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1 **Stoichiometric regulation of phytoplankton toxins**

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21 contributed substantially to revisions.

22 **Abstract**

23 Ecological Stoichiometry theory predicts that the production, elemental structure, and cellular
24 content of biomolecules should depend upon the relative availability of resources and the
25 elemental composition of their producer organism. We review the extent to which carbon- and
26 nitrogen-rich phytoplankton toxins are regulated by nutrient limitation and cellular
27 stoichiometry. Consistent with theory, we show that nitrogen limitation causes a reduction in the
28 cellular quota of nitrogen-rich toxins, while phosphorus limitation causes an increase in the most
29 nitrogen-rich paralytic shellfish poisoning toxin. In addition, we show that the cellular content of
30 nitrogen-rich toxins increases with increasing cellular N:P ratios. Also consistent with theory,
31 limitation by either nitrogen or phosphorus promotes the cellular quota, or toxicity, of the
32 investigated carbon-rich toxins. These observed relationships may assist in predicting and
33 managing toxin-producing phytoplankton blooms. Such a stoichiometric regulation of toxins is
34 likely not restricted to phytoplankton, and may well apply to carbon- and nitrogen-rich secondary
35 metabolites produced by bacteria, fungi, and plants.

36

37 *Key words:* Ecological Stoichiometry, harmful cyanobacteria, Harmful Algal Blooms, natural
38 toxins, nutrient limitation, secondary metabolites.

39

40

41 **Introduction**

42 It is well-established that the elemental composition of both prokaryotic and eukaryotic cells
43 strongly depends upon the supplies of chemical elements, such as the key nutrients nitrogen (N)
44 and phosphorus (P) (Sterner & Elser 2002; Klausmeier *et al.* 2004). Primary producers use light
45 energy to convert CO₂ and nutrients into an enormous variety of organic compounds, including
46 fatty acids, amino acids, and nucleic acids. These three key groups of biomolecules differ
47 significantly in their elemental composition, notably in their relative carbon (C), N, and P
48 contents. Similarly, there are major differences in the C:N:P stoichiometry of secondary cellular
49 metabolites, which are known to serve as signaling molecules as well as deterrents against
50 pathogens and herbivores (Keller & Surette 2006; Iason *et al.* 2013). Some of the secondary
51 metabolites produced by planktonic aquatic primary producers (unicellular and colonial
52 cyanobacteria and eukaryotic algae, collectively termed “phytoplankton” hereafter) are toxic to
53 invertebrates, fish, birds, and mammals. Blooms of such toxic phytoplankton are of increasing
54 global concern because their appearance and proliferation are driven by high anthropogenic
55 loadings of N and P to surface waters (eutrophication) (Anderson *et al.* 2002; Smith 2003). Thus,
56 there is a strong need to gain a better understanding of how the absolute and relative supplies of
57 key nutrients affect the production of toxic compounds by bloom-forming phytoplankton.

58 Toxins produced by phytoplankton are stoichiometrically diverse, ranging from N-rich
59 alkaloids to C-based polyketides (Table 1). Previous studies involving particular phytoplankton
60 taxa have demonstrated that the production and cellular contents (internal cell "quota") of C- and
61 N-rich toxins respond strongly to the relative availability of nutrients (Granéli *et al.* 1998;
62 Sivonen & Jones 1999; Granéli & Flynn 2006; Van de Waal *et al.* 2009). Here, we present the
63 first quantitative analysis of the regulation of phytoplankton toxins by nutrient limitation. We

64 analyzed >500 published studies and compiled a total of 180 datasets, covering 31 phytoplankton
65 species representing 19 different genera (Fig. S1; Table S1). Using these data, we analyzed the
66 toxin cell quotas or toxicities of phytoplankton cells grown under either N- or P-limitation, and
67 we compared these responses with cells grown in nutrient-sufficient controls. Based on
68 stoichiometric theory (Sterner & Elser 2002) and current understanding of phytoplankton
69 physiology, we predicted that production of C-rich toxic compounds would be elevated under
70 both N- and P-limitation because, under such conditions, cellular C is by definition in excess and
71 can accumulate in secondary pools. Following similar reasoning, we also predicted that
72 production of N-rich toxins would be inhibited under N-limitation (because N is shunted into
73 intracellular pools to support active growth), but amplified under P-limitation (because N is in
74 excess and accumulates in secondary intracellular pools).

75

76 **Material and Methods**

77 Data was obtained by ISI Web of Science searches with the terms: ("phytoplankton" or
78 "cyanobacteri*" or "dinoflagellate*") and "*toxin*" and ("nutrient*" or "nitr*" or "phosph*")
79 (Fig. S1; Tables S1 and S2). Estimates of toxin quota or toxicity of phytoplankton grown under
80 low N and/or low P were recovered from the stationary growth phase of batch culture
81 experiments, and from steady state conditions of continuous cultures. Toxin quotas or toxicities
82 of cells grown under nutrient-sufficient conditions, or at maximum growth rates during the
83 exponential phase, served as controls. Studies reporting earlier published data were not included.
84 We made a distinction between N-rich and C-rich toxins based on elemental composition. Toxins
85 with a molar C:N ratio <6.6 (i.e. less than the Redfield ratio) were considered "N-rich", whereas
86 toxins with a higher molar C:N ratio were considered "C-rich" (Table 1). For each study we took

87 toxin quotas or toxicities and calculated the average response ratio (RR) by normalizing toxin
88 quotas or toxicities from the low N and low P conditions to the control. Furthermore, we derived
89 cellular N:P ratios expressed as molar ratios.

90 Toxins were found to be represented by varying numbers of studies (Fig. S1). Some
91 toxins were produced by multiple species, and these toxins were represented by different
92 numbers of species (Fig. S1). To correct for biases that may result from such unbalanced and
93 nested data structure, we worked with weighted means and variances. First, we calculated the
94 average response and variance for each toxin that was represented by at least three or more
95 independent studies (i.e. $n>3$). In case a toxin was produced by multiple species, and one species
96 was represented by more than two studies, we first calculated the mean of this species and then
97 calculated the average response of the toxin across species. In case multiple species were
98 represented by multiple studies, the weighted average response of a toxin across species could be
99 calculated according to (Borenstein *et al.* 2009):

$$100 \quad M_{RR,W} = \frac{\sum_{i=1}^n M_{RR} W_i}{\sum_{i=1}^n W_i} \quad (1)$$

101 with weight W_i being the reciprocal of the variance $V_{tox, sp}$, i.e. the variance among studies for
102 each species i , calculated as:

$$103 \quad V_{tox,sp} = \frac{1}{n-1} \sum_{i=1}^n (RR_i - M_{RR})^2 \quad (2)$$

104 Similarly, the overall weighted average response of toxins across the means of all N- or C-rich
105 toxins was calculated. We assessed the statistical significance of differences between the
106 calculated RRs and their respective controls with a one sample t-test comparing the weighted
107 mean RR with 1, which represents the absence of a response ($\alpha<0.05$).

108

109 **Results**

110 The assembled studies yielded 100 datasets with N-rich toxins (49 with N-limitation and 51 with
111 P-limitation) and 80 datasets with C-rich toxins (37 with N-limitation and 43 with P-limitation;
112 Fig. S1). N-rich toxins predominantly consisted of Paralytic Shellfish Poisoning (PSP) toxins
113 produced by marine dinoflagellates ($n=57$), followed by microcystins (MCs) produced by
114 freshwater cyanobacteria ($n=34$). Data on C-rich toxins comprised 12 compounds, groups of
115 compounds, or toxicities associated to C-rich toxins. The C-rich toxins were almost exclusively
116 found in marine ecosystems and consisted of various toxins produced by dinoflagellates
117 (including Diarrhetic Shellfish Poisoning (DSP) toxins, $n=18$, karlotoxins (KMTX), $n=11$, and
118 Neurotoxic Shellfish Poisoning (NSP) toxins, $n=10$), diatoms (Amnesic Shellfish Poisoning
119 (ASP) toxins, $n=9$), and prymnesiophytes (hemolytic activity (HA), $n=12$). Various toxins were
120 only represented by a limited number of studies ($n<3$) and were therefore excluded from our
121 analyses. For most of the toxins, experiments with N-limitation and P-limitation tended to be
122 equally well-represented (Fig. S1).

123 The impact of nutrient limitation on toxin quota or toxicity varied greatly and depended
124 strongly on the stoichiometric nature of the toxins. Consistent with theoretical predictions, our
125 analysis revealed that N-rich toxins generally decreased upon N-limitation while C-rich toxins
126 generally increased upon limitation by either N or P (Figs. 1 and 2). More specifically, cellular
127 quota of both N-rich PSP toxins and MCs decreased significantly upon N-limitation, irrespective
128 of the producing taxonomic group. Upon P-limitation, cellular quota of dinoflagellate PSP toxins
129 increased by 32%, while MCs decreased by 75% (Fig. 1; Table 2). Relative changes in C-rich
130 toxins were generally larger than the N-rich toxins, particularly under P-limitation, although
131 variances within toxins were also higher. Significant increases in cellular toxin quota ranged

132 from 99% in NSP toxins under N-limitation to 215% in KMTX under P-limitation (Fig. 2; Table
133 2).

134 Under balanced, non-limiting growth conditions, molar phytoplankton N:P ratios
135 typically converge upon the so-called ‘Redfield ratio’ of N:P=16 (Sterner & Elser 2002). Indeed,
136 in the control treatments of the experiments we assembled, cellular N:P ratios resembled the
137 Redfield ratio, whereas cellular N:P stoichiometry was almost always lower than Redfield under
138 N-limitation and higher than Redfield under P-limitation (Fig. 3). N-rich toxins showed a
139 positive linear relationship with cellular N:P ratios. The response ratios of the N-rich PSP toxins
140 and MCs decreased below 1 at N:P ratios below the Redfield ratio, but increased beyond 1 when
141 N:P ratios exceeded the Redfield ratio (Fig. 3a). In contrast, the C-rich DSP toxins and HA
142 increased at N:P ratios both below and above the Redfield ratio (Fig. 3b).

143

144 **Discussion**

145 Our results demonstrate that the synthesis of N-rich and C-rich toxins is regulated by the relative
146 availabilities of N and P (Figs. 1 and 2). Upon N-limitation, cellular quota of the N-rich PSP
147 toxins and microcystins decreased, while upon P-limitation, cellular quota of the most N-rich
148 PSP toxins increased. Cellular quota of microcystin, however, showed a strong decrease, and
149 nodularin did not change upon P-limitation (Fig. 1). These contrasting responses to P-limitation
150 may reflect the fact that, unlike eukaryotic algae, cyanobacteria can shunt excess N into the
151 storage compound cyanophycin (Allen 1984), a process that likely prevents N from
152 accumulating primarily as microcystin. C-rich toxins generally increased upon limitation by both
153 N and P (Fig. 2). Most of the C-rich toxins were produced by eukaryotic algae and are
154 polyketides, whose synthesis is tightly coupled with cellular C acquisition (Staunton &

155 Weissman 2001). Thus, under conditions of nutrient limitation, the relative excess of energy and
156 newly-synthesized organic C cannot be used for cell growth and instead appears to be shunted
157 into C-rich molecules such as lipids and polyketides, including those that have toxic effects. We
158 also observed a general increase of N-rich toxins with cellular N:P ratio (Fig. 3a), while C-rich
159 toxins showed an initial decrease with N:P ratios until reaching the Redfield ratio, after which
160 their content increased (Fig. 4b). These findings are consistent with the ‘V-shaped’ response to
161 N:P ratios that has been reported previously for hemolytic activity associated with C-rich
162 compounds (Granéli & Flynn 2006; Granéli *et al.* 2012).

163 While most of the selected datasets reported cellular toxin quotas, a few (~6%) assessed
164 toxicities relative to a standard toxin analogue, for instance as saxitoxin equivalents (Table S1).
165 Changes in these relative toxicities are generally associated with changes in cellular toxin quota,
166 yet may also relate to a shift in the toxin composition (Cembella 1998; Sivonen & Jones 1999).
167 Because the few selected studies reporting relative toxicities generally showed none, or only
168 minor shifts in toxin composition, we considered the observed changes in relative toxicities to
169 mainly reflect changes in the N-rich toxin quota. All reported hemolytic activities of
170 prymnesiophytes were assessed by bioassays and have been ascribed to various C-rich
171 compounds, such as glycolipids and the polyether prymnesin (Edwardsen & Imai 2006; Granéli
172 *et al.* 2012). Although hemolytic activity showed a high average response to nutrient limitation,
173 the variance was also very high (Table 2), which may reflect a higher inherent variability of
174 bioassay data relative to direct chemical measurements of the cellular quota of a specific
175 compound. Nevertheless, our analysis reveals that the response of hemolytic activity by
176 prymnesiophytes towards both N- and P-limitation is consistent with the general response of C-
177 rich toxins, and therefore presumably reflects changes in cellular quotas of a C-rich compound.

178 Toxins produced by various harmful phytoplankton taxonomic groups have been shown
179 to exhibit a broad range of potential functions, including grazer deterrence, prey capture, and
180 resource uptake (see also Granéli & Turner 2006; Glibert & Burkholder 2011). The question
181 remains whether our results reflect a possible role of toxins in acquiring N and P, or if the toxin
182 quota merely responds to the relative availability of N and C within the cells. Production of N-
183 rich toxins generally decreased with N-limitation, suggesting a cost rather than a benefit under
184 N-limitation. Cellular contents of N-rich cyanobacterial toxins strongly decreased or remained
185 unaltered upon P-limitation, whereas the quota of N-rich dinoflagellate PSP toxins increased. C-
186 rich toxins in dinoflagellates and diatoms, as well as hemolytic activity caused by
187 prymnesiophytes, also increased with P-limitation, and the dinoflagellate karlotoxin and NSP
188 toxins increased significantly with N-limitation. These findings suggest that toxin synthesis may
189 be related to resource acquisition, specifically C-rich toxins for acquiring P. Indeed, C-rich
190 toxins produced by prymnesiophytes seem to be involved in prey capture (Skovgaard *et al.* 2003;
191 Tillmann 2003). Furthermore, nutrient limitation may stimulate feeding by mixotrophic
192 dinoflagellates and prymnesiophytes (Legrand *et al.* 2001; Smalley *et al.* 2003), though it is not
193 clear whether this is related to their toxicity (Stoecker *et al.* 2006). Thus, whether toxins
194 stimulated by nutrient limitation are indeed involved in resource acquisition and prey capture, or
195 whether they merely respond to a relative excess of non-limiting resources required for their
196 synthesis, needs to be further elucidated.

197 Our findings illustrate that the extent to which phytoplankton are able to produce toxins,
198 and thereby exploit their putative function, will partially depend on the relative availabilities of
199 N and P in the environment. Because N and P often limit phytoplankton growth in both
200 freshwater and marine ecosystems (Elser *et al.* 2007), the cellular production of C-rich toxins

201 avoids resource allocation conflicts between population growth and secondary metabolite
202 synthesis. Production of N-rich toxins, however, does decrease with N-limitation, suggesting that
203 N is allocated to growth rather than secondary metabolites. Growth and the synthesis of
204 biochemicals together will determine the elemental stoichiometry of phytoplankton, which
205 shows distinct differences between taxonomic groups. For instance, dinoflagellates exhibit
206 higher C:N and C:P ratios as compared to prymnesiophytes and diatoms (Quigg *et al.* 2003),
207 while cyanobacteria have generally higher optimal N:P ratios than diatoms (Hillebrand *et al.*
208 2013). Most of the C-rich toxins studied here were indeed produced by dinoflagellates (i.e. 10
209 out of 12 toxins; Fig. S1), and the reported N-rich toxins were produced by cyanobacteria (i.e.
210 MC, PSP, NOD and CYN) or seem to have a common origin with cyanobacteria (i.e. PSP;
211 Moustafa *et al.* 2009; Hackett *et al.* 2013). We did not observe differences in the elemental
212 composition between taxonomic groups, yet our results demonstrate that the cellular quota of the
213 N-rich PSP toxins and microcystins are strongly correlated with N:P stoichiometry, and this
214 relationship is irrespective of the producing taxonomic group, both at the genus as well as at the
215 species level (Fig. 3). To what extent the stoichiometry of a taxonomic group may influence the
216 stoichiometry of the toxins that can be produced remains to be elucidated. Obviously, cellular
217 elemental ratios strongly reflect the intracellular content of major biochemicals such as proteins
218 and nucleic acids (Sterner & Elser 2002). Here, we show that they may also determine, at least
219 partially, the stoichiometry of secondary metabolites that can be synthesized by the cell.

220 The strong association of phytoplankton toxins with N:P stoichiometry has important
221 implications for interpreting and predicting the responses of phytoplankton-associated toxins to
222 human-induced changes in the magnitude and ratios of N and P supplies to surface waters. One
223 of the clearest and most predictable responses of both freshwater and marine ecosystems to

224 increased nutrient loading is the profound stimulation of phytoplankton biomass, often by orders
225 of magnitude (Smith 2003; Dolman *et al.* 2012; Orihel *et al.* 2012). Total toxin concentrations in
226 natural waters will therefore tend to increase with eutrophication-driven increases in total
227 phytoplankton biomass, but the magnitude of this response will be amplified if nutrient
228 enrichment also selects for dominance by toxin-producing taxa (Anderson *et al.* 2002; Heisler *et*
229 *al.* 2008; Dolman *et al.* 2012; Scott *et al.* 2013) or clones (Kardinaal *et al.* 2007; Touzet *et al.*
230 2007) [ENREF 25](#) [ENREF 12](#) and if nutrient limitation promotes the internal accumulation of toxic
231 molecules, as shown in our analysis. These insights also provide a means for anticipating which
232 toxins will be triggered as a function of N- or P-limitation during bloom termination. Thus, the
233 stoichiometric relationships we have uncovered may provide a practical basis for better
234 forecasting the potential occurrence of toxic compounds in the aquatic environment, possibly
235 allowing the creation of an “early warning system” for more detailed chemical analyses of
236 suspended particulate matter, both in drinking water supplies and in surface waters that support
237 fisheries and heavy recreational use.

238 Our analysis demonstrates that the production of stoichiometrically distinct toxins can be
239 explained, at least partially, by the availabilities of key limiting nutrients in the environment and
240 within the cell. However, we note that the production of toxins may also be affected by other
241 environmental factors. In particular, C-rich toxins may be sensitive to changes in CO₂ and light
242 availability, as these resources provide phytoplankton with C and energy. Indeed, enhanced CO₂
243 availabilities have been shown to increase the production of C-rich toxins such as domoic acid
244 (Sun *et al.* 2011) and karlotoxin (Fu *et al.* 2010), particularly under P-limited conditions.
245 However, there seems no general response of C-rich toxins to increasing light intensities. More
246 specifically, increasing light intensities were shown to cause an increase (Bates 1998; Cusack *et*

247 *al.* 2002) or a decrease (Auro & Cochlan 2013) in the production of domoic acid, while the
248 sensitivity of hemolytic activity (Baker *et al.* 2007; Granéli *et al.* 2012) and DSP toxins (Nielsen
249 *et al.* 2013) to light intensity seems to be marginal or even absent. Enhanced light intensities
250 were shown to cause an increase in the production of the N-rich PSP toxins (Parkhill & Cembella
251 1999; Etheridge & Roesler 2005) and microcystins (Wiedner *et al.* 2003; Tonk *et al.* 2005) at
252 limiting light levels, but caused a decrease at saturating light levels. Generally, the response of
253 N- and C-rich toxins to light availability seems less pronounced as compared to nutrient
254 limitation, while enhanced CO₂ availabilities may further promote the production of C-rich
255 toxins under nutrient limiting conditions.

256 Our results provide a broad picture of how the synthesis of toxins in response to nutrient
257 limitation might be modified by internal allocation processes that shunt excess C and nutrients
258 not to toxins but towards storage compounds, as in the case of N in cyanobacteria. In higher
259 plants, nutrient limitation may result in a surplus of fixed C as well, which can also be allocated
260 either to storage compounds or to C-rich secondary metabolites (Koricheva *et al.* 1998).
261 Likewise, the availability of C and nutrients has been shown to affect synthesis of C- or N-rich
262 secondary metabolites in bacteria and fungi (Sanchez & Demain 2002; Cornforth & Foster
263 2013). Even though secondary metabolites are highly diverse in function among producers, the
264 synthesis of these molecules generally has a flexible component that at least partially depends on
265 the interplay between their own elemental composition and the relative availability of key
266 resources. Thus, toxin production can be expected to behave in broadly predictable ways across a
267 wide variety of biota.

268 Ongoing anthropogenic loading of nutrients will lead to deterioration of water quality not
269 only by promoting biomass accumulation of phytoplankton, but by affecting both the quantities

270 and the biochemical composition of the toxins they can produce, especially when nutrient
271 loading leads to strong stoichiometric imbalances of N and P. For instance, nutrient inputs from
272 nonpoint sources such as atmospheric N deposition or release of sediment-bound P (Carpenter *et*
273 *al.* 1998; Elser *et al.* 2009) may shift phytoplankton from N- to P-limitation, or vice versa, and
274 thereby alter their toxicity. Thus, application of stoichiometric theory, together with knowledge
275 of the phytoplankton species and the stoichiometric composition of the toxins they produce, may
276 help us predict whether nutrient loading will cause an increase, decrease, or no change in overall
277 toxicity in natural waters (Anderson *et al.* 2002; Heisler *et al.* 2008), with important potential
278 consequences for the various services provided by freshwater and marine ecosystems.

279

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427 **Supporting Information**

428 The following Supporting Information is available for this article:

429

430 Figure S1. Overview of investigated datasets grouped according to toxin stoichiometry, type of
431 experimental nutrient limitation, habitat of origin, identity of toxin or toxin group, and
432 taxonomic group (species or genus). Numbers between parentheses indicate for each
433 category the number of datasets that were available for analysis.

434 Table S1. Toxin quota and toxicities of various phytoplankton species grown under low N, low P
435 and nutrient-sufficient conditions.

436 Table S2. C:N:P stoichiometry of various phytoplankton species grown under low N, low P and
437 nutrient-sufficient conditions.

438

439

440 **Table 1.** Overview of toxins grouped according to their C:N ratio.

Group	Toxins	Molecular formula	C:N ratio*	
N-rich	PSP	Paralytic Shellfish Poisoning toxins (incl. saxitoxin, STX)	C ₁₀ H ₁₉ N ₇ O ₄	1.5
	MC	Microcystin (incl. microcystin-LR; microcystin-RR)	C ₄₉ H ₇₄ N ₁₀ O ₁₂ (MC-LR); C ₄₉ H ₇₅ N ₁₃ O ₁₂ (MC-RR)	4.3
	CYN	Cylindrospermopsin	C ₁₅ H ₂₁ N ₅ O ₇ S	3.0
	NOD	Nodularin	C ₄₁ H ₆₀ N ₈ O ₁₀	5.1
C-rich	ANTX	Anatoxin a	C ₁₀ H ₁₅ NO	10
	ASP	Amnesic Shellfish Poisoning toxin (incl. domoic acid, DA)	C ₁₅ H ₂₁ NO ₆	15
	GYM	Gymnodimine	C ₃₂ H ₄₅ NO ₄	32
	SPX	Spirolide	C ₄₂ H ₆₁ NO ₇	42
	PLTX	Palytoxin	C ₁₂₉ H ₂₂₃ N ₃ O ₅₄	43
	OVTX	Ovatoxin (incl. OVTX-a)	C ₁₂₉ H ₂₂₃ N ₃ O ₅₂	43
	DSP	Diarrhetic Shellfish Poisoning toxins (incl. okadaic acid, OA; dinophysins toxins, DTX-1, DTX-2)	C ₄₄ H ₆₈ O ₁₃ (OA, DTX-2); C ₄₅ H ₇₀ O ₁₃ (DTX-1);	-
	NSP	Neurotoxic Shellfish Poisoning toxin (incl. brevetoxin, PbTx)	C ₄₉ H ₇₀ O ₁₃	-
	KMTX	Karlotoxins (incl. KMTX-1-1, KMTX-2)	C ₆₇ H ₁₂₀ O ₂₄ (KMTX-1-1); C ₆₇ H ₁₂₁ ClO ₂₄ (KMTX-2)	-
	CTX	Ciguatoxin	C ₆₁ H ₈₈ O ₁₉	-
	MTX	Maitotoxin	C ₁₆₄ H ₂₅₆ O ₆₈ S ₂ Na ₂	-
	HA	Hemolytic activity based on bioassays		

441 *Values refer to the described toxins, or to the average of the shown analogues.

442

443 **Table 2.** Results of one-sample t-tests comparing relative changes in N- and C-rich toxin quotas
 444 or toxicities between a treatment and its control, with sample size (*n*), mean change, 95%
 445 confidence interval, and *P*-value at a significance level of 0.05 (indicated in bold).

N-rich					
	<i>n</i>	Mean	Variance	95% CI	<i>P</i> -value
N-limitation					
PSP	5*	0.65	0.009	0.46 to 0.83	0.011
MC	2*	0.49	0.003	0.38 to 0.60	0.035
All	2†	0.53	0.002	0.44 to 0.62	0.033
P-limitation					
PSP	5*	1.32	0.004	1.19 to 1.44	0.004
MC	3*	0.25	0.008	0.08 to 0.43	0.008
NOD	5	0.93	0.886	0.11 to 1.76	0.440
All	3†	0.96	0.003	0.86 to 1.06	0.271
C-rich					
	<i>n</i>	Mean	Variance	95% CI	<i>P</i> -value
N-limitation					
DSP	2*	1.69	1.95	-1.04 to 4.43	0.353
KMTX	5	2.77	0.93	1.92 to 3.61	0.008
NSP	5	1.99	0.15	1.65 to 2.33	0.002
ASP	2*	1.04	1.02	-0.37 to 2.44	0.484
HA	2*	4.41	6.47	0.88 to 7.93	0.155
All	5†	2.00	0.11	1.36 to 2.64	0.019
P-limitation					
DSP	2*	2.04	4.40	-0.34 to 4.41	0.241
KMTX	6	3.15	3.72	1.61 to 4.70	0.021
NSP	5	2.30	0.34	1.79 to 2.82	0.004
ASP	2*	6.25	4.87	1.93 to 10.58	0.127
HA	2*	6.48	25.69	0.74 to 12.21	0.101
ANTX	3	1.10	0.16	0.65 to 1.56	0.351
All	6†	1.66	0.10	1.04 to 2.29	0.002

446 *Analysis based on means or weighted means across species; †Analysis based
 447 on weighted means across toxins.

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 449

450 **Figure legends**

451 **Fig. 1.** Relative changes of N-rich toxins in response to nutrient limitation. **(a)** N-limitation and
452 **(b)** P-limitation. White symbols show weighted means for individual toxins with error bars
453 indicating SE across means of species; grey symbols show the overall weighted means of N-rich
454 toxins with error bars indicating SE across means of toxins. The horizontal line indicates absence
455 of response. Asterisks indicate significances $P<0.01$ (**) and $P<0.05$ (*).

456
457 **Fig. 2.** Relative changes of C-rich toxins in response to nutrient limitation. **(a)** N-limitation and
458 **(b)** P-limitation. White symbols show weighted means for individual toxins with error bars
459 indicating SE across means of species; grey symbols show the overall weighted means of C-rich
460 toxins with error bars indicating SE across means of toxins. The horizontal line indicates absence
461 of response. Asterisks indicate significances $P<0.01$ (**) and $P<0.05$ (*).

462
463 **Fig. 3.** Relative changes of N- and C-rich toxins in response to cellular N:P ratios. **(a)** N-rich
464 toxins and **(b)** C-rich toxins. Symbols show weighted means for individual toxins with error bars
465 indicating SE across means of species. The horizontal line indicates an absence of response, and
466 the vertical line illustrates the Redfield N:P ratio (N:P=16). The solid grey line in **(a)** shows the
467 linear fit to all data ($R^2=0.989$, $n=6$, $P<0.001$).