Supporting Information:

Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys

Matthew J. Harke[1], Timothy W. Davis[2], Susan B. Watson[3], Christopher J. Gobler[1]*

[1] Stony Brook University, School of Marine and Atmospheric Sciences, Stony Brook, NY 11794

[2] NOAA Great Lakes Environmental Research Laboratory, 4840 S. State Road, Ann Arbor, MI 48108

[3] Canadian Centre for Inland Waters, Environment Canada, Burlington, ON, L7R 4A6, Canada

* Corresponding author: christopher.gobler@stonybrook.edu

18 Pages

7 Supplemental Tables

12 Supplemental Figures

Supplemental Tables

Table S1 Physical, chemical, and community characterizing parameters measured at each station during September (A) and October (B) transects of the western basin of Lake Erie (Fig 1, Fig S8). Values in parenthesis are the standard deviation between two biological replicates. Values below detection limits are denoted with BD. For SRP, detection limit was 0.00254 µM.

	9/12/2013					
A	**L1**	**L15**	**L16**	**L17**	**L18**	**L19**
Latitude	41.705450	41.725032	41.710016	41.677711	41.692743	41.672816
Longitude	-83.446490	-83.406564	-83.297111	-83.235626	-83.007609	-82.897967
Temperature [°C]	23.4	23.6	23.1	23	23.2	23.3
Dissolved Oxygen [mg L^{-1}]	5.19	6.87	6.62	6.86	7.60	7.17
Secchi depth [m]	0.4	0.4	0.5	0.6	1.7	1.9
DIN [µM]	27.33 (0.85)	10.83 (0.47)	1.90 (0.04)	1.63 (0.02)	8.83 (0.25)	4.68 (0.27)
SRP [µM]	1.11 (0.02)	0.44 (0.06)	0.28 (0.07)	0.24 (0.03)	BD	0.20 (0.07)
Microcystis [cells mL^{-1}]	29,967 (6,643)	51,633 (7,683)	51,317 (5,921)	51,450 (10,765)	29,433 (8,171)	15,900 (3,518)
Fv/Fm	0.30 (0.05)	0.31 (0.04)	0.27 (0.02)	0.35 (0.06)	0.46 (0.01)	0.45 (0.01)
in vivo chlorophyll [RFU]	0.87 (0.07)	1.02 (0.05)	0.77 (0.05)	0.76 (0..08)	0.43 (0.03)	0.32 (0.04)
Extracted chlorophyll [µg L^{-1}]	20.01 (5.51)	36.45 (3.01)	21.86 (1.36)	23.04 (9.52)	10.32 (0.40)	8.86 (0.49)
APA [nmol mL^{-1} hr^{-1}]	0.22 (0.01)	0.28 (0.00)	0.32 (0.01)	0.37 (0.01)	0.81 (0.01)	0.54 (0.01)
Fluoroprobe Bluegreen [µg L^{-1}]	21.08 (2.10)	34.88 (6.12)	22.79 (0.66)	23.86 (1.08)	14.99 (0.73)	8.22 (0.51)
Phycocyanin [RFU]	17.20 (1.47)	26.97 (5.34)	20.83 (1.82)	18.53 (0.51)	15.03 (2.89)	10.77 (0.46)
Microcystin [µg L^{-1}]	2.31 (0.76)	6.95 (0.19)	3.87 (0.40)	8.17 (0.69)	0.33 (0.08)	0.82 (0.07)

	10/8/2013						
B	**LET7**	**LET6**	**LET5**	**LET4**	**LET3**	**LET2**	**LET1**
Latitude	41.698889	41.739444	41.766667	41.725556	41.702778	41.670833	41.722778
Longitude	-83.458889	-83.375000	-83.308611	-83.110556	-83.000556	-82.849722	-82.751389
Temperature [°C]	19.8	17.6	18.3	18.8	18.6	18.9	19.4
Dissolved Oxygen [mg L^{-1}]	10.01	8.41	6.27	6.65	6.27	6.06	6.58
Secchi depth [m]	NA	0.75	1.1	1.8	2.8	1.8	1.2
DIN [µM]	50.14 (1.71)	5.76 (0.08)	1.35 (0.01)	6.54 (0.79)	14.74 (0.70)	1.76 (0.22	3.14 (0.06)
SRP [µM]	1.61 (0.16)	0.19 (0.02)	0.06 (0.01)	BD	0.05 (0.01)	0.07 (0.00)	0.25 (0.01)
Microcystis bioamass [µg L^{-1}]	147	10,812	6,480	1,605	4,139	2,089	3,561
Extracted chlorophyll [µg L^{-1}]	26.88 (0.86)	24.99 (1.95)	6.85 (0.55)	4.59 (0.58)	3.43 (0.23)	5.26 (0.36)	6.97 (0.21)
Microcystin [µg L^{-1}]	0.90 (0.21)	1.51 (0.24)	0.91 (0.26)	0.74 (0.19)	0.92 (0.09)	0.78 (0.15)	0.63 (0.24)

Table S2 Nutrient concentrations [μM] for each station of the September and October transects of the western basin of Lake Erie. Stations are ordered from west to east as in Figure 1. Values below detection limits are indicated with BD. Detection limits were 0.00697 μM for urea, 0.00254 μM DIP, and 0.00243 μM for DOP. Values represent the average of duplicate samples with standard deviations in parenthesis.

	9/12/2013						10/8/2013						
	L1	**L15**	**L16**	**L17**	**L18**	**L19**	**LET7**	**LET6**	**LET5**	**LET4**	**LET3**	**LET2**	**LET1**
NO_x	24.69	9.53	0.21	0.11	7.11	3.08	40.70	4.91	0.38	5.48	12.50	0.28	0.84
	(0.81)	(0.42)	(0.02)	(0.01)	(0.27)	(0.24)	(1.29)	(0.08)	(0.01)	(0.12)	(0.12)	(0.02)	(0.01)
NH_4	2.64	1.29	1.69	1.52	1.72	1.60	9.44	0.85	0.97	1.06	2.25	1.48	2.30
	(0.04)	(0.05)	(0.02)	(0.01)	(0.03)	(0.04)	(0.42)	(0.00)	(0.03)	(0.08)	(0.82)	(0.24)	(0.07)
Urea	1.60	0.62	1.98	0.18	0.20	0.08	3.61	0.21	BD	1.27	4.53	BD	0.46
	(0.03)	(0.01)	(0.19)	(0.01)	(0.04)	(0.01)	(0.04)	(0.01)		(0.01)	(0.26)		(0.09)
TN	53.79	46.83	45.76	42.57	36.47	25.10	60.88	52.37	27.80	48.44	39.18	23.57	23.99
	(4.58)	(0.44)	(0.66)	(0.95)	(0.23)	(1.83)	(0.13)	(4.92)	(0.46)	(0.54)	(0.01)	(0.36)	(1.78)
TDN	44.61	31.39	28.64	20.02	17.72	11.17	32.52	34.44	13.33	16.31	29.64	10.87	16.32
	(4.26)	(0.19)	(1.06)	(0.20)	(0.39)	(0.14)	(1.99)	(0.93)	(0.99)	(0.06)	(0.36)	(0.68)	(1.05)
DON	19.92	21.86	28.43	19.91	10.61	8.09	BD	29.53	12.94	10.83	17.15	10.59	15.48
	(5.07)	(0.23)	(1.04)	(0.21)	(0.12)	(0.37)		(0.84)	(1.00)	(0.93)	(0.23)	(0.66)	(1.05)
PN	9.18	15.44	17.12	22.55	18.75	13.93	28.36	17.93	14.48	32.13	9.54	12.70	7.68
	(0.32)	(0.25)	(1.72)	(1.15)	(0.16)	(1.96)	(1.86)	(5.84)	(0.53)	(0.60)	(0.34)	(1.04)	(2.84)
SRP	1.11	0.44	0.28	0.24	BD	0.20	1.61	0.19	0.06	BD	0.05	0.07	0.25
	(0.02)	(0.06)	(0.07)	(0.03)		(0.07)	(0.16)	(0.02)	(0.01)		(0.01)	(0.00)	(0.01)
TP	6.22	6.47	3.58	4.60	1.54	1.27	10.11	4.44	1.85	2.34	2.10	1.98	3.43
	(0.06)	(0.21)	(0.04)	(0.15)	(0.19)	(0.06)	(0.32)	(0.22)	(0.08)	(0.59)	(0.05)	(0.07)	(0.12)
TDP	0.65	1.28	0.49	0.26	0.24	0.17	1.72	1.19	0.41	0.53	0.98	0.52	0.33
	(0.03)	(0.63)	(0.05)	(0.03)	(0.09)	(0.05)	(0.22)	(0.28)	(0.04)	(0.37)	(0.23)	(0.08)	(0.09)
DOP	BD	0.84	0.22	0.03	0.27	BD	0.11	1.00	0.34	0.53	0.94	0.45	0.08
		(0.57)	(0.12)	(0.06)	(0.12)		(0.37)	(0.29)	(0.04)	(0.37)	(0.23)	(0.08)	(0.10)
PP	5.57	5.19	3.09	4.33	1.29	1.10	8.39	3.25	1.44	1.81	1.11	1.47	3.09
	(0.03)	(0.84)	(0.09)	(0.13)	(.010)	(0.01)	(0.54)	(0.50)	(0.11)	(0.96)	(0.28)	(0.01)	(0.21)

Table S3 Transcriptomic sequencing results. Alignment results are the number of reads aligning to the *Microcystis aeruginosa* NIES-843 genome using Bowtie2 within RSEM. Each station or treatment has two biological replicates.

Station or Treatment	Number of reads	Aligned 0 times	Aligned exactly 1 time	Aligned >1 time	Overall alignment rate
LET1	27,903,522	24,945,541	1,659,152	1,298,829	10.60%
	27,536,041	24,284,725	1,444,752	1,806,564	11.81%
LET2	36,997,191	26,746,690	4,204,127	6,046,374	27.71%
	29,833,053	22,659,861	2,156,750	5,016,442	24.04%
LET3	28,864,110	23,924,801	2,316,711	2,622,598	17.11%
	28,650,471	23,552,927	2,043,243	3,054,301	17.79%
LET4	35,597,296	21,792,004	4,869,108	8,936,184	38.78%
	27,014,116	19,210,720	3,625,526	4,177,870	28.89%
LET5	25,556,343	17,968,910	4,150,242	3,437,191	29.69%
	25,153,667	18,465,371	3,093,990	3,594,306	26.59%
LET6	31,451,508	20,254,955	4,011,289	7,185,264	35.60%
	33,128,987	32,764,260	191,220	173,507	1.10%
LET7	38,595,755	37,911,204	156,426	528,125	1.77%
	33,928,689	32,938,676	113,686	876,327	2.92%
+P	25,734,772	21,133,075	3,170,522	1,431,175	17.88%
	30,634,245	24,635,004	3,748,488	2,250,753	19.58%
+NH4	36,319,164	29,604,594	4,557,190	2,157,380	18.49%
	32,820,933	26,160,377	4,705,489	1,955,067	20.29%
+Urea	36,497,773	28,856,115	4,602,858	3,038,800	20.94%
	28,528,820	22,765,343	3,605,361	2,158,116	20.20%
Control	33,983,026	27,619,927	4,156,741	2,206,358	18.72%
	37,595,286	30,176,832	4,697,085	2,721,369	19.73%
Initial	32,420,679	25,081,626	4,671,776	2,667,277	22.67%
	32,000,000	24,144,624	5,275,028	2,580,348	24.55%

Table S4 Transcriptomic sequencing results. Alignment results are the number of reads aligning to the *Anabaena* sp. PCC7108 genome using Bowtie2 within RSEM. Each station or treatment has two biological replicates.

Treatment or Station	Number of Reads	Aligned 0 times	Aligned exactly 1 time	Aligned >1 time	Overall alignment rate
Initial (L16)	32,420,679	32,301,184	47,013	72,482	0.37%
	31,858,827	31,722,341	60,070	76,416	0.43%
+P	25,734,772	25,686,336	8,500	39,936	0.19%
	30,634,245	30,595,894	10,372	27,979	0.13%
+NH4	36,319,164	36,281,206	11,106	26,852	0.10%
	32,820,933	32,768,476	19,206	33,251	0.16%
+Urea	36,497,773	36,461,942	11,309	24,522	0.10%
	28,528,820	28,503,313	9,030	16,477	0.09%
Control	33,983,026	33,948,716	13,789	20,521	0.10%
	37,595,286	37,547,609	14,757	32,920	0.13%
LET1	27,903,522	27,886,920	5,456	11,146	0.06%
	27,536,041	27,512,968	7,029	16,044	0.08%
LET2	36,997,191	36,979,812	3,135	14,244	0.05%
	29,833,053	29,819,785	2,927	10,341	0.04%
LET3	28,864,110	28,855,616	819	7,675	0.03%
	28,650,471	28,637,702	912	11,857	0.04%
LET4	35,597,296	35,588,672	1,235	7,389	0.02%
	27,014,116	26,996,707	1,388	16,021	0.06%
LET5	25,556,343	25,544,824	1,902	9,617	0.05%
	25,153,667	25,124,403	2,410	26,854	0.12%
LET6	31,451,508	31,439,930	1,088	10,490	0.04%
	33,128,987	32,941,384	122,110	65,493	0.57%
LET7	38,595,755	38,438,228	105,090	52,437	0.41%
	33,928,689	33,756,246	116,697	55,746	0.51%

Table S5 Transcriptomic sequencing results. Alignment results are the number of reads aligning to the *Planktothrix agardhii* NIVA-CYA 15 genome using Bowtie2 within RSEM. Each station or treatment has two biological replicates.

Treatment or Station	Number of Reads	Aligned 0 times	Aligned exactly 1 time	Aligned >1 time	Overall alignment rate
Initial (L16)	32,420,679	32,383,565	16,153	20,961	0.11%
	31,858,827	31,827,593	18,704	12,530	0.10%
+P	25,734,772	25,682,548	16,632	35,592	0.20%
	30,634,245	30,596,304	18,071	19,870	0.12%
+NH4	36,319,164	36,278,491	22,331	18,342	0.11%
	32,820,933	32,779,619	21,632	19,682	0.13%
+Urea	36,497,773	36,461,668	21,926	14,179	0.10%
	28,528,820	28,500,991	17,992	9,837	0.10%
Control	33,983,026	33,952,384	20,332	10,310	0.09%
	37,595,286	37,549,421	24,755	21,110	0.12%
LET1	27,903,522	27,888,104	10,243	5,175	0.06%
	27,536,041	27,518,397	8,300	9,344	0.06%
LET2	36,997,191	36,960,342	27,142	9,707	0.10%
	29,833,053	29,811,914	14,688	6,451	0.07%
LET3	28,864,110	28,842,315	17,098	4,697	0.08%
	28,650,471	28,627,157	14,574	8,740	0.08%
LET4	35,597,296	35,553,217	40,135	3,944	0.12%
	27,014,116	26,973,973	28,119	12,024	0.15%
LET5	25,556,343	25,516,138	31,521	8,684	0.16%
	25,153,667	25,106,056	22,813	24,798	0.19%
LET6	31,451,508	31,416,940	24,518	10,050	0.11%
	33,128,987	32,222,541	875,006	31,440	2.74%
LET7	38,595,755	37,906,634	664,944	24,177	1.79%
	33,928,689	33,363,190	542,576	22,923	1.67%

Table S6 Nutrient concentrations [μM] for each treatment for the nutrient amendment experiment. Values in parentheses represent the standard deviation between biological replicates (n=2). Values below detection limits are denoted with BD. For DOP, detection limit was 0.00243 μM.

	Initial (Station L16)	Control	+P	+NH_4	+Urea
NO_x	0.21 (0.02)	0.19 (0.06)	0.23 (0.05)	0.14 (0.02)	0.16 (0.06)
NH_4	1.69 (0.02)	1.25 (0.19)	1.42 (0.40)	1.23 (0.10)	1.11 (0.10)
Urea	1.98 (0.19)	0.36 (0.09)	0.11 (0.03)	0.30 (0.14)	0.28 (0.04)
TN	45.76 (0.66)	40.07 (1.38)	38.92 (2.14)	46.38 (3.65)	42.33 (5.54)
TDN	28.64 (1.06)	19.14 (2.43)	20.98 (0.47)	21.79 (1.36)	19.13 (1.66)
DON	28.43 (1.04)	18.95 (2.44)	20.75 (0.49)	21.65 (1.35)	18.97 (1.62)
PN	17.12 (1.72)	20.94 (2.78)	17.94 (2.17)	24.59 (3.46)	23.21 (4.69)
SRP	0.28 (0.07)	0.22 (0.07)	0.25 (0.03)	0.17 (0.09)	0.19 (0.01)
TP	3.58 (0.04)	5.17 (0.99)	4.98 (0.51)	3.56 (0.37)	5.16 (1.18)
TDP	0.49 (0.05)	0.75 (0.64)	0.30 (0.24)	0.46 (0.28)	0.38 (0.17)
DOP	0.22 (0.12)	0.57 (0.65)	BD	0.29 (0.31)	BD
PP	3.09 (0.09)	4.42 (0.78)	4.68 (0.45)	3.10 (0.37)	4.78 (1.19)

Table S7 The number of significantly differentially expressed genes for *Microcystis* within each functional category relative to the control.

Category	+P	+NH4	+Urea
Amino acid biosynthesis	6	2	4
Biosynthesis of cofactors, prosthetic groups, and carriers	6	8	7
Cell envelope	5	9	3
Cellular processes	3	6	12
Central intermediary metabolism	4	2	1
Energy metabolism	32	7	14
Fatty acid, phospholipid and sterol metabolism	8	2	2
Photosynthesis and respiration	53	42	29
Purines, pyrimidines, nucleosides, and nucleotides	12	4	3
Regulatory functions	35	12	9
DNA replication, restriction, modification, recombination, and repair	16	5	6
Transcription	6	7	6
Translation	22	38	53
Transport and binding proteins	51	18	12
Unknown	150	52	54
RNA	0	0	0
Other categories	489	179	158
Hypothetical	377	167	131

Supplemental Figures

Figure S1 Composite of MODIS Cyanobacterial Index images from the NOAA Experimental Lake Erie Harmful Algal Bloom Bulletin (http://www.glerl.noaa.gov/res/Centers/HABS/lake_erie_hab/lake_erie_hab.html) for 2013. Grey indicates cloud cover or missing data. Black represents no cyanobacteria detected. Colored pixels indicate the presence of cyanobacteria. Cooler colors (blue and purple) indicate low concentrations and warmer colors (red, orange, and yellow) indicate high concentrations.

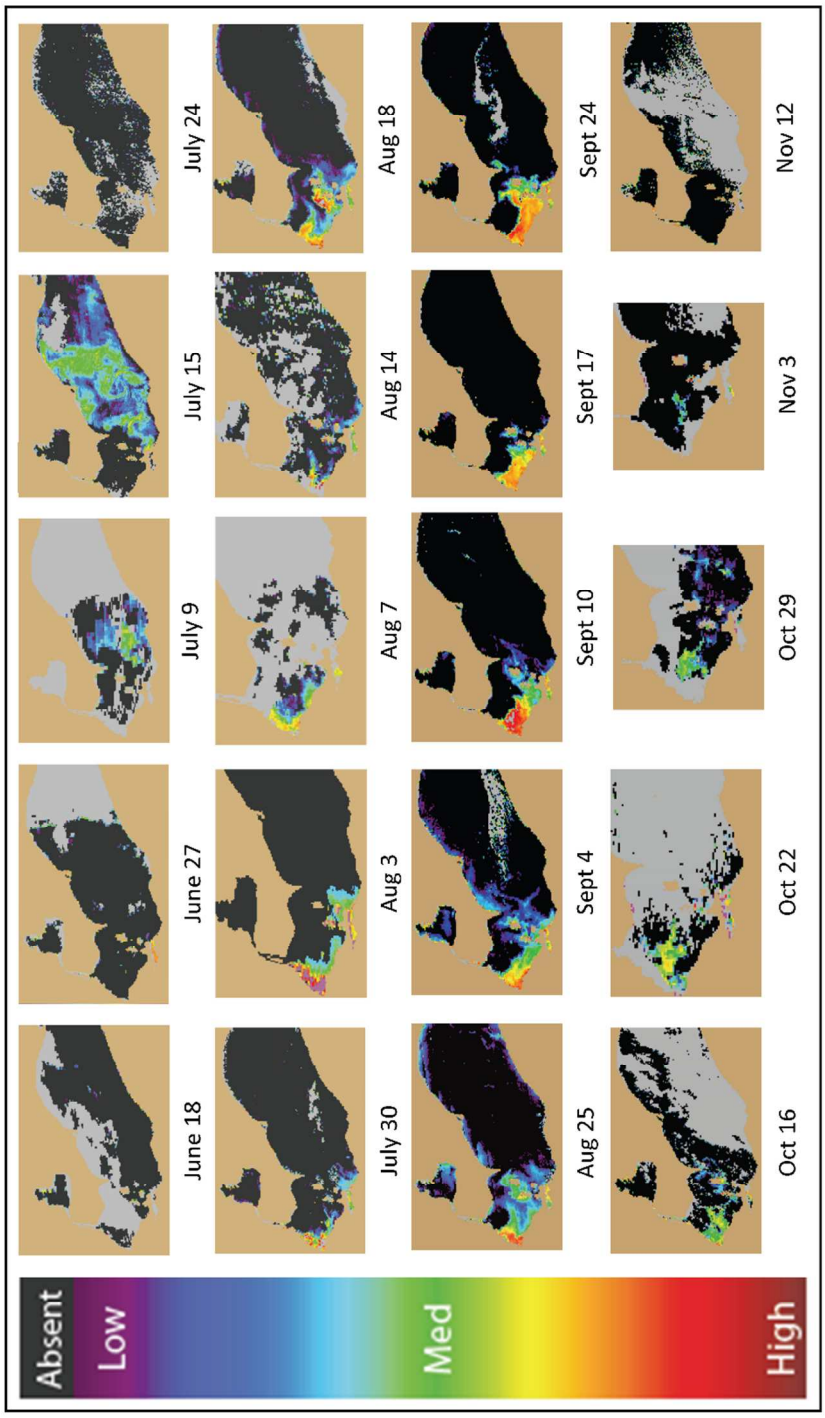


Figure S2 A) The number of significant differentially expressed genes for *Microcystis* at each station relative to station LET7 and B) relative to the control after 48hr. incubation. Blue denotes increases in transcript abundance and yellow denotes decrease in transcript abundance. Stations are ordered from west to east as appearing in Figure 1.

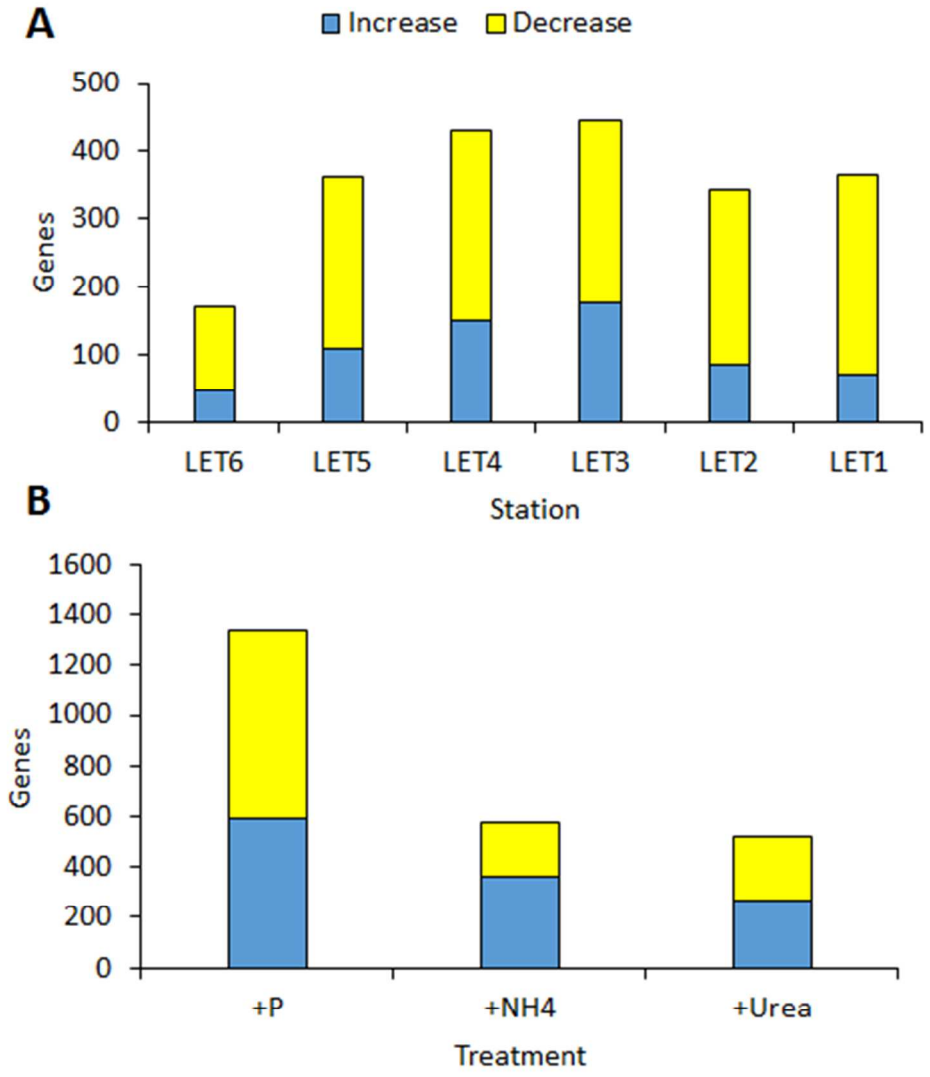
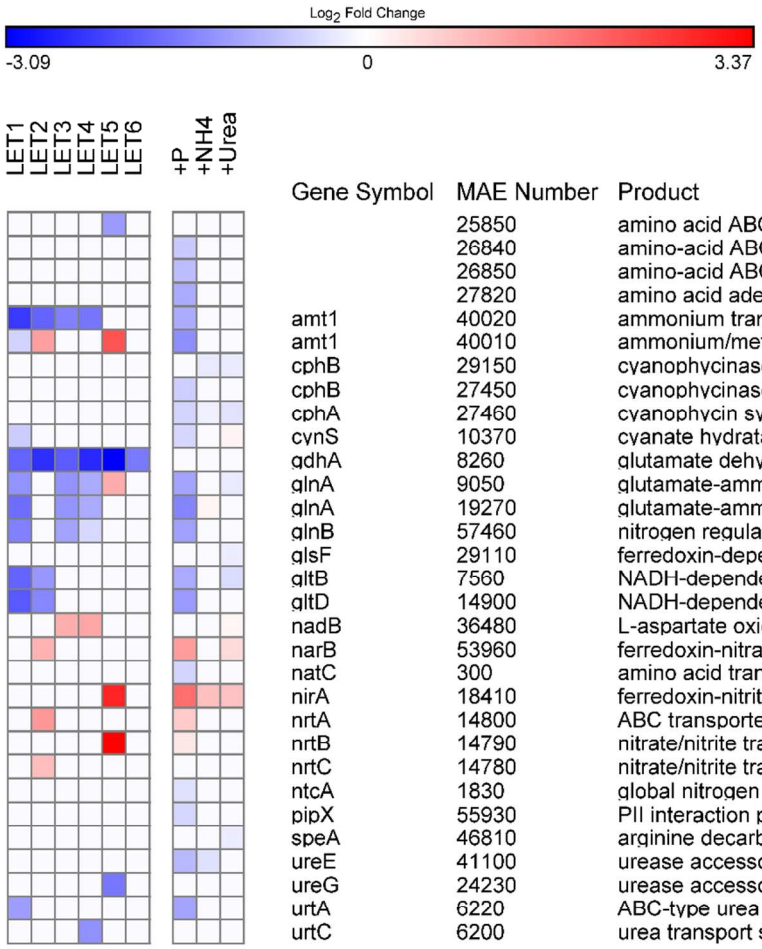
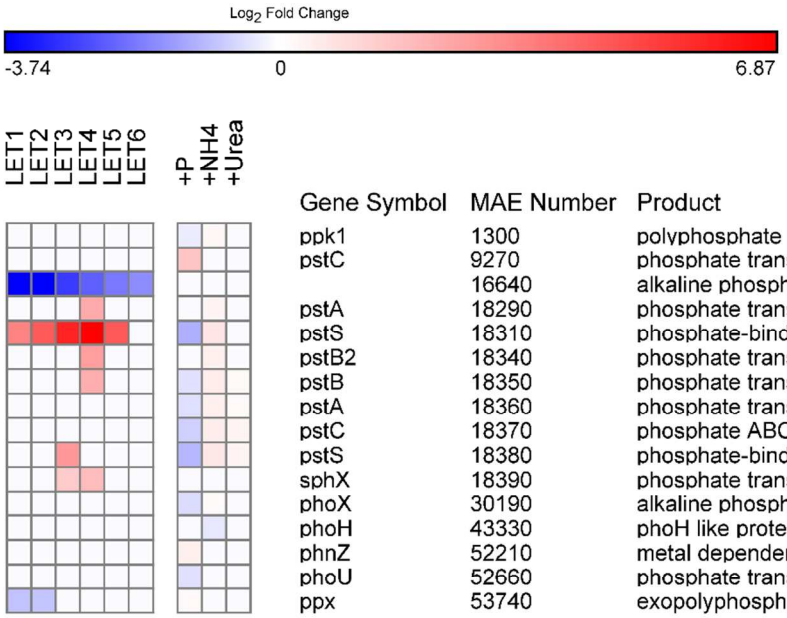


Figure S3 Heat map of genes involved in nitrogen transport and metabolism and their significant differential expression at each station relative to LET7 and under each treatment relative to the control for *Microcystis*. Values are the $\log_2$ fold change in gene expression. Blue colors correspond to a decrease in transcript abundance while red colors correspond to an increase in transcript abundance. White denotes no difference from the reference condition.



Gene Symbol	MAE Number	Product
	25850	amino acid ABC-transporter permease protein
	26840	amino-acid ABC-transporter ATP-binding protein
	26850	amino-acid ABC-transporter permease protein
	27820	amino acid adenylation
amt1	40020	ammonium tranport protein
amt1	40010	ammonium/methylammonium permease
cphB	29150	cyanophycinase
cphB	27450	cyanophycinase
cphA	27460	cyanophycin synthetase
cynS	10370	cyanate hydratase
gdhA	8260	glutamate dehydrogenase (NADP+)
glnA	9050	glutamate-ammonia ligase
glnA	19270	glutamate-ammonia ligase
glnB	57460	nitrogen regulatory protein P-II
glsF	29110	ferredoxin-dependent glutamate synthase
gltB	7560	NADH-dependent glutamate synthase large subunit
gltD	14900	NADH-dependent glutamate synthase small subunit
nadB	36480	L-aspartate oxidase
narB	53960	ferredoxin-nitrate reductase
natC	300	amino acid transport system permease protein
nirA	18410	ferredoxin-nitrite reductase
nrtA	14800	ABC transporter nitrate-binding protein
nrtB	14790	nitrate/nitrite transport system permease protein
nrtC	14780	nitrate/nitrite transport system ATP-binding protein
ntcA	1830	global nitrogen regulatory protein Ycf28
pipX	55930	PII interaction protein X
speA	46810	arginine decarboxylase
ureE	41100	urease accessory protein E
ureG	24230	urease accessory protein G
urtA	6220	ABC-type urea transport system substrate-binding protein
urtC	6200	urea transport system permease protein

Figure S4 Heat map of genes involved in phosphorus transport and metabolism and their significant differential expression at each station relative to LET7 and under each treatment relative to the control for *Microcystis*. Values are the $\log_2$ fold change in gene expression. Blue colors correspond to a decrease in transcript abundance while red colors correspond to an increase in transcript abundance. White denotes no difference from the reference condition.



Gene Symbol	MAE Number	Product
ppk1	1300	polyphosphate kinase
pstC	9270	phosphate transport system permease protein
	16640	alkaline phosphatase
pstA	18290	phosphate transport system permease protein
pstS	18310	phosphate-binding periplasmic protein
pstB2	18340	phosphate transport ATP-binding protein
pstB	18350	phosphate transport ATP-binding protein
pstA	18360	phosphate transport system permease protein
pstC	18370	phosphate ABC transporter permease
pstS	18380	phosphate-binding periplasmic protein
sphX	18390	phosphate transport system substrate-binding protein
phoX	30190	alkaline phosphatase
phoH	43330	phoH like protein
phnZ	52210	metal dependent phosphohydrolase HD region
phoU	52660	phosphate transport system regulatory protein
ppx	53740	exopolyphosphatase

Figure S5 Heat map of transposase genes and their significant differential expression at each station relative to LET7 for *Microcystis*. Values are the $\log_2$ fold change in gene expression. Blue colors correspond to a decrease in transcript abundance while red colors correspond to an increase in transcript abundance. White denotes no difference from the reference condition.

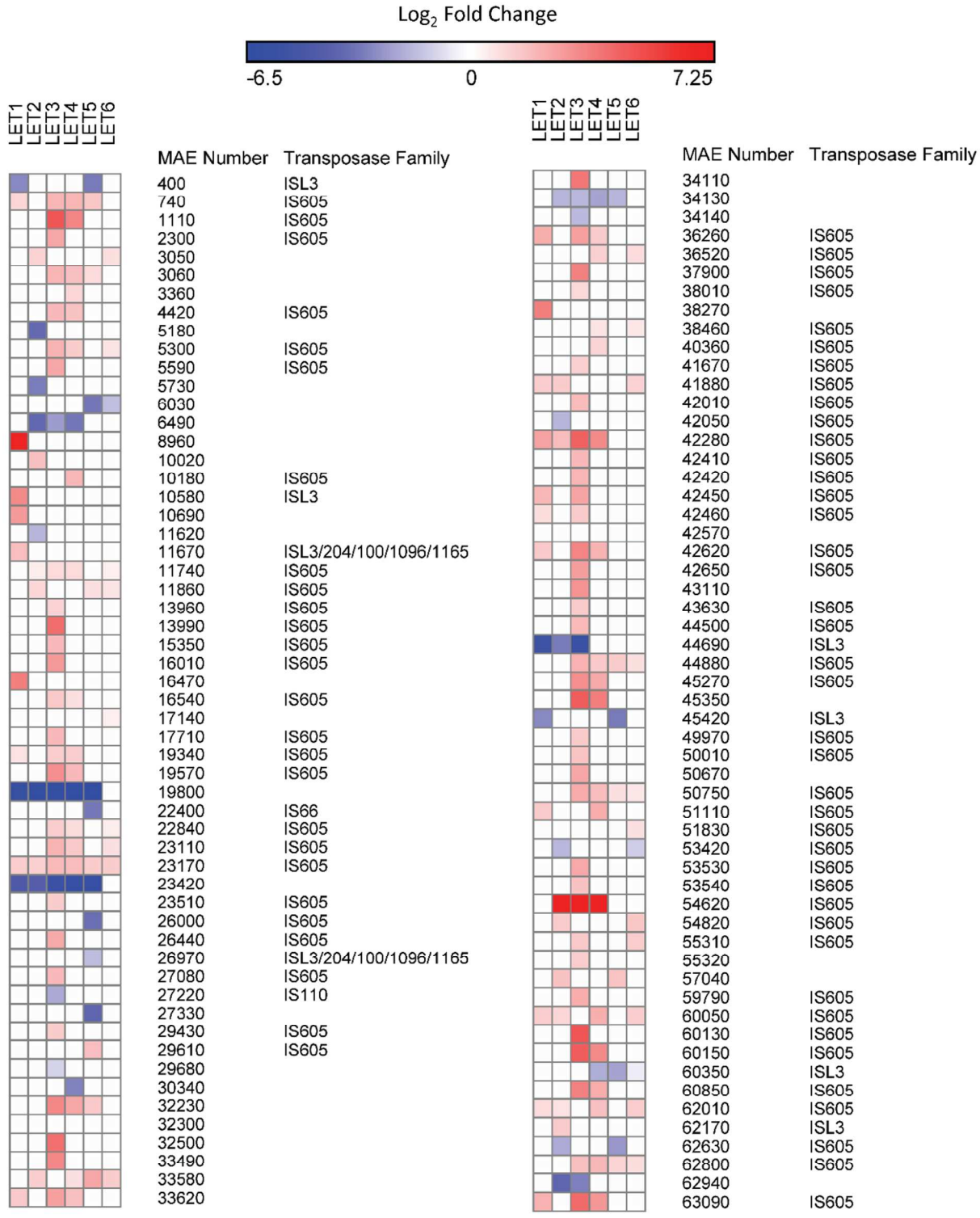


Figure S6 Cyanobacterial abundance during the October transect of the western basin of Lake Erie for the three genera discussed. Stations are ordered from west to east as appearing in Figure 1.

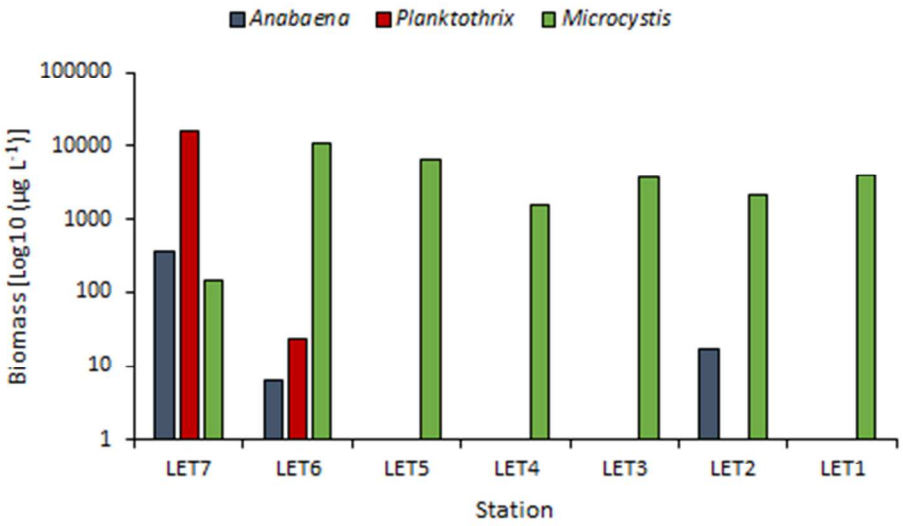


Figure S7 The number of significant differentially expressed genes in *Planktothrix agaradhii* NIVA-CYA 15 relative to station LET7 (A), relative to the control (B), and relative to the initial (C). Blue denotes an increase in transcript abundance and yellow denotes a decrease in transcript abundance. Stations are ordered from west to east as appearing in Figure 1.

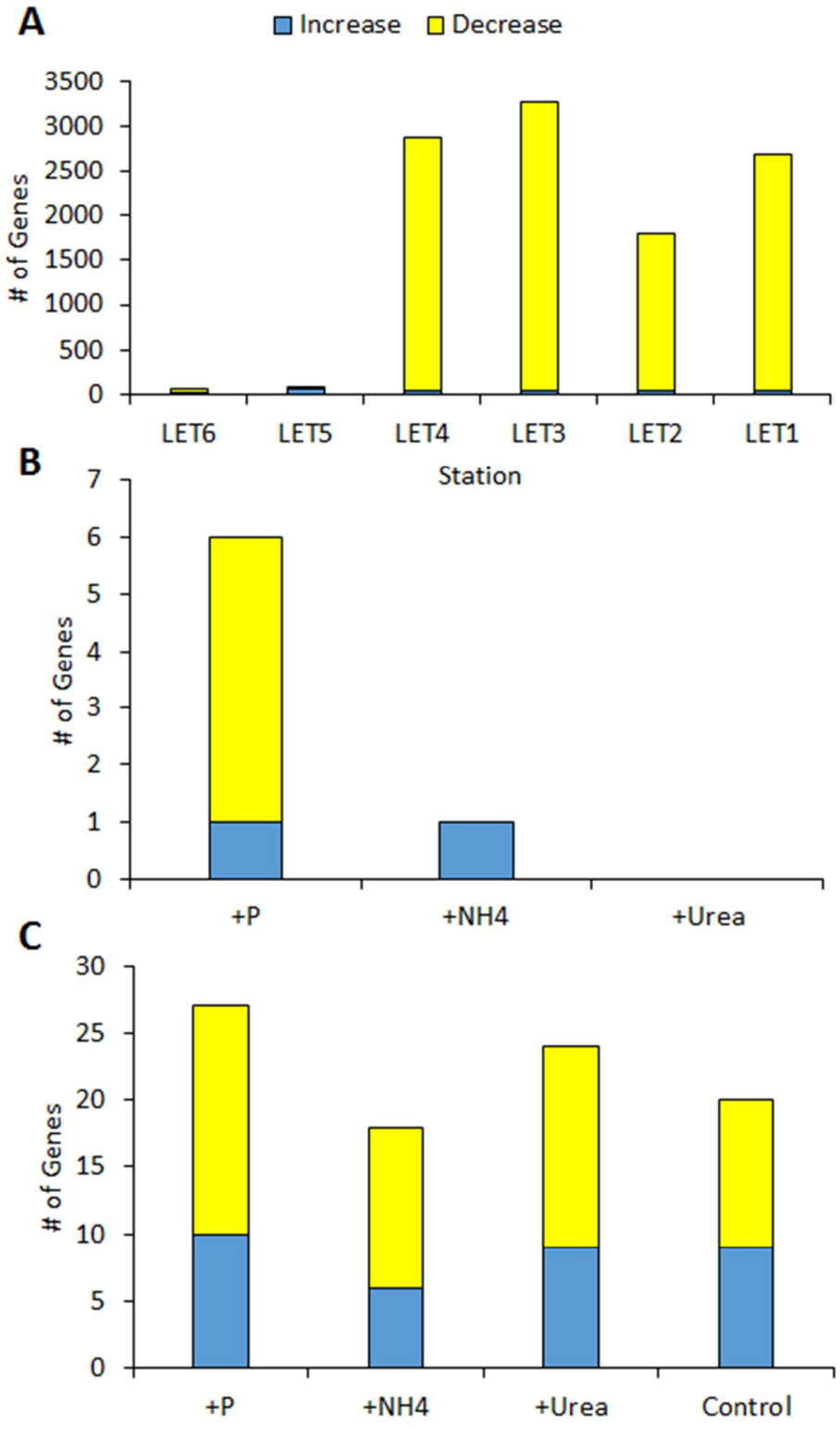


Figure S8 The number of significant differentially expressed genes in *Anabaena* sp. PCC7108 relative to station LET7 (A), relative to the control (B), and relative to the initial (C). Blue denotes an increase in transcript abundance and yellow denotes a decrease in transcript abundance. Stations are ordered from west to east as appearing in Figure 1.

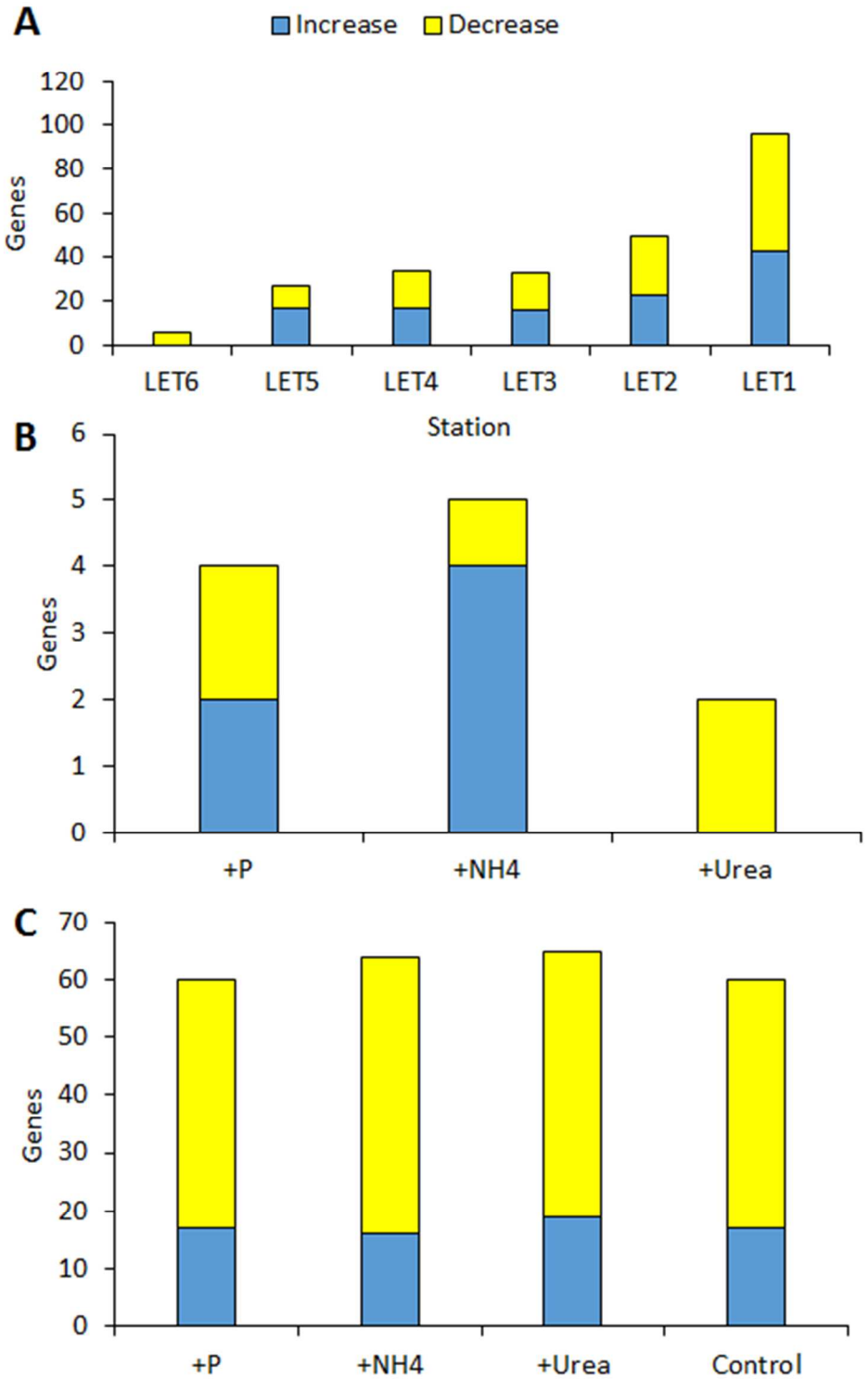


Figure S9 Heat map of transposase genes and their significant differential expression relative to the control for *Microcystis*. Values are the $\log_2$ fold change in gene expression. Blue colors correspond to a decrease in transcript abundance while red colors correspond to an increase in transcript abundance. White denotes no difference from the reference condition.

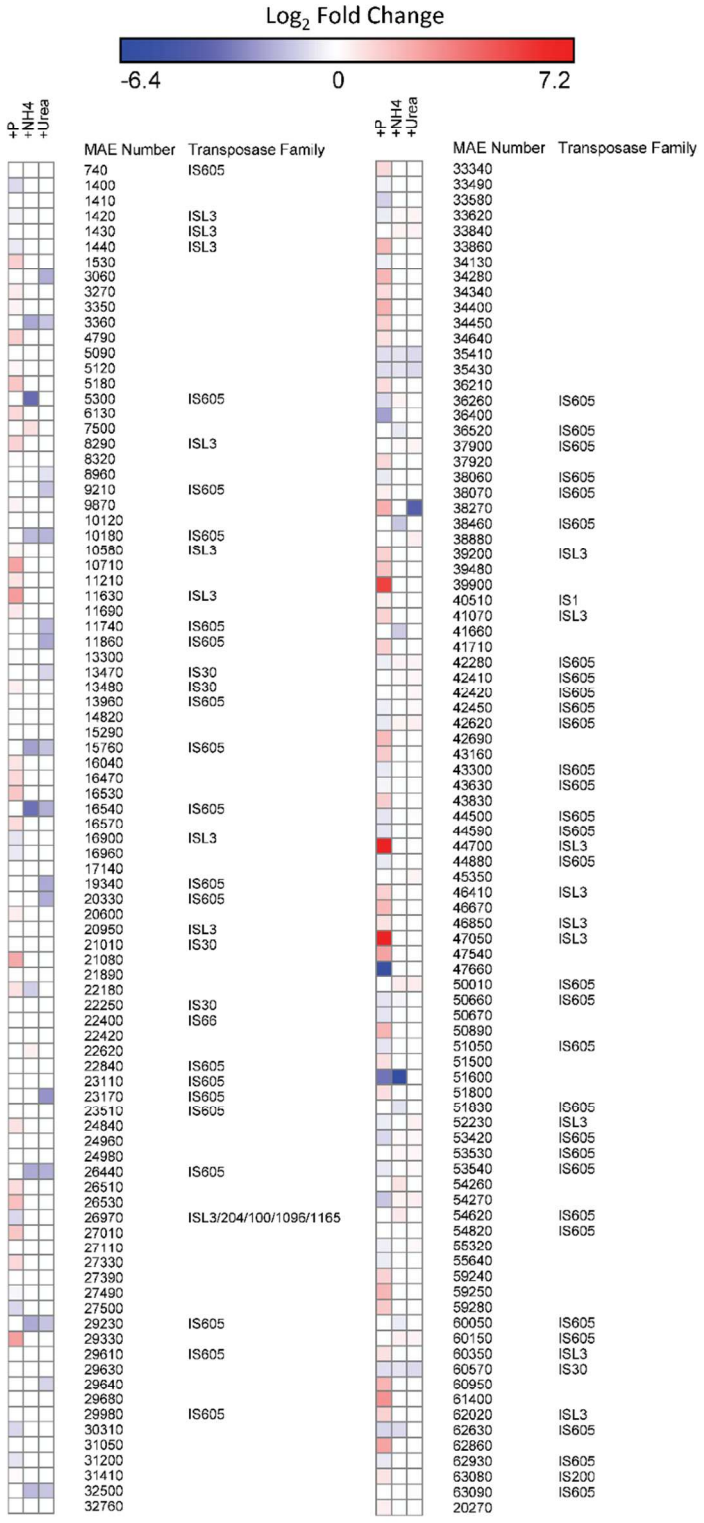


Figure S10 Heat map of genes involved in DNA replication, restriction, modification, recombination, and repair and their significant differential expression at each station relative to LET7 and under each treatment relative to the control for *Microcystis*. Values are the $\log_2$ fold change in gene expression. Blue colors correspond to a decrease in transcript abundance while red colors correspond to an increase in transcript abundance. White denotes no difference from the reference condition.

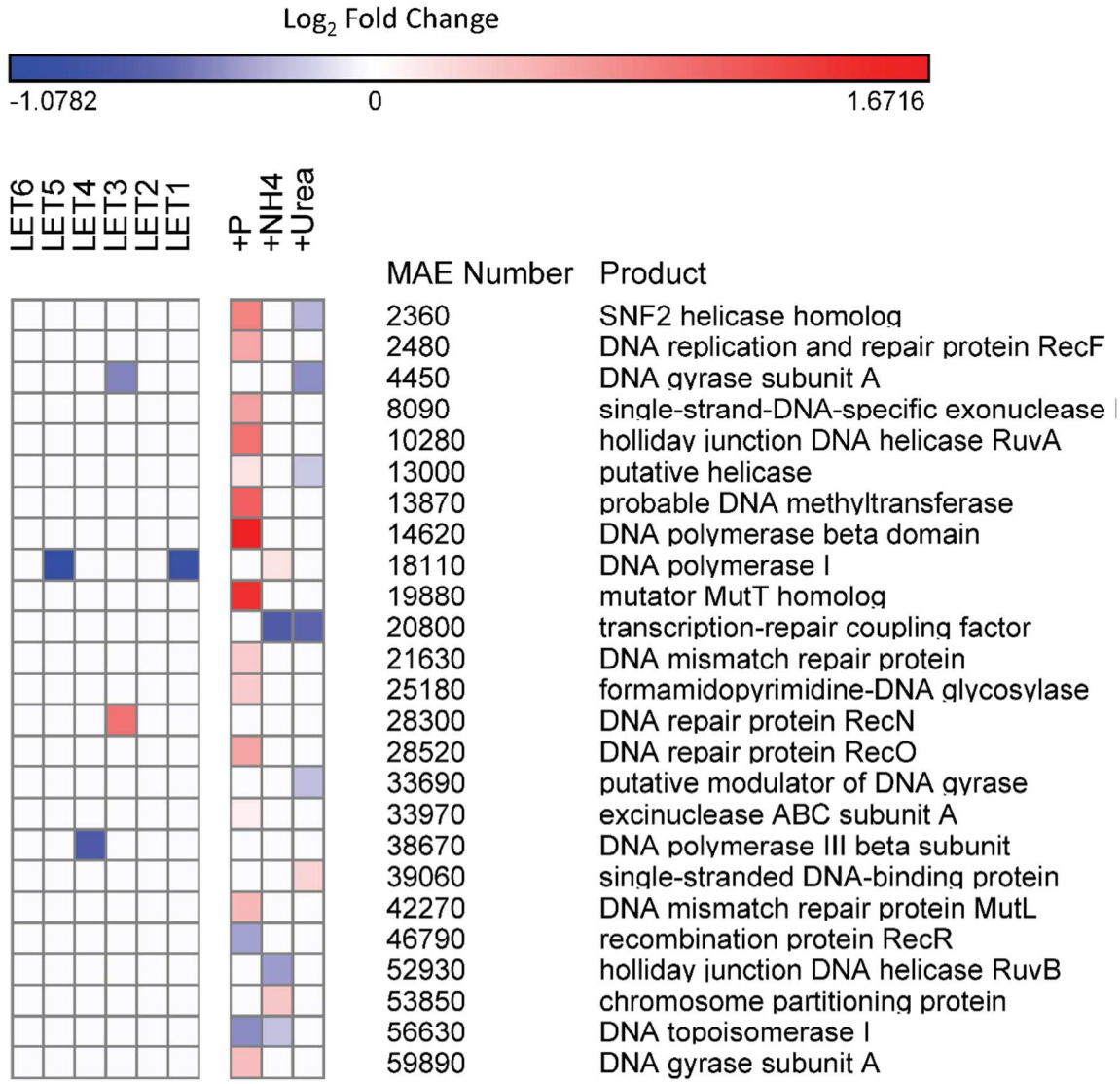


Figure S11 Community analysis via Metaphlan displaying the average % abundance across two biological replicates for the nutrient enrichment experiments.

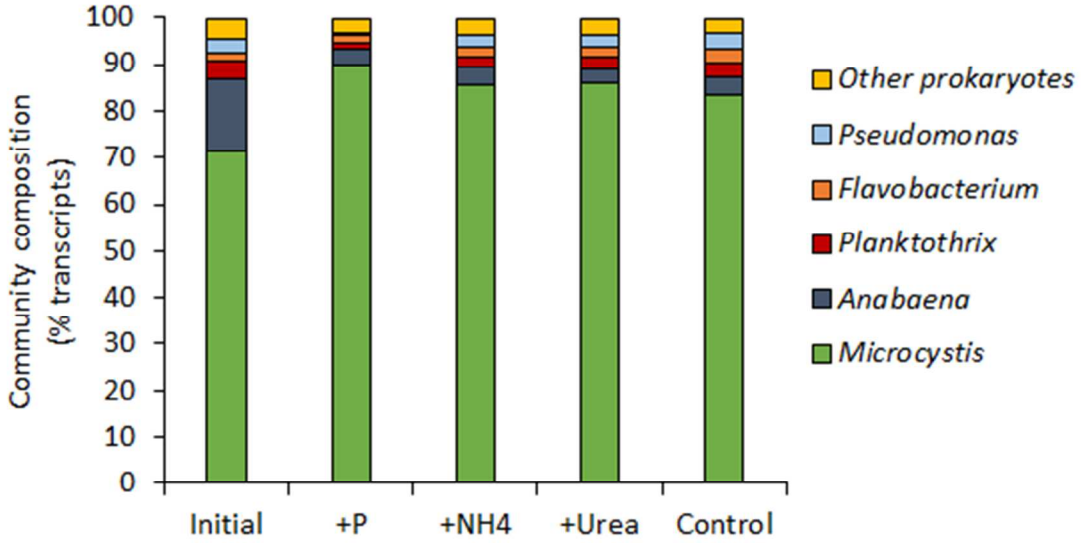


Figure S12 The number of significant differentially expressed transposase genes from Harke, M. J., & Gobler, C. J. (2013). Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. PLoS ONE, 8(7), e69834. doi: 10.1371/journal.pone.0069834.