Supporting Information (SI) for manuscript # es-2016-062961:

l

Community Biological Ammonium Demand: A Conceptual Model for Cyanobacteria Blooms in Eutrophic Lakes

Wayne S. Gardner[1*], Silvia E. Newell[2], Mark J. McCarthy[1,2], Daniel K. Hoffman[2], Kaijun Lu[1], Peter J. Lavrentyev[3], Ferdi L. Hellweger[4], Steven W. Wilhelm[5], Zhanfei Liu[1], Denise Bruesewitz[6], and Hans W. Paerl[7]

June 15, 2017

[1]The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373 United States

[*]Corresponding Author: wayne.gardner@utexas.edu Office 361-749-6823; Cell 361-332-6703

[2]Wright State University, Department of Earth & Environmental Sciences, 3640 Col Glenn Hwy, Dayton, OH 45435 USA United States

[3]The University of Akron, Department of Biology, Akron, OH 44325 United States

[4]Northeastern University, Department of Civil and Environmental Engineering, Boston, MA 02115 United States

[5]The University of Tennessee, Department of Microbiology, Knoxville, TN 37919 United States

[6] Colby College, Waterville, ME 04901 United States

[7]The University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, NC , United States
and Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, College of Environment, Hohai University, Nanjing 210098 China

The SI is 10 pages long and includes three verbal explanations, one table, four figures, and a list of references.

Supporting Information (SI)

1. Phosphorus remains oxidized and, except for advective and physical removal processes, is conserved within aquatic ecosystems. In contrast, the quantity and composition of nitrogen (N) forms are affected by redox conditions and biogeochemical exchanges with atmospheric N_2 via N fixation and denitrification, which add or remove available N, respectively. The relative importance of N and P in CyanoHAB dynamics in lakes is the subject of unresolved debate (*1–7*), although evidence is mounting for the importance of dual nutrient management strategies.
2. The stoichiometric approach (*8*) reflects the recent history of plankton growth conditions (e.g., *9*). It identifies the apparent 'limiting' nutrient(s), but does not account for co-limitation (i.e., balanced growth) varying in space and time (*4*,*10*). Moreover, it does not account for the fact that organisms have genus (and often species) specific demands and abilities to assimilate nutrients (*11*,*12*). Nutrient addition and dilution experiments that overcome algal growth deficiency issues can provide meaningful insights into nutrient limitation and/or co-limitation at specific periods. However, experiments are time consuming and do not capture some ecosystem processes involved in controlling algal blooms (e.g., seasonal changes, sediment-water column nutrient exchange, predation, *etc*.).
3. Water samples (ca. 10 ml) were collected from each bottle and filtered (0.2 µm pore size Nylon filter) immediately before and after each incubation. The first three ml of sample, at minimum, rinsed the filter, and the remaining seven ml of filtrate were frozen in an NH_4^+-free 8 ml glass vial (Wheaten; *13*) for analysis by AIRTS-HPLC (*14*). Maumee Bay samples from 2015 were analyzed using OX/MIMS (*15*).

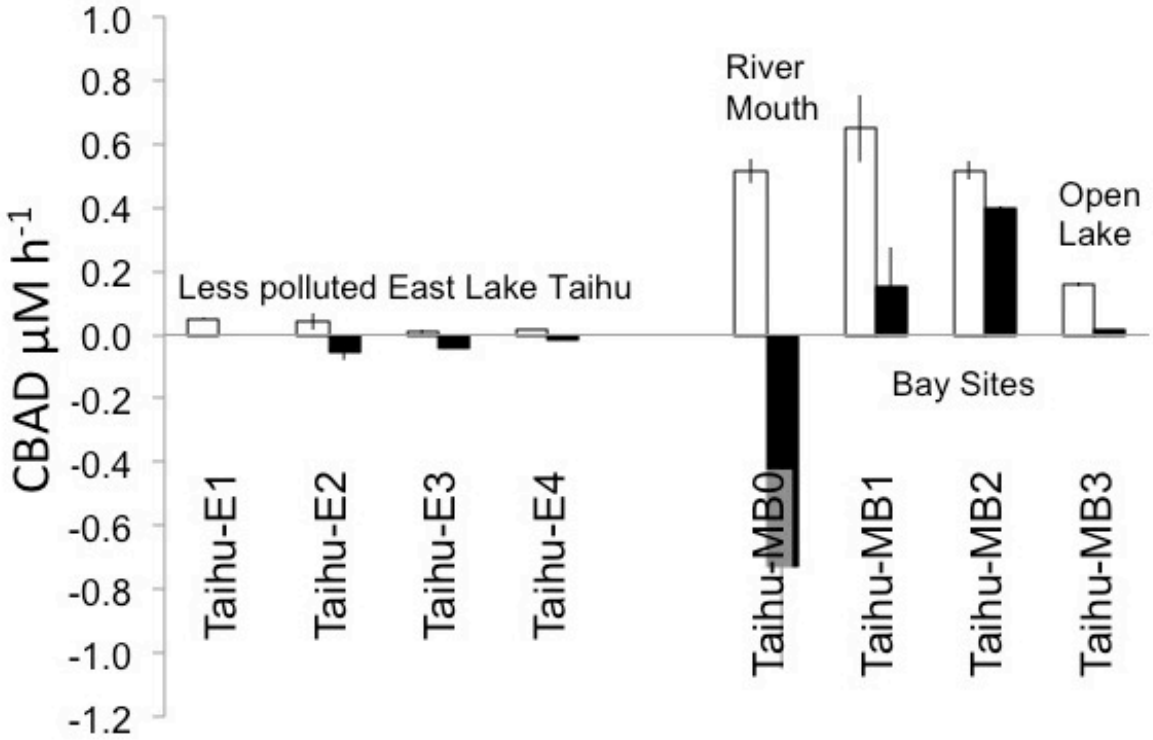
SI Table 1. Data used to compare the effects of temperature on CBAD measurements.

Site Name	Station	Date	Chl (mg/L)	Temp (C)
Lake Maracaibo	1.1	Sept 1995	22.0	31.2
Lake Maracaibo	1.3	Sept 1995	13.5	30.8
Lake Maracaibo	2.1	Sept 1995	4.1	31.2
Lake Maracaibo	2.5	Sept 1995	4.5	30.5
Lake Maracaibo	3.1	Sept 1995	3.5	30.3
Lake Maracaibo	3.5	Sept 1995	3.7	30.3
Lake Maracaibo	4.5	Sept 1995	ND	30.6
Lake Maracaibo	5.1	Sept 1995	7.3	30.4
Lake Maracaibo	5.5	Sept 1995	5.0	30.3
Taihu Lake	River	May 2004	10.2	22.6
Taihu Lake	Mid Bay	May 2004	16.5	22.1
Taihu Lake	Lower Bay	May 2004	11.7	21.8
Taihu Lake	Open Lake	May 2004	7.8	21.3
Taihu Lake	E1	May 2004	ND	ND
Taihu Lake	E2	May 2004	ND	23.5
Taihu Lake	E3	May 2004	ND	25.0
Taihu Lake	E4	May 2004	ND	24.0
W. Lake Erie	River	July 2004	4.5	26.5
W. Lake Erie	Mid Bay	July 2004	6.8	22.1
W. Lake Erie	Open Lake	July 2004	2.0	23.5
Lake Erie	HE	Sept 2005	5.3	20.8
Lake Erie	AS	Sept 2005	ND	20.9
Lake Erie	AE	Sept 2005	3.7	19.4
Lake Erie	BW	Sept 2005	ND	19.3
Lake Erie	958	Sept 2005	3.7	ND
W. Lake Erie	WLE-6-S	June 2015	1.63	23.6
W. Lake Erie	WLE-6-B	June 2015	1.60	23.4
W. Lake Erie	WLE-2-S	June 2015	3.84	23.3
W. Lake Erie	WLE-2-B	June 2015	2.83	23
W. Lake Erie	WLE-4-S	June 2015	1.01	21.4
W. Lake Erie	WLE-4-B	June 2015	1.01	21.4
W. Lake Erie	WLE-6-S	July 2015	26.00	22.3
W. Lake Erie	WLE-6-B	July 2015	25.03	22.3
W. Lake Erie	WLE-6-S	July 2015	6.59	23.5
W. Lake Erie	WLE-6-B	July 2015	5.45	23.6
W. Lake Erie	WLE-2-S	July 2015	5.93	21.8
W. Lake Erie	WLE-2-B	July 2015	3.18	21.6
W. Lake Erie	WLE-2-S	July 2015	2.88	21.9
W. Lake Erie	WLE-2-B	July 2015	2.56	21.9
W. Lake Erie	WLE-4-S	July 2015	119.72	24.2

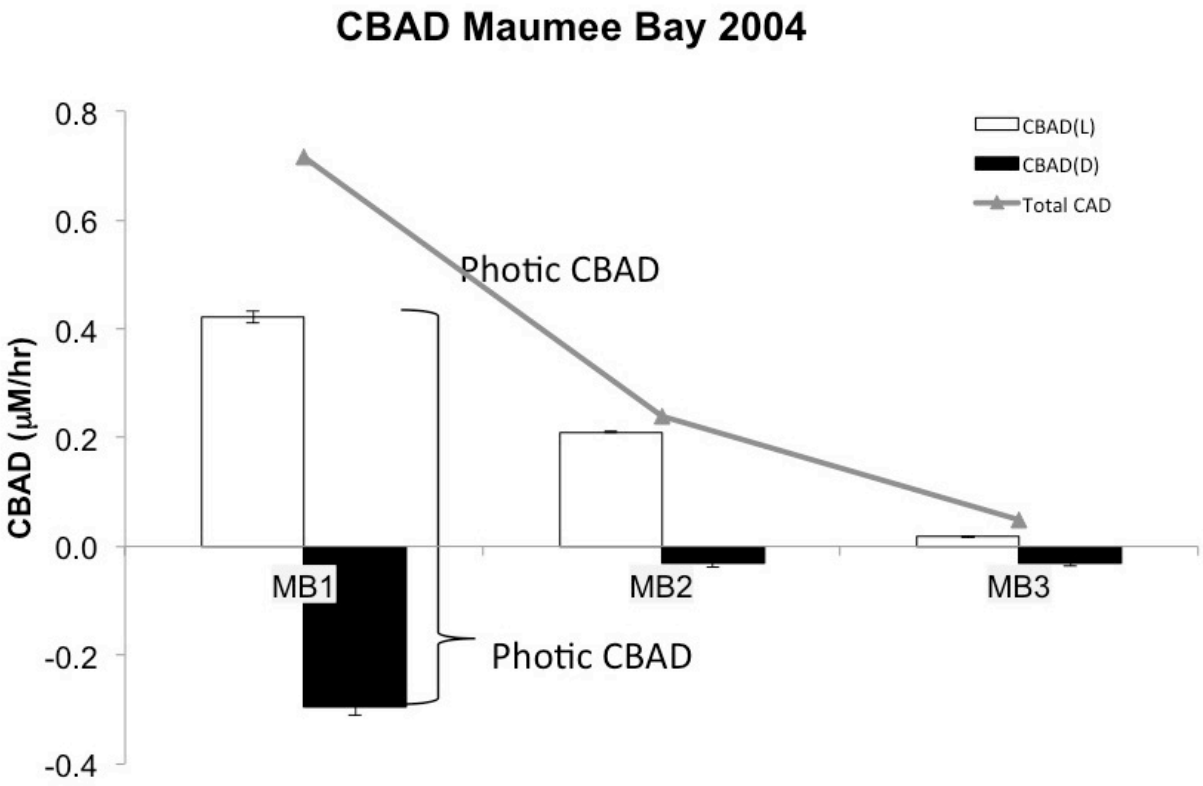
W. Lake Erie	WLE-4-B	July 2015	ND	24.2
W. Lake Erie	WLE-6-S	August 2015	352	24.1
W. Lake Erie	WLE-6-B	August 2015	ND	ND
W. Lake Erie	WLE-2-S	August 2015	187	23.9
W. Lake Erie	WLE-2-B	August 2015	81.3	23.9
W. Lake Erie	WLE-4-S	August 2015	86.3	24.3
W. Lake Erie	WLE-4-B	August 2015	ND	ND
W. Lake Erie	WLE-6-S	Sept 2015	54.99	20.5
W. Lake Erie	WLE-6-B	Sept 2015	54.99	20.5
W. Lake Erie	WLE-2-S	Sept 2015	27.95	20.5
W. Lake Erie	WLE-2-B	Sept 2015	28.21	20.6
W. Lake Erie	WLE-4-S	Sept 2015	22.15	21.2
W. Lake Erie	WLE-4-B	Sept 2015	22.15	21.2
Old Woman Creek	Stream	July 2004	1.500	20.4
Old Woman Creek	Stream	July 2005	1.800	22.5
Old Woman Creek	Wetland	July 2004	10.700	22.9
Old Woman Creek	Mouth	July 2005	29.100	23.4
Old Woman Creek	Mouth	July 2004	22.800	22.6
Old Woman Creek	Lake	July 2005	1.400	20.2
Old Woman Creek	Lake	July 2004	30.600	22.9
Missisquoi Bay	PRM	July 2008	29.61	23.2
Missisquoi Bay	PRM	July 2009	ND	19.8
Missisquoi Bay	PRM	July 2008	10.9	23.9
Missisquoi Bay	PRM	Aug 2008	28.24	ND
Missisquoi Bay	PRM	Oct 2008	6.785	ND
Missisquoi Bay	MB	May 2008	ND	14.5
Missisquoi Bay	MB	June 2008	8.6	16
Missisquoi Bay	MB	June 2007	8.5	20.7
Missisquoi Bay	MB	June 2008	19.44	21.5
Missisquoi Bay	MB	July 2008	9.05	23.1
Missisquoi Bay	MB	July 2009	17.6	20.9
Missisquoi Bay	MB	Aug 2008	8.64	23.7
Missisquoi Bay	MB	Aug 2008	66.69	22.7
Missisquoi Bay	MB	Aug 2007	2.619	ND
Missisquoi Bay	MB	Seot 2009	6.2	ND
Missisquoi Bay	MB	Oct 2008	16.063	11.4
Lake Rotorua	7m	Jan 2006	28	20.6
Lake Rotorua	7m	Jan 2006	28	20.6
Lake Rotorua	7m	Jan 2006	28	20.6
Lake Rotorua	20m	Jan 2006	25	20.8
Lake Rotorua	20m	Jan 2006	25	20.8
Lake Rotorua	20m	Jan 2006	25	20.8
Lake Rotoiti	4.5m	Jan 2006	11.6	22.1

Lake Rotoiti	4.5m	Jan 2006	11.6	22.1
Lake Rotoiti	4.5m	Jan 2006	11.6	22.1
Lake Rotoiti	100m	Jan 2006	2.1	21.2
Lake Rotoiti	100m	Jan 2006	2.1	21.2
Lake Rotoiti	100m	Jan 2006	2.1	21.2
Lake Michigan	M15-5	March 1999	1.5	1.3
Lake Michigan	M45-5	March 1999	1.39	2.4
Lake Michigan	S15-5	March 1999	2.36	ND
Lake Michigan	J15-5	March 1999	3.41	1
Lake Michigan	J20-5	March 1999	3.24	0.9
Lake Michigan	J60-5	March 1999		ND
Lake Michigan	J80-5	March 1999	2.34	2.4
Lake Michigan	G20-5	March 1999	0.92	1.1
Lake Michigan	C15-5	March 1999	0.79	0.6
Lake Michigan	C80-5	March 1999	2.36	1.9
Lake Michigan	R15-5	March 1999	2.13	1.4
Lake Michigan	R80-5	March 1999	2.42	2.4
Lake Michigan	SJRM-5	March 2000	7.43	5.5
Lake Michigan	J15-5	March 2000	1.73	3.2
Lake Michigan	J30-5	March 2000	1.14	3
Lake Michigan	NB20-5	March 2000	1.85	3.7
Lake Michigan	G15-5	March 2000	0.91	4.2
Lake Michigan	G45-5	March 2000	1.52	2.5
Lake Michigan	C15-5	March 2000	0.47	2.8
Lake Michigan	C80-5	March 2000	1.48	3
Lake Michigan	M15-5	June 1999	1.58	11.3
Lake Michigan	M110-5	June 1999	1.29	10.2
Lake Michigan	M110-DCL	June 1999	2.12	7
Lake Michigan	J15-5	June 1999	4.99	16.6
Lake Michigan	J80-5	June 1999	0.76	14
Lake Michigan	J80-DCL	June 1999	2.49	6
Lake Michigan	C80-DCL	June 1999	2.78	6.1
Lake Michigan	M45-5	May 2000	2.1	9.3
Lake Michigan	M45-DCL	May-00	1.93	8
Lake Michigan	SJRM-5	May-00	18.36	17.5
Lake Michigan	J15-5	May-00	0.99	12.2
Lake Michigan	J30-5	May-00	0.72	9.7
Lake Michigan	NB15-5	May-00	0.8	11.5
Lake Michigan	G15-5	May-00	1.17	11.5
Lake Michigan	G45-5	May-00	1.37	8

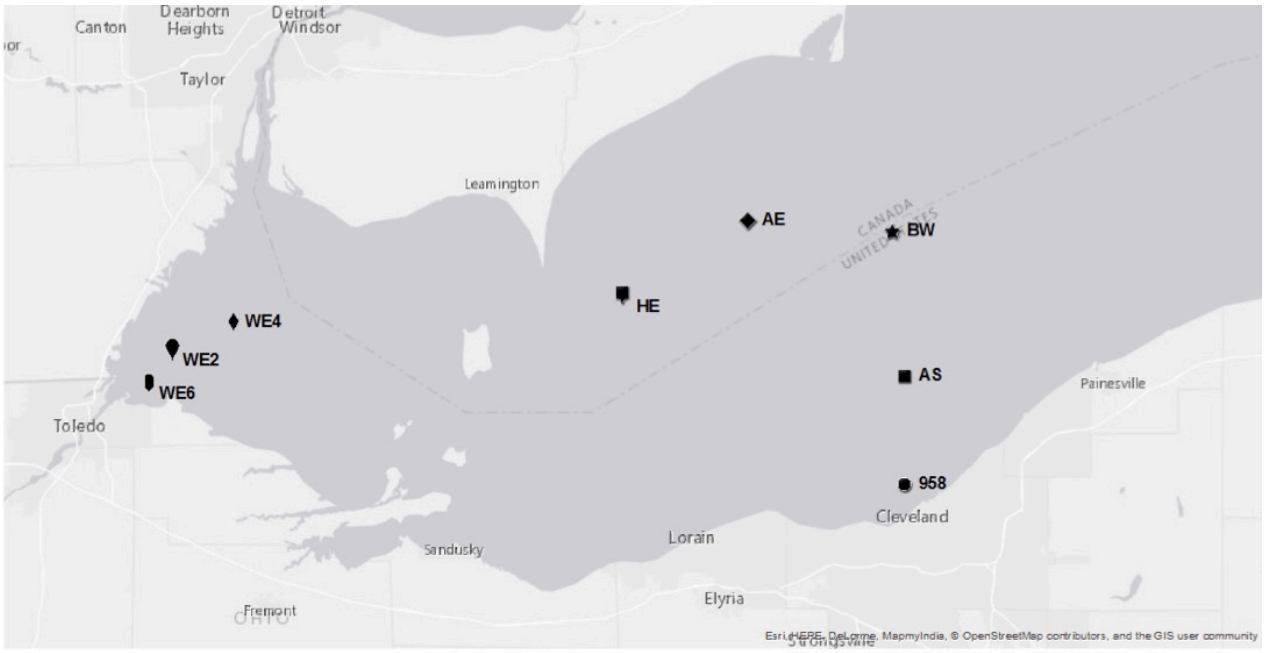
SI Figure 1. Light (open bars)- and dark (closed bars)-CBAD from Lake Taihu sites in May 2004.



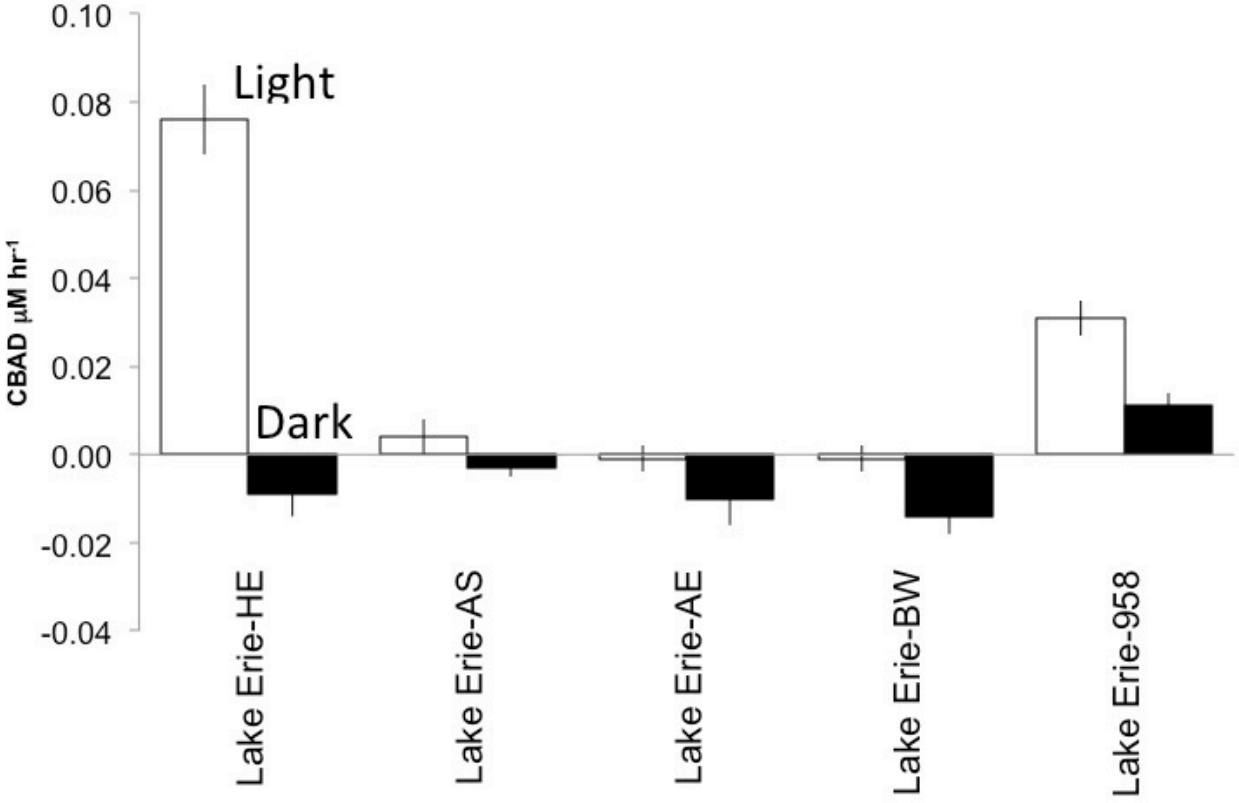
SI Figure 2. Natural-light and dark CBAD at three sites in Maumee Bay, Lake Erie, July 2004. MB1 = River Mouth; MB2 = mid bay; MB3 = Toward open lake. Note that the negative values of CBAD indicate that on average more NH_4^+ was regenerated in the dark than the net (but not total) amount taken up during the normal light-dark cycle. Photic CBAD is calculated as Light CBAD minus Dark CBAD, assuming that natural-light and dark regeneration rates are equal.



SI Figure 3. Site Map for Lake Erie Stations for September 2005 (HE, ZD, BW, AS, and 958) and the temporal data taken surrounding the 2015 CyanoHAB in Maumee Bay (WE6, WE 2, and WE4). Depths (m): WE2: 5.0, WE6: 2.75, WE4: 8.0, AE: 21.8, AS: 21.1, BW: 24.0, HE: 15.6, 958: 10.5.



Lake Erie SI Figure 4. Light and Dark CBAD from Lake Erie sites in September 2005. Note, the negative dark CBAD values, indicate higher dark LOM-N mineralization rates (to NH_4^+) than net (but not total) light NH_4^+ uptake rates. They reflect active NH_4^+ cycling dynamics. CBAD was not distinguishable from zero at central sites AS, AE, and BW, suggesting that NH_4^+ was not limiting community production in these offshore lake stations. In contrast, Sites HE and Site 958 (located near Cleveland, Ohio) had significant light CBAD values.



Supporting Information References

(1) Schindler, D. W.; Vallentyne, J. R. *The algal bowl: overfertilization of the world's freshwaters and estuaries*, Rev. and expanded ed.; University of Alberta Press: Edmonton, 2008.

(2) Lewis, W. M.; Wurtsbaugh, W. A.; Paerl, H. W. Rationale for Control of Anthropogenic Nitrogen and Phosphorus to Reduce Eutrophication of Inland Waters. *Environ. Sci. Technol.* **2011**, *45* (24), 10300–10305.

(3) Paerl, H. W.; Xu, H.; Hall, N. S.; Zhu, G.; Qin, B.; Wu, Y.; Rossignol, K. L.; Dong, L.; McCarthy, M. J.; Joyner, A. R. Controlling Cyanobacterial Blooms in Hypertrophic Lake Taihu, China: Will Nitrogen Reductions Cause Replacement of Non-N2 Fixing by N2 Fixing Taxa? *PLoS ONE* **2014**, *9* (11), e113123.

(4) Paerl, H. W.; Gardner, W. S.; Havens, K. E.; Joyner, A. R.; McCarthy, M. J.; Newell, S. E.; Qin, B.; Scott, J. T. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. *Harmful Algae* **2016**, *54*, 213–222.

(5) Kane, D. D.; Conroy, J. D.; Peter Richards, R.; Baker, D. B.; Culver, D. A. Re-eutrophication of Lake Erie: Correlations between tributary nutrient loads and phytoplankton biomass. *J. Gt. Lakes Res.* **2014**, *40* (3), 496–501.

(6) Belisle, B. S.; Steffen, M. M.; Pound, H. L.; Watson, S. B.; DeBruyn, J. M.; Bourbonniere, R. A.; Boyer, G. L.; Wilhelm, S. W. Urea in Lake Erie: Organic nutrient sources as potentially important drivers of phytoplankton biomass. *J. Gt. Lakes Res.* **2016**, *42* (3), 599–607.

(7) Schindler, D. W.; Carpenter, S. R.; Chapra, S. C.; Hecky, R. E.; Orihel, D. M. Reducing Phosphorus to Curb Lake Eutrophication is a Success. *Environ. Sci. Technol.* **2016**, *50* (17), 8923–8929.

(8) Redfield, A. C. The influence of organisms on the composition of sea-water. *The Sea* **1963**, 26–77.

(9) Smith, V. H. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis1. *Limnol. Oceanogr.* **1982**, *27* (6), 1101–1111.

(10) Paerl, H. W.; Hall, N. S.; Calandrino, E. S. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Sci. Total Environ.* **2011**, *409* (10), 1739–1745.

(11) Steffen, M. M.; Belisle, B. S.; Watson, S. B.; Boyer, G. L.; Wilhelm, S. W. Status, causes and controls of cyanobacterial blooms in Lake Erie. *J. Gt. Lakes Res.* **2014**, *40* (2), 215–225.

(12) Steffen, M. M.; Belisle, B. S.; Watson, S. B.; Boyer, G. L.; Bourbonniere, R. A.; Wilhelm, S. W. Metatranscriptomic Evidence for Co-Occurring Top-Down and Bottom-Up Controls on Toxic Cyanobacterial Communities. *Appl. Environ. Microbiol.* **2015**, *81* (9), 3268–3276.

(13) McCarthy, M. J.; Lavrentyev, P. J.; Yang, L.; Zhang, L.; Chen, Y.; Qin, B.; Gardner, W. S. Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China). *Hydrobiologia* **2007**, *581* (1), 195–207.

(14) Gardner, W. S.; Bootsma, H. A.; Evans, C.; John, P. A. S. Improved chromatographic analysis of 15N:14N ratios in ammonium or nitrate for isotope addition experiments. *Mar. Chem.* **1995**, *48* (3–4), 271–282.

(15) Yin, G.; Hou, L.; Liu, M.; Liu, Z.; Gardner, W. S. A Novel Membrane Inlet Mass Spectrometer Method to Measure 15NH4+ for Isotope-Enrichment Experiments in Aquatic Ecosystems. *Environ. Sci. Technol.* **2014**, *48* (16), 9555–9562.