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**Pelagic phytoplankton community change-points across  
nutrient gradients and in response to invasive mussels**

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**Running title:** Phytoplankton change-points across nutrient gradients and in response to invasive  
mussels

**Key words:** community thresholds; eutrophication; *Dreissena*; algae; water quality

**Summary**

1. Phytoplankton communities can experience non-linear responses to changing nutrient concentrations, but the nature of species shifts within phytoplankton is not well understood and few studies have explored responses of pelagic assemblages in large lakes.
2. Using pelagic phytoplankton data from the Great Lakes, we assess phytoplankton assemblage change-point responses to nutrients and invasive *Dreissena*, characterizing community responses in a multi-stressor environment, and determine whether species responses to *in situ* nutrients can be approximated from nutrient loading.

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- 30 3. We demonstrate assemblage shifts in phytoplankton communities along major stressor  
31 gradients, particularly prominent in spring assemblages, providing insight into  
32 community thresholds at the lower end of the phosphorus gradient and species-stressor  
33 responses in a multi-stressor environment. We show that responses to water nutrient  
34 concentrations could not be estimated from large-scale nutrient loading data likely due to  
35 lake-specific retention time and long-term accumulation of nutrients.
- 36 4. These findings highlight the potential for significant accumulation of nitrates in ultra-  
37 oligotrophic systems, non-linear responses of phytoplankton at nutrient concentrations  
38 relevant to current water quality standards, and system-specific (e.g. lake or ecozone)  
39 differences in phytoplankton responses likely due to differences in nutrient co-limitation  
40 and effects of dreissenids.

## 41 42 **Introduction**

43 Cycling of nitrogen and phosphorus, the two most limiting nutrients for primary  
44 production, has been greatly altered by human activities (Falkowski et al. 2008, Galloway et al.  
45 2008, Canfield et al. 2010, Bouwman et al. 2013). Primary producers are strongly affected by  
46 nutrient limitation (*e.g.* Tilman 1982, Wetzel 2001), and changes in their assemblages in  
47 response to nutrients propagate up the food web through a multitude of pathways including  
48 decreased diversity, increased biomass turn-over, and overwhelming contribution of bloom taxa  
49 to the overall energy flow (McCann and Rooney 2009). In the pelagic zones of lakes,  
50 phytoplankton directly respond to many stressors associated with human development such as  
51 excess nutrients, and can therefore be one of the early-warning signals for ecosystem change in  
52 response to stress (McCormick and Cairns 1994).

53 There is evidence that algal communities can experience non-linear changes in response  
54 to increasing nutrient concentrations (Smucker et al. 2013a). The abrupt transition from  
55 macrophyte-dominated to an algal-dominated state is one of the classic examples of alternative  
56 stable states (Scheffer and Jeppesen 1998); however, the nature of species shifts within the  
57 phytoplankton assemblage is not as well documented. In addition to nutrient loading,  
58 proliferation of invasive filter feeders has been linked to major shifts in primary producer  
59 community composition due to grazing and the resulting broader changes in food web structure  
60 and nutrient cycling across aquatic ecosystems such as the shift from mostly pelagic to benthic–

61 littoral energy pathways (Higgins and Vander Zanden 2010, Karatayev et al. 2015a, Gallardo et  
62 al. 2016). Understanding the degree of non-linearity in phytoplankton assemblage shifts and  
63 identifying key species responding to these anthropogenic stressors is necessary to relate changes  
64 in water quality, nutrient cycling and precipitation patterns to the rest of the food web. This  
65 approach can contribute strong ecology-based evidence for developing nutrient criteria (Smucker  
66 et al. 2013a) and is particularly important since there are few biotic indicators available for the  
67 pelagic zones of large lakes, making it difficult to monitor assemblage responses to such large-  
68 scale stressors as invasive species and nutrient loading.

69 The Laurentian Great Lakes have a long history of anthropogenic stress affecting  
70 phytoplankton, often resulting in undesirable effects such as cultural eutrophication (Stoermer  
71 1978). Excess phosphorus loading has most commonly been cited as the major causal agent in  
72 water quality and algal problems (Conley et al. 2009). Nitrogen concentrations in the Great  
73 Lakes region has also increased greatly over the past century (Han and Allan 2012) and is likely  
74 implicated in changes throughout the ecosystem (e.g. Elser et al. 2010). Effects of nitrogen  
75 loading on pelagic phytoplankton dynamics received less attention than effects of phosphorus  
76 (but see Reavie et al. 2014a); but there is evidence of nitrogen limitation and co-limitation of  
77 coastal algal biofilms (Cooper et al. 2016). More recently, the extensive invasion of zebra and  
78 quagga mussels (*Dreissena polymorpha* and *D. bugensis*: Dreissenidae) throughout most of the  
79 basin has greatly impacted primary production in the lakes by shunting energy flow from the  
80 pelagic to the benthic food web (Hecky et al. 2004, Bunnell et al. 2014). This has further altered  
81 phytoplankton communities, particularly in Lake Michigan and Lake Huron (Reavie et al.  
82 2014b), but the mechanistic processes that drive these phytoplankton changes are still under  
83 discussion (e.g. Kerfoot et al. 2010). This dreissenid invasion was ranked as the top  
84 environmental threat out of the comprehensive list of 50 stressor variables in the Great Lakes,  
85 according to a recent expert survey (Smith et al. 2015), and is probably the most important  
86 stressor relevant to pelagic autotrophs along with atmospheric nutrient deposition.

87 We examined responses of phytoplankton communities to *Dreissena* abundance and  
88 nitrogen (N) loading as well as *in situ* water nitrate and total phosphorus concentrations, known  
89 to be relevant in-water predictors for phytoplankton assemblages based on previous studies  
90 (Reavie et al. 2014a). Some of the predictors examined in our study are not necessarily stressors  
91 from the perspective of phytoplankton; however, these predictors are often referred to as

92 stressors for consistency with other studies and to reflect the general perception of nutrient  
93 loading. Our major goals were to (1) test whether there is a relationship between nitrogen loading  
94 and water nitrate concentrations, (2) determine whether there is evidence of non-linear changes  
95 in phytoplankton assemblage composition in response to *in situ* nutrient concentrations and  
96 estimates of offshore environmental stress (nitrogen loading and *Dreissena* abundance), and (3)  
97 examine key species responses along the stressor gradients, characterize assemblage responses in  
98 a multi-stressor environment, and test whether large-scale estimates of nutrient loading can be  
99 related to similar assemblage responses as *in situ* water nutrient concentrations.

100

## 101 **Materials and Methods**

### 102 *Data collection and processing*

103 The pelagic zones of the Laurentian Great Lakes, which encompass lakes Erie, Ontario,  
104 Huron, Michigan and Superior, range from ultra-oligotrophic in the northern GL to eutrophic-  
105 mesotrophic in the south. The standard operating procedure for phytoplankton collection and  
106 analysis is described in detail in the published procedures (USEPA, 2010) and a comprehensive  
107 summary is provided by Reavie et al. (2014a). Briefly, water samples were collected during  
108 biannual synoptic sampling (“spring” - April, “summer” - August) from 72 standard pelagic  
109 stations throughout the Great Lakes (Fig. 1), with each station sampled each year in April and  
110 August for a total of 8 samples per station (2007-2010). Integrated water samples were collected  
111 from the rosette sampler onboard the *R/V Lake Guardian*. In the laboratory, water samples were  
112 digested by acid persulfate and measured by a Lachat QuikChem AE autoanalyzer (Hach  
113 Company, Loveland, CO, USA) for total phosphorus (Barbiero et al. 2006). Nitrate + nitrite  
114 ( $\text{NO}_x$ ) concentrations, hereafter referred to as nitrate due to much lower nitrite concentrations in  
115 open water, were determined by diazotizing with sulfanilamide dihydrochloride after nitrate was  
116 reduced by copper-coated cadmium (GLNPO, 2010). We used  $\text{NO}_x$  because organic N and  
117 ammonia were a very minor component of the TN budget (<0.2%) and were no longer being  
118 measured at the pelagic stations during the years of this study. We used TP, because most of  
119 phosphorus is bound in seston, the inorganic compartment is generally very small (<1%, Wetzel  
120 2001) and the overall productivity is determined by TP and the rate of cycling between the  
121 inorganic and particulate P compartments. Because mean values of water quality parameters can

122 be skewed by extreme values (Helsel and Hirsch, 2002), we used median water quality from  
123 2007 to 2010 in all analyses.

124 Phytoplankton samples were composites of water sampled at discrete depths from the  
125 euphotic zone of the water column (the entire water column during isothermal spring conditions  
126 and the epilimnion during summer). Samples were analyzed for the whole phytoplankton  
127 assemblage. Analysis of soft algae used the quantitative Utermöhl method (Utermöhl, 1958)  
128 while acid digestions were used to isolate diatom valves which were then plated on slides and  
129 counted using oil immersion (1000X or higher; valve counts: at least 500 per sample, soft algae:  
130 at least 250 "entities" [colonies, filaments, cells] per sample). Dead diatoms (empty frustules)  
131 were not included in final counts. As with water quality, each station was sampled twice each  
132 year in April and August. Only 2008-2010 data were used because previous studies demonstrated  
133 strong changes in assemblage composition prior to this period (Reavie and Barbiero 2013).

134 We used environmental stressor data from the Great Lakes Environmental Assessment  
135 and Mapping project (GLEAM), which overall aimed to synthesize the cumulative impact of 34  
136 stressors across the Great Lakes (Allan et al. 2013). For nitrogen loading, 2003-2007  
137 atmospheric deposition of nitrate was compiled from the National Atmospheric Deposition  
138 Program (NADP) for wet nitrate deposition (via rain, fog and snow) and Clean Air Status and  
139 Trends Network (CASTNET) for dry deposition; dry:wet deposition ratios were used to estimate  
140 5-year averages of total deposition of nitrate (dry + wet) across the surfaces of the lakes (Han  
141 and Allan 2012, Allan et al. 2013). Nitrogen loading also included estimates of tributary loadings  
142 in addition to N atmospheric deposition; however, for most off-shore pelagic stations, total  
143 inputs were dominated by deposition because tributary loading rapidly declined away from the  
144 coastline, to 10% of initial levels at 15 km. Although water nitrate concentrations were  
145 determined contemporaneously with phytoplankton assessment (2007-10), whereas N loading  
146 was estimated from earlier data (2003-07), large-scale N loading is unlikely to have changed  
147 greatly during this time period. Unlike N loading, it was not possible to compare assemblage  
148 responses to water TP vs. P loading, because tributary loading data were highly skewed (little  
149 tributary P was detectable offshore) and atmospheric deposition was minor and not well-  
150 quantified (Allan et al. 2013). For invasive mussels, point observations (2000-2006 time range)  
151 in numbers per m<sup>2</sup> (e.g., Lozano et al. 2001, Dermott and Geminiuc 2003, Nalepa et al. 2007,  
152 Watkins et al. 2007, Nalepa et al. 2014; unpublished data) were kriged to produce continuous

153 maps of zebra and quagga mussel densities across the surfaces of the lakes (Allan et al. 2013).  
154 Both raster layers were transformed with a cumulative distribution function to express the data as  
155 percentiles relative to all other pixels in the five Great Lakes.

156 Multi-year data on environmental variables and phytoplankton assemblage composition  
157 were averaged by station to avoid pseudoreplication of the nutrient loading data; averaging was  
158 done separately for each season to reflect distinct seasonal dynamics of these assemblages (e.g.,  
159 Reavie et al. 2014a). To link GLEAM stress variables with phytoplankton data, we created a 5-  
160 km buffer (diameter = 10 km) around each phytoplankton sampling station, and averaged the N  
161 loading and *Dreissena* abundance values within the buffer. This buffer distance was chosen for  
162 its water quality/biological relevance (P. Yurista, pers. comm.) and to account for the fact that  
163 the actual pixel/cell that a sampling site falls on can be slightly misaligned from projection  
164 conversions. The stressor layers are estimated via propagation from point data, so averaging over  
165 a larger area reduces the risk that an individual pixel might be an outlier.

166

#### 167 *Analytical approaches*

168 Community responses were analyzed using Thresholds Indicator Taxa Analysis (TITAN;  
169 King and Baker, 2010). This approach combines Indicator Species Analysis (Dufrene and  
170 Legendre, 1997) and change-point analysis to determine indicator values for each candidate  
171 change-point along the stressor gradient and then uses bootstrapping to identify reliable indicator  
172 taxa. Community threshold is identified by the synchronous change in abundance of many taxa,  
173 an approach that is more sensitive than methods considering composite metrics (richness,  
174 diversity) or combining sensitive and tolerant taxa, because it does not aggregate taxa responses  
175 prior to analysis (Baker and King, 2010; King and Baker, 2010). Species present in fewer than  
176 four stations (~10% of observed species) were removed from the analysis. Species data were  
177  $\log(x+1)$ -transformed prior to analysis. The following criteria were used to judge evidence for  
178 community thresholds: synchronous shifts in many species and strong responses of individual  
179 species (large standardized change in abundance, narrow bootstrapped quantile intervals). It is  
180 acknowledged that change-point identification is inherently subjective and may depend on  
181 frequency of observations across the stressor gradient, sample size and total number of taxa  
182 (Daily et al. 2012, Kovalenko et al. 2014). The degree of change (z-score) values of significant  
183 and reliable indicators in response to each stressor were combined in a heatmap visualization

184 (*NMF* package in R, Gaujoux and Seoighe 2010), and cluster analysis was used to highlight taxa  
185 with similar responses and identify overlaps among assemblages responding to different  
186 stressors. For instance, if spring and summer assemblage responses to dreissenids were similar,  
187 we would expect these two seasonal assemblage responses to cluster close together. Analyses  
188 were based on density data (cells/mL), which is appropriate for analyses of community  
189 composition using these approaches. All analyses were done in R (version 3.0.2; R Development  
190 Core Team, Vienna, Austria).

191 Human activity across large scales is unavoidably confounded with geography, and in the  
192 Great Lakes most of the agricultural activity and settlement have occurred in the southern part of  
193 the basin (Danz et al. 2007). Due to the resulting differences in stressor distribution as well as  
194 differences in phytoplankton assemblage composition, we conducted separate analyses for the  
195 northern Great Lakes (NGL: Superior, Huron, Michigan) and southern Great Lakes (SGL: Erie,  
196 Ontario). Linear regressions were used to check the relationships among stressors within each  
197 ecoregion. Because overall nutrient budgets reflect nutrient loading and water retention time, we  
198 used ANCOVA to relate nitrate dynamics to lake retention time. In addition, we tested lake-  
199 specificity of the key species responding to stressor gradients to ensure community changes were  
200 not driven by biogeographic limitations in species distributions by determining the proportion of  
201 species within each ecoregion which were confined to a single lake.

202

## 203 **Results**

204 Relationships between the stressors, particularly N loading, water nitrate concentrations  
205 and *Dreissena* abundance, were dependent on ecoregion, season and lake water retention time  
206 (SI Table 1). In NGL (Superior, Huron, Michigan), there was a significant *negative* relationship  
207 between water nitrate and large-scale estimates of N loading (Fig. 2,  $r = -0.83$  and  $-0.78$  in spring  
208 and summer, respectively,  $p < 0.001$ ). This relationship was mostly due to high water nitrate  
209 concentrations but low N loading rates in Lake Superior, although without this lake, it was still  
210 marginally significant and negative ( $r = -0.38$ ,  $p = 0.060$  in spring and  $r = -0.41$ ,  $p = 0.040$  in  
211 summer). ANCOVA demonstrated that this relationship between water and atmospheric-  
212 deposited nitrate was highly correlated with lake retention time in spring ( $p < 0.001$ ), but only  
213 marginally so in the summer (retention time effect  $p = 0.11$ , see SI Table 1 for model details).  
214 *Dreissena* were absent in pelagic Lake Superior, but even without this lake, there was still a

215 significant relationship between large-scale estimates of *Dreissena* abundance and water nitrate  
216 concentrations, particularly in the summer ( $r = -0.41$ ,  $p = 0.044$  in spring,  $r = -0.94$ ,  $p < 0.001$  in  
217 summer), as well as spring TP (Fig. 3,  $r = 0.76$ ,  $p < 0.001$  spring,  $p = 0.48$  summer). In SGL  
218 (Erie, Ontario), patterns were quite different: there was a positive relationship between N loading  
219 and spring but not summer water nitrate concentrations ( $r = 0.63$ ,  $p < 0.001$  spring,  $p = 0.19$   
220 summer), and a positive relationship between *Dreissena* and water nitrate (Spearman's  $D = 1436$   
221  $p = 0.002$  in spring, and  $D = 1778$ ,  $p = 0.007$  in summer). The effect of water residence time was  
222 not tested due to relatively negligible differences in residence time between Erie and Ontario (2.7  
223 and 6 years, compared with 173, 62 and 21 years for Superior, Michigan and Huron,  
224 respectively). Stations with the highest nitrate and *Dreissena* densities were primarily in the  
225 Western Basin of Lake Erie. Water TP was not related to any other stressors in the SGL (Fig. 3,  
226 SI Table 2a).

227 Despite these distinct stressor profiles even within ecoregions, indicator lake-specificity  
228 analyses demonstrated that only 8 of 88 (9%) indicator species responsible for changes in  
229 assemblages were confined to a single lake in the NGL (SI Table 3). Nearly half of the species  
230 were present at 60% of the sampling stations. In the SGL, 30% of 115 indicator species were  
231 lake-specific. It is therefore likely that observed species responses were in fact driven by  
232 stressors and not biogeographic limitations as many of the sensitive and tolerant species had  
233 broad geographic distributions. Overall, phytoplankton species richness had variable  
234 relationships with the stressors depending on region and season. The strongest trends were the  
235 decline in richness with increasing *Dreissena* and TP in spring NGL assemblages, and increase  
236 with increasing TP and nitrate concentrations in spring SGL assemblages (Fig. 4, SI Table 2b).

237 There were change-point shifts in phytoplankton assemblages in response to major  
238 nutrients (water TP and water nitrate) and *Dreissena* abundance (Figs. 5-7). In general, responses  
239 consistent with the threshold model can be characterized by a large number of individual  
240 indicator species and narrow quantile intervals around their change-points. In many cases,  
241 evidence for assemblage threshold response was not very strong, particularly for sensitive taxa.  
242 Stronger evidence of thresholds was observed for nitrate-spring-NGL-sensitive and tolerant,  
243 *Dreissena*-spring-NGL-sensitive and tolerant, and *Dreissena*-summer-NGL-sensitive (Fig. 5, SI  
244 Fig. 1a; SI Table 4 for change-point locations). Stronger assemblage responses to the above-  
245 mentioned stressors were observed in spring rather than summer, as indicated by the more



246 pronounced assemblage thresholds (more synchronous responses of a greater number of species,  
247 Fig. 5-6) in the NGL, whereas in the SGL this trend was not obvious. Many taxa with  
248 preferences for higher nutrient concentrations increased in response to TP and nitrate, but  
249 particularly with TP this change was more gradual.

250 Assemblages in the NGL responded more strongly to water nitrate and *Dreissena* in  
251 terms of the number of sensitive and tolerant species changing in abundance with increasing  
252 levels of stress. Highest levels of these stressors were associated with distinct assemblages (see  
253 below). Many more tolerant taxa increased at higher nutrient concentrations, particularly TP,  
254 than declined at the sensitive species threshold; this was most clearly visible with the greater  
255 number of tolerant (red) than sensitive (green) blocks within each TP column (Fig. 7).

256 Based on cluster analysis of species-stressor responses (Fig. 7, top), responses to  
257 nutrients in the southern basin are a distinct property of the Great Lakes phytoplankton  
258 assemblages (leftmost 4 variables in cluster analysis). Nitrate responses in the northern basin  
259 (right-most 2 variables) also stand out. Based on cluster analysis of the species-specific  
260 responses to multiple variables (Fig. 7, clustering on the left) we characterized 10 distinct groups  
261 of taxa with unique combinations of responses along the various gradients of pelagic stressors  
262 and water quality data. We briefly present and discuss these groups below, acknowledging that  
263 smaller taxonomic groups within these groups are possible. We provide the details of the  
264 clustering results (Fig. 7) so that readers may further characterize autecological data for taxa, if  
265 desired.

- 266 1. *N tolerant (NGL, spring and summer), dreissenid sensitive (NGL, summer)*. This group of  
267 taxa is tolerant of higher N concentrations in the water. These taxa also tend to occur in  
268 areas that have been less influenced by the dreissenid invasion. *Cosmarium phaseolus*,  
269 *Monoraphidium irregulare*, *Crucigenia quadrata* and *Synedra filiformis* var. *exilis* were  
270 among the species with the strongest stressor responses in this group.
- 271 2. *Dreissenid sensitive (NGL, spring)*. Unlike the group above, these taxa are more  
272 specifically sensitive to dreissenids in the spring, including *Kephyrion cupuliformae* and  
273 *Stephanodiscus conspicueporus*.
- 274 3. *N and P tolerant (SGL, spring)*. This group of nutrient-tolerant taxa includes species that  
275 are well-known indicators of nutrient pollution, such as *Cyclotella meneghiniana* (e.g.  
276 Kwandrans et al. 1998) and *Gomphonema olivaceum* (Kelly and Whitton 1995).

277 *Cyclotella operculata* and *Cymbella minuta* var. *silesiaca* responded the strongest along  
278 the nutrient gradient.

279 4. *Dreissenid tolerant (NGL), P tolerant (NGL, spring), N sensitive (NGL)*. This small  
280 group of taxa includes *Cyclotella michiganiana*, *Fragilaria crotonensis*, *Rhodomonas*  
281 *lens* and *Diatoma tenue* var. *elongatum*. Based on recent phytoplankton data from Lake  
282 Michigan (the lake with the greatest dreissenid abundance), *Rhodomonas lens* (a  
283 cryptophyte) and *Fragilaria crotonensis* (a pennate diatom), for instance, comprise a  
284 relatively large proportion of the summer assemblage, despite losses due to the dreissenid  
285 advance the decade prior (Reavie et al. 2014b).

286 5. *Diverse responses but generally P and dreissenid tolerant (spring) and N sensitive (NGL,*  
287 *spring)*. These taxa apparently have multiple, simultaneous responses along the  
288 environmental gradients tested, but a number of known nutrient-tolerant taxa are  
289 included, such as *Stephanodiscus alpinus* and *S. binderanus* (Reavie et al. 2014a).

290 6. *Dreissenid tolerant and N sensitive (NGL, summer)*. This is another group that seems  
291 limited by water nitrate concentrations, which includes taxa that are known to occur in  
292 the dreissenid-heavy Lake Michigan in the summer (e.g. *Pseudokephyrion millerense*;  
293 Reavie et al. 2014a).

294 7. *Stressor-specific*. While individual taxa have stressor-specific responses, for the most part  
295 these taxa had few responses or did not adequately fit within other groups. These taxa  
296 may hold promise for stressor-specific monitoring.

297 8. *P tolerant (SGL, summer)*. This group was largely defined by a unique tolerance to higher  
298 summer phosphorus in the southern GL. Several taxa in this list (e.g. *Aulacoseira*  
299 *granulata*, *Cyclostephanos dubius*, *Microcystis aeruginosa* and *Aphanizomenon flos-*  
300 *aquae*) are well-known indicators of phosphorus-enriched summer conditions in Lake  
301 Erie (Reavie et al. 2014b). *Nitzschia subacicularis*, *N. inconspicua* and *Scenedesmus*  
302 *opoliensis* were among the most strongly responding species.

303 9. *Dreissenid tolerant and N sensitive (NGL, spring)*. These taxa, including *Stephanodiscus*  
304 *hantzschii* f. *hantzschii* and *Rhodomonas minuta*, were intolerant of higher N  
305 concentrations. However, these tend to be spring taxa that occur in areas of the NGL with  
306 many dreissenids.

307 10. *N and P tolerant (SGL)*. These taxa are broadly tolerant to higher nutrients in the SGL,  
308 including the known eutrophication indicator *Cyclostephanos invisitatus* (Mortensen  
309 2013; Reavie et al. 2014a).

310

## 311 **Discussion**

312 Using spatial relationships between phytoplankton assemblage composition and  
313 environmental variables, this study demonstrates assemblage shifts in phytoplankton  
314 communities along major stressor gradients and identifies species which are most responsive on  
315 large scales. Many nutrient-tolerant algal species abruptly and concurrently increased in  
316 abundance, while oligotrophic species declined, near the change-points in nutrient gradients and  
317 *Dreissena* abundance; however, in some cases evidence of community thresholds was not very  
318 strong, and responses to water nutrient concentrations could not be estimated from large-scale  
319 nutrient loading data. Similar change-points in community composition have been previously  
320 observed in several groups of aquatic organisms in response to urbanization in the surrounding  
321 watersheds and eutrophication (Baker and King 2010, Hilderbrand et al. 2010, King et al. 2011,  
322 Smucker et al. 2013b, Kovalenko et al. 2014).

323 The most distinct clustering was associated with NGL (Superior, Huron, Michigan) vs.  
324 SGL (Erie, Ontario) partition, with many more species responding to stressor gradients in the  
325 NGL than in the SGL, likely reflecting the unique characteristics of nutrient dynamics and co-  
326 limitation in the northern and southern regions (details below). Stronger assemblage responses in  
327 spring than in the summer could be due to spring nutrients being a better predictor of algal  
328 assemblage dynamics early in the ice-free season. By summer, the uptake of available nutrients  
329 by algae minimizes this signal by having responses limited to a smaller subset of taxa that are  
330 tolerant of nutrient limitation (e.g. cyanophytes in Lake Erie). In addition, summer  
331 phytoplankton assemblages may be more affected by zooplankton grazing. *Dreissena* are also  
332 known to elicit the greatest responses in spring phytoplankton (Fahnenstiel et al. 2010, Rowe et  
333 al. 2015).

334

### 335 *Nitrogen loading, nitrate concentrations and Dreissena abundance*

336 Water nitrate concentrations were negatively correlated with N loading in the more  
337 oligotrophic northern ecoregion. This may not be surprising because nitrate loading in NGL has

338 increased over the past century (Han and Allan 2012) and this is on the scale of water retention  
339 time in these lakes (100+ years in Superior). Indeed, our ancillary analysis shows that the  
340 relationship between nitrate loading from the atmosphere and nitrate concentrations in the water  
341 are dependent on water retention time. In addition, very low phosphorus availability and lower  
342 primary production in NGL (Evans et al. 2011), particularly in Lake Superior, could result in  
343 lower nitrate assimilation and reduction, resulting in higher *in situ* nitrate concentrations despite  
344 the lower loading rates, although this was not tested directly in this study. This highlights the  
345 potential for significant accumulation of nitrates in ultra-oligotrophic systems with long water  
346 residence times even under conditions of low atmospheric inputs. The complex relationship  
347 between N loading and pelagic N dynamics underscores previously discussed difficulty in  
348 predicting nitrogen budgets for large lakes due to uneven historic trends in the major input  
349 compartments (Han and Allan 2012). The NGL are experiencing increasing nitrate  
350 concentrations, particularly prominent in Lake Superior with documented 5-fold increases in the  
351 past century, due to nitrate loading as well as *in situ* nitrification (Sterner et al. 2007) and a long  
352 residence time (Small et al. 2014); therefore, it is critical to understand phytoplankton responses  
353 along the nitrogen gradient in the context of other nutrient limitations.

354 Our results demonstrate that distinct phytoplankton assemblages are associated with low  
355 and high ends of the nitrogen gradient. The negative relationship between *in situ* nitrate and  
356 *Dreissena* densities in the north could indicate that the N cycle is affected by invasive filter-  
357 feeding mussels. These invasive mussels are significant players in nitrogen remineralization as  
358 well as sequestration and biodeposition (Mellina et al. 1995, Bruesewitz et al. 2008, Ozersky et  
359 al. 2015), yet the balance among those processes is likely dependent on mussel densities, growth  
360 rates and lake morphometry. High dreissenid densities in areas with greater N loadings could  
361 result in higher N sequestration and lower availability of dissolved inorganic nitrogen, but our  
362 study was not designed to investigate this connection in more detail. *Dreissena* also has direct  
363 effects on phytoplankton communities, particularly noticeable in reduction of spring  
364 phytoplankton blooms (Rowe et al. 2015) and shifting assemblage composition. Changes in  
365 assemblage composition occur due to direct filtering of algae from the water column and mussel  
366 preferences for certain types of algae (e.g. *Cyclotella*), whereas others are rejected as  
367 pseudofeces (e.g. *Microcystis*, Lavrentyev et al. 1995, Makarewicz et al. 1999). Algal sinking  
368 rates could also be important (Bramburger and Reavie 2016). Previous studies demonstrated

369 spring decline in diatom taxa likely as a result of reduced pelagic nutrients and selective  
370 consumption (Reavie et al. 2014b). As dreissenid densities (and filtering rates) increase above a  
371 certain threshold, pelagic phytoplankton could be shifting to more buoyant and/or more tolerant  
372 of lower pelagic nutrients (e.g. *Chrysolykos planktonicus*). Overall, different phytoplankton  
373 community responses to water nitrate concentrations indicate potential system-specific  
374 differences in N cycling, nutrient co-limitation and effects of dreissenids, and it would be  
375 important to disentangle independent effects of *Dreissena* and nitrates in future studies.

376

### 377 *Phosphorus*

378 Observed community responses to TP concentrations are interesting because they provide  
379 insight into potential change-points at the lower end of the phosphorus gradient. Non-linear algal  
380 responses to phosphorus have been previously observed in systems with higher nutrient  
381 concentrations such as small lakes (e.g. Scheffer et al. 1997, Smith 2014, Giblin et al. 2014,  
382 Wang et al. 2014) and streams (Smucker et al. 2013b). However, many of the traditional  
383 alternative stable states studies did not closely examine species shifts within the phytoplankton  
384 assemblage, and considering individual species change-points may be helpful for anticipating  
385 community-wide shifts. It is interesting that our community change-points, although not very  
386 prominent, particularly for sensitive species, were below the proposed target values based on the  
387 GL Water Quality Agreement: 8 µg/L TP for spring phytoplankton in SGL vs. 10 µg/L proposed  
388 as the target for Ontario and Eastern and Central Erie (DePinto et al. 2006). We also note that the  
389 dominant community change-points along the phosphorus gradient vary between spring and  
390 summer, which is not surprising given the substantial differences between the seasons in terms of  
391 assemblage composition and water condition. While mechanisms for these seasonal differences  
392 in change-points are probably quite complex, this finding emphasizes that one must account for  
393 temporal variation when characterizing assemblage responses based on synoptic data.

394 Phosphorus is a well-known driver of phytoplankton abundance and composition in the  
395 Great Lakes (e.g. Makarewicz 1993, Shaw Chraïbi et al. 2014). Our results indicate that the  
396 abundance of several taxa is clearly correlated with variables other than (or in addition to)  
397 phosphorus (e.g. *Cyclotella ocellata* is tolerant of high P, but is apparently sensitive to higher  
398 dreissenid abundance). Future developments may use such information to refine indicator models  
399 by accounting for or eliminating species with no apparent response along the gradient of interest

400 (e.g. Racca et al. 2003) or better yet, considering the context of multiple stressors that  
401 simultaneously determine patterns in the observed phytoplankton assemblages.

402

### 403 *Indicator species*

404 Despite the lake-specificity of water quality and GLEAM stressors, some general species  
405 responses were observed across large areas of the basin. In NGL, lake-specific differences in  
406 assemblages were not the primary driving force behind assemblage shifts along the stressor  
407 gradients, as indicated by a low proportion of lake-specific indicators. This comparison is more  
408 complicated in the SGL because the number of stations in the more nutrient-limited Lake Ontario  
409 was smaller than in Lake Erie, the latter also being known for its unique species composition  
410 (among the GLs) and community dynamics, such as dense spring diatom blooms (central basin)  
411 and summer cyanobacterial blooms (western basin) (Reavie et al. 2014b). In addition, Lake Erie  
412 experienced complex dynamics of zebra and quagga mussel populations over the past few years  
413 (Karatayev et al. 2015b).

414 We identified clusters of species that could be related to particular stressors, and there is  
415 potential to use this information to better understand driving factors behind recent shifts in  
416 phytoplankton assemblages. Consider the recent rise in the relative abundance of the centric  
417 diatoms *Cyclotella sensu lato* in the Great Lakes (Shaw Chraïbi et al. 2014) and other stratifying  
418 northern lakes worldwide (Rühland et al. 2008). This phenomenon has been repeatedly  
419 associated with recent atmospheric and water warming, but additional and often related factors  
420 such as atmospheric nutrient deposition, water clarity and water column stability also need to be  
421 considered (Saros et al. 2013). As noted in nearby lakes (e.g. Siskiwit Lake on Isle Royale, Lake  
422 Superior; Saros et al. 2012), the role of N in determining *Cyclotella* abundance in the Great  
423 Lakes is complex. The simple correlation between warming and *Cyclotella* has been recognized,  
424 but given concurrent increases in N loading in many regions (Holtgrieve et al. 2011), teasing  
425 apart driving stressors is difficult. From our data, it is clear that lumping all *Cyclotella* as  
426 preferring higher N would be erroneous as some taxa are tolerant of higher concentrations (e.g.  
427 *Cyclotella ocellata*) while others are sensitive (e.g. *Cyclotella comensis*).

428

### 429 *Summary*

430 We show that pelagic Great Lakes phytoplankton communities exhibit change-point  
431 responses to *in situ* nutrient concentrations and abundance of invasive mussels across large  
432 geographic areas as taxa adapted to low-nutrient conditions are replaced, along the nutrient  
433 gradient, by species with higher nutrient optima. The relationship between water nitrate  
434 concentrations and nutrient loading was dependent on ecoregion, season and lake water retention  
435 time, indicating limited ability to forecast small-scale nutrient dynamics. Potential management  
436 applications of this approach and the present findings include development of biologically  
437 relevant nutrient criteria and more complete understanding of assemblage responses in a multi-  
438 stressor environment. This approach can be adapted for future impacts of stressors such as  
439 nutrient loads, invasive species and anthropogenic climate change, as well as more precise  
440 characterization of assemblage responses during state shifts.

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453

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683 **Figure legends**

684

685 Fig. 1. Map of pelagic sampling stations in the Great Lakes superimposed on estimates of nitrate  
686 loading (percentile/quintiles) across the basin. See main text and Allan et al. (2013) for more  
687 details on nitrogen deposition and USEPA (2010) and Reavie et al. (2014a) for more details on  
688 the sampling stations

689 Fig. 2. Relationship between water nitrate and N loading and *Dreissena* densities in Northern  
690 (SU – Superior; HU – Huron, MI – Michigan) and Southern Great Lakes (ER – Erie, ON –  
691 Ontario). Transformed *Dreissena* and N loading estimates are presented as unitless measures.

692 Fig. 3. Relationship between total phosphorus and *Dreissena* and N loading in NGL (SU –  
693 Superior; HU – Huron, MI – Michigan) and SGL (ER – Erie, ON – Ontario). Note the large  
694 differences in scale in the two regions. Lake Superior was excluded from statistical testing of TP  
695 vs. *Dreissena* relationship. Transformed *Dreissena* and N loading estimates are presented as  
696 unitless measures.

697 Fig. 4. Relationships between phytoplankton species richness and *Dreissena*, TP and nitrate in  
698 NGL (SU – Superior; HU – Huron, MI – Michigan) and SGL (ER – Erie, ON – Ontario).

699 Fig. 5. Phytoplankton community thresholds in NGL (Superior, Huron, Michigan) in response to  
700 *Dreissena* densities, water nitrate and TP in a) spring and b) summer. Change-points (0.05–0.95  
701 bootstrap quantile intervals) are shown for significant sensitive (filled, left side) and tolerant  
702 (open) taxa. Indicator value p-value cut-off = 0.05; purity and reliability cut-offs = 0.8  
703 (consistency with which indicator taxa are assigned to each indicator group and consistency of  
704 significant IndVal scores across bootstrap replicates). See SI Table 5 for species codes and  
705 authorities. Note that x-axis is adjusted to display the entire range of observed stressor values for  
706 each scenario.

707 Fig. 6. Phytoplankton community thresholds in SGL (Erie, Ontario) in response to *Dreissena*,  
708 water nitrate and TP in a) spring and b) summer. Change-points (0.05–0.95 bootstrap quantile  
709 intervals) are shown for significant sensitive (filled, left side) and tolerant (open) taxa.

710 Fig. 7. Heatmap of species response to stressors, including TP, nitrate, N loading and *Dreissena*,  
711 by NGL and SGL. Color values reflect relative change in abundance (standardized indicator  
712 values, or z-scores) for each species and stressor, ranging from strongly responding sensitive or  
713 declining (green) to tolerant (red) species. Species overlaps across stressors are shown using



714 cluster analysis, which highlights groups of species with similar responses, e.g. right-most four  
715 columns are a cluster of species declining in response to nitrate/increasing in response to  
716 *Dreissena* (cluster numbers match those referred to in the text). Each column is a result of a  
717 separate TITAN analysis. Note that standardization is within-test (within-column), so  
718 comparison of slight variations in color intensity (i.e. strength of and specificity of response) is  
719 meaningful within each column but less so across columns. See Figs. 5-6 for change-point  
720 locations for individual taxa. Horizontal axes stressor abbreviations are a combination of stressor  
721 ( $\text{NO}_x$  – water nitrate concentrations, Nload – N loading, TP – *in situ* total phosphorus, Dreis –  
722 *Dreissena* densities), season (spr – spring, sum – summer) and region (N –NGL, S – SGL).  
723 Zoomable version is available as SI Fig. 2.

724

725

## 726 **Supplemental Information**

727

728 SI Fig. 1. Plot of community-level sums of taxon-specific change points along nitrate, TP and  
729 *Dreissena* gradients.

730 SI Fig. 2. Zoomable heatmap of species responses to stressors.

731

732 SI Table 1. Details of ANCOVA model

733 SI Table 2. Summary of statistical comparisons for TP and species richness

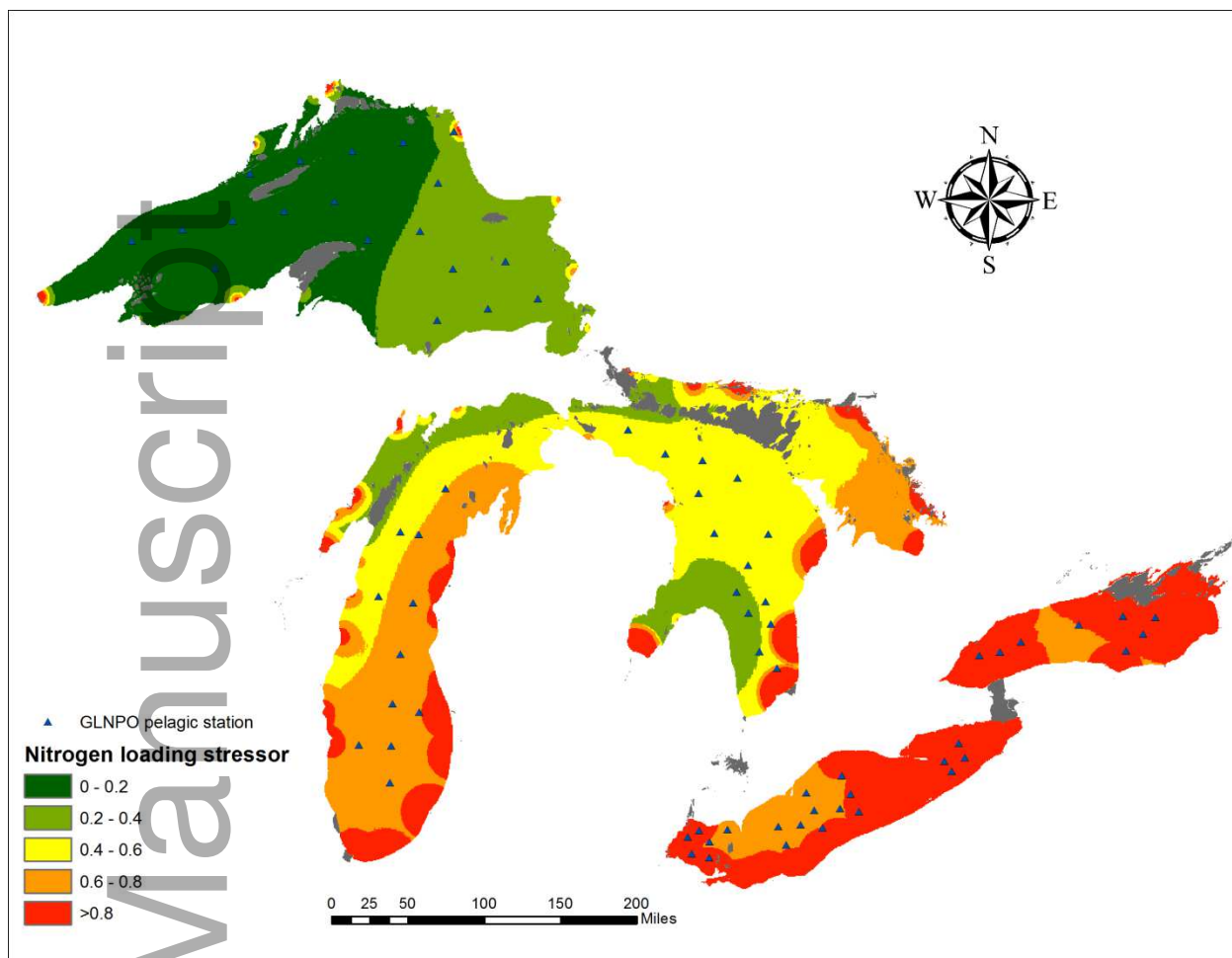
734 SI Table 3. List of lake-specific species

735 SI Table 4. Summary of phytoplankton change-point locations with respect to all stressors

736 SI Table 5. Species codes, Latin names and reference sources

737 Fig. 1

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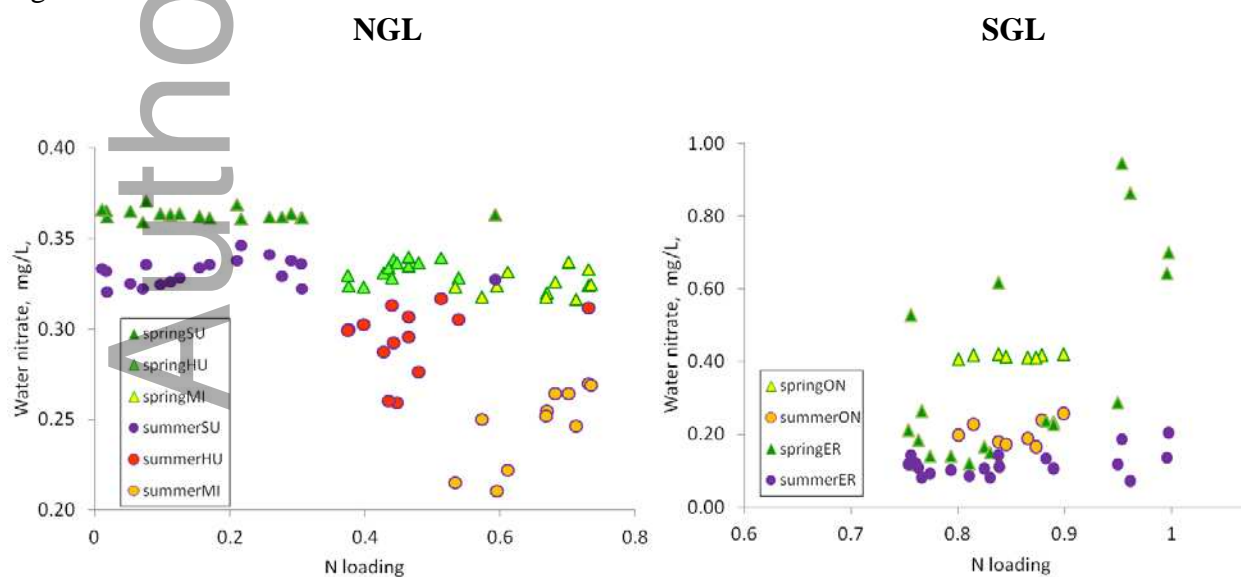
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742 Fig. 2

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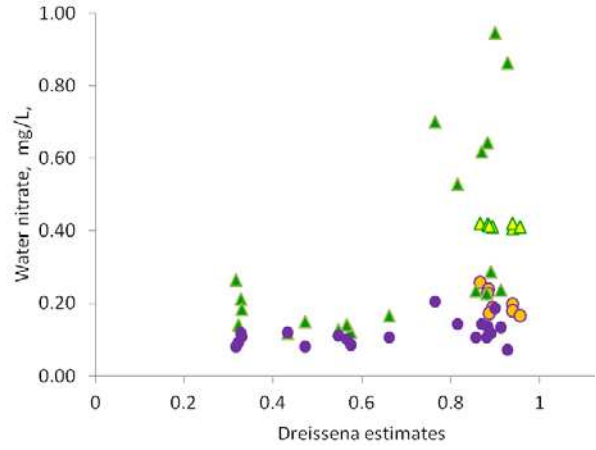
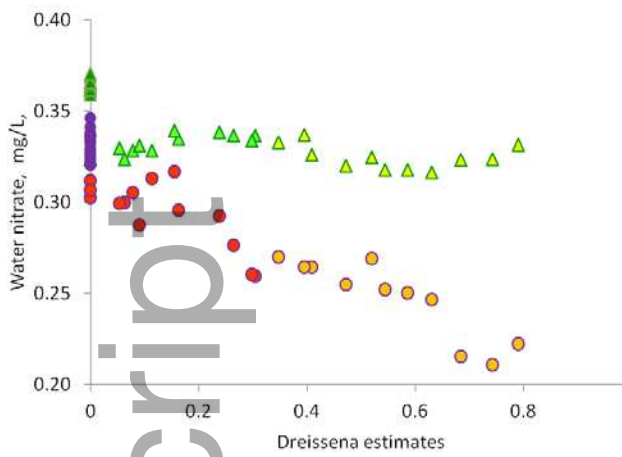
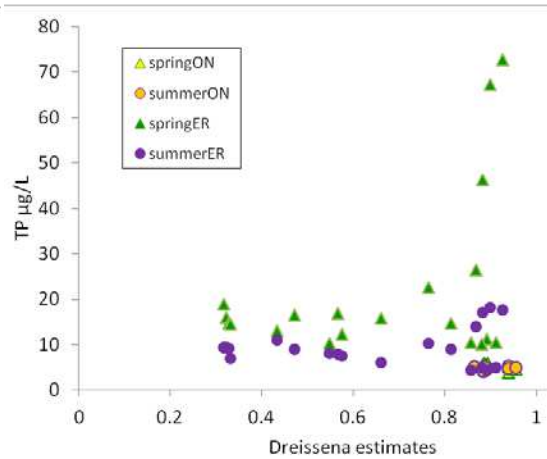
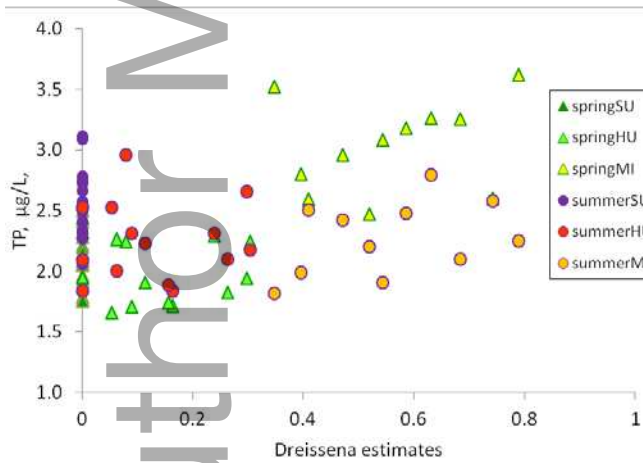


Fig. 3

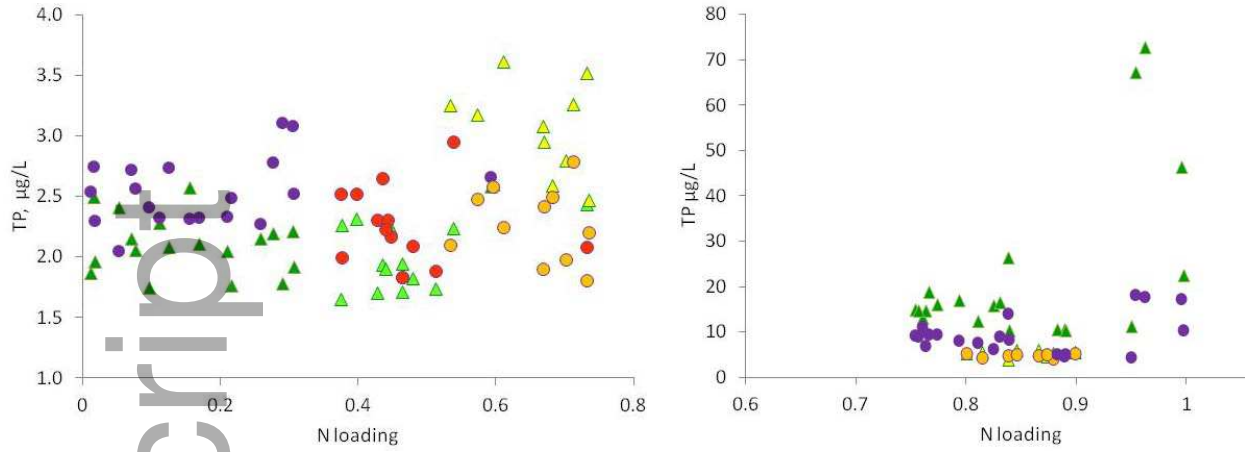
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**SGL**



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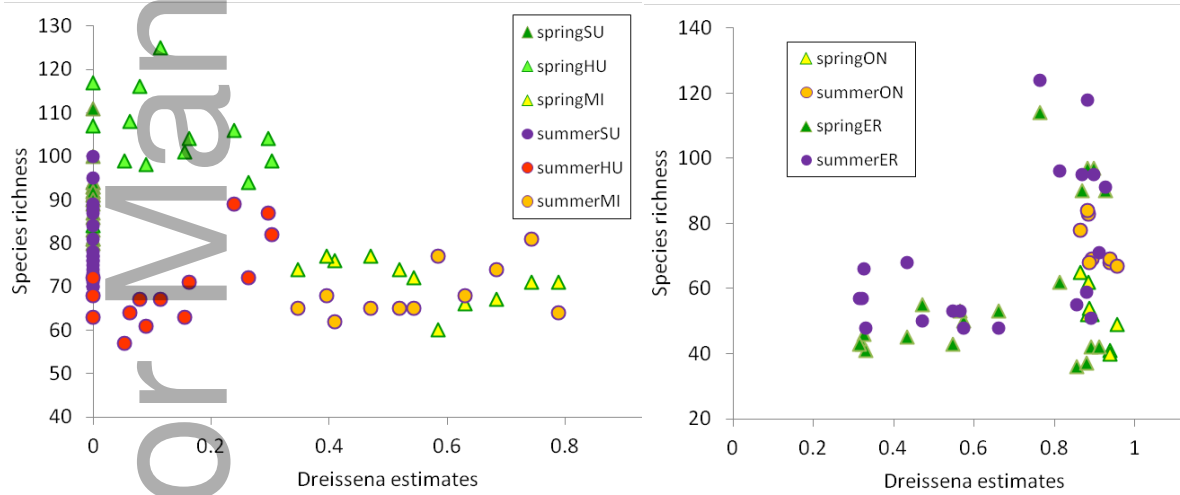


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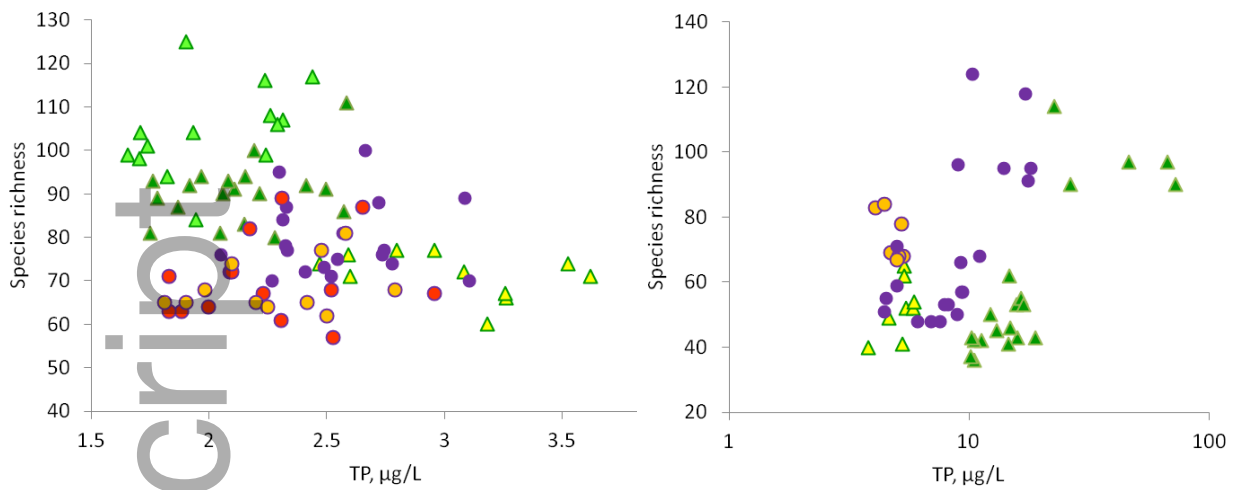
Fig. 4

**NGL**

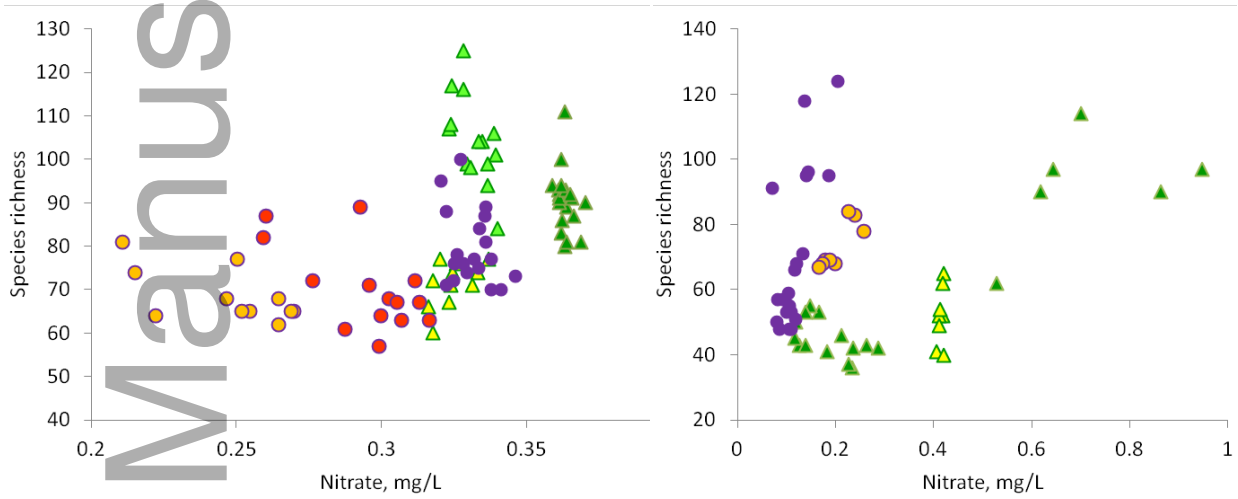
**SGL**



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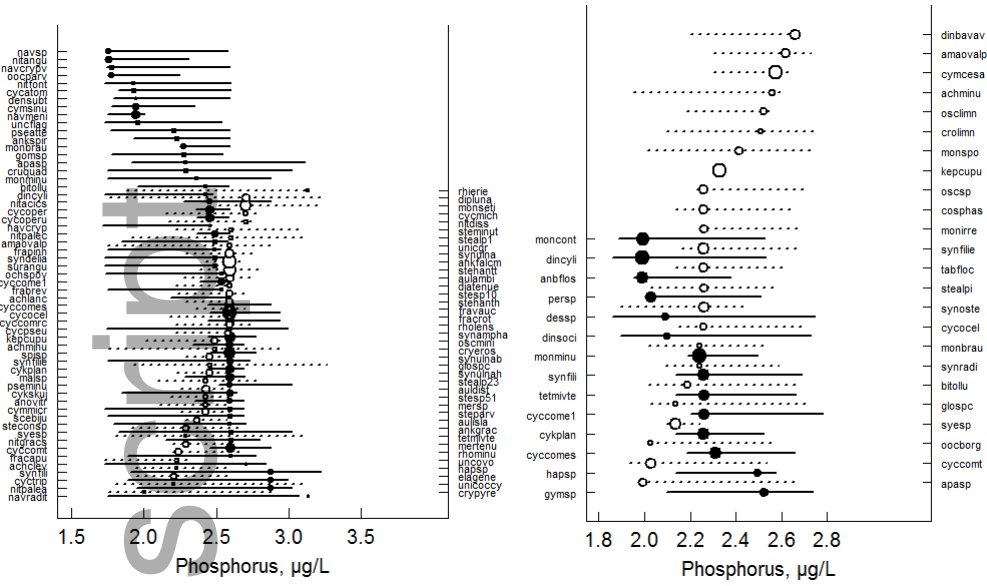
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766 Fig. 5

767 a)

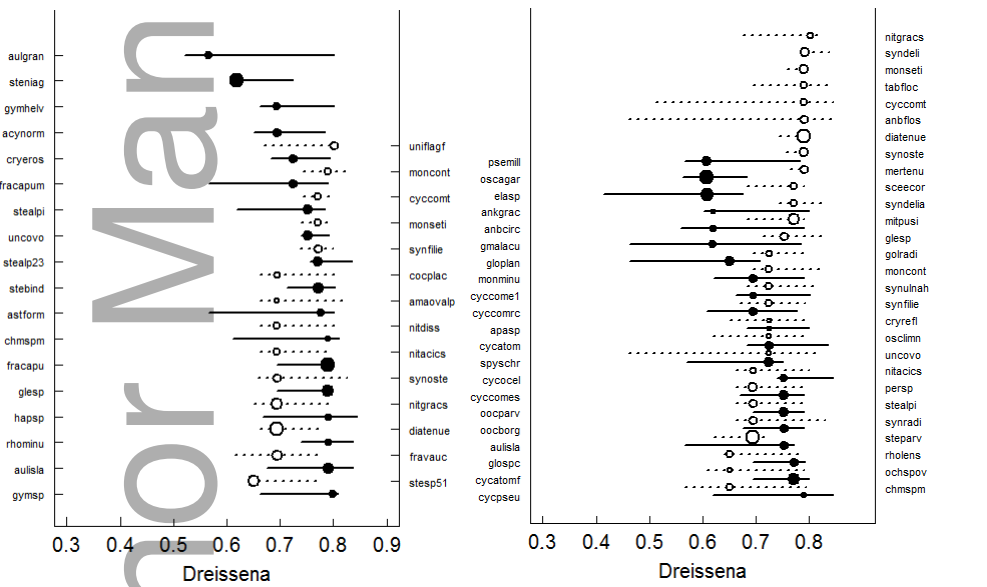
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771 Fig. 6. SGL



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