Supplemental Information

Phytoplankton Responses to Nitrogen Enrichment in Pacific Northwest Mountain Lakes
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Section 1 – Phytoplankton Community Responses to Nutrient Addition

Methods

Phytoplankton community changes in response to N and P enrichment were visualized with nonmetric multidimensional scaling (NMDS) plots and tested for statistical significance using permutation multivariate analysis of variance (PERMANOVA) (Anderson, 2001a, 2001b). NMDS and PERMANOVA analyses were applied to the control, N-only, P-only, and N+P treatments. The metaMDS function from the Vegan package in R was used to construct a similarity matrix of pairwise Bray-Curtis distances after $\log10(x+1)$ transformation of taxa cell densities. Data were then visualized using 2-dimensional NMS. NMDS plots were constructed for each lake, and again after combining data across lakes. Significance of phytoplankton community changes were tested statistically using PERMANOVA (Anderson, 2001a, 2001b). Within each lake, a two-way PERMANOVA was used to test for main effects of N and P, and NxP interactions. A nested two-way PERMANOVA was also applied to limitation treatment data from all lakes with lake as the nesting factor to test for significant effects of N and P addition across lakes. PERMANOVA analyses used 999 permutations and were performed on Bray-Curtis similarity matrices constructed after $\log10(x+1)$ transformation of cell densities, were applied at the taxa level (the level at which taxa were identified and enumerated), and were performed using Adonis from the vegan package (version 2.2-1) in R (Oksanen et al., 2015).

Results

NMDS plots developed using control, N-only, P-only, and N+P treatments (Figure S1) qualitatively indicated changes in phytoplankton community structure in all lakes except Copper Lake (NOCA). Plots indicate community composition changes due to N+P addition, and distinct effects of N and N+P addition (Figure S1). NMDS plots suggest community structure was generally similar in control and P-only treatments, which is consistent with patterns in Figure S2. Qualitative patterns in NMDS plots were consistent with PERMANOVA results. In two-way PERMANOVA analyses applied to each lake, there were significant (< 0.05) N and P main effects and NxP interactions in all lakes except Copper (NOCA) and Heather (OLYM). In Copper Lake, there were no significant main effects of interactions. In Heather Lake, there was a significant main effect of N, but no significant effect of P or NxP interaction. There was also a significant main effect and interaction in a nested two-way PERMANOVA applied to data from all lakes with lake as the nesting factor. Clustering of lakes in NMDS plots indicated phytoplankton community differences between lakes were larger than differences in phytoplankton community composition within lakes across treatments in some cases (Figure S1).
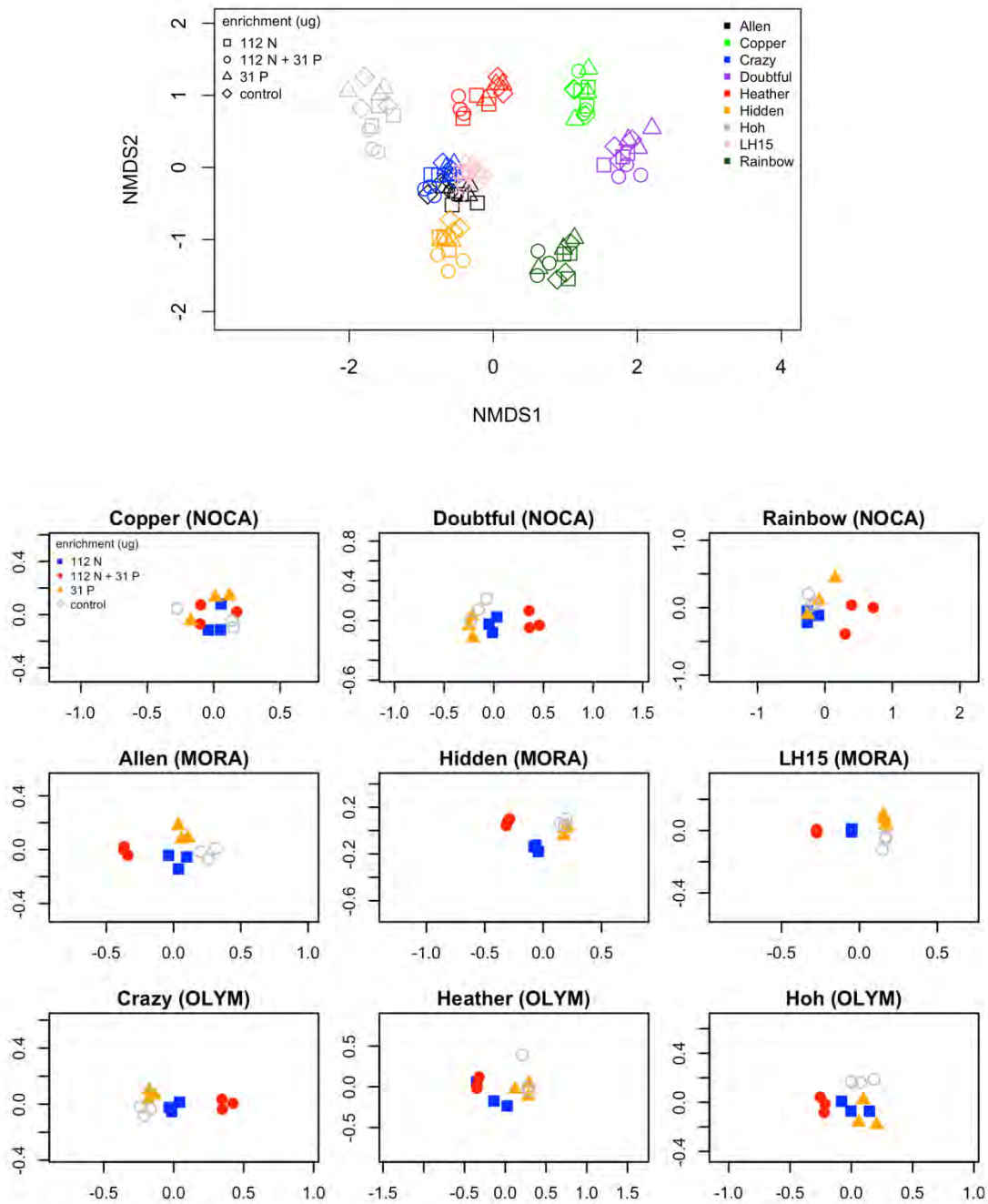
Figure S1. Nonmetric multidimensional scaling (NMDS) of phytoplankton community responses in control, N only, P only, and N+P treatments when for lakes plotted together (top) and for individual lakes (bottom). Distances between shapes reflect community similarity, with greater distances indicating larger differences.
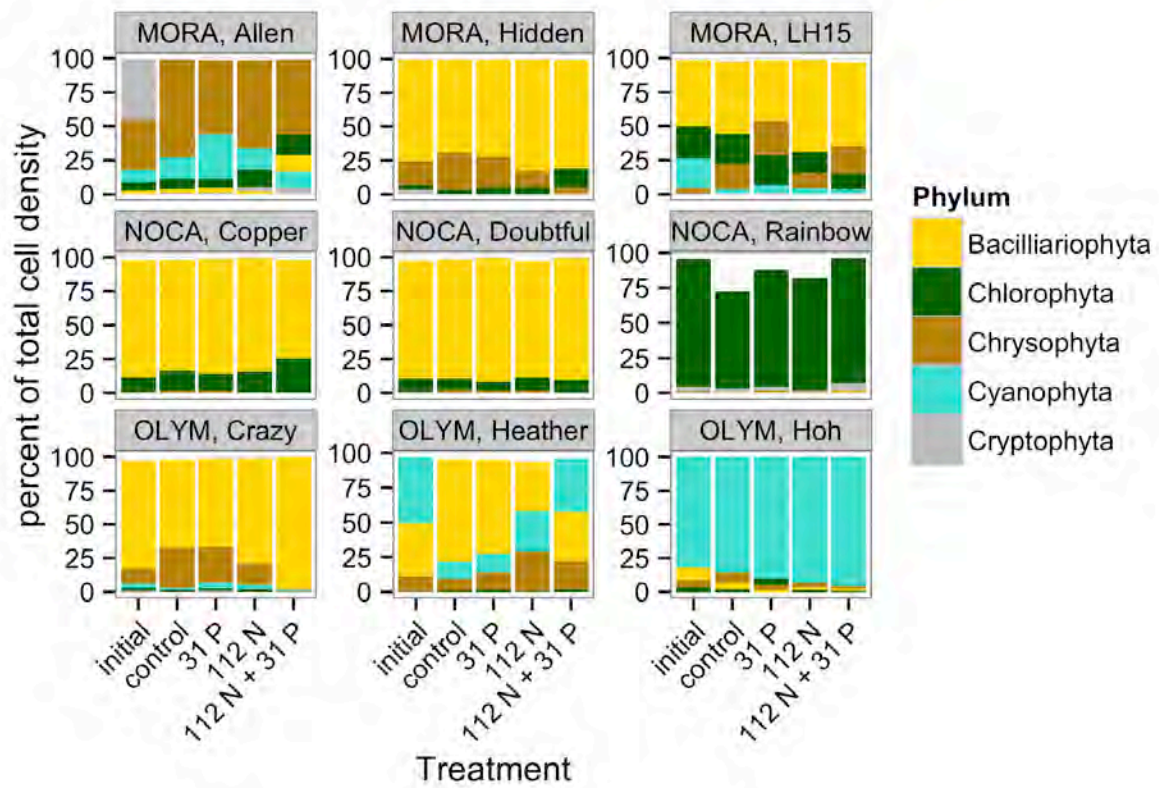
Figure S2. Relative proportions of common phytoplankton groups in each lake.

Section 2 – Taxa Responses to Nutrient Enrichment

Table S1. Relative responses (RR), nutrient limitation classification, and Monod parameters for abundant taxa (> 1% lake total cell density). Monod parameters are provided if taxa growth was N-limited or co-limited and a Monod curve could be constructed.

Lake	Phylum[a]	Taxa	RR-encl[b]	RR-N[b]	RR-P[b]	RR-NP[b]	Limitation Type[c]	μ_{max} (d^{-1}) ± SE	K_s (µg N L^{-1}) ± SE
Copper	BAC	*Aulacoseira alpigena*	0.8	0.8	1.0	1.0	none		
Crazy	BAC	*Aulacoseira alpigena*	1.6	2.0	0.8	2.0	none		
Heather	BAC	*Aulacoseira alpigena*	0.8	0.6	1.0	1.0	none		
LH15	BAC	*Aulacoseira alpigena*	4.1	4.2	1.6	4.8	N	0.49 ± 0.05*	27.17 ± 6.64 *
Heather	BAC	*Aulacoseira nivalis*	1.0	1.0	1.0	1.9	none		
Heather	BAC	*Aulacoseira pusilla*	0.3	2.3	1.1	6.3	S. co-lim.	-0.07 ± 0.02*	-1.82 ± 0.53*
Hoh	BAC	*Aulacoseira pusilla* < 5 µm	0.6	0.7	0.3	0.3	constrained		
Allen	BAC	*Aulacoseira pusilla* 6 µm	1.2	1.1	1.4	2.6	S. co-lim.	0.12 ± 0.02*	3.90 ± 3.31
Crazy	BAC	*Aulacoseira pusilla* 6 µm	0.9	3.1	0.6	128	serial N		
LH15	BAC	*Aulacoseira pusilla* 6 µm	1.4	4.0	1.2	7.5	serial N		
Crazy	BAC	*Cyclotella* < 7 µm cf *distinguenda*	0.8	10.0	0.7	186	serial N		
Doubtful	BAC	*Cyclotella* < 7 µm cf *distinguenda*	1.0	1.2	1.0	8.6	S. co-lim.		
LH15	BAC	*Cyclotella* < 7 µm cf *distinguenda*	0.9	3.3	0.7	6.0	serial N		
Heather	BAC	*Discostella glomerata*	0.9	6.7	1.4	14.8	N		
Crazy	BAC	*Discostella glomerata* 7-8 µm	0.9	3.8	2.0	3.1	N	0.06 ± 0.03	9.46 ± 5.76
LH15	BAC	*Discostella glomerata* 7-8 µm	2.1	10.1	1.8	22.8	I. co-lim.	3.73 ± 3.22	782 ± 761
Doubtful	BAC	*Discostella stelligera*	1.2	2.0	1.1	11.9	serial N		
Crazy	BAC	*Fragilaria capucina*	28.4	1.0	0.6	10.0	S. co-lim.	0.51 ± 0.01*	1.50 ± 0.21*
Hidden	BAC	*Fragilaria capucina var. parminuta*	0.6	2.9	1.3	2.6	N	0.01 ± 0.01	-0.11 ± 0.04*
Allen	BAC	*Fragilaria crotonensis*	2.0	1.0	2.0	18.2	S. co-lim.	0.63 ± 0.10*	32.1 ± 13.5*
Crazy	BAC	*Fragilaria crotonensis*	2.3	3.2	1.0	12.0	serial N	0.30 ± 0.01*	6.25 ± 0.83*
Hidden	BAC	*Fragilaria crotonensis*	1.0	1.2	0.6	4.6	S. co-lim.		
Crazy	BAC	*Fragilaria crotonensis* type 2	1.6	1.0	3.5	74.0	serial P		
Crazy	BAC	*Fragilaria tenera*	1.3	3.7	0.8	19.0	serial N	0.34 ± 0.02*	24.7 ± 4.76*
Hidden	BAC	*Fragilaria tenera*	0.5	2.1	0.7	4.7	serial N		
Hoh	BAC	*Fragilaria tenera*	3.6	1.1	0.7	6.3	S. co-lim.	0.42 ± 0.04*	14.5 ± 3.61*
Hidden	BAC	*Fragilaria* undiff 25-30 µm	1.3	4.5	0.4	2.4	none		
Heather	BAC	Small *Fragilaria* undiff.	0.8	3.2	1.6	2.5	none		

NOTE: * indicates a statistically significant Monod parameter (p < 0.05)

[a]: BAC = Bacilliarophyta, CHL = Chlorophyta, CHR = Chrysophyta, CRYP = Cryptophyta, CYA = Cyanophyta, PYR = Pyrrhophyta, UND = Undetermined

[b]: RR-encl = (control mean density / initial mean density); RR-N = (N-only treatment mean density / control mean density);); RR-P = (P-only treatment mean density / control mean density);); RR-NP = (N+P treatment mean density / control mean density)

[c]: 'S. co-lim.' = simultaneous co-limitation; 'I-co-lim.' = independent co-limitation

Table S1 (page 2). Relative responses (RR), nutrient limitation classification, and Monod parameters for abundant taxa (> 1% lake total cell density). Monod parameters are provided if taxa growth was N-limited or co-limited and a Monod curve could be constructed.

Lake	Phylum[a]	Taxa	RR-encl[b]	RR-N[b]	RR-P[b]	RR-NP[b]	Limitation Type[c]	μ_{max} (d^{-1}) ± SE	K_s (µg N L^{-1}) ± SE
Allen	CHL	*Ankistrodesmas sp.*	0.4	1.6	1.5	34.3	S. co-lim.	0.64 ± 0.54	77.6 ± 128
Copper	CHL	*Chlorophyceae sp.*	1.1	0.6	0.7	1.9	none		
Doubtful	CHL	*Chlorophyceae sp.*	0.7	1.8	1.0	2.9	S. co-lim.		
Allen	CHL	Coccoid green algae	17.4	2.7	1.0	3.7	N	0.51 ± 0.04*	0.05 ± 0.05
LH15	CHL	Coccoid green algae < 5 µm	1.4	2.3	1.3	3.2	N	0.28 ± 0.04*	9.95 ± 5.87
Copper	CHL	*Gloeomonas sp.*	1.0	1.3	0.8	1.2	none		
Hidden	CHL	*Gloeomonas sp.*	0.7	1.8	1.3	0.4	none		
Rainbow	CHL	*Gloeomonas sp.*	0.6	1.1	1.2	1.1	none		
Rainbow	CHL	*Gonium* colony	1.0	2.1	1.3	151	S. co-lim.		
Hidden	CHL	*Gonium sp.*	0.1	29.3	2.3	326	serial N	0.45 ± 0.15*	10.2 ± 12.4
Heather	CHL	Nonmotile unicellular green algae sp.	0.9	1.4	1.3	5.6	S. co-lim.	0.21 ± 0.04*	13.49 ± 6.62
Rainbow	CHL	*Oocystis sp.*	0.5	1.3	0.4	0.4	none		
Allen	CHL	*Schroederia sp.*	1.1	0.9	0.9	3.4	S. co-lim.	0.17 ± 0.06*	16.4 ± 18.8
LH15	CHL	*Schroederia sp.*	2.3	2.7	0.9	3.9	N	0.40 ± 0.06*	56.9 ± 17.4*
Doubtful	CHL	*Tetraspora sp.*	0.9	2.4	1.1	105	S. co-lim.		
LH15	CHL	*Tetraspora sp.*	0.8	1.6	0.3	2.7	S. co-lim.	0.02 ± 0.07*	0.57 ± 16.5
Rainbow	CHL	*Tetraspora sp.*	1.0	1.8	0.3	0.5	none		
Allen	CHR	*Chrysolepidomonas sp.*	3.9	2.1	2.1	1.8	I. co-lim.	0.25 ± 0.02*	0.02 ± 0.04
Crazy	CHR	*Chrysolepidomonas sp.*	5.0	0.9	0.7	0.2	constrained		
Hidden	CHR	*Chrysolepidomonas sp.*	15.4	0.8	1.0	0.8	none		
LH15	CHR	*Chrysolepidomonas sp.*	36.1	15	1.6	8.7	S. co-lim.	0.82 ± 0.12*	27.4 ± 9.95*
Heather	CHR	*Mallomonas sp.*	0.4	2.6	1.3	0.9	none		
Allen	CHR	Small golden w/o flagella	1.8	1.2	0.5	1.0	constrained		
Crazy	CHR	Small golden w/o flagella	4.3	1.6	1.0	0.4	serial N	-0.05 ± 0.04	-0.09 ± 2.97
Heather	CHR	Unknown unicellular Chrysophyta 5-7 µm	0.3	8.2	2.1	13.5	N		
Hidden	CHR	Small golden w/o flagella	0.4	0.9	0.3	0.0	constrained		
LH15	CHR	Unknown unicellular Chrysophyta 5-7 µm	1.7	2.9	1.7	1.5	N	0.15 ± 0.04*	36.7 ± 24.0
Allen	CHR	Unknown unicellular Chrysophyta 10 µm	6.5	1.8	1.5	4.9	serial N	0.40 ± 0.03*	0.06 ± 0.05*
Hoh	CHR	*Synura* colony	8.2	1.1	0.7	1.9	S. co-lim.		

NOTE: * indicates a statistically significant Monod parameter (p < 0.05)

[a]: BAC = Bacilliarophyta, CHL = Chlorophyta, CHR = Chrysophyta, CRYP = Cryptophyta, CYA = Cyanophyta, PYR = Pyrrhophyta, UND = Undetermined

[b]: RR-encl = (control mean density / initial mean density); RR-N = (N-only treatment mean density / control mean density);); RR-P = (P-only treatment mean density / control mean density);); RR-NP = (N+P treatment mean density / control mean density)

[c]: 'S. co-lim.' = simultaneous co-limitation; 'I-co-lim.' = independent co-limitation

Table S1 (page 3). Relative responses (RR), nutrient limitation classification, and Monod parameters for abundant taxa (> 1% lake total cell density). Monod parameters are provided if taxa growth was N-limited or co-limited and a Monod curve could be constructed.

Lake	Phylum[a]	Taxa	RR-encl[b]	RR-N[b]	RR-P[b]	RR-NP[b]	Limitation Type[c]	μ_{max} (d^{-1}) ± SE	K_s (µg N L^{-1}) ± SE
Allen	CRYP	Cryptomonad 15-30 µm	0.5	1.8	0.6	9.0	S. co-lim.		
Rainbow	CRYP	Cryptomonad 7-15 µm	0.9	0.2	1.0	2.0	none		
Allen	CRYP	*Rhodomonas sp.*	0.0	6.1	1.1	4.6	N	-0.40 ± 0.06*	-0.40 ± 0.04*
Allen	CYA	Coccoid cyanobacteria	0.4	5.3	2.3	14.0	S. co-lim.	0.26 ± 0.10	18.3 ± 22.7
LH15	CYA	Cyanotetras	0.7	1.1	1.3	2.0	none		
Allen	CYA	*Microcystis* colony	2.9	1.4	2.5	1.2	P		
Crazy	CYA	*Microcystis* colony	0.8	6.4	1.4	2.9	N	0.41 ± 0.31	129 ± 162
Heather	CYA	*Microcystis* colony	0.0	20	8.6	28	I. co-lim.	-0.10 ± 0.03*	-14.5 ± 1.76*
Heather	CYA	*Synechocystis sp.*	0.1	5.2	1.1	13.8	serial N		
Hoh	CYA	*Synechocystis sp.*	1.6	2.4	1.5	9.0	serial N	0.38 ± 0.03*	14.13 ± 2.51*
Heather	PYR	*Peridinium* sp.	1.2	2.5	1.2	2.5	none		
Rainbow	UND	Chrysolepidomonas/Chlorophyceae	29.4	0.6	0.2	0.1	constrained		
Heather	UND	Large yellow balls w/or flagella	0.4	2.6	1.2	3.2	S. co-lim.		
Allen	UND	Large yellow synurophyte	7.7	0.8	0.6	1.2	none		
Rainbow	UND	Round, ciliated 3-pack	0.1	2.2	11.9	3.1	none		

NOTE: * indicates a statistically significant Monod parameter (p < 0.05)

[a]: BAC = Bacilliarophyta, CHL = Chlorophyta, CHR = Chrysophyta, CRYP = Cryptophyta, CYA = Cyanophyta, PYR = Pyrrhophyta, UND = Undetermined

[b]: RR-encl = (control mean density / initial mean density); RR-N = (N-only treatment mean density / control mean density);); RR-P = (P-only treatment mean density / control mean density);); RR-NP = (N+P treatment mean density / control mean density)

[c]: 'S. co-lim.' = simultaneous co-limitation; 'I-co-lim.' = independent co-limitation

Section 3 – Monod Growth Curves for N-responsive Taxa. See manuscript Table 4 for corresponding Monod parameters.
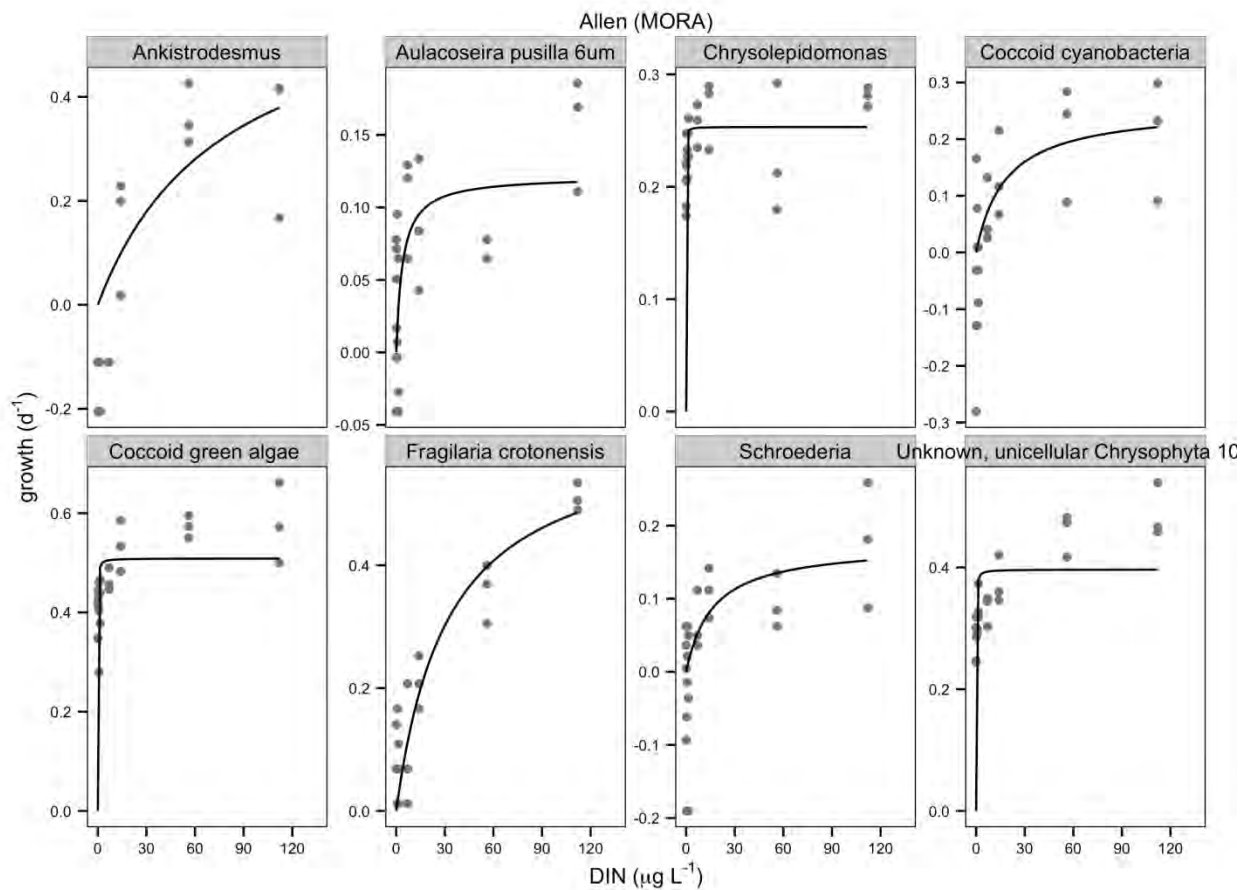

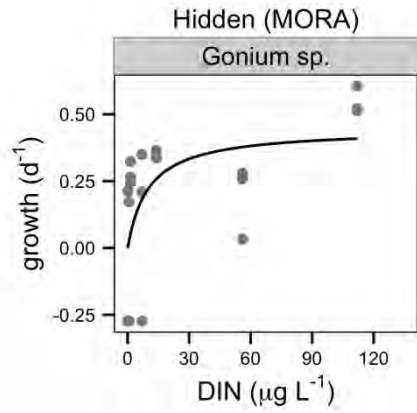**Figure S3.** Monod curves for N-responsive taxa in Lake Allen (MORA).

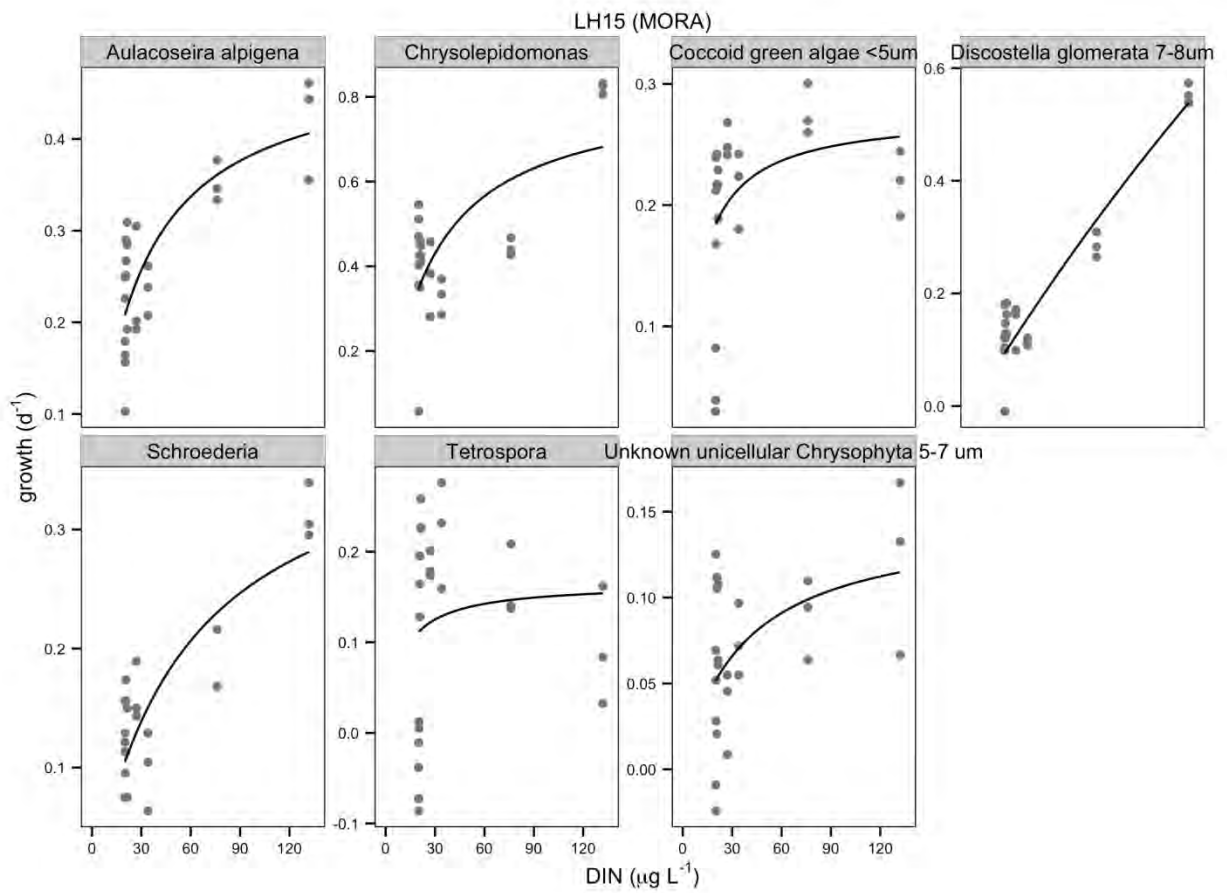**Figure S4.** Monod curve for N-responsive taxa in Hidden Lake (MORA).

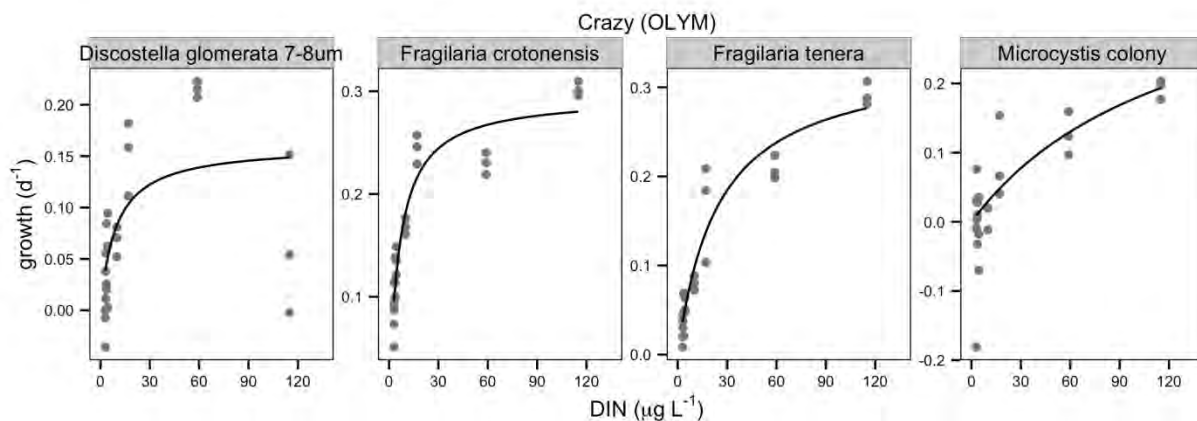**Figure S5.** Monod curves for N-responsive taxa in Lake LH15 (MORA).

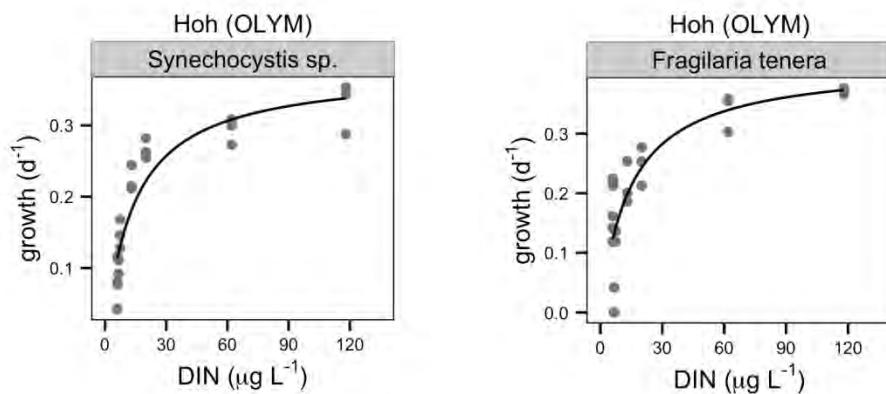**Figure S6.** Monod curves for N-responsive taxa in Crazy Lake (OLYM)

Figure S7. Monod curves for N-responsive taxa in Hoh Lake (OLYM).

Works Cited

Anderson, M. J., 2001a. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson, M. J., 2001b. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58: 626–639.

Oksanen, J., Blanchet, F.G., Kind, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens. M.H.H., Wagner, H. 2015. Vegan community ecology package version 2.2-1. https://cran.r-project.org/web/packages/vegan/index.html