for 1 March through 30 June for years 2010, 2011, and 2012 using the tributary loading tool provided by the Heidelberg University National Center for Water Quality Research (NCWQR) (downloaded from: http://www.heidelberg.edu/academiclife/distinctive/ncwqr/data, accessed 29 January, 2013). Cumulative discharge and loads were calculated from 1 March through 30 June because this time period was the best predictor of cyanobacterial bloom magnitude in Lake Erie (Stumpf et al., 2012). Total N load was calculated as the sum of total Kjeldahl N (TKN) and nitrate loads.

Field methods

This research was conducted at site MB18 (N 41°44′51″, W 83°24′5″) in Maumee Bay from early June to late September in 2010, 2011, and 2012. Site MB18 has a depth of 2.5 m. Water was collected over the entire water column using a metal-free, 2-meter long integrated tube sampler constructed from PVC tubing. Water for nutrient analysis was transferred to 250-mL acid-washed polyethylene bottles and kept on ice during transportation back to the laboratory. Water for nutrient enrichment bioassays was poured into 20-L acid-washed polyethylene containers and kept in a large dark cooler.

Vertical profiles of underwater photosynthetic active radiation (PAR) were recorded, as in Chaffin et al. (2011). The PAR profiles were used to determine the light attenuation coefficient (K_d). We then calculated mean PAR (Guildford et al., 2005) using K_d, the light intensity at the lake surface, and the depth of site MB18 (2.5 m) rather than the lesser mixing depth because site MB18 does not thermally stratify. Mean PAR is presented as percent of surface light (Guildford et al., 2005). During 2012 PAR profiles were not completed on every sample trip, but Secchi disk depth was measured on all trips. Mean PAR for these dates was calculated based on the relationship between Secchi disk depth and the light attenuation coefficient at site MB18 (Bridgeman unpublished data).

Nutrient analysis

Total phosphorus (TP) and total Kjeldahl nitrogen (TKN) concentrations were determined on unfiltered water. Dissolved inorganic nutrient [dissolved reactive P (DRP), NO_3^-, NH_4^+] concentrations were determined on water samples filtered through a 0.45-μm membrane filter. After filtering, all nutrient samples were stored at −20 °C until analyses at the National Center for Water Quality Research (NCWQR) at Heidelberg University (Tiffin, Ohio, USA) using USA Environmental Protection Agency protocols (Richards et al., 2010). Details on methods and minimum detection concentrations are available from NCWQR (at http://www.heidelberg.edu/academiclife/distinctive/ncwqr).

Bioassays

Phytoplankton nutrient limitation was determined monthly (June, July, August, September) in 2010 and 2011 and 12 times during 2012 by P- and N-enrichment bioassays (Schelske, 1984). For the incubations, 200 mL of lake water were poured into acid-washed 250-mL polycarbonate flasks. Treatments included the enrichment of 10 μmol/L P (+P; KH_2PO_4), 520 μmol/L N (+N; 500 μmol/L $NaNO_3$ and 20 μmol/L NH_4^+ [$(NH_4)_2SO_4$]), and combination P and N enrichment (+P&N). Controls were used in which only deionized water was added to lake water at a volume that matched the volume of nutrient additions. Each treatment was replicated in three separate flasks. Flasks were incubated in a growth chamber (Percival model: E-36HO, Fontana, Wisconsin, USA) at lake temperature (19.1 °C to 27.5 °C) at the time of collection under a light intensity of 300–350 μmol photon/m^2/s on a 12:12 h light:dark cycle. This light intensity approximates the mean PAR of western Lake Erie (Chaffin et al., 2011), and previous Lake Erie bioassays conducted in incubation chambers used similar light intensities (Moon and Carrick, 2007). Flasks were inverted several times to prevent settling and randomly rearranged in the growth chamber daily (Moon and Carrick, 2007).

Phytoplankton abundance was estimated as chlorophyll (chl) *a* on initial samples and after 48 h of incubation. During 2010 and 2011 chl *a* was extracted using dimethylsulfoxide and quantified by absorbance (Chaffin et al., 2012). During 2012 chl *a* was extracted from the filters using N-N-dimethylformamide and quantified by fluorometry (Speziale et al., 1984). These two methods gave very similar results from split water samples that were analyzed for chl *a* during 2007 and 2008 (Chaffin, 2009).

Microcystis biovolume

Microcystis biovolume was measured to compare bloom intensity among the three years. Data for 2010 and 2011 were accessed from Bridgeman et al. (2013) and data for 2012 were determined following the methods of Bridgeman et al. (2013).

Data analysis

Final chl *a* concentration of each nutrient enrichment experiment was subjected to a normality test. Normally distributed data were analyzed with a one-way analysis of variance (ANOVA) and post hoc Tukey test. Non-normally distributed data were first log transformed

then analyzed with one-way ANOVA and post hoc Tukey test. SPSS (version 20) was used for all statistical analyzes.

For each of the 20 nutrient enrichment experiments, average final and initial chl *a* levels were used to calculate phytoplankton growth rates (growth = $chla_{48}$ / $chla_0$ / 2d; where $chla_{48}$ is the chl *a* concentration after 48 h of growth and $chla_0$ is the initial chl *a* concentration). Then the growth rate response to P enrichment (R_{+P}) and N enrichment (R_{+N}) was determined by dividing the growth rates of the enrichment treatment by the growth response of the control. This approach allowed for comparing across all experiments by accounting for differences in phytoplankton abundance across seasons and years. *R* values of 1.0 would indicate that nutrient enrichment did not increase phytoplankton growth rate relative to non-enriched controls and an ambient nutrient concentration sufficient to support phytoplankton growth, whereas *R* values greater than 1.0 indicate that nutrient enrichment increased growth and that ambient nutrient concentration was limiting growth. To investigate concentrations of N that induced N-limitation, R_{+N} was plotted against ambient dissolved inorganic N ($NO_3^- + NH_4^+$), TN, and TN:TP at the time the samples for experimental incubation were collected, while R_{+P} was plotted against DRP, TP, and TN:TP. The response to nutrient enrichment should not be expected to follow a continuous relationship with concentration, because above some concentration of limiting nutrient, limitation should not exist (as another factor becomes limiting). A goal of this manuscript is to determine the N and P concentrations that result in phytoplankton growth limitation, hence, we are looking for a threshold effect. Furthermore, plots of *R* vs. nutrient concentration were heteroscedastic due to unequal variances across the observed nutrient concentrations (see the Results section), which violated assumptions of correlation or regression models. Because threshold concentrations were desired and regression models were not acceptable with this data set, a two-dimensional Kolmogorov–Smirnov test (2DKS; Garvey et al., 1998) was used to determine if a relationship existed between severity of limitation and nutrient concentration. The 2DKS test gives a D_{BKS} statistics that can be interpreted as a threshold level (Garvey et al., 1998). 2DKS tests were performed using the software program developed by Garvey et al. (1998) and freely available on the internet (http://www.zoology.siu.edu/garvey/2dks.html; accessed 7 April, 2013). Plots of R_{+P} vs. P concentration were less heteroscedastic than N, and also were analyzed using linear regression.

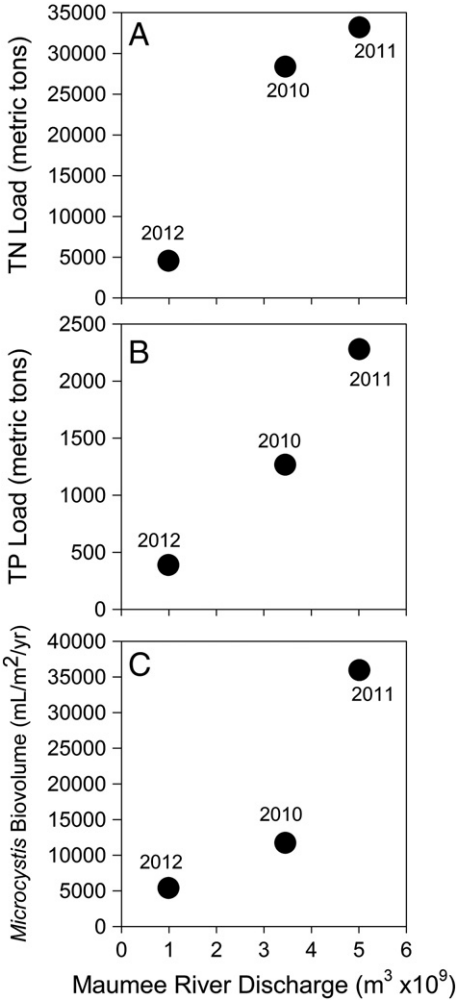

Fig. 1. A) Cumulative total nitrogen load, B) total phosphorus load, and from the Maumee River and C) *Microcystis* biovolume in western Lake Erie as a function of Maumee River discharge from 1 March to 30 June for 2010, 2011, and 2012.

Results

Since 1995, the average cumulative discharge volume from the Maumee River between 1 March and 30 June was 2.73×10^9 m^3. The year 2011 had the largest cumulative discharge volume (5.01×10^9 m^3), whereas 2012 had the lowest discharge (0.99×10^9 m^3). The year 2010 had a cumulative discharge intermediate between 2011 and 2012 but greater than the long-term average (3.45×10^9 m^3). The TN cumulative load during the intermediate discharge year (2010) was similar to the TN load of the high discharge year (2011; Fig. 1A). The TP load during the intermediate year was intermediate to the TP load of the low (2012) and the high discharge years (Fig. 1B). These TN and TP loads resulted in the intermediate discharge year having the highest TN:TP load, whereas the low and high discharge years had lower TN:TP values. *Microcystis* biovolume was greatest during the high discharge year and lowest during the low discharge year (Fig. 1C).

Nitrate and TN concentrations were highest during June and decreased throughout summer in all three years. In the low discharge year of 2012, NO_3^- and TN were 2 to 4 times lower in June and July compared to intermediate and high discharge years (2010 and 2011; Table 1). Furthermore, by 9 July NO_3^- concentration in the low discharge year was below 0.7 μmol/L, which was a month earlier than intermediate and high discharge years. Ammonium concentration was greatest in the high discharge year and ranged between 7.0 μmol/L and 9.4 μmol/L. Highest DRP and TP levels were recorded in June of high discharge years, with concentrations of 2.3 and 6.2 μmol/L, respectively. TN:TP was, in general, highest in June and then decreased throughout the summer.

Ambient chl *a* concentration ranged from 6.7 to 48.3 μg/L, and these concentrations also represent the initial chl *a* levels in the enrichment experiment (Table 2). Chlorophyll *a* concentration in the controls increased from initial levels by 10% to 78% in 8 of the 11 experiments conducted during June and July across all three years, and the control chl *a* concentration did not increase during August and September of all years. Phosphorus enrichment (without N) resulted in chl *a* concentrations that were 9% to 61% greater than that of the control in July 2010 and 23 May through 27 June during 2012 ($p < 0.05$). Nitrogen enrichment (without P) resulted in chl *a* concentrations that were 12% to 80% greater than that of the control in August of 2010 and 2011 and 9 July through 11 September 2012, excluding 2 August 2012 ($p < 0.05$). The P and N enrichment resulted in chl *a* concentrations that were equal to or greater than that of the chl *a* response to either P or N.

Plotting the growth rate response of N enrichment relative to control (R_{+N}) against N concentration allowed for determination of the N concentration that induced a growth limitation (Fig. 2). High $NO_3^- + NH_4^+$ and TN concentrations and high TN:TP resulted in R_{+N} values near 1.0, whereas low $NO_3^- + NH_4^+$ and TN concentration and low TN:TP resulted in R_{+N} values with high variance that ranged between 1.16 and 3.50 (Fig. 2). The 2DKS test confirmed a relationship between R_{+N} and $NO_3^- + NH_4^+$, TN, and TN:TP ($p < 0.05$; Table 1), and D_{BKS} (the threshold

Table 1
Ambient concentrations of nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (TN), dissolved reactive phosphorus (DRP), total phosphorus (TP), the ratio of TN to TP (TN:TP), Secchi disk depth, light attention coefficient (K_d), and mean PAR at site MB18 in Maumee Bay observed on dates of the nutrient enrichment experiment.

Sample date	NO_3^-	NH_4^+	TN	DRP	TP	TN:TP	Secchi disk depth	K_d	Mean PAR
	μmol/L	μmol/L	μmol/L	μmol/L	μmol/L	mol/mol	cm	/m	% of surface
21 Jun. 2010	241.26	3.35	305.20	1.31	2.94	103.9	120	0.942	38.4
15 Jul. 2010	99.21	6.85	149.43	0.50	1.74	85.7	130	0.876	40.5
10 Aug. 2010	0.71	2.00	38.13	0.17	1.21	31.6	90	1.514	25.8
20 Sep. 2010	2.86	4.43	57.00	0.17	2.44	23.4	40	3.340	12.0
20 Jun. 2011	159.89	7.07	251.15	2.34	6.16	40.8	28	3.512	11.4
11 Jul. 2011	103.50	7.85	153.70	0.64	1.84	83.4	77	1.868	21.2
12 Aug. 2011	0.71	9.42	68.38	0.10	2.23	30.7	46	2.242	18.5
14 Sep. 2011	16.42	7.57	64.47	0.87	2.51	25.7	51	2.042	21.1
23 May 2012	63.53	1.93	104.02	0.15	1.27	82.0	120	1.297	29.6
4 Jun. 2012	37.12	3.35	66.18	0.50	1.90	34.8	60	1.786	22.1
13 Jun. 2012	28.55	5.35	93.68	0.22	1.46	64.3	80	1.698	23.2
20 Jun. 2012	37.12	5.92	85.63	0.76	2.32	36.9	140	0.948	38.3
27 Jun. 2012	8.57	2.00	57.92	0.01	1.41	41.1	80	1.698	23.2
9 Jul. 2012	0.00	1.36	52.37	0.12	2.61	20.1	65	2.080	19.1
16 Jul. 2012	0.00	2.36	26.85	0.05	0.96	27.9	120	0.796	43.4
25 Jul. 2012	0.00	2.36	53.81	0.53	3.25	16.5	60	2.308	17.3
2 Aug. 2012	0.71	2.21	59.43	1.38	4.92	12.1	100	1.366	28.3
13 Aug. 2012	0.71	0.93	51.39	0.05	1.30	39.6	110	1.661	23.7
27 Aug. 2012	6.42	9.78	53.10	0.03	0.95	55.9	130	1.089	34.3
11 Sep. 2012	0.71	2.21	56.96	0.55	3.24	17.6	60	2.652	15.1

between N-replete growth and N-limited growth) occurred at $NO_3^- + NH_4^+$ of 7.29 μmol/L, TN of 57.00 μmol/L, and TN:TP of 31.58 (Table 3).

The 2DKS failed to show a relationship with the growth rate response of P enrichment relative to control (R_{+P}) and concentrations of DRP and TP and the TN:TP ($p > 0.05$; Table 1). However, the variance in R_{+P} was highest at low concentrations and decreased at higher concentrations (Figs. 3A and B) and the 2DKS between R_{+P} and TP was nearly significant ($p = 0.087$, $D_{BKS} = 1.91$ μmol/L). Regressions between R_{+P} and DRP and TP were not significant ($p = 0.609$ and 0.468, respectively), however linear regression between R_{+P} and TN:TP was significant ($p = 0.040$, $r^2 = 0.214$).

Discussion

River discharge and phytoplankton nutrient limitation

Since 1995, the cumulative discharge volume, TN load, and TP load from the Maumee River observed during 2011 were the largest on record, while 2012 had the lowest on record (Stumpf et al., 2012). The high discharge and nutrient load were the likely drivers of the high *Microcystis* bloom during 2011, and Stumpf et al. (2012) showed that cyanobacteria abundance in Lake Erie increases exponentially with the Maumee River TP load. Furthermore, the low *Microcystis* bloom year of 2012 corresponded with low discharge and loads of TN and TP. The

Table 2
Chlorophyll *a* concentration (μg/L) of the 20 nutrient enrichment bioassays conducted using water from site MB18 in Maumee Bay. Ambient (Amb.) also represents the initial chl *a* concentration. Control (no nutrients added), +P (phosphorus enrichment 10 μmol/L), +N (nitrogen enrichment 520 μmol/L), and +P+N (phosphorus and nitrogen enrichment) indicate chl *a* concentration following 48 h of incubation. Values are mean of three replicates and standard error in parenthesis. The F and P values represent the main treatment effects ANOVA with degrees of freedom = 3,11. Bold values indicate significant ($p < 0.05$) differences greater than control indicated by Tukey test.

Sample date	Amb.	Control	+P	+N	+P+N	F	p
21 Jun. 2010	16.73	42.5 (0.3)	46.8 (1.5)	47.1 (1.9)	50.8 (2.8)	3.396	0.074
15 Jul. 2010	9.34	27.8 (1.3)	**51.5 (1.8)**	29.2 (2.1)	**55.7 (0.5)**	87.82	<0.001
10 Aug. 2010	15.25	16.9 (1.8)	15.3 (1.2)	**39.6 (1.2)**	**71.1 (1.5)**	233.8	<0.001
20 Sep. 2010	16.31	19.4 (0.7)	**23.3 (1.0)**	22.0 (0.3)	22.0 (0.1)	7.115	0.012
20 Jun. 2011	33.45	110.0 (0.9)	108.7 (1.9)	112.6 (0.7)	112.8 (1.0)	3.194	0.084
11 Jul. 2011	7.59	21.6 (1.4)	21.7 (0.1)	22.3 (1.7)	24.3 (0.4)	1.251	0.354
12 Aug. 2011	14.80	14.7 (0.4)	19.4 (0.3)	**46.0 (0.9)**	**61.0 (1.6)**	900.1	<0.001
14 Sep. 2011	9.89	15.4 (0.4)	14.6 (0.6)	15.1 (0.3)	15.7 (1.0)	0.457	0.720
23 May 2012	17.21	22.9 (1.2)	**58.9 (1.7)**	22.9 (0.8)	**64.0 (2.4)**	188.9	<0.001
4 Jun. 2012	10.97	34.4 (0.9)	**48.5 (0.7)**	37.1 (0.4)	**54.0 (0.9)**	147.9	<0.001
13 Jun. 2012	10.68	25.1 (1.2)	**46.1 (0.2)**	28.9 (0.7)	**56.1 (2.1)**	130.8	<0.001
20 Jun. 2012	10.84	51.6 (2.4)	**66.6 (4.6)**	56.8 (2.6)	**75.6 (4.8)**	8.152	0.008
27 Jun. 2012	25.21	29.5 (0.2)	**38.1 (2.7)**	30.1 (0.7)	**137.7 (8.3)**	144.1	<0.001
9 Jul. 2012	23.88	12.5 (0.1)	12.3 (0.1)	**64.8 (0.6)**	**89.3 (0.9)**	4852.	<0.001
16 Jul. 2012	6.71	8.6 (0.2)	6.6 (0.7)	**14.9 (0.7)**	**40.6 (0.2)**	1034	<0.001
25 Jul. 2012	26.87	18.6 (0.3)	19.3 (0.5)	**65.3 (1.2)**	**86.1 (0.9)**	1401.	<0.001
2 Aug. 2012	26.36	17.9 (0.3)	23.8 (0.5)	26.4 (1.9)	**61.6 (0.4)**	370.1	<0.001
13 Aug. 2012	31.20	33.1 (0.4)	21.6 (0.1)	**39.9 (1.1)**	**111.7 (0.3)**	516.1	<0.001
27 Aug. 2012	26.13	34.2 (0.2)	**39.8 (0.4)**	**39.9 (0.6)**	**41.4 (1.9)**	10.89	0.003
11 Sep. 2012	48.34	40.6 (0.3)	42.5 (1.3)	**79.4 (1.8)**	**96.8 (1.6)**	407.8	<0.001

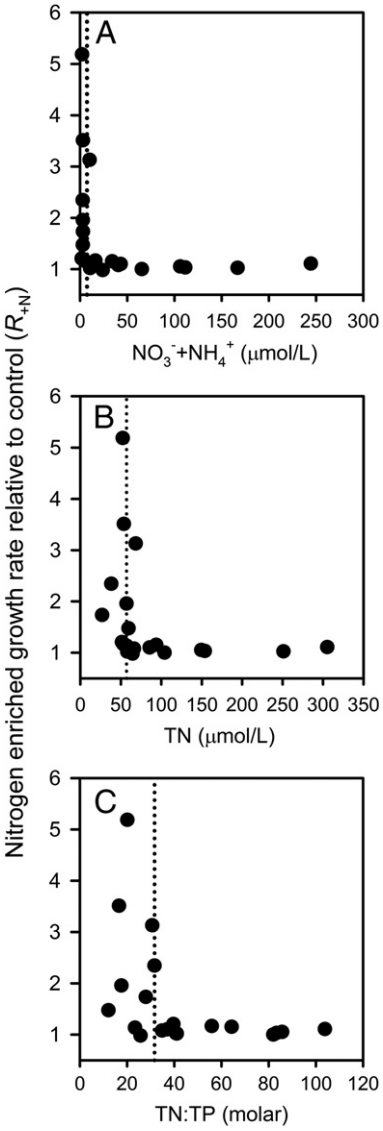

Fig. 2. Growth rate response of N enrichment relative to control (R_{+N}) plotted against nitrate plus ammonium (NO_3^- + NH_4^+, A), total nitrogen (B) and the total N-to-total P ratio (TN:TP, C) of 20 enrichment assays conducted at site MB18 of Maumee Bay during summers of 2010, 2011, and 2012. The dotted vertical line is the threshold concentration and indicates that N-limited growth occurs at lower concentrations (left of the line), whereas N-replete growth occurs at greater concentrations. Relative growth values greater than 1.0 indicated that phytoplankton growth was stimulated by N enrichment, whereas values of 1.0 or less indicated no effect of N enrichment.

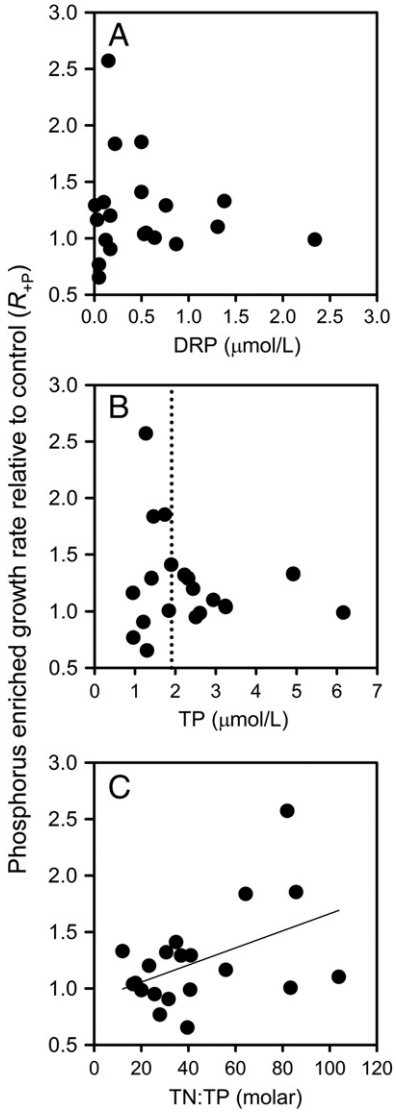

Fig. 3. Growth rate response of P enrichment relative to control (R_{+P}) plotted against dissolved reactive P (DRP, A), total phosphorus (B) and the total N-to-total P ratio (TN:TP, C) of 20 enrichment assays conducted at site MB18 of Maumee Bay during summers of 2010, 2011, and 2012. The dotted vertical line on plot B is the threshold concentration and indicates that P-limited growth occurs at lower concentrations (left of the line), whereas P-replete growth occurs at greater concentrations. Solid line on plot C is the linear regression. Relative growth rates greater than 1.0 indicated that phytoplankton growth was stimulated by P enrichment, whereas values of 1.0 or less indicated no effect of P enrichment.

Table 3
Results of the 2DKS test for the effects nitrate + ammonium (NO_3^- + NH_4^+), total nitrogen (TN), and ratio of TN to total P (TN:TP) on the growth rate response of N enrichment relative to control (R_{+N}) (left half) and the effects of dissolved reactive P (DRP), total P (TP), and TN:TP on growth rate response of P enrichment relative to control (R_{+P}).

R_{+N}	p value	Threshold concentration D_{BKS}
NO_3^- + NH_4^+	0.0346	7.29 μmol/L
TN	0.0026	57.00 μmol/L
TN:TP	0.0384	31.58 mol/mol
R_{+P}	p value	D_{BKS}
DRP	0.7615	0.51 μmol/L
TP	0.0870	1.91 μmol/L
TN:TP	0.7703	23.35 mol/mol

bioassay results suggested that phytoplankton growth during the high discharge year was nutrient-replete then became N-limited, whereas in the low discharge year phytoplankton growth was P-limited and then became N-limited. These results confirm our hypothesis that increased nutrient loading via the Maumee River discharge alleviated algal nutrient limitation during early-summer; however, increased river loading did not affect late-summer N-limitation.

During June of the intermediate discharge year (2010) and June and July of the high discharge year (2011) high growth yields were observed in the control flasks and P enrichment did not stimulate chl *a* production relative to the control. These results indicated that ambient DRP concentrations between 1.3 and 2.3 μmol/L supported phytoplankton growth that was unconstrained by P availability, and suggest that other factors, such as grazing or light availability, could have constrained phytoplankton biomass. In contrast, concentrations of DRP were less than 0.50 μmol/L during the low discharge year (2012) and chl *a* concentrations increased with P enrichment indicating P-limitation. In short, high

discharge-loading corresponded with P-replete algal growth whereas low discharge-loading resulted in P-limited algal growth. Furthermore, because cyanobacteria comprised about 66% of the total chl *a* concentration during the high discharge year (Chaffin, 2013), and because the *Microcystis* bloom of the low P-load year was almost 7× smaller than the bloom in the high discharge year, the current cyanobacterial bloom problems in western Lake Erie might be alleviated with further P loading constraints.

Nitrogen enrichment increased chl *a* concentration in the experiments in August of all three years and in July, August, and September of the low discharge year (2012; Table 2) indicating N-limitation. The 2DKS test showed that the growth rate response of N enrichment relative to control (R_{+N}) was dependent on N concentration (Table 3) and the threshold between N-replete and N-limited growth (D_{BKS}) occurred at 7.29 μmol/L for $NO_3^- + NH_4^+$, 57.00 μmol/L for TN, and a TN:TP molar value of 31.58 (Table 3). At concentrations above the threshold N-limitation is unlikely to be observed; while at concentrations below the threshold, N-limitation should be expected, but the severity of limitation could be tempered by other limiting factors besides N. The low TN loading during the low discharge year likely resulted in the lower N concentrations observed during early summer and led to earlier NO_3^- depletion and N-limitation. During the seasonal decline in N concentrations, these thresholds were crossed all three years of the study and indicate that N-limitation can occur during high discharge and low discharge years and in cyanobacterial bloom and non-bloom years.

Nitrogen limitation in Maumee Bay occurred at $NO_3^- + NH_4^+$ concentrations below 7.29 μmol/L and TN less than 57.00 μmol/L. Following N-limitation, the N-fixing cyanobacterium *Anabaena* appeared and began to replace *Microcystis* (Chaffin and Bridgeman, 2014), and the *Anabaena* bloom demonstrated measurable N-fixation in 2010 and 2011 (Bade, unpublished data). However, it is unclear if N-fixation can supply sufficient N to maintain phytoplankton biomass compared to N-replete conditions in Maumee Bay and elsewhere (Schindler et al., 2008; Scott and McCarthy, 2011). Only a few studies on N-fixation have been conducted in Lake Erie (Howard et al., 1970; Mague and Burris, 1973), and it remains to be determined whether N-fixing cyanobacteria can completely compensate for N deficiencies in Maumee Bay.

In some experiments there was very little chl *a* response to enrichments of only P and only N when ambient concentrations of DRP and $NO_3^- + NH_4^+$ were relatively low, as indicated in the lower left area of the graphs (Figs. 2, 3). In experiments with low ambient DRP and $NO_3^- + NH_4^+$, the largest chl *a* responses were observed when both P and N were added (Table 2) indicating co-limitation by both P and N. Co-limitation was observed during August of all three years and 10 of the 12 experiments during the low discharge year because of low dissolved N and P concentrations. Furthermore, the heteroscedasticity in the *R* vs. nutrient concentration plots is likely due to P and N co-limitation and the high variance at low nutrient concentrations. For example, at $NO_3^- + NH_4^+$ concentrations less than 10 μmol/L, R_{+N} ranged from 1.16 to 3.50, whereas at $NO_3^- + NH_4^+$ concentrations 10 to 250 μmol/L, R_{+N} was near 1.0. Thus, the variance across the X-axis was not consistent and regression models not acceptable. The 2DKS test was able to determine the threshold effect from the heteroscedastic data which is more important for assessing at what nutrient concentration induces growth limitation.

Physical factors such as light, mixing, temperature, and iron concentration can also affect phytoplankton growth. The shallowness of Maumee Bay (less than 2.5 m) maintains mean PAR ranging from 11.4% to 43.4% of surface light intensity even during high turbidity events. Using theoretical cloud-free surface PAR (calculated from NOAA Earth System Research Laboratory, http://www.esrl.noaa.gov/gmd/grad/surfrad/, accessed 9 May 2014) instantaneous mean PAR of the water column at midday, would have ranged from 250.6 to 891.0 μmol/m^2/s (11%–43% of midday PAR). Fahnenstiel et al. (1989) reported that I_k (PAR at onset of light saturation) values of 600 to 700 μmol/m^2/s are characteristic of Great Lakes phytoplankton indicating that PAR could be limiting photosynthetic rates throughout the day for many of the samples analyzed, especially in the high discharge year of 2011. Further into the western basin, light may become even more of a limiting factor for phytoplankton photosynthesis as mixed depth increases (to nearly 10 m) while high concentrations of suspended sediments maintain high rate of light attention (Chaffin et al., 2012). However, weak vertical circulation can allow positively buoyant cyanobacteria to become dominant by rising toward the lake surface while negatively buoyant phytoplankton would become light-limited in turbid conditions (Huisman et al., 2004). Positive buoyancy can provide an advantage to cyanobacteria when light limits photosynthesis of many other phytoplankton taxa. This trait may explain why, following the heavy spring nutrient and turbidity loading of 2011, weak lake circulation was observed favoring the initiation of the record-breaking cyanobacterial bloom (Michalak et al., 2013). The potential role of seasonal light limitation should receive close attention in future investigations of the cyanobacterial blooms in the Maumee River plume. Temperature limitation is not a likely factor, because these experiments were conducted during the summer growing season and water temperatures ranged from 19.1 °C to 27.5 °C. Iron is essential for NO_3^- assimilation (Flores and Herrero, 2005), and low concentrations of dissolved iron can result in N-limitation (North et al., 2007). However, iron and nitrate enrichment of Maumee Bay water gave growth responses equal to those shown for nitrate-alone additions (Chaffin and Bridgeman, 2014). The Maumee River has high levels of dissolved iron (Havens et al., 2012) suggesting that Maumee Bay likely has a high enough dissolved iron concentration to support NO_3^- assimilation.

Implications of findings

Some of the data presented here could be interpreted as support for and against a dual nutrient management strategy (Paerl et al., 2011) calling for P and N abatement in order to control eutrophication (Conley et al., 2009; Lewis and Wurtsbaugh, 2008). The *Microcystis* bloom of the low discharge year (2012) was 15% that of the bloom in the high discharge year (2011, Fig. 1D), while the TN load of the low discharge year was only 14% that of the high discharge year. Furthermore, N-limitation occurred earlier in the summer during the low discharge year than the high discharge year. The low TN loading and earlier N-limitation may have interacted and led to a small *Microcystis* bloom in the low discharge year. However, our data can also support the P-only abatement strategy (Schindler and Hecky, 2009; Schindler et al., 2008). Phytoplankton growth during the start of the low discharge year was P-limited and N-replete in contrast to the high discharge year which high P and N concentrations met the growth demand. Thus, the only difference between the cyanobacterial bloom year and non-bloom year, in regard to phytoplankton nutrient status at the start of the growing season, was P-limitation during the non-bloom year. Furthermore, the 2DKS test gave a $NO_3^- + NH_4^+$ threshold concentration of 7.29 μmol/L when N-limitation occurred. In order to induce N-limitation at the start of the growing season, NO_3^- concentration would have to be decreased by at least 73% (when compared to 2012) or as much as 93% (compared to 2010). Similar decreases in TN also would be needed. In contrast, P-limitation was observed at low DRP concentration, and at the beginning of the low discharge year DRP concentrations were low enough for P-limitation. Therefore, inducing P-limitation in Maumee Bay will be easier to achieve. Moreover, there are numerous examples when P-only abatement was successful at reversing eutrophication (Schelske, 2009; Schindler, 2012), including Lake Erie (DePinto et al., 1986). However, increasing N inputs may exacerbate late summer cyanobacterial blooms.

Cyanobacterial blooms have the potential to produce compounds that have toxic effects on animals and people (Huisman et al., 2005). The main cyanobacterial toxin of concern in Lake Erie is microcystin,

and microcystin has been detected at concentrations that far exceed acceptable levels for drinking and recreational use (Michalak et al., 2013). Microcystins are N-rich compounds with N accounting for 14% of the molecule by mass. *Microcystis* grown in laboratory experiments have showed that transcripts of the microcystin synthase genes are greatly reduced under low-N conditions (Harke and Gobler, 2013). The N-limitation observed in late-summer may result in less-toxic blooms compared to N-replete blooms in early-summer. Further work is needed to determine if low N concentrations in Maumee Bay constrain toxin production by cyanobacteria.

Results of this study suggest that increased spring-time Maumee River discharge alleviated phytoplankton P-limitation and led to large cyanobacterial blooms. Since 1995, the volume of water discharged from the Maumee River during 2011 was the largest on record and was nearly twice that of the long-term average. Higher than average discharges and large cyanobacterial blooms were reported during summers of 2008 and 2009 (Stumpf et al., 2012). Furthermore, Michalak et al. (2013) determined that the weather patterns observed during 2011 which generated the large discharge and loading were consistent with expected future climate change scenarios. Thus, Lake Erie may continue to experience large summer-time cyanobacterial blooms if nutrient limitation is alleviated due to large spring-time discharges.

Decreasing P loading to Lake Erie is paramount to restore water quality. Analysis of recent Maumee River TP loading with cyanobacterial bloom magnitude suggests that if loading can be minimized to less than 1000 metric tonnes that blooms will be greatly reduced (Stumpf et al., 2012). Confirming this idea was the fact that only 387.4 metric tonnes of P was loaded via the Maumee during spring 2012 and the bloom was small (Fig. 1). However, that low P loading was the result of low discharge due to a drought; but it did demonstrate that substantial improvements in Maumee Bay water quality could be realized fairly quickly because the relatively small bloom of 2012 immediately followed the worst-ever bloom in 2011.

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