

# Supplementary Material

## Shotgun Metagenomic Sequencing to Assess Cyanobacterial Community Composition following Coagulation of Cyanobacterial Blooms

Kim Thien Nguyen Le <sup>1,\*</sup>, Juan Francisco Guerra Maldonado <sup>1</sup>, Eyerusalem Goitom <sup>2</sup>, Hana Trigui <sup>3</sup>, Yves Terrat <sup>4</sup>, Thanh-Luan Nguyen <sup>1</sup>, Barry Husk <sup>5</sup>, B. Jesse Shapiro <sup>4,6,7</sup>, Sébastien Sauvé <sup>8</sup>, Michèle Prévost <sup>1</sup> and Sarah Dorner <sup>1</sup>

<sup>1</sup> Department of Civil, Geological and Mining Engineering, Polytechnique de Montréal, Montréal, QC H3C 3A7, Canada

<sup>2</sup> Department of Geography and Environmental Studies, Toronto Metropolitan University, Toronto, ON M5B 2K3, Canada

<sup>3</sup> Institut National de Santé Publique de Québec, Montréal, QC H2P 1E2, Canada

<sup>4</sup> Department of Biological Sciences, University of Montréal, Montréal, QC H2V 0B3, Canada

<sup>5</sup> BlueLeaf Inc., 310 Chapleau Street, Drummondville, QC J2B 5E9, Canada

<sup>6</sup> McGill Genome Centre, McGill University, Montréal, QC H3A 0G1, Canada

<sup>7</sup> Department of Microbiology and Immunology, McGill University, Montréal, QC H3A 2B4, Canada

<sup>8</sup> Department of Chemistry, University of Montréal, Montréal, QC H3C 3J7, Canada

\* Correspondence: thien-kim.le-nguyen@polymtl.ca

**Table S1.** Removal effectiveness (%) of taxonomic cell counts of individual cyanobacterial genus after 48 hours (Mean $\pm$ Standard deviation) in Missisquoi Bay (MB) and Petit Lac St. François (PLSF). Adapted from the author of [1].

Event	Treat	Total cell counts	<i>Aphanizomenon</i>	<i>Aphanocapsa</i>	<i>Aphanothecae</i>	<i>Chroococcus</i>	<i>Coelosphaerium</i>	<i>Dolichospermum</i>	<i>Merismopedia</i>	<i>Microcystis</i>	<i>Pseudanabaena</i>
September 10-12, 2018	20 mgFe/L	99.96 $\pm$ 0.04	97.84 $\pm$ 3.05	100.0 $\pm$ 0.00	98.30 $\pm$ 0.21	100.0 $\pm$ 0.00	NA	99.88 $\pm$ 0.04	100.0 $\pm$ 0.00	99.97 $\pm$ 0.01	99.97 $\pm$ 2.81
	35 mgFe/L	99.94 $\pm$ 0.04	97.69 $\pm$ 1.12	100.0 $\pm$ 0.00	97.27 $\pm$ 0.11	100.0 $\pm$ 0.00	NA	99.81 $\pm$ 0.05	100.0 $\pm$ 0.00	99.65 $\pm$ 0.05	97.75 $\pm$ 0.79
September 24-26, 2018	20 mgFe/L	71.91 $\pm$ 5.38	68.55 $\pm$ 1.94	89.27 $\pm$ 6.55	64.38 $\pm$ 2.83	NA	NA	75.22 $\pm$ 7.48	100.0 $\pm$ 0.00	86.92 $\pm$ 2.14	72.69 $\pm$ 3.93
	35 mgFe/L	96.39 $\pm$ 1.29	80.58 $\pm$ 7.53	76.56 $\pm$ 2.31	69.09 $\pm$ 1.63	NA	NA	98.51 $\pm$ 1.19	100.0 $\pm$ 0.00	93.98 $\pm$ 2.61	71.59 $\pm$ 8.54
August 13- 15, 2019	20 mgFe/L	94.27 $\pm$ 1.67	93.91 $\pm$ 1.64	NA	87.73 $\pm$ 6.22	NA	100.0 $\pm$ 0.00	97.17 $\pm$ 3.14	NA	86.55 $\pm$ 1.62	NA
	35 mgFe/L	99.35 $\pm$ 0.11	99.26 $\pm$ 0.15	NA	96.51 $\pm$ 1.44	NA	100.0 $\pm$ 0.00	99.97 $\pm$ 0.01	NA	100.0 $\pm$ 0.00	NA
PLSF June 26-28, 2019	20 mgFe/L	85.22 $\pm$ 6.43	77.17 $\pm$ 8.16	NA	-37.5 $\pm$ 1.67	NA	35.11 $\pm$ 5.01	84.66 $\pm$ 4.07	NA	92.66 $\pm$ 8.64	NA
	35 mgFe/L	98.99 $\pm$ 1.06	98.66 $\pm$ 1.44	NA	88.50 $\pm$ 4.58	NA	100.0 $\pm$ 0.00	99.03 $\pm$ 0.95	NA	95.82 $\pm$ 4.36	NA
PLSF July 24-26, 2019	20 mgFe/L	51.98 $\pm$ 6.21	76.76 $\pm$ 4.51	NA	-13.5 $\pm$ 1.24	100.0 $\pm$ 0.00	19.36 $\pm$ 1.14	66.57 $\pm$ 4.71	77.5 $\pm$ 0.28	66.99 $\pm$ 0.95	NA
	35 mgFe/L	99.11 $\pm$ 0.11	97.93 $\pm$ 0.28	NA	92.65 $\pm$ 0.96	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	NA
PLSF August 05- 07, 2019	20 mgFe/L	78.21 $\pm$ 6.72	72.22 $\pm$ 6.32	NA	79.47 $\pm$ 4.98	100.0 $\pm$ 0.00	75.86 $\pm$ 4.33	77.97 $\pm$ 5.04	92.14 $\pm$ 3.59	96.51 $\pm$ 5.39	NA
	35 mgFe/L	99.72 $\pm$ 0.65	98.22 $\pm$ 0.65	NA	93.46 $\pm$ 2.34	NA	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	100.0 $\pm$ 0.00	99.97 $\pm$ 0.01	NA

$$\text{Removal effectiveness percentage (\%)} = \frac{T_0 - T_{48}}{T_0} \times 100. \text{ NA: no value.}$$

**Table S2.** Removal effectiveness (%) of taxonomic cell counts of individual cyanobacterial genus after 48 hours on August 08-10, 2018, in Missisquoi Bay (Mean  $\pm$  Standard deviation).

Event	Treat	Total cell counts	<i>Aphanocapsa</i>	<i>Aphanothece</i>	<i>Dolichospermum</i>	<i>Microcystis</i>
<b>August 08-10, 2018</b>	20 mgFe/L	50.01 $\pm$ 0.04	100.0 $\pm$ 0.00	53.36 $\pm$ 0.21	77.88 $\pm$ 0.04	99.97 $\pm$ 0.01
	35 mgFe/L	96.94 $\pm$ 0.04	100.0 $\pm$ 0.00	67.27 $\pm$ 0.11	97.81 $\pm$ 0.05	

$$\text{Removal effectiveness percentage (\%)} = \frac{T_0 - T_{48}}{T_0} \times 100. \text{ NA: no value}$$

**Table S3.** Mean  $\pm$  standard deviation value for environmental conditions of the sampled water in the control mesocosms on August 08-10, 2018, in Missisquoi Bay (n=2).

Parameters	August 08 2018	August 10 2018
<b>Chlorophyll-a (RFU)</b>	20.01 $\pm$ 2.45	-
<b>Phycocyanin (RFU)</b>	155.16 $\pm$ 2.96	-
<b>pH</b>	8.61 $\pm$ 0.07	-
<b>DO (mg/L)</b>	9.11 $\pm$ 0.21	-
<b>Temp (°C)</b>	26.70 $\pm$ 0.21	-
<b>TOC (mg C/L)</b>	2387.5 $\pm$ 45.96	185.51 $\pm$ 4.01
<b>DOC (mg C/L)</b>	38.41 $\pm$ 0.27	36.10 $\pm$ 1.46
<b>TN (mg N/L)</b>	14.44 $\pm$ 0.83	14.85 $\pm$ 0.69
<b>TP (µg P/L)</b>	3155.01 $\pm$ 67.45	1769.02 $\pm$ 89.39
<b>DN (mg N/L)</b>	0.10 $\pm$ 0.001	5.46 $\pm$ 0.57
<b>DP (µg P/L)</b>	328.22 $\pm$ 2.07	168.58 $\pm$ 5.79

- : No data.

**Table S4.** Environmental conditions of lake water samples in control mesocosms (n = 6) at T0 (Mean  $\pm$  standard deviation). Adapted from author of [1].

Parameters	Missisquoi Bay			Petit Lac St. François		
	(A) 10 September 2018	(B) 24 September 2018	(C) 13 August 2019	(a) 26 June 2019	(b) 24 July 2019	(c) 05 August 2019
Total cell counts (cells/mL)	998,183 $\pm$ 35,034	547,325 $\pm$ 16,578	17,408,158 $\pm$ 138,898	6,033,197 $\pm$ 316,425	109,193 $\pm$ 3578	235,723 $\pm$ 5986
Chlorophyll-a (RFU)	-	-	62.22 $\pm$ 1.03	3.97 $\pm$ 0.04	5.14 $\pm$ 0.06	6.27 $\pm$ 0.08
Phycocyanin (RFU)	-	-	93.43 $\pm$ 0.52	16.51 $\pm$ 1.54	1.77 $\pm$ 0.04	4.83 $\pm$ 0.13
pH	6.5 $\pm$ 0.08	6.4 $\pm$ 0.07	8.05 $\pm$ 0.24	8.08 $\pm$ 0.07	7.84 $\pm$ 0.03	8.01 $\pm$ 0.01
TDS (mg/L)	101 $\pm$ 0.00	100 $\pm$ 0.00	98.00 $\pm$ 0.00	122.50 $\pm$ 0.71	116.00 $\pm$ 0.00	115.00 $\pm$ 0.00
Temp (°C)	21.8 $\pm$ 0.01	18.7 $\pm$ 0.12	26.89 $\pm$ 0.21	25.96 $\pm$ 0.49	25.14 $\pm$ 0.12	24.43 $\pm$ 0.24
TOC (mg C/L)	15.22 $\pm$ 0.25	5.55 $\pm$ 0.07	885.00 $\pm$ 33.34	175.00 $\pm$ 9.50	11.10 $\pm$ 0.38	10.76 $\pm$ 0.13
DOC (mg C/L)	7.50 $\pm$ 0.05	5.00 $\pm$ 0.00	19.34 $\pm$ 1.67	9.80 $\pm$ 0.11	9.83 $\pm$ 0.13	11.34 $\pm$ 1.58
TN (mg N/L)	5.55 $\pm$ 0.65	2.75 $\pm$ 0.08	7.84 $\pm$ 2.95	12.85 $\pm$ 0.57	1.11 $\pm$ 0.05	1.29 $\pm$ 0.008
TP (µg P/L)	360.51 $\pm$ 0.01	292.11 $\pm$ 13.28	4336.92 $\pm$ 74.65	723.55 $\pm$ 24.97	110.38 $\pm$ 1.89	131.78 $\pm$ 7.71
DN (mg N/L)	0.45 $\pm$ 0.004	0.52 $\pm$ 0.009	2.66 $\pm$ 0.12	0.73 $\pm$ 0.08	0.60 $\pm$ 0.02	0.50 $\pm$ 0.02
DP (µg P/L)	17.05 $\pm$ 0.07	17.99 $\pm$ 0.45	302.17 $\pm$ 12.98	139.06 $\pm$ 3.35	23.92 $\pm$ 4.91	42.86 $\pm$ 1.13

- : No data.

**Table S5.** Environmental conditions of lake water samples in control mesocosms at T48 (Mean  $\pm$  standard deviation). Adapted from author of [1].

Parameters	Missisquoi Bay			Petit-Lac-St-François		
	Event A September 12 2018	Event B September 26 2018	Event C August 15 2019	Event a June 28 2019	Event b July 26 2019	Event c August 07 2019
Chlorophyll-a (RFU)	-	-	64.72 $\pm$ 0.59	3.37 $\pm$ 0.32	5.63 $\pm$ 0.16	7.88 $\pm$ 0.17
Phycocyanin (RFU)	-	-	169.21 $\pm$ 0.34	16.42 $\pm$ 0.15	0.87 $\pm$ 0.03	6.78 $\pm$ 0.16
pH	7.47 $\pm$ 0.07	-	6.33 $\pm$ 0.09	9.93 $\pm$ 0.06	8.36 $\pm$ 0.07	7.37 $\pm$ 0.15
TDS (mg/L)	105 $\pm$ 0.00	-	140.0 $\pm$ 4.24	151.0 $\pm$ 5.65	118.5 $\pm$ 0.71	121.0 $\pm$ 0.00
Temp (°C)	22.7 $\pm$ 0.17	-	22.09 $\pm$ 0.17	27.81 $\pm$ 0.41	25.37 $\pm$ 0.34	25.25 $\pm$ 0.02
TOC (mg C/L)	19.97 $\pm$ 0.24	5.46 $\pm$ 0.00	700.0 $\pm$ 23.19	11.39 $\pm$ 0.23	10.57 $\pm$ 0.21	10.19 $\pm$ 0.44
DOC (mg C/L)	12.17 $\pm$ 0.39	5.08 $\pm$ 0.05	73.48 $\pm$ 1.45	9.83 $\pm$ 0.06	10.07 $\pm$ 0.83	9.81 $\pm$ 0.07
TN (mg N/L)	5.46 $\pm$ 0.69	1.68 $\pm$ 0.01	6.84 $\pm$ 1.78	11.21 $\pm$ 1.07	1.01 $\pm$ 0.15	1.28 $\pm$ 0.05
TP (µg P/L)	320.92 $\pm$ 4.48	177.49 $\pm$ 21.03	2074.60 $\pm$ 20.22	603.01 $\pm$ 3.17	72.08 $\pm$ 5.32	89.29 $\pm$ 4.81
DN (mg N/L)	2.02 $\pm$ 0.001	0.48 $\pm$ 0.009	1.56 $\pm$ 0.11	0.91 $\pm$ 0.25	0.58 $\pm$ 0.00	0.63 $\pm$ 0.03
DP (µg P/L)	40.47 $\pm$ 1.17	15.43 $\pm$ 0.25	215.01 $\pm$ 11.61	108.06 $\pm$ 2.22	16.46 $\pm$ 1.16	21.81 $\pm$ 2.13

- : No data.

**Table S6.** Environmental conditions of lake water samples in mesocosms with dose of 20 mgFe/L at T48 (Mean  $\pm$  standard deviation).

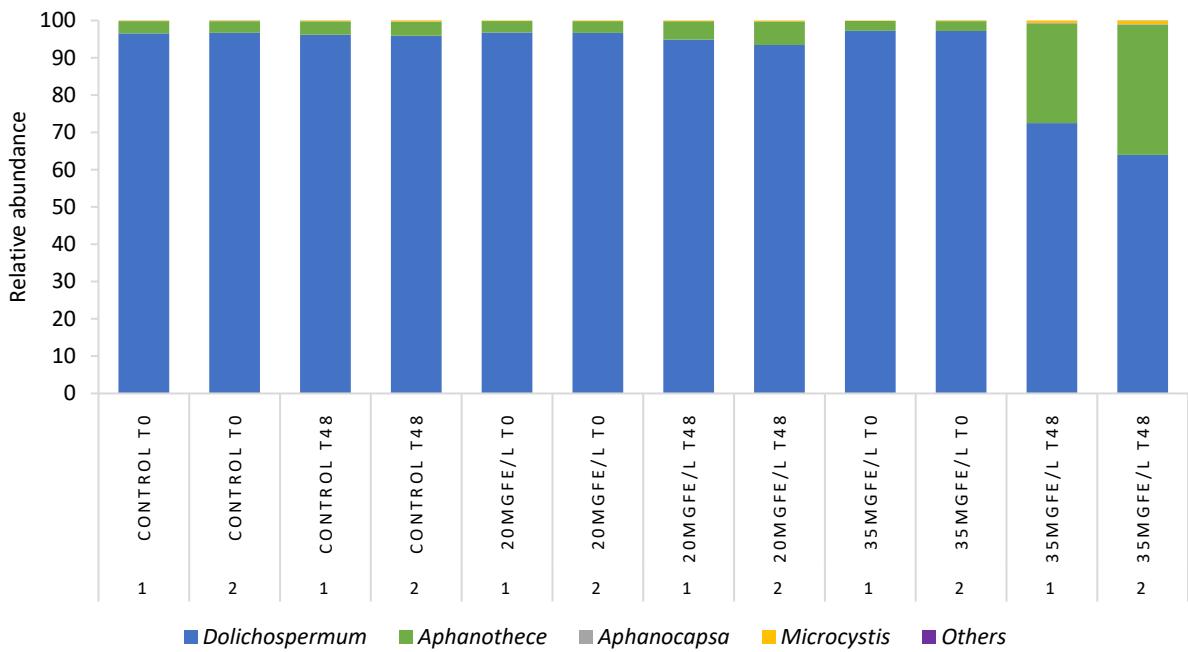
Parameters	Missisquoi Bay			Petit-Lac-St-François		
	Event A September 12 2018	Event B September 26 2018	Event C August 15 2019	Event a June 28 2019	Event b July 26 2019	Event c August 07 2019
Chlorophyll-a (RFU)	-	-	1.04 $\pm$ 0.002	0.08 $\pm$ 0.00	1.18 $\pm$ 0.01	0.82 $\pm$ 0.07
Phycocyanin (RFU)	-	-	33.69 $\pm$ 5.21	0.49 $\pm$ 0.05	0.54 $\pm$ 0.00	1.03 $\pm$ 0.05
pH	4.9 $\pm$ 0.04	-	5.08 $\pm$ 1.01	6.09 $\pm$ 0.01	7.70 $\pm$ 0.19	5.66 $\pm$ 0.02
TDS (mg/L)	138 $\pm$ 0.00	-	152.00 $\pm$ 0.00	130.00 $\pm$ 0.00	139.00 $\pm$ 0.00	146.00 $\pm$ 0.00
Temp (°C)	21.8 $\pm$ 0.01	-	22.95 $\pm$ 0.04	23.92 $\pm$ 0.02	25.21 $\pm$ 0.01	25.03 $\pm$ 0.01
TOC (mg C/L)	4.52 $\pm$ 0.15	2.22 $\pm$ 0.09	23.76 $\pm$ 4.52	17.56 $\pm$ 2.71	3.81 $\pm$ 0.51	3.69 $\pm$ 0.11
DOC (mg C/L)	3.21 $\pm$ 0.03	2.39 $\pm$ 0.03	22.62 $\pm$ 1.92	15.10 $\pm$ 2.87	3.63 $\pm$ 0.24	3.44 $\pm$ 0.01
TN (mg N/L)	0.67 $\pm$ 0.01	1.29 $\pm$ 0.01	4.16 $\pm$ 1.01	3.71 $\pm$ 0.48	0.55 $\pm$ 0.007	0.35 $\pm$ 0.009
TP (µg P/L)	8.47 $\pm$ 0.35	126.02 $\pm$ 14.54	167.73 $\pm$ 6.34	130.02 $\pm$ 14.49	20.12 $\pm$ 1.15	14.73 $\pm$ 0.71
DN (mg N/L)	0.64 $\pm$ 0.001	0.39 $\pm$ 0.01	3.28 $\pm$ 0.29	1.28 $\pm$ 0.04	0.36 $\pm$ 0.02	0.23 $\pm$ 0.001
DP (µg P/L)	5.51 $\pm$ 0.02	7.14 $\pm$ 0.14	60.08 $\pm$ 12.79	19.34 $\pm$ 1.44	6.72 $\pm$ 1.56	3.56 $\pm$ 0.21

**Table S7.** Environmental conditions of lake water samples in mesocosms with dose of 35 mgFe/L at T48 (Mean  $\pm$  standard deviation).

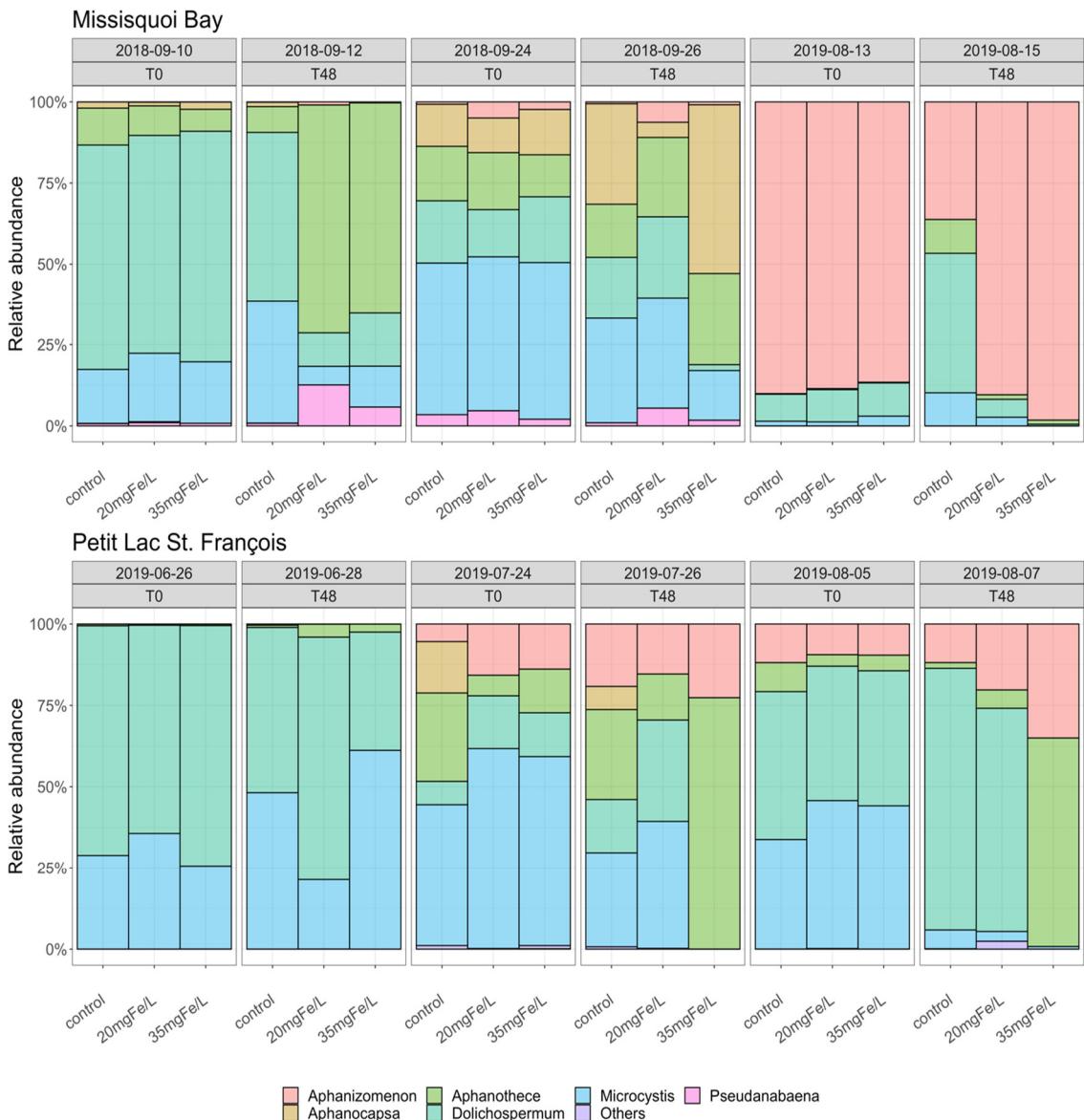
Parameters	Missisquoi Bay			Petit-Lac-St-François		
	Event A September 12 2018	Event B September 26 2018	Event C August 15 2019	Event a June 28 2019	Event b July 26 2019	Event c August 07 2019
Chlorophyll-a (RFU)	-	-	1.05 $\pm$ 0.002	0.01 $\pm$ 0.00	0.09 $\pm$ 0.007	0.01 $\pm$ 0.00
Phycocyanin (RFU)	-	-	29.69 $\pm$ 1.13	0.05 $\pm$ 0.00	0.11 $\pm$ 0.00	0.015 $\pm$ 0.007
pH	3.95 $\pm$ 0.08	-	4.01 $\pm$ 0.13	4.47 $\pm$ 0.15	4.1 $\pm$ 0.02	4.01 $\pm$ 0.05
TDS (mg/L)	198 $\pm$ 0.00	-	221.5 $\pm$ 9.81	285.01 $\pm$ 7.12	228.00 $\pm$ 0.00	296.50 $\pm$ 2.12
Temp (°C)	21.05 $\pm$ 0.01	-	22.83 $\pm$ 0.12	27.73 $\pm$ 0.33	25.26 $\pm$ 0.03	24.69 $\pm$ 0.33
TOC (mg C/L)	4.01 $\pm$ 0.06	2.18 $\pm$ 0.09	22.02 $\pm$ 0.41	5.68 $\pm$ 0.34	2.27 $\pm$ 0.31	1.68 $\pm$ 0.01
DOC (mg C/L)	3.71 $\pm$ 0.01	2.41 $\pm$ 0.03	21.07 $\pm$ 0.91	7.31 $\pm$ 0.10	1.87 $\pm$ 0.21	1.87 $\pm$ 0.10
TN (mg N/L)	0.67 $\pm$ 0.01	1.07 $\pm$ 0.02	4.06 $\pm$ 0.15	0.95 $\pm$ 0.01	0.28 $\pm$ 0.007	0.29 $\pm$ 0.007
TP (µg P/L)	8.83 $\pm$ 0.56	109.97 $\pm$ 23.21	146.44 $\pm$ 7.81	29.29 $\pm$ 0.73	6.58 $\pm$ 1.71	4.86 $\pm$ 0.82
DN (mg N/L)	0.63 $\pm$ 0.001	0.41 $\pm$ 0.007	2.93 $\pm$ 0.08	0.72 $\pm$ 0.04	0.25 $\pm$ 0.001	0.27 $\pm$ 0.00
DP (µg P/L)	7.11 $\pm$ 0.04	6.92 $\pm$ 0.04	64.71 $\pm$ 4.23	17.99 $\pm$ 2.02	4.49 $\pm$ 0.11	4.01 $\pm$ 0.19

**Table S8.** Pairwise Kruskal-Wallis test, showing differences in changing of the species richness and Shannon indices at genus level between control mesocosms and mesocosms with dose of 20 mgFe/L, control mesocosms and mesocosms with dose of 35 mgFe/L, mesocosms with dose of 20 mgFe/L and 35 mgFe/L after 48 hours in Missisquoi Bay and Petit Lac St. François (p-value< 0.05).

		<i>df</i>	<i>chi-squared</i>	<i>p</i> -value
Missisquoi Bay	<i>Richness</i>	1	1.218	0.264
	<i>Shannon</i>	1	0.561	0.452
Petit Lac St. François	<i>Richness</i>	1	1.334	0.248
	<i>Shannon</i>	1	0.044	0.833

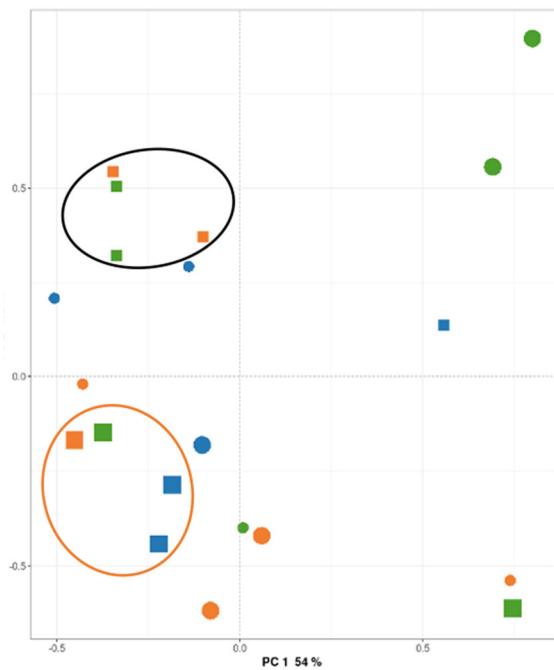


**Figure S1.** Cyanobacterial relative abundance at genus level in the control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay in August 08 (T0) and August 10 (T48), 2018.

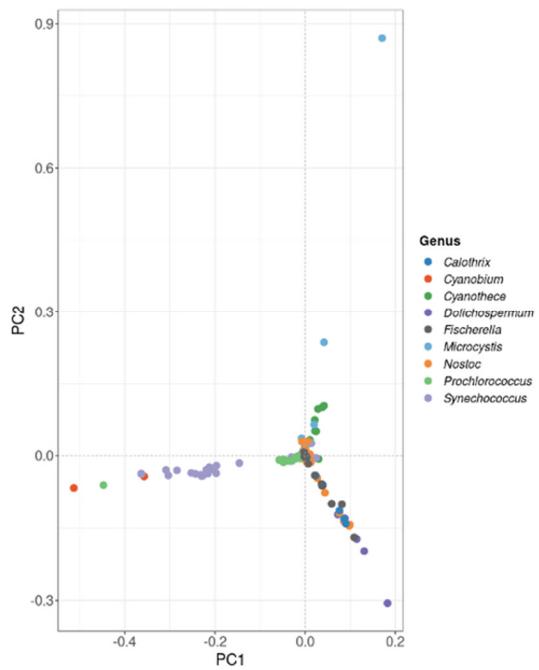


**Figure S2.** Cyanobacterial relative abundance at genus level in the control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay and Petit Lac St. François. Adapted from [1].

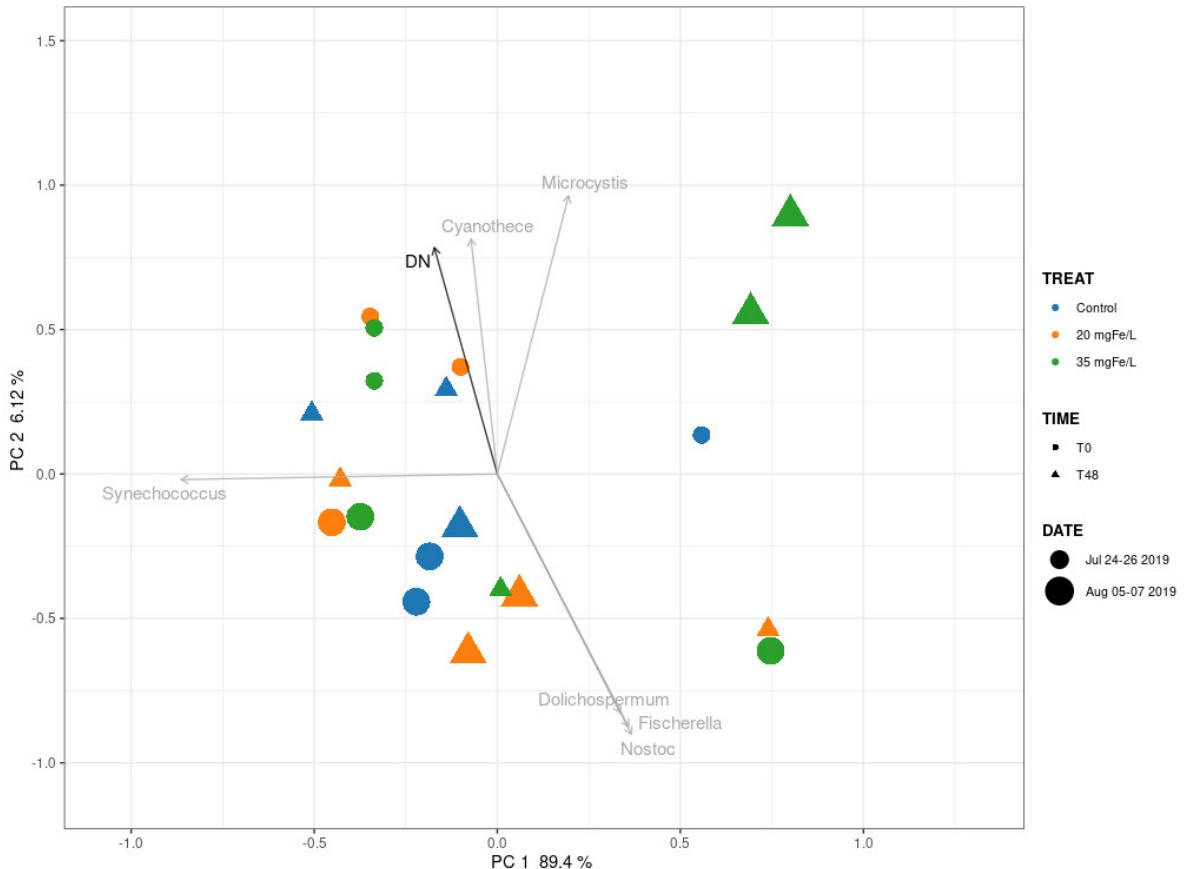
A)



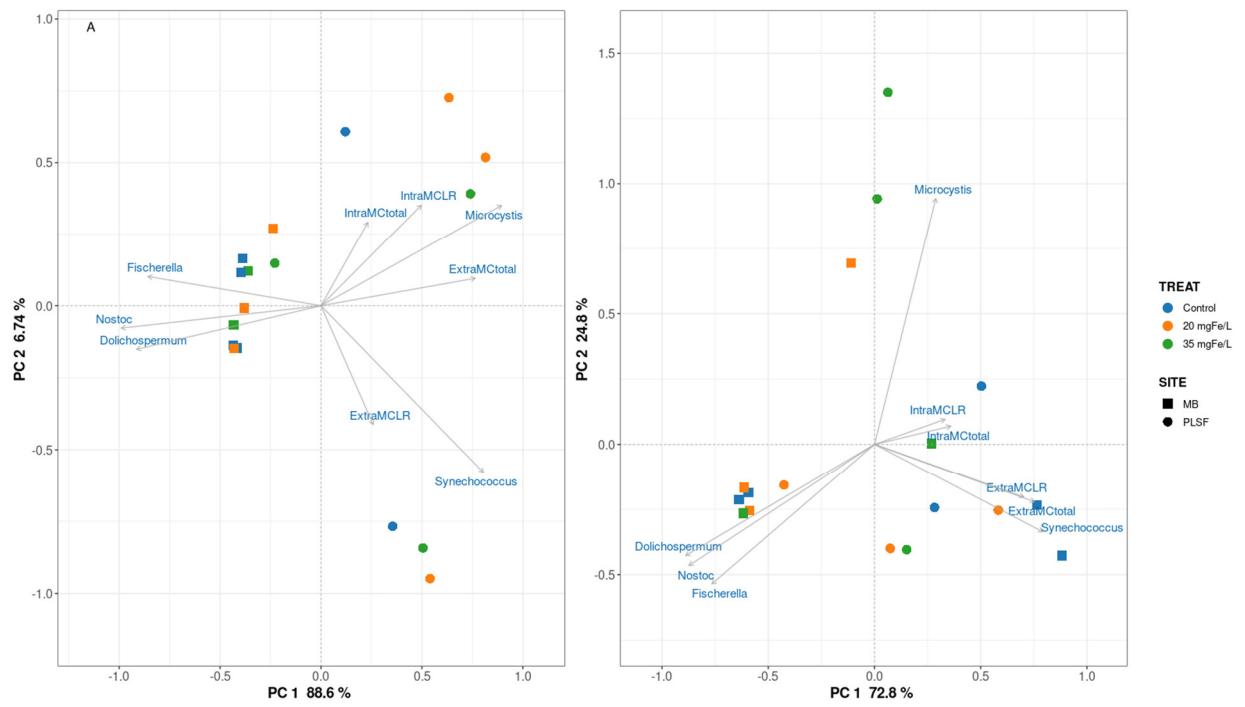
B)



**Figure S3.** Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to genus abundance in Petit Lac St. François. (a) PCA analysis of cyanobacterial community following coagulation; (b) Data are plotted following the genus-level classification.



**Figure S4.** Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to genus abundance in Petit Lac St. François (PLSF). (a) PCA analysis of cyanobacterial community following coagulation; (b) Data are plotted following the genus-level classification.



**Figure S5.** Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to intra- and extracellular total microcystins, MC-LR in Missisquoi Bay and Petit Lac St. François at T0 (left panel) and T48 (right panel).

## References

- Le, K.T.; Goitom, E.; Trigui, H.; Sauvé, S.; Prévost, M.; Dorner, S. The Effects of Ferric Sulfate ( $\text{Fe}_2(\text{SO}_4)_3$ ) on the Removal of Cyanobacteria and Cyanotoxins: A Mesocosm Experiment. *Toxins* **2021**, *13*, 753. <https://doi.org/10.3390/toxins13110753>.