Characterizing and predicting cyanobacterial blooms in an 8-year amplicon sequencing time-course

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Summary

Cyanobacterial blooms occur in lakes worldwide, producing toxins that pose a serious public health threat. Eutrophication caused by human activities and warmer temperatures both contribute to blooms, but it is still difficult to predict precisely when and where blooms will occur. One reason that prediction is so difficult is that blooms can be caused by different species or genera of cyanobacteria, which may interact with other bacteria and respond to a variety of environmental cues. Here we used a deep 16S amplicon sequencing approach to profile the bacterial community in eutrophic Lake Champlain over time, to characterize the composition and repeatability of cyanobacterial blooms, and to determine the potential for blooms to be predicted based on time-course sequence data. Our analysis, based on 143 samples between 2006 and 2013, spans multiple bloom events. We found that the microbial community varies substantially over months and seasons, while remaining stable from year to year. Bloom events significantly alter the bacterial community but do not reduce overall diversity, suggesting that a distinct microbial community – including non-cyanobacteria – prospers during the bloom. Blooms tend to be dominated by one or two genera of cyanobacteria: *Microcystis* or *Dolichospermum*. Blooms are thus relatively repeatable at the genus level, but more unpredictable at finer taxonomic scales (97% operational taxonomic units; OTUs). We therefore used probabilistic assemblages of OTUs (rather than individual OTUs) to classify our samples into bloom or non-bloom bins, achieving up to 92% accuracy (86% after excluding cyanobacterial sequences). Finally, using symbolic regression, we were able to predict the start date of a bloom with 78-91% explained variance over tested data (depending on the data used for model training), and found that sequence data was a better predictor than environmental factors.

Introduction

Cyanobacterial blooms occur in freshwaters around the world, and are both a nuisance and a public health threat (Zingone and Enevoldsen, 2000; Paerl *et al*., 2013). These blooms are defined by a massive accumulation of cyanobacterial biomass, generally formed through growth, migration, and physical–chemical forces (Paerl, 1996). In temperate eutrophic lakes, blooms tend to occur annually, specifically during the summer when water temperatures are warmer (Kanoshina *et al*., 2003, Havens, 2008). In the context of accelerated eutrophication due to climate change and increased nutrient input from human activities (O'Neil *et al*., 2012; Winder, 2012), the frequency and intensity of these blooms is increasing over time (Johnson *et al*., 2010; Posch *et al*., 2012). Attempts have been made to predict blooms using mathematical models based on environmental parameters (Recknagel *et al*., 1997; Downing *et al*., 2001; Oh *et al*., 2007). Nevertheless, these models have been limited in their ability to accurately predict cyanobacterial dynamics (Downing *et al*., 2001; Taranu *et al*., 2012), perhaps because blooms can be composed of various species or genera of cyanobacteria. These species or genera may interact with other bacteria (Eiler and Bertilsson, 2004) and respond to different environmental cues, resulting in different temporal and biological dynamics.

Recent studies have shown that many aquatic microbial communities are temporally dynamic (Kara et al., 2013; Fuhrman *et al*., 2015), often with predictable patterns of community structure (Fuhrman *et al*., 2006; Fuhrman *et al*., 2015). For example, seasonal variation in microbial community composition is greater in surface waters, reflecting the seasonal changes in the environment (Gilbert *et al*., 2009; 2012). Seasonality appears to be a common feature of many aquatic microbial environments, often with long-term stability of the microbial community (Shade *et al*., 2007; Kara *et al*., 2013; Cram *et al*., 2015; Fuhrman *et al*., 2015). A one-year study

71 in the eutrophic Lake Taihu, where cyanobacterial blooms occur frequently, suggested a
72 periodicity in community structure (Li *et al*., 2015). However, as highlighted by Fuhrman *et al*.,
73 (2015) data should be collected over several consecutive years to properly identify bacterial
74 dynamics, and to assess if community structure follows a predictable seasonal pattern.

75 Temporal dynamics of bacterial communities in eutrophic lakes are poorly known, and
76 the impact of cyanobacterial blooms on these dynamics remain unclear. Blooms are likely to have
77 a major impact on microbial community composition and dynamics, through both direct (*e.g*.
78 microbe-microbe interactions) and indirect effects (*e.g*. changes to lake chemistry). Intense
79 cyanobacterial blooms could reduce carbon dioxide, increase pH to extreme levels, and alter the
80 distribution of biomass across the length and depth of a lake (da Rosa *et al*., 2005; Huisman *et*
81 *al*., 2005, Havens, 2008). Such bloom-induced changes in water chemistry could then impact the
82 structure and diversity of microbial communities (Bouvy *et al*., 2001; Eiler and Bertilsson, 2004;
83 Bagatini *et al*., 2014; Li *et al*., 2015). Therefore, identifying repeatable patterns in bacterial
84 community dynamics and characterizing the ecological successions pre- and post-bloom are
85 needed to determine if blooms can be predicted based on bacterial community structure.

86 Here, we present an 8-year time-course study of the bacterial community structure of a
87 large eutrophic North American lake, Lake Champlain, where cyanobacterial blooms are
88 observed nearly every summer. Samples were collected from 2006 to 2013 and analyzed using
89 high-throughput 16S amplicon sequencing. We tracked the bacterial community composition in
90 143 time-course samples to determine how the community varies over time and how it is
91 influenced by cyanobacterial blooms. We then asked to what extent the bloom is repeatable and
92 predictable based on amplicon sequence data. As expected, we find that blooms are highly
93 seasonal, but surprisingly, they do not reduce non-cyanobacterial diversity. Blooms in this lake
94 are consistently dominated by two cyanobacterial genera, *Microcystis* and *Dolichospermum*, but

95 are much less repeatable at finer taxonomic scales. Although it is clear that large-scale events like
96 climate change, droughts, floods, and the associated changes in nutrient inputs, are impacting the
97 prevalence of blooms over seasons and decades, we demonstrate that community sequence
98 surveys can provide predictions over finer time scales of weeks or months.
99
100
101 **Materials and Methods**
102
103 **Sampling**
104 A total of 150 water samples were collected from the photic zone (0-1 meter depth) of
105 Missisquoi Bay, Lake Champlain, Quebec, Canada (45°02'44.86"N, 73°07'57.60"W). Between 12
106 and 27 (median 17) samples were collected each year between 2006 and 2013. Samples were
107 taken from both littoral (78 samples) and pelagic (72 samples) zones. Between 50 and 250 ml of
108 lake water was filtered depending on the density of the planktonic biomass using 0.2-µm
109 hydrophilic polyethersulfone membranes (Millipore). Physico-chemical measurements, as
110 described in Fortin *et al*. (2015), were also taken during most sampling events. These
111 environmental data included water temperature, average air temperature over one week,
112 cumulative precipitation over one week, microcystin toxin concentration, total and dissolved
113 nutrients (phosphorus and nitrogen).
114
115 **DNA extraction, purification and sequencing**
116 DNA was extracted from frozen filters by a combination of enzymatic lysis and phenol-
117 chloroform purification as described by Fortin *et al*. (2010). Each DNA sample was resuspended
118 in 250 µl of TE (Tris-Cl, 10 mM; EDTA, 1 mM; pH 8) and quantified with the PicoGreen®

119 dsDNA quantitation assay (Invitrogen). DNA libraries for paired-end Illumina sequencing were
120 prepared using a two-step 16S rRNA gene amplicon PCR as described in Preheim *et al*. (2013).
121 We amplified the V4 region, then confirmed the library size by agarose gels and quantified DNA
122 with a Qubit v.2.0 fluorometer (Life Technologies). DNA libraries were pooled and denatured as
123 described in the Illumina protocol. We performed two sequencing runs using MiSeq reagent Kit
124 V2 (Illumina) on a MiSeq instrument (Illumina). Each run included negative controls and two
125 mock communities composed of 16S rRNA clones libraries from other lake samples (Preheim *et*
126 *al*., 2013). Details of the library preparation protocol are described in Supplementary Methods.
127

128 **Sequence analysis and OTU picking**

129 Sequences were processed with the default parameters of the SmileTrain pipeline
130 (https://github.com/almlab/SmileTrain/wiki/) that included chimera filtering, paired-end joining
131 and, de-replication. *De novo* distribution-based clustering using the dbOTUcaller algorithm
132 (Preheim *et al*., 2013) (https://github.com/spacocha/dbOTUcaller), which is also included in
133 SmileTrain, was performed to cluster sequences into Operational Taxonomic Units (OTUs) by
134 taking into account the sequence distribution across samples. The OTU table generated was then
135 filtered using QIIME (Caporaso *et al*., 2010) (version 1.8, http://qiime.org/) scripts to remove
136 OTUs observed less than 10 times and minimize false OTUs. Seven samples with less than 150
137 sequences were removed from the OTU table, yielding a final dataset of 143 samples. Taxonomy
138 was assigned with the 97% reference OTU collection of the GreenGenes database release 13_8
139 (http://greengenes.lbl.gov) using QIIME and biom-metadata scripts (http://biom-format.org/). We
140 removed OTUs that were not prokaryotes but still present in the database (Cryptophyta,
141 Streptophyta, Chlorophyta and Stramenopiles orders). Overall, 7,349,035 sequences were
142 obtained from our 143 lake samples, which were clustered into 4069 OTUs (excluding mock

143 communities and controls).

144 To evaluate the quality of the pipeline used, we compared the number and identity of
145 OTUs obtained for a mock community using another approach based on reference clustering
146 (QIIME: pick_open_reference_otus.py). In this case, sequences were processed using illumina-
147 utils (https://github.com/meren/illumina-utils) with the --enforce-Q30-check option to enforce
148 sequence quality control. Chimeras were removed using QIIME scripts and USEARCH61.
149 SmileTrain (using the dbOTUcaller algorithm) recovered 100% of the expected OTUs in the
150 mock community, and suffered from fewer false positive OTUs than the QIIME script "pick open
151 reference otus" (Table S1).

152

153 **Diversity analysis**

154 To calculate the alpha diversity, indexes known for their robustness to sequencing depth variation
155 were used: Shannon (Shannon and Weaver, 1949) and Based-Weighted-abundance Phylogenetic
156 Diversity (BWPD) (McCoy and Matsen IV, 2013). To assess the impact of variable sequencing
157 depth on these diversity measures, rarefaction curves were made with multiple rarefactions from
158 the lowest to the deepest sequencing depth, at intervals of 3000 sequences, with replacement and
159 100 iterations (Fig S1). Alpha diversity was then calculated using the mean of the 100 iterations
160 of the deepest sequencing depth for each sample. This approach was used to avoid losing data,
161 and to estimate alpha diversity as accurately as possible. The Shannon index, which accounts for
162 both OTU richness and evenness, was calculated using QIIME. The BWPD index that captures
163 both the phylogeny (summed branch length) and the abundance species was calculated using the
164 guppy script with *fpd* subcommand
165 (http://matsen.github.io/pplacer/generated_rst/guppy_fpd.html). The phylogenetic tree was
166 generated using FastTree 2.1.8 (Price *et al*., 2009) (http://meta.microbesonline.org/fasttree/).

167 To calculate the beta diversity between groups of samples (*e.g*. months or seasons), we
168 used a non-rarefied OTU table to calculate two metrics that are robust to sequencing depth
169 variation: weighted Unifrac (Lozupone and Knight, 2005) and Jensen-Shannon divergence (JSD)
170 (Fuglede and Topsoe 2004; Preheim *et al*., 2013). We used the Phyloseq R package (McMurdie
171 and Holmes, 2013) (https://joey711.github.io/phyloseq/) to first transform the OTU table into
172 relative abundance, then to calculate the two different metrics and finally to generate principal
173 coordinates analysis (PCoA) and Nonmetric multidimensional scaling (NMDS) plots.
174 Differences between groups (*e.g*. bloom vs. non-bloom samples) in term of community structure
175 were tested using: (i) analysis of similarity using the anosim() function (Clarke, 1993); (ii) and
176 permutational multivariate analysis of variance (PERMANOVA)(Anderson, 2001) with the
177 adonis() function. The adonis test can be sensitive to dispersion, so we tested for dispersion in the
178 data by performing an analysis of multivariate homogeneity (PERMDISP) with the permuted
179 betadisper() function (Anderson, 2006). In our analysis, we observed a significant dispersion
180 effect in most of the beta diversity analyses that included cyanobacteria. Nevertheless, this effect
181 disappeared when we removed this phylum, meaning that the cyanobacterial community was
182 mainly responsible for the differences in dispersion between groups. The ANOSIM test was
183 performed to determine the degree of difference in community composition between groups. If
184 the anosim() function returns an R value of 1, this indicates that the groups do not share any
185 members of the bacterial community. PERMANOVA, PERMDISP and ANOSIM were
186 performed using the vegan package (Oksanen, 2005), with 999 permutations. Beta diversity
187 analyses were also performed using a rarefied OTU table (rarefied to 10,000 reads per sample)
188 and similar results were observed (data not shown).
189
190 **Rhythmicity and seasonality in community structure**

191 To track changes in community composition over time, we first calculated the Bray-Curtis
192 dissimilarity between all pairs of samples. Bray-Curtis is sensitive to sequencing depth variation
193 so we used OTU tables rarefied to 10,000 reads. We then plotted the mean dissimilarity of
194 samples versus the amount of time separating the samples. Rhythmicity of each OTU over years
195 was also analyzed using JTK-CYCLE (https://github.com/alanlhutchison/empirical-
196 JTK_CYCLE-with-asymmetry/blob/master/jtk7.py) as described in Hutchinson *et al*. (2015)
197 using season as the period, and a cosine waveform. We define seasons as the calendar seasons
198 (*e.g*. summer spans June 21 to September 20). Rhythmicity is defined as the likelihood of
199 observing a correlation between a reference waveform (generated previously by Hutchinson *et*
200 *al*.) and the relative abundance of an OTU over time. OTUs with a Q-value under 0.05 after
201 Benjamini-Hochberg correction were considered rhythmic. In order to avoid any possible bias
202 due to sequencing depth variation, we used littoral (3419 OTUs) and pelagic (3306 OTUs) OTU
203 tables rarefied to 10,000 reads.
204
205 **Taxa-environment relationships**
206 To investigate taxa-environment relationships, we performed a redundancy analysis
207 (RDA) with community matrices standardized by Hellinger transformation (Legendre and
208 Gallagher, 2001) as response variables to determine the best set of environmental variables that
209 relates with the bloom community structure. Environmental matrix variables were composed of
210 total phosphorus in μg/L (TP), total nitrogen in mg/L (TN), dissolved phosphorus in μg/L (DP),
211 dissolved nitrogen in mg/L (DN), 1-week-cumulative precipitation in mm, 1-week-average air
212 temperature in Celsius and microcystin concentration in μg/L. These data were log-transformed
213 and standardized using the decostand() function. The collinearity between environmental
214 variables was first tested by calculating variance inflation factors using the corvif() function

215 (Zuur *et al*., 2009). From this test, we concluded that all environmental variables could be used in
216 the RDA analysis. Environmental parameters were then pre-selected using the adonis() function.
217 RDA was performed using the rda() function (Legendre and Legendre, 1998) and with only the
218 environmental variables that were found to be highly significant ($p<0.01$). PERMANOVA, RDA
219 and standardization were performed using the vegan package, with 9,999 permutations. The low
220 variance explained by the RDA suggests that a horseshoe effect is unlikely to be a source of bias
221 in our analyses.
222

223 **Differential OTU abundance analysis**

224 We first used LEfSe version 1.0 (Segata *et al*., 2011) with modified parameters
225 (normalization value of 1,000,000; minimum linear discriminant analysis score of 4.0; 100
226 bootstraps for linear discriminant analysis) on filtered genera tables (we removed taxa with a
227 relative abundance of less than 0.1 after summing all the samples) to identify genera associated
228 with the blooms. We repeated the same analysis with an LDA score of 2.5 to expand the list of
229 bloom biomarkers. Taxa with a Q-value under 0.05 after false discovery rate (FDR) correction
230 were considered biomarkers.
231

232 **Heatmaps**

233 We normalized the OTU table using metagenomeSeq's CSS approach (Paulson *et al*.,
234 2013). Then we measured the following ratio: $Mean(Xi)_{bloom} \div Mean\ (Xi)_{no_bloom}$ where Xi is
235 the relative abundance of one OTU or one genus. We used the heatmap2() R function (Warnes *et*
236 *al*., 2015) (gplots R package) to generate heatmaps associated with a hierarchical cluster analysis
237 at the OTU and genus levels.
238

239 **Bloom classification**

240 To classify bloom and non-bloom samples, we used the Bayesian inference of microbial
241 communities (BIOMICO) model described by Shafiei *et al*., (2015). This supervised machine
242 learning approach infers communities based on microbial assemblages. We defined the bloom
243 here as an environmental parameter for samples that showed a cyanobacterial relative abundance
244 higher than 20%, above which the Shannon diversity begins to decline (Figure S2). We trained
245 the model with two different approaches: (i) with 2/3 of the total data, selected at random, and (ii)
246 with two distinctive years: 2007, a year with only a short-lived fall bloom, and 2009, a year with
247 a very significant bloom. In the training stage, BIOMICO learns how OTU assemblages
248 contribute to community structure, and what assemblages tend to be present during blooms. In
249 the testing stage, the model classifies the rest of the data (not used during training), and we assess
250 accuracy as the percentage of correctly classified samples.

251

252 **Bloom prediction**

253 We attempted to predict the timing of blooms using sequence data. As many OTUs or
254 genera may have such low abundances that they might be missed in some samples, and might
255 also increase the probability of finding spurious correlations, we pre-filtered the OTU table by
256 removing taxa with summed relative abundances (over the 143 samples) lower than 0.1. Our goal
257 was to predict the timing until the next bloom, using sequencing and/or environmental data from
258 before a bloom event. Samples taken during a bloom were not used in these analyses. We defined
259 the time (in days) from each non-bloom sample to the next bloom of the year as the response
260 variable. In these analyses, we used either OTUs, genera, OTUs combined with metadata, or
261 genera combined with metadata as predictor variables. We also calculated the trend in all
262 predictor variables from one sample to the next by subtracting the latter values from the former

263 and dividing by the number of days that separated both sample dates. In this way, we obtained a
264 trend value for each predictor variable.

265 Genetic programming, namely in the form of symbolic regression (SR) (Koza, 1992), is a
266 particular derivation of genetic algorithms that searches the space of mathematical equations
267 without any constraints on their form, hence providing the flexibility to represent complex
268 systems, such as lake microbial communities. Contrary to traditional statistical techniques,
269 symbolic regression searches for both the formal structure of equations and the fitted parameters
270 simultaneously (Schmidt and Lipson 2009). Using SR, (Cardoso *et al*. 2015) we were able to
271 "distill" free-form equations and models that consistently outperformed and were more
272 intelligible than the ones resulting from rigid methods such as GLM or "black-boxes" such as
273 maximum entropy or neural networks. We used the software Eureqa
274 (http://www.nutonian.com/products/eureqa/) to implement SR, using 75% of the data for model
275 training and 25% for testing. As building blocks of the equations we used all predictor variables
276 (including trends), random constants, algebraic operators (+, –, ÷, ×) and analytic function types
277 (exponential, log and power). Given the inherent stochasticity of the process, ten replicate runs
278 were conducted for each analysis. All runs were stopped when the percentage of convergence
279 was 100, meaning that the formulas being tested were similar and were no longer evolving. Each
280 run produces multiple formulas along a Pareto front (see Cardoso *et al*. 2015.). For each formula,
281 we calculated the Akaike information criterion (AIC) and the corrected AIC for small sample
282 sizes. Formulas with the lowest AICs for each analysis were retained.

283

284 **Statistical analysis**

285 R version 3.1.3 (http://www.r-project.org/) and IBM SPSS version 22 were used for all
286 subsequent analysis.

287

288 **Results**

289

290 *Rhythmic seasonal dynamics*

291 To survey microbial diversity over time, we sequenced each of the 143 lake samples to an
292 average depth of 51,392 reads per sample and clustered the sequences into 4,069 operational
293 taxonomic units (OTUs). We first asked how the lake microbial community varied over time by
294 comparing diversity at different time scales: days, months and years. Overall levels of microbial
295 diversity were stable over time. No significant differences in alpha diversity (either Shannon or
296 BWPD) were observed between years, and only slight differences were observed between months
297 or seasons (Figure S3, Table S3).

298 Taxonomic richness and evenness (alpha diversity) can remain stable despite significant
299 changes in taxonomic composition (beta diversity) of the community. To track changes in beta
300 diversity over time, we compared the community composition by calculating the Bray-Curtis
301 dissimilarity between samples separated by increasing amounts of time. We observed an
302 oscillating pattern without any upward or downward trend, suggesting long-term stability of the
303 community composition (Figure 1). We also noted that the bacterial community could change
304 very quickly – in less than one week (Figure S4). Over longer time scales (Figures 1 and S5),
305 Bray Curtis dissimilarity clearly oscillates, reaching a plateau with an average between 0.5 and
306 0.75. This rhythmic pattern suggests that the community is dynamic over time, yet it does not
307 diverge without bounds: we did not observe any tendency for the community to become more
308 dissimilar over time, suggesting a long-term stability of the bacterial community on the time
309 scale of years in both the littoral and pelagic sampling sites (Figure S6). Bacterial taxonomic

310 composition was highly similar between years, for both littoral and pelagic samples (Weighted
311 Unifrac: ANOSIM, $R<0.1$, $P<0.01$; PERMANOVA, $R^2=0.011$, $P>0.05$).

312 To identify bacterial taxa that might explain the rhythmic pattern, we measured the
313 rhythmicity of each OTU by fitting its abundance over time to wave functions (Methods). After
314 correcting for multiple tests, we found that 951 out of 3,419 OTUs (28%) were significantly
315 rhythmic in the littoral zone, and 718 out of 3,306 OTUs in the pelagic zone (22%). This result
316 suggests that a substantial fraction of OTUs are rhythmic.

317 We next asked if the rhythmicity could be due to seasonal changes in community
318 structure. Indeed, samples that belong to the same season cluster significantly together (Figure 2;
319 PERMANOVA, $R^2=0.157$, $P<0.001$). We also tested changes in community composition at time
320 scales of months and years (Table S2, Figures S7 and S8). The PERMANOVA R^2 for months
321 was highest (Table S2), meaning that monthly dynamics provide the most relevant time scale of
322 variance in community structure. On the contrary, years were not significantly different from one
323 another (PERMANOVA, $R^2=0.011$, $P>0.05$). As seasonality is associated with environmental
324 changes, we sought to determine why months were the most explanatory temporal variable. We
325 compared the concentrations of TP and TN over months and seasons and found that both
326 environmental factors vary significantly by month but neither varies by season (Figure S9).
327 Therefore, months appear to be the best temporal predictor because it captures most of the
328 variation in environmental variables.

329 Lake Champlain is a eutrophic lake where cyanobacterial blooms are observed almost
330 every summer. To determine if the observed seasonal pattern (Figure 2) was driven by
331 cyanobacterial blooms, we repeated the beta diversity analysis after removing all cyanobacterial
332 sequences. A significant clustering by month and season was also observed without
333 cyanobacteria (Table S2). These results indicate that seasonality is not entirely driven by

334 cyanobacterial blooms. Rather, the entire bacterial community is involved in seasonal changes.
335 Together, these results show how the community – including both cyanobacteria and other
336 bacteria – is seasonal over months and seasons, but stable over years.
337
338 *Blooms change community composition without reducing diversity*
339 The observation that the whole community, not just cyanobacteria, changes seasonally
340 suggests that cyanobacterial blooms might impact the diversity and community composition of
341 other lake bacteria. To assess the impact of the bloom on the microbial community, we first
342 needed to define bloom events. A bloom is generally defined as a dramatic increase in the
343 abundance of cyanobacteria above a specific cell density. The World Health Organization
344 (WHO) has proposed three different guidelines to connect blooms to potential health risks. The
345 first level (low health risk probability) is set at 20,000 cyanobacterial cells/mL (WHO,
346 Guidelines for safe recreational water environments, 2003). We estimated the relative abundance
347 of cyanobacteria based on 16S rRNA gene amplicon data, which was significantly correlated
348 with *in situ* cyanobacterial cell counts from a limited number of samples (Figure S10; R^2=0.336;
349 $F_{1,29}$, P<0.001). We propose that a biologically relevant bloom definition should reflect the
350 impact of cyanobacteria on the microbial community, as well as the risk for human health. We
351 observed that increasing cyanobacterial dominance was associated with a decline in alpha
352 diversity in the community, and drew a cutoff at 20% cyanobacteria. Above 20% cyanobacteria,
353 Shannon diversity begins to decline (Figure S2). We therefore used a 20% cutoff to bin our
354 samples into "bloom" or "no-bloom" (Table S4). In our samples, 20% cyanobacteria corresponds
355 to approximately 3450 +/- 1509 cells/mL (Figure S10), which is below the bloom threshold set
356 by WHO but appears to be biologically relevant, given the decline in community diversity.
357 We found that bloom samples had significantly higher phylogenetic diversity (BWPD)

358 compared to no-bloom samples (Figure 3A). In contrast, there is reduced taxonomic (Shannon)
359 diversity in bloom samples (Figure 3B). These result suggests that cyanobacterial blooms lead to
360 (i) an increase in phylogenetic diversity by adding additional, relatively long cyanobacterial
361 branches to the phylogeny, and (ii) a decrease of Shannon diversity due to the dominance of
362 cyanobacteria, reducing taxonomic evenness. However, when we repeated the same analysis after
363 removing all cyanobacterial OTUs, we found that blooms did not alter the diversity of the
364 remaining (non-cyanobacterial) community (Figure 3C and D). Thus, blooms decrease
365 community diversity by increasing the amount of cyanobacteria, but not by reducing the diversity
366 of other bacteria.

367 Despite their limited impact on non-cyanobacterial diversity, we found that blooms
368 clearly alter the community composition of the lake. In a beta diversity analysis, we found a
369 significant clustering of bloom and no-bloom samples (PERMANOVA, R^2=0.316; P<0.001),
370 meaning that bloom samples have a similar bacterial composition to one another (Figure 4A).
371 When we removed the Cyanobacteria counts from the OTU table (Figure 4B), we still observed a
372 significant clustering (PERMANOVA, R^2=0.059; P<0.001). We confirmed this observation using
373 another beta diversity distance, JSD (Table S2 and Figure S11). This result suggests that even
374 excluding Cyanobacteria (the bloom-defining feature), the bloom community still differs
375 significantly from the non-bloom community.

376

377 *Nutrient association with blooms*

378 A subset of our samples was associated with environmental measurements that might
379 explain bloom events. We performed an RDA analysis to identify environmental variables that
380 could explain the clustering of bloom and no-bloom communities, and found total nitrogen (TN),
381 total phosphorus (TP), microcystin concentration, and to a lesser extent dissolved phosphorus

382 (DP), to be most explanatory of the bloom (Figure 5; adjusted R^2=0.232; ANOVA, $F_{6,74}$=5.028,
383 P<0.001). DN and temperature explain less of the bloom variation and act in opposing directions,
384 perhaps because higher temperatures favour the growth of microbes that rapidly consume
385 dissolved nitrogen (Hong *et al*., 2014). The RDA results are consistent with many previous
386 studies describing the environmental factors responsible for blooms (Owens and Esaias, 1976;
387 Hecky and Kilham, 1988). For example, cyanobacterial growth is optimal at higher temperatures,
388 between 15 and 30°C (Konoka and Brock, 1978). Together, these environmental variables
389 explain 22.864% of the variation between bloom and no-bloom samples (axis 1: 16.539%; axis 2:
390 6.325%) suggesting that unknown physico-chemical or biological factors also play an important
391 role in the onset of blooms.
392
393 *Blooms are repeatably dominated by Microcystis and Dolichospermum*
394 To further explore potential biological factors involved in bloom formation, we attempted
395 to identify taxonomic biomarkers of bloom or no-bloom samples. To do so, we first performed a
396 LEfSe analysis to identify the genera that are most enriched in bloom samples. We found 30
397 significant biomarkers (LDA score > 4; Figure S12). As expected, the strongest bloom
398 biomarkers belonged to the phylum Cyanobacteria (Figure S12 and Table S5). The two strongest
399 genus-level biomarkers were *Microcystis* (Microcystacae) and *Dolichospermum* (Nostocaceae),
400 both genera of Cyanobacteria. These two bloom-forming genera are associated with lake
401 eutrophication (O'Neil *et al*., 2012) and are also known to produce cyanotoxins (Gorham and
402 Carmichael *et al*., 1979; Carmichael, 1981). We performed a more permissive LEfSe analysis to
403 identify genera associated with blooms (LDA score > 2.5; Table S5) and found 12 additional
404 biomarkers, including genera within the Pseudanabaenales and Cytophagales orders, previously
405 found to be associated with cyanobacterial blooms (Rashidan and Bird, 2001; O'Neil *et al*.,

406 2012). In summary, blooms tend to be dominated by one or two genera of Cyanobacteria:
407 *Microcystis* or *Dolichospermum* (Figures S12 and S13). Therefore, blooms seem to be quite
408 repeatable when viewed at the genus level.
409
410 *Blooms are less repeatable at finer taxonomic levels*
411 We next asked whether blooms were also repeatable at finer taxonomic scales, down to
412 the OTU level. Our OTU table contains 14 distinct *Microcystis* and 53 distinct *Dolichospermum*
413 OTUs. We calculated the "bloom ratio" of each cyanobacterial OTU as the ratio of its relative
414 abundance in bloom versus no-bloom samples, averaged within each year (Methods). Plotting a
415 heatmap with OTUs clustered according to their profile of bloom ratios over years revealed a
416 cluster of relatively repeatable bloom-associated OTUs (Figure 6, right panel). This cluster
417 contained all *Microcystis* OTUs (blue), and approximately half the *Dolichospermum* OTUs
418 (purple). These "relatively repeatable" OTUs were associated with the bloom in several, but not
419 all years. The "less repeatable" cluster of OTUs contained no *Microcystis,* several
420 *Dolichospermum*, and other cyanobacterial OTUs. Many of these OTUs were associated with the
421 bloom for only one or two years. In contrast, at the genus level, *Microcystis* and *Dolichospermum*
422 were associated with the bloom in nearly every year, with the exception of 2007, when no bloom
423 occurred (Figure 6, left panel). These results show that the bloom community is repeatable at the
424 genus level, but more unpredictable at finer taxonomic scales. Even within the dominant genera,
425 *Microcystis* OTUs were more consistently bloom-associated than *Dolichospermum* OTUs.
426
427 *Blooms can be accurately classified based on non-cyanobacterial sequence data*
428 Given the observation that bloom samples have distinct cyanobacterial and non-
429 cyanobacterial communities (Figure 4), we hypothesized that blooms could be classified based on

430 their bacterial community composition. We trained a machine-learning model (BIOMICO) on a
431 portion of the samples, and tested its accuracy in classifying the remaining samples (Methods).
432 BIOMICO was able to correctly classify samples with ~92% accuracy (Table 1). Such high
433 accuracy is expected because blooms are defined as having >20% cyanobacteria, so the model
434 should be able to easily classify samples based on cyanobacterial abundance. More impressively,
435 BIOMICO was able to classify samples with 83-86% accuracy after excluding cyanobacterial
436 sequences. This result supports the existence of a characteristic non-cyanobacterial community
437 repeatably associated with the bloom. Two different training approaches (Methods) yielded
438 similar classification accuracy (Table 1), but found different bloom-associated assemblages.
439 When we compared the best assemblages obtained with the two different trainings, focusing only
440 on the 50 best OTU scores, 11 OTUs were found in both trainings (Table S6). This result
441 suggests that data can be classified into bloom or no-bloom samples, but different assemblages
442 (containing different sets of OTUs) can be found with similarly high classification accuracy. This
443 is consistent with the general lack of repeatability of blooms at the OTU level (Figure 6, right
444 panel), but that there exist combinations of OTUs and higher-order taxa that are highly
445 characteristic of blooms.
446
447 *Blooms can be predicted by sequence data*
448 The existence of microbial taxa and assemblages characteristic of blooms suggests that
449 blooms could, in principle, be predicted based on amplicon sequence data. Although blooms can
450 be accurately classified based on sequence data (Table 1), we consider prediction to be a distinct
451 task: based on one sample, we wish to predict the number of days until a bloom occurs. We
452 therefore used symbolic regression (SR) to model the response variable "days until bloom" as a
453 function of OTU- or genus-level relative abundances, their interactions, and their trends over time

454 (Methods). To achieve true prediction, not simply classification, we used data collected prior to
455 each bloom event in order to predict the number of days until the bloom. We based our analysis
456 on 54 samples, ranging from 7 to 112 days before a bloom sample. Using OTUs or genera, we
457 were able to predict the timing of the next bloom event with 80.5% or 78.2% explained variance
458 on tested data, respectively (Table 2). Using a subset of 21 samples with a full complement of
459 environmental data, we were able to compare the predictive power of sequence data (OTU or
460 genus level) versus environmental data. The analysis based on 21 instead of 54 samples yielded
461 better predictions from both OTUs and genera (Table 2), possibly due to over-fitting. However,
462 bloom prediction based on genus-level sequence data clearly outperformed predictions based on
463 environmental data. Predictions based on OTU-level sequence data explained less variance,
464 consistent with OTUs being more variable and less reliable bloom predictors. Therefore,
465 sequence data appear are potentially more informative than environmental data in predicting
466 future blooms. One taxon – an unknown genus within the family Oxalobacteraceae, was
467 consistently found in every predictive formula (Table 2). We observed that Oxalobacteraceae are
468 significantly and negatively correlated with *Microcystis* and *Dolichospermum* (Figures S14, S15),
469 and positively associated with days until a bloom event (Figure S16).
470

471 **Discussion**

472 We used a deep 16S rRNA amplicon sequencing approach to profile the bacterial community in
473 Lake Champlain over eight years, spanning multiple cyanobacterial blooms. We found that the
474 microbial community varied over short time scales, oscillating from days to months (Figures 1,
475 S4 and S5). To explain this result, we found that two of the main environmental factors, TP and
476 TN, varied only among months, but not among seasons (Figure S9). However, on the long term,

477 community structure and diversity remained stable over years (Figures 1, S3 and S6). In
478 agreement with previous observations in eutrophic lakes (Shade *et al*., 2007), Lake Champlain
479 appears to return to a steady-state, despite dramatic bloom events. Various studies have already
480 shown temporal patterns in microbial communities (Kara et al., 2013; Fuhrman *et al*., 2015), but
481 ours does so in the context of cyanobacterial blooms. Blooms could potentially push the bacterial
482 community out of equilibrium and into a new steady-state; however, this does not appear to be
483 the case, suggesting that the lake bacterial community is relatively robust to perturbation by
484 blooms.

485 In contrast with an earlier time course study in another temperate lake, which found
486 increasing microbial diversity from spring to autumn (Kara et al., 2013), we observed relatively
487 stable diversity across seasons (Figure S3), and that cyanobacterial blooms were a major driver of
488 diversity (Figures 3 and 4). It was previously reported that intense blooms could temporarily
489 impact the microbial community, but these reports were based on a limited number of time points
490 (Bouvy *et al*., 1999; Li *et al*., 2015; Louati *et al*., 2015). In this study, we found that blooms
491 affected community diversity by increasing the relative amount of cyanobacteria, but not by
492 reducing the diversity of other bacteria. The diverse bloom-associated community is significantly
493 different from the non-bloom community, and could include bacteria that prey on or engage in
494 metabolic mutualism with Cyanobacteria (Paerl *et al*., 2001; Louati *et al*., 2015).

495 We confirmed that cyanobacterial blooms respond significantly to total phosphorus and
496 total nitrogen as previously described (Fogg, 1969; Jacoby *et al*., 2000; Paerl and Huisman, 2008;
497 Paerl and Huisman, 2009, Fortin *et al* 2015, Isles *et al*., 2015). Temperature was also an
498 important factor shaping the lake microbial community, as previously documented (Shade *et al*.,
499 2007). However, in this study, we observed that these predictors explained only a part of the
500 variation between bloom and no-bloom samples. Other predictors might include water column

501 stability and mixing, and the interactions of predictors, especially nutrients and temperature
502 (Taranu *et al*., 2012).

503 In addition to environmental factors, we showed that biological factors, in the form of
504 bacterial OTUs or genera, could also help to characterize the bloom. Using machine learning, we
505 were able to classify bloom samples with high accuracy based on microbial assemblages,
506 confirming that there is a specific microbial community associated with blooms. We identified
507 two bloom-forming Cyanobacteria, *Microcystis* and *Dolichospermum*, present in all bloom
508 assemblages (Table S6). Cyanobacterial blooms alter the local environment, likely altering the
509 surrounding microbial community (Louati *et al*., 2015). As a result, these assemblages likely
510 include bacteria that are reliant on cyanobacterial metabolites and biomass. For example, bloom
511 assemblages included potential cyanobacterial predators from the order Cytophagales and the
512 genus Flavobacterium (Table S6), both associated with bloom termination (Rashidan and Bird,
513 2001; Kirchman, 2002).

514 The bloom community composition was clearly repeatable at the genus level, with
515 *Microcystis* and *Dolichospermum* as main actors nearly every year (Figures 6 and S13). At finer
516 taxonomic levels, we observed much more variability in bloom-associated OTUs, meaning that
517 the OTU-level composition of blooms is more difficult to predict. It has been previously
518 demonstrated that some OTUs could be mostly rare, but abundant for short periods of time
519 (Shade *et al*., 2014). We hypothesized that many of the *Dolichospermum* OTUs were
520 conditionally rare, being bloom-associated in some years but not in others (Figure 6). *Microcystis*
521 OTUs, on the other hand, were more consistently bloom-associated, suggesting that the two
522 dominant bloom-forming genera might use different ecological strategies, or respond differently
523 to environmental or biological variables. OTUs within both *Microcystis* and *Dolichospermum*
524 may correspond to ecologically distinct species or ecotypes, which could be elucidated with

525 population genomics rather than single marker genes.

526 Finally, we show the potential for bloom events to be predicted based on amplicon
527 sequence data. We acknowledge that long-term environmental processes such as global warming,
528 and punctual seasonal events such as floods and droughts, are major determinants of whether a
529 bloom will occur in a given year. For example, no bloom occurred in 2007, likely due to a spring
530 drought which dramatically reduced nutrient run-off into the lake. However, sequence data might
531 be useful to predict bloom dynamics on shorter time scales of days, weeks or months. We
532 demonstrated that it is possible to use pre-bloom sequence data to predict the number of days
533 until a bloom event with good accuracy. Sequence data appears to be a strong predictor, similar
534 or better than prediction with environmental variables (Table 2). This shows that, although
535 blooms in Lake Champlain (and other temperate lakes) are clearly correlated with seasonality
536 (*i.e*. blooms occur mainly during summer, at warmer temperatures), the state of the microbial
537 community may contain more information than environmental factors alone about the likelihood
538 of an impending bloom. This could be because one microbial taxon contains information about
539 numerous environmental parameters, resulting in parsimonious predictive models based on a
540 small number of taxonomic biomarkers. This result is consistent with a recent study suggesting
541 that abiotic environmental factors could be crucial to initiate blooms, but that biotic interactions
542 might also be important in the exact timing and dominant members of the bloom (Needham and
543 Fuhrman, 2016).

544 Surprisingly, we never found cyanobacteria as a bloom predictor in any of the predictive
545 models (Table 2). This means that the models are not simply tracking a positive trend in
546 cyanobacterial abundance. Instead, we always found an Oxalobacteraceae genus in the predictive
547 equations, and this genus was negatively correlated with the two bloom-forming cyanobacterial
548 genera (Figures S14, S15). This result could be explained by an ecological succession between

549 the Oxalobacteraceae genus and *Microcystis*/*Dolichospermum*. The fact that Oxalobacteraceae
550 was chosen as a better predictor than Cyanobacteria suggests that Oxalobacteraceae begins to
551 decline before any detectable increase in Cyanobacteria, providing a potential early warning sign
552 (Figures S16).

553 We have shown that cyanobacterial blooms contain highly (but not exactly) repeatable
554 communities of Cyanobacteria and other bacteria. It appears that the community begins to change
555 before a full-blown bloom, suggesting that sequence-based surveys could provide useful early
556 warning signals. It remains to be seen to what extent bloom and pre-bloom communities – which
557 show repeatable dynamics within one lake – are also repeatable across different lakes, and to
558 what extent predictors could be universal or lake-specific.

559

560 **Data availability**

561

562 Raw sequence data and OTUs tables will be deposited in the Qiita database.

563

564 **Author information**

565 The authors declare no competing financial interest.

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730 **Figures and Tables**

731

732 **Figure 1. Bacterial community similarity over time.** We calculated the mean Bray-Curtis
733 dissimilarity of all pairs of samples separated by 1 to 30 days (first circle), 31 to 60 days (second
734 circle), and so on. This analysis was performed with samples taken from the littoral sampling site.
735 Error bars correspond to the standard deviation. A mean value close to 0 means that samples have
736 similar bacterial communities. The results for the pelagic samples are presented in Figure S5.

737

738 **Figure 2. Community composition differs by season.** Each point in the PCoA plot represents a
739 sample, with distances between samples calculated using weighted UniFrac as a measure of
740 community composition. Different shapes indicate different seasons. The same data plotted using
741 JSD as an alternative measure of community similarity are presented in Figure S8. As described
742 in the Methods section, we observed a significant dispersion effect in most of the beta diversity
743 analyses that included cyanobacteria. Nevertheless, this effect disappeared when we removed the
744 cyanobacterial phylum. This observation indicates that the cyanobacterial community was mainly
745 responsible for the differences in dispersion between groups (Table S2).

746

747 **Figure 3. Comparison of alpha diversity between bloom and no-bloom states.** Two alpha
748 diversity metrics were employed: the Shannon index and BWPD (Methods) to compare the alpha
749 diversity within samples that belong to bloom or no-bloom samples (A-B). We repeated the same
750 analysis after removing Cyanobacteria (C-D). Comparisons were performed using a Mann-
751 Whitney test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

752

753 **Figure 4. Bloom samples have similar community composition.** Each point in the PCoA plot

754 represents a sample, with distances between samples calculated using weighted UniFrac as a
755 measure of community composition. Bloom samples are shown with the black triangle, no-bloom
756 samples with the empty circle. (A) Samples with all OTUs included. (B) Samples excluding
757 OTUs from the phylum Cyanobacteria.
758
759 **Figure 5. Redundancy analysis of environmental predictors**. Environmental parameters were
760 preselected as potential predictors of the bloom (Adonis, $p<0.01$). TN, TP are the environmental
761 factors that best explain the bloom. Air temperature and DN are additional factors shaping the
762 microbial community.
763
764 **Figure 6. Bloom-associated OTUs and genera vary from year to year.** The heatmap is colored
765 according to the bloom ratio (the relative abundance of each taxon in bloom vs. no-bloom
766 samples), averaged within each year. Note that no bloom occurred in 2007. Taxa are clustered
767 according to their profile of bloom ratios across years. Left: cyanobacterial genera. Right:
768 cyanobacterial OTUs. Top row: Violet color indicates *Dolichospermum* taxa; blue color indicates
769 *Microcystis* taxa. Black color indicates the absence of the taxa.
770
771 **Table 1. Bloom classification results.** We used a supervised machine learning approach
772 (BioMico) to determine if samples can be classified into bloom bins based on microbial
773 assemblages (Methods). Accuracy was calculated as the percentage of correctly classified
774 samples (true positives + true negatives) relative to the total number of samples in the testing set.
775
776 **Table 2. Predicting bloom timing with symbolic regression (SR).** The best formula is shown
777 for each category of predictor variables. SR was performed on two datasets. First, OTUs and

genera were used as predictor variables, using the maximum number of non-bloom samples (N = 54). Second, in order to determine the impact of including environmental data as predictor variables, we used only samples with a full set of metadata (N = 21).

781 **Table 1.**

782

Training set	Testing set	Accuracy	False positives	False negatives	True negatives	True positives
2/3 of all samples	1/3 of all samples	91.84 %	4	0	33	12
2007 & 2009 samples	All other samples	92.52%	8	0	73	26
2/3 of all samples, without cyanobacteria	1/3 of all samples, without cyanobacteria	85.71%	6	1	31	11
2007 & 2009 samples, without cyanobacteria	All other samples, without cyanobacteria	83.18%	9	9	72	17

Table 2.

Predictor variables	Best response formula days to bloom=	R^2	Components	Number of samples used	Mean squared error	AIC	Corrected AIC
OTU	18.264 + 2179.337 × f__Cryomorphaceae|Unknown_genus|seq436 + 2007.048 × f__Oxalobacteraceae|Unknown_genus|seq413	0.805	4	54	117.540	265.406	266.222
Genera	19.780 + 2057.652× f_Oxalobacteraceae|Unknown_genus + 703.606 × f_Armatimonadaceae| Unknown_genus - 2599.909 × genus_Arcobacter-7598.106 × genus_Rickettsiella	0.782	6	54	131.134	275.316	277.103
OTU	15.941 + 49774.285 × trend(f_Cerasicoccaceae|Unknown_genus|seq548) + 2511.838 × f__Oxalobacteraceae|Unknown_genus|seq413	0.826	4	21	83.845	101.008	103.508
Genera	21.185 + 2646.333 × f_Oxalobacteraceae| Unknown_genus - 13323.212 × genus_Flavobacterium - 16288.058 × o_Ellin329| Unknown_genus	0.914	5	21	31.776	82.633	86.633
Environmental data	111.528 + 201.846 × trendMeanT + 149.714 × DN - 0.330 × TP - 6.867 × MeanT - 253.937 × DN × trendMeanT - 57.017 × DN^2	0.859	8	21	52.034	98.989	110.989

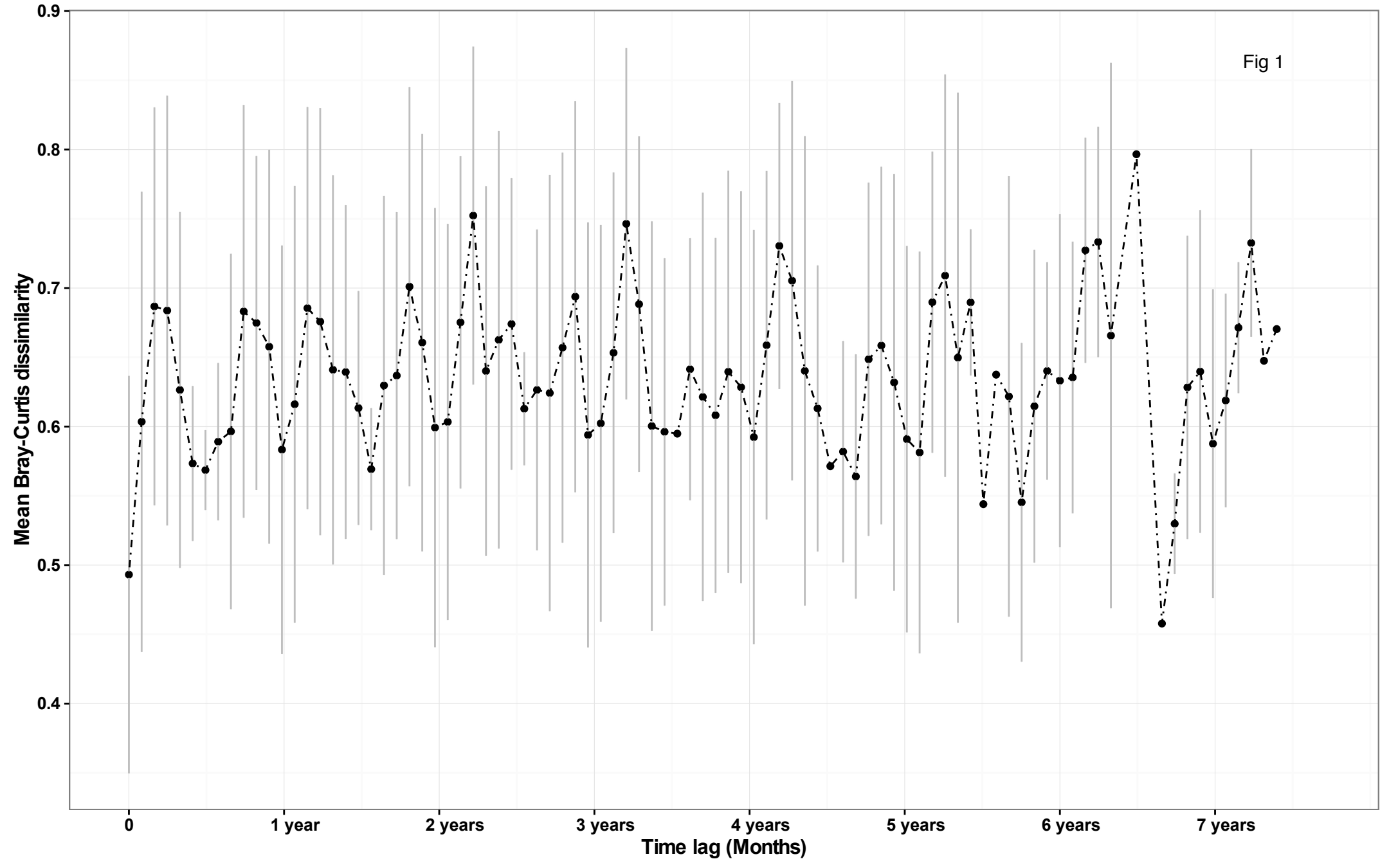


Fig 1
Mean Bray-Curtis dissimilarity
0.9
0.8
0.7
0.6
0.5
0.4
0
1 year
2 years
3 years
4 years
5 years
6 years
7 years
Time lag (Months)

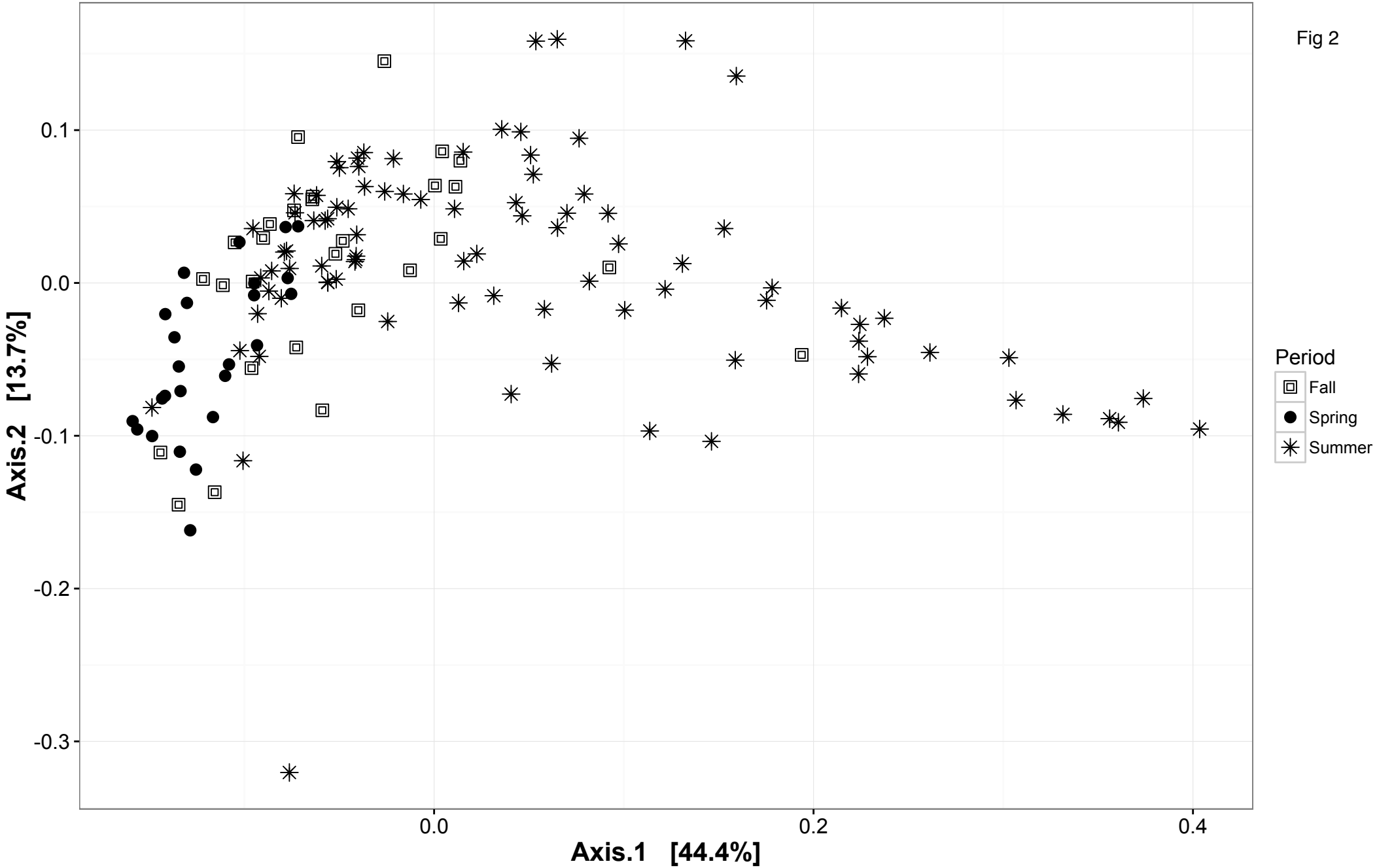
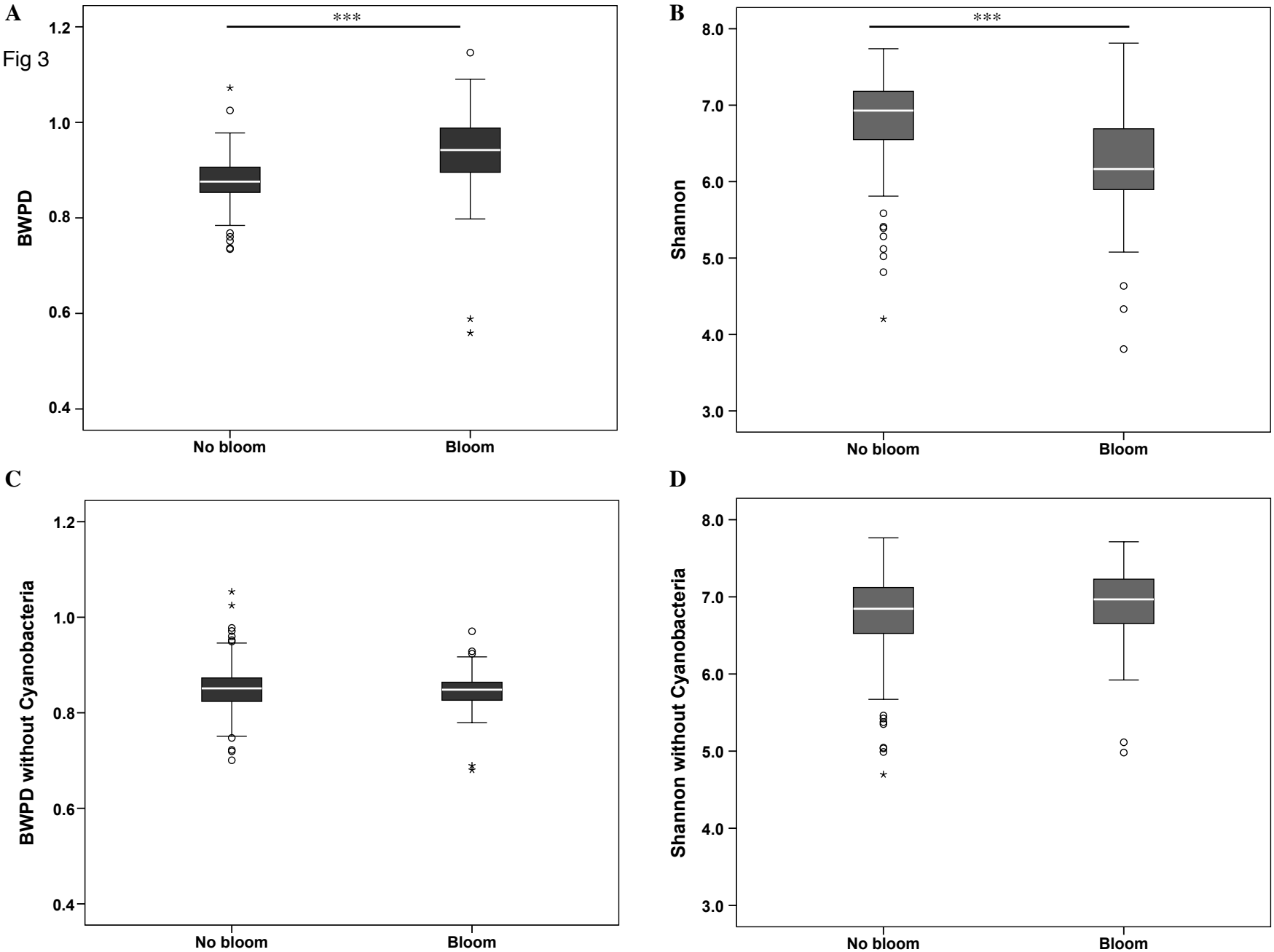


Fig 2
Axis.2 [13.7%]
Axis.1 [44.4%]
0.1
0.0
-0.1
-0.2
-0.3
0.0
0.2
0.4
Period
Fall
Spring
Summer

Fig 3


A
***
BWPD
1.2
1.0
0.8
0.6
0.4
No bloom
Bloom
B
***
Shannon
8.0
7.0
6.0
5.0
4.0
3.0
No bloom
Bloom
C
BWPD without Cyanobacteria
1.2
1.0
0.8
0.6
0.4
No bloom
Bloom
D
Shannon without Cyanobacteria
8.0
7.0
6.0
5.0
4.0
3.0
No bloom
Bloom

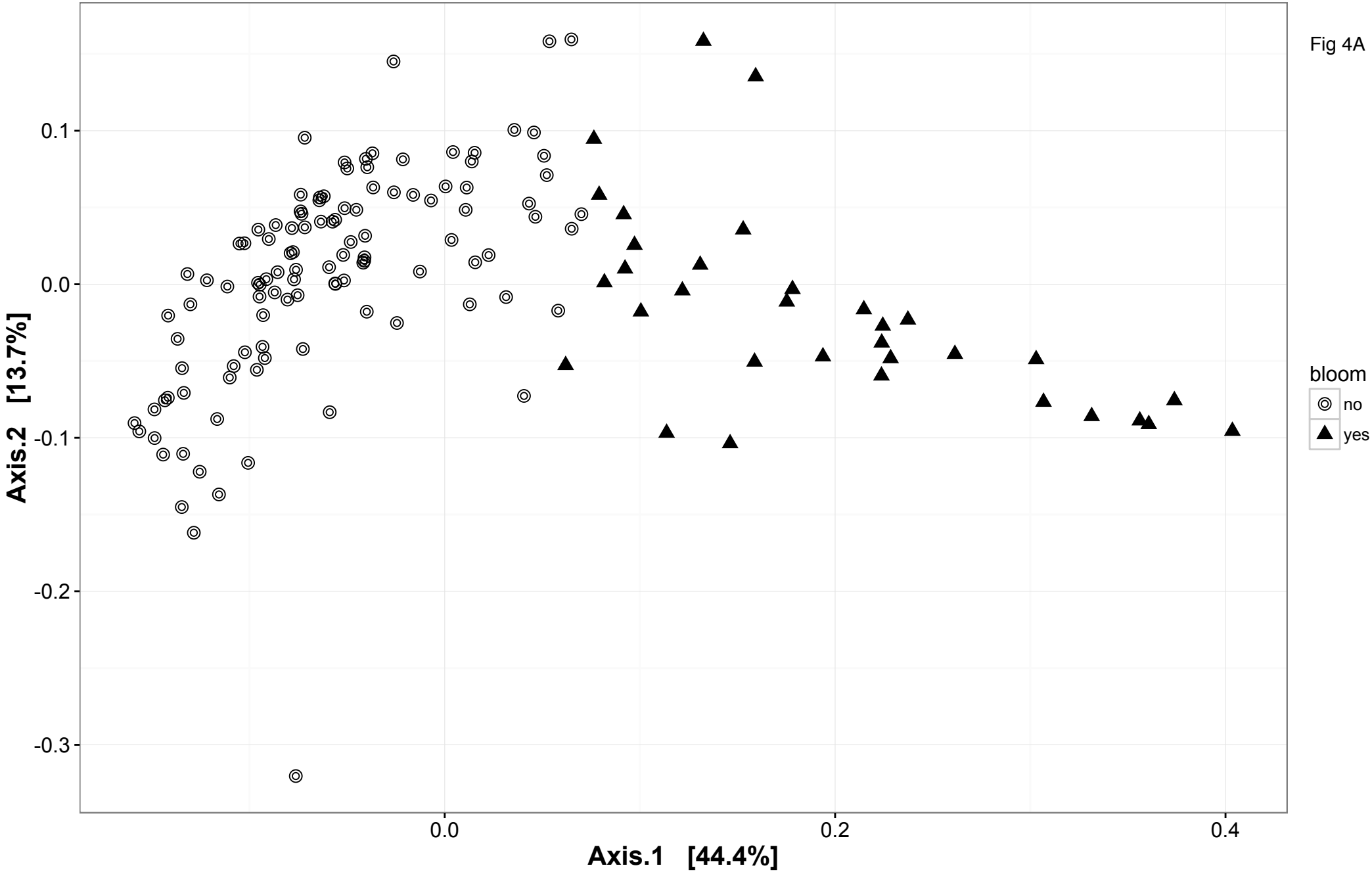


Fig 4A
0.1
0.0
-0.1
-0.2
-0.3
Axis.2 [13.7%]
0.0
0.2
0.4
Axis.1 [44.4%]
bloom
no
yes

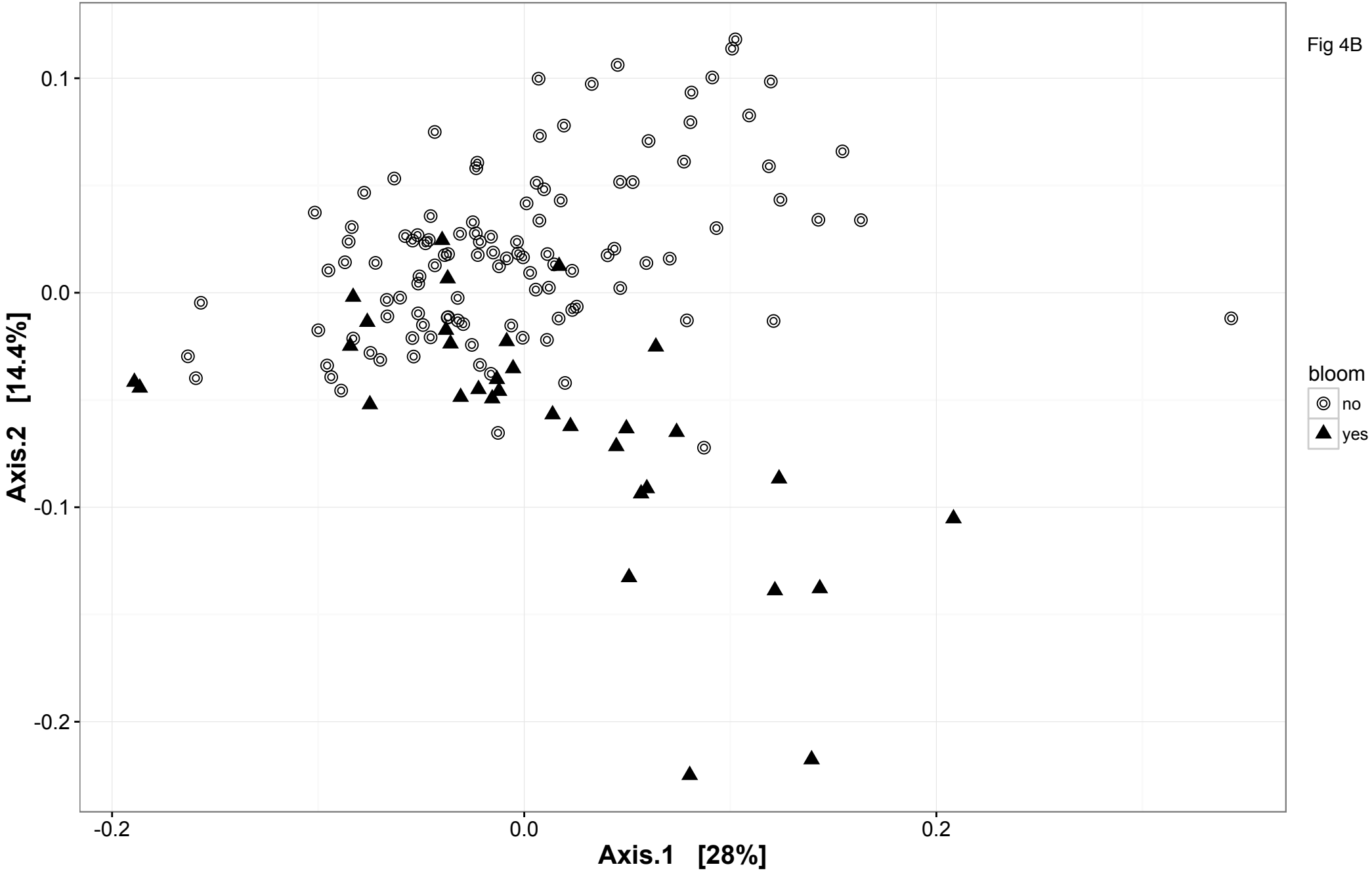


Fig 4B
0.1
0.0
-0.1
-0.2
Axis.2 [14.4%]
-0.2
0.0
0.2
Axis.1 [28%]
bloom
no
yes

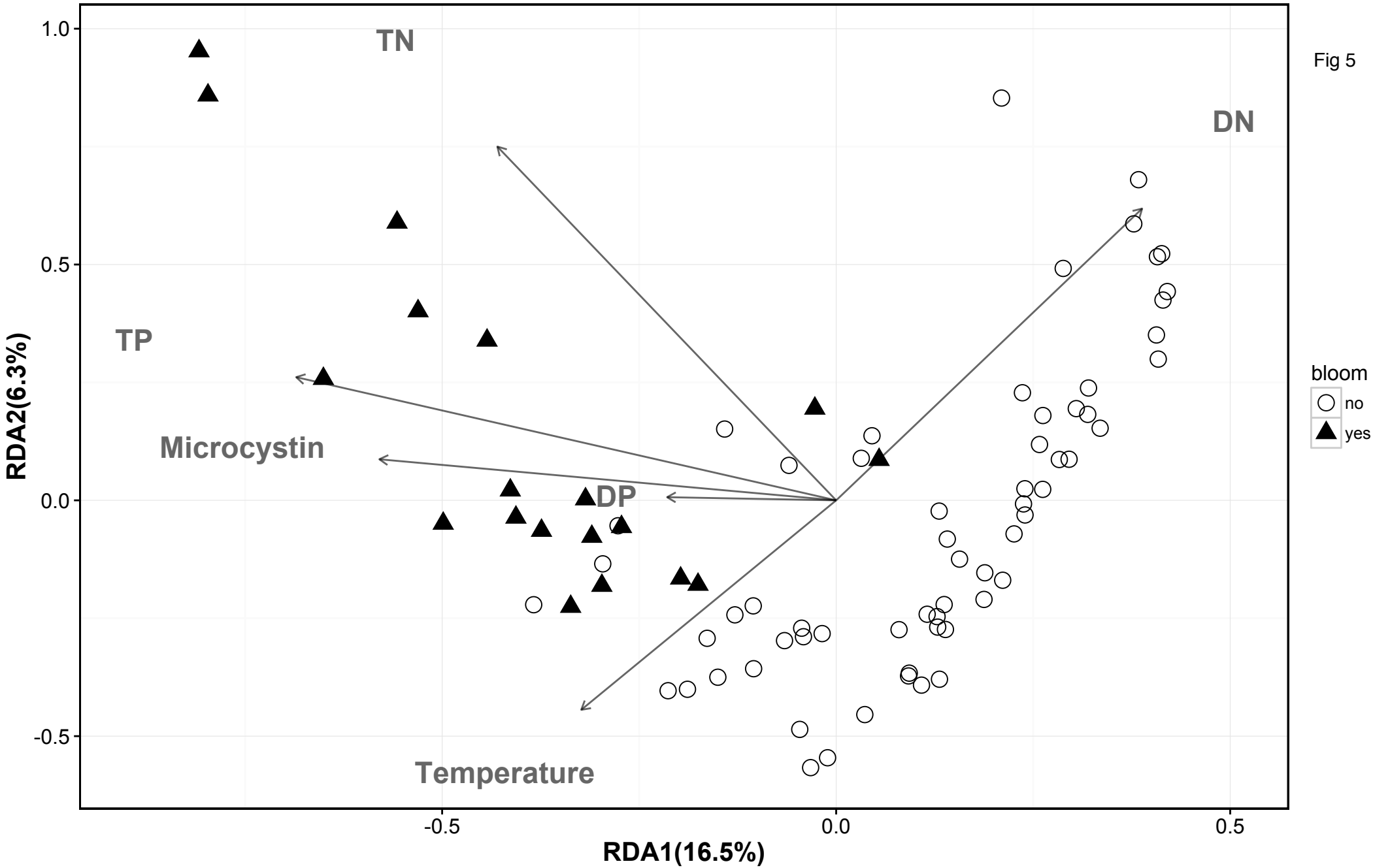
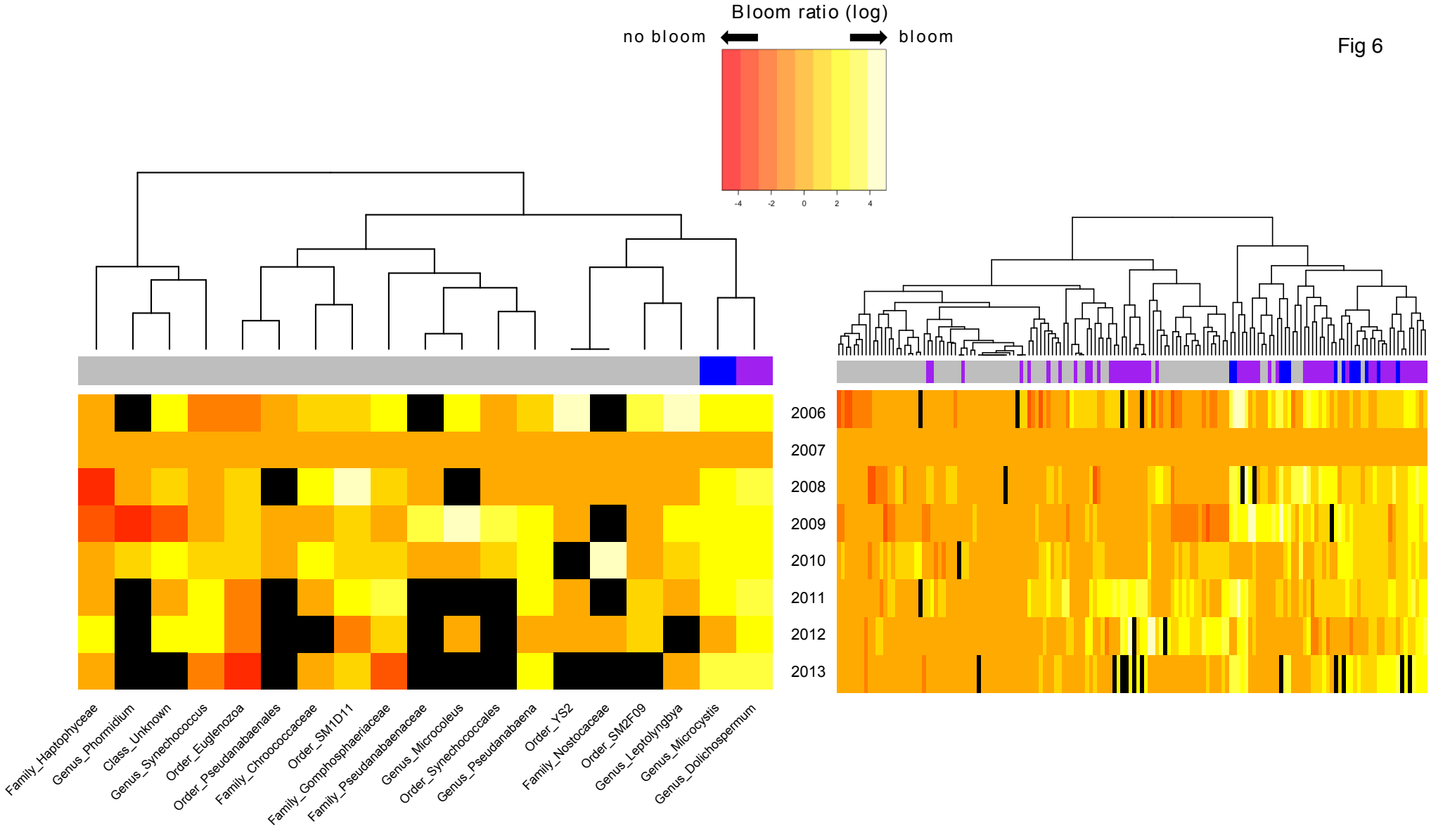


Fig 5
TN
DN
TP
Microcystin
DP
Temperature
bloom
no
yes
1.0
0.5
0.0
-0.5
-0.5
0.0
0.5
RDA2(6.3%)
RDA1(16.5%)

Fig 6


Bloom ratio (log)
no bloom
bloom
-4
-2
0
2
4
2006
2007
2008
2009
2010
2011
2012
2013
Family_Haptophyceae
Genus_Phormidium
Class_Unknown
Genus_Synechococcus
Order_Euglenozoa
Order_Pseudanabaenales
Family_Chroococcaceae
Order_SM1D11
Family_Gomphosphaeriaceae
Family_Pseudanabaenaceae
Genus_Microcoleus
Order_Synechococcales
Genus_Pseudanabaena
Order_YS2
Family_Nostocaceae
Order_SM2F09
Genus_Leptolyngbya
Genus_Microcystis
Genus_Dolichospermum