1   # Characterizing and predicting cyanobacterial blooms in an 8-year

2   # amplicon sequencing time-course

3   ## Authors

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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 135 samples between 2006 and 2013, spans multiple bloom events. We found that bloom events significantly alter the bacterial community without reducing overall diversity, suggesting that a distinct microbial community – including non-cyanobacteria – prospers during the bloom. We also observed that the community changes cyclically over the course of a year, with a repeatable pattern from year to year. This suggests that, in principle, bloom events are predictable. We used probabilistic assemblages of OTUs to characterize the bloom-associated community, and to classify samples into bloom or non-bloom categories, achieving up to 92% classification accuracy (86% after excluding cyanobacterial sequences). Finally, using symbolic regression, we were able to predict the start date of a bloom with 78-92% accuracy (depending on the data used for model training), and found that sequence data was a better predictor than environmental variables.

Introduction

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47   Cyanobacterial blooms occur in freshwaters systems around the world, and are both a

48   nuisance and a public health threat (Zingone and Enevoldsen, 2000; Paerl *et al*., 2013). These

49   blooms are defined by a massive accumulation of cyanobacterial biomass, formed through

50   growth, migration, and physical–chemical forces (Paerl, 1996). In temperate eutrophic lakes,

51   blooms tend to occur annually, specifically during the summer when water temperatures are

52   warmer (Kanoshina *et al*., 2003, Havens, 2008). The frequency and intensity of these blooms is

53   increasing over time (Johnson *et al*., 2010; Posch *et al*., 2012), likely due to increased

54   eutrophication, climate change, and increased nutrient input from human activities (O'Neil *et al*.,

55   2012; Winder, 2012).

56   Attempts have been made to predict blooms using hydrodynamic-ecosystem models

57   (Allen, 2008, Qing *et al*., 2014), artificial neural networks models (Maier *et al*., 2000; 2001), or

58   statistical models such as on linear regression (Dillion and Rigler, 1974, Onderka, 2007).

59   Nevertheless, these models have been limited in their ability to accurately predict cyanobacterial

60   dynamics (Downing *et al*., 2001; Taranu *et al*., 2012), perhaps because they mainly used abiotic

61   factors (*e.g.* temperature, pH, nutrients, etc.) to predict blooms, while largely ignoring biotic

62   factors (Recknagel *et al*., 1997; Downing *et al*., 2001; Oh *et al*., 2007). It is known that

63   cyanobacteria interact with their biotic environment in a variety of ways, ranging from predator-

64   prey interactions to mutualistic interactions (Rashidan and Bird, 2001; Eiler and Bertilsson, 2004;

65   Berg *et al*., 2008; Li *et al*., 2012; Mou *et al*., 2013; Louati *et al*., 2015; Woodhouse *et al.* 2016).

66   Biotic factors, such as the composition of the surrounding bacterial community, could therefore

67   help refine bloom prediction. Previous studies have predicted the distribution of other bacteria

68   based on community structure (Larsen *et al* 2012; Kuang *et al*., 2016) but to our knowledge this

69   has not been attempted to predict freshwater cyanobacterial blooms. Prediction based on biotic

70   factors is attractive because the composition of the microbial community can be thoroughly

71   measured through culture-independent, high-throughput sequencing, whereas it is not always

72   clear which are the relevant (or most predictive) abiotic factors that should be measured.

73   Moreover, the microbial community composition may contain information about both measured

74   and unmeasured abiotic variables, insofar as these variables impact the community.

75   For bloom prediction based on biotic factors to be successful, there must be some degree

76   of repeatability in the changes to lake bacterial community composition that precede blooms.

77   Several studies have shown that many aquatic microbial communities are temporally dynamic

78   (Pernthaler *et al*., 1998; Hofle *et al*., 1999; Lindstrom *et al*., 2000; Crump et al., 2003; Kent *et al*.,

79   2004; Shade *et al*., 2007 ; Kara *et al*., 2013; Fuhrman *et al*., 2015), often with repeatable patterns

80   of community structure (Fuhrman *et al*., 2006; Fuhrman *et al*., 2015). Recent studies have

81   tracked the dynamics of microbial communities in bloom-impacted lakes using culture-

82   independent sequencing methods (Eiler *et al*., 2012; Li *et al*., 2015; Woodhouse *et al*., 2016). Li

83   *et al*. (2015) found that a bloom-impacted lake returned to its initial community composition after

84   a period of one year. However, all these studies were carried out over one year or less, making it

85   difficult to generalize the results and make robust predictions. As highlighted by Fuhrman *et al*.,

86   (2015) data should be collected over several consecutive years to assess the repeatability of

87   bacterial community dynamics, and to assess if community structure follows a predictable

88   pattern, and over what time scales.

89   Blooms can be operationally defined in numerous ways. A classic definition is simply

90   when algal biomass is high enough to be visible (Reynolds and Walsby, 1975). Other bloom

91   definitions rely on chlorophyll concentrations (≥ 20 µg/L), or dominance of cyanobacteria ($>50\%$)

92   over other phytoplankton (Molot *et al*., 2014). An attractive alternative is to view cyanobacterial

93   blooms as a biological disturbance, measurable by their impact on the surrounding microbial

94   community (Shade *et al*., 2012). Blooms can have a major impact on the microbial community

95   through both direct (e.g. microbe-microbe interactions) and indirect effects (e.g. changes to lake

96   chemistry). For example, blooms can reduce carbon dioxide concentrations, increase pH, and

97   alter the distribution of biomass across the length and depth of a lake (Verspagen *et al.,* 2014;

98   Sandrini *et al*., 2016). Such bloom-induced changes in water chemistry could then impact the

99   structure and diversity of microbial communities (Bouvy *et al*., 2001; Eiler and Bertilsson, 2004;

100   Bagatini *et al*., 2014; Li *et al*., 2015, Woodhouse *et al*., 2016). For example, as cyanobacteria

101   decompose, they release metabolites that can be utilized by other taxa, such as Cytophagaceae

102   (Rashidan and Bird, 2001; O'Neil *et al*., 2012), which we therefore expect to be observed in

103   association with blooms. Positive associations have been observed between the genus

104   *Phenylobacterium* or members of the order Rhizobiales with the cyanobacterial genus

105   *Microcystis* (Louati *et al*., 2015). However, the reasons for these interactions, as well as their

106   repeatability (over time) and generality (across different lakes) remain unknown.

107   Here, we present an eight-year time-course study of the bacterial community structure of a

108   large eutrophic North American lake, Lake Champlain, where cyanobacterial blooms are

109   observed nearly every summer. Samples were collected from 2006 to 2013 and analyzed using

110   high-throughput 16S amplicon sequencing. We tracked the bacterial community composition in

111   135 time-course samples to determine how the community varies over time and how it is

112   impacted by blooms. Considering blooms as a disturbance to the surrounding microbial

113   community (Shade *et al.* 2012), we defined bloom events as a relative abundance of

114   cyanobacteria above which community diversity begins to decline. Blooms are characterized both

115   by a dominance of cyanobacteria, but also a characteristic surrounding bacterial community. We

116   show that the community composition does not vary considerably from year to year, but does

117   vary within a year, on time scales of days to months. As a result, community dynamics are

118   largely repeatable from year to year, and are in principle predictable. Finally, exploiting the

119   repeatable dynamics of the lake community, we showed that bloom events can be predicted

120   several weeks in advance based on the microbial community composition, with slightly greater

121   accuracy than predictions based on abiotic factors.

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123

124   **Materials and Methods**

125

126   **Sampling**

127   A total of 150 water samples were collected from the photic zone (0-1 meter depth) of

128   Missisquoi Bay, Lake Champlain, Quebec, Canada (45°02'45"N, 73°07'58"W). Between 12 and

129   27 (median 17) samples were collected each year, from 2006 to 2013, between April and

130   November of each year. Samples were taken from both littoral (78 samples) and pelagic (72

131   samples) zones (Supplementary Methods). Between 50 and 250 ml of lake water was filtered

132   depending on the density of the planktonic biomass using 0.2-μm hydrophilic polyethersulfone

133   membranes (Millipore). Physico-chemical measurements, as described in Fortin *et al*. (2015),

134   were   also   taken   during   most   sampling   events   (Supplementary   File:

135   File_S1_Environmental_Table.txt). These environmental data included water temperature,

136   average air temperature over one week, cumulative precipitation over one week, microcystin

137   toxin concentration, total and dissolved nutrients (phosphorus and nitrogen). Details of the

138   sampling protocol are described in Supplementary Methods.

139

140   **DNA extraction, purification and sequencing**

141   DNA was extracted from frozen filters by a combination of enzymatic lysis and phenol-

142   chloroform purification as described by Fortin *et al*. (2010). Each DNA sample was resuspended

143   in 250 μl of TE (Tris-Cl, 10 mM; EDTA, 1 mM; pH 8) and quantified with the PicoGreen®

144   dsDNA quantitation assay (Invitrogen). DNA libraries for paired-end Illumina sequencing were

145   prepared using a two-step 16S rRNA gene amplicon PCR as described in Preheim *et al*. (2013).

146   We amplified the V4 region, then confirmed the library size by agarose gels and quantified DNA

147   with a Qubit v.2.0 fluorometer (Life Technologies). Libraries were pooled and denatured as

148   described in the Illumina protocol. We performed two sequencing runs using MiSeq reagent Kit

149   V2 (Illumina) on a MiSeq instrument (Illumina). Each run included negative controls and two

150   mock communities composed of 16S rRNA clones libraries from other lake samples (Preheim *et*

151   *al*., 2013). Details of the library preparation protocol are described in Supplementary Methods.

152

153   **Sequence analysis and OTU picking**

154   Sequences were processed with the default parameters of the SmileTrain pipeline

155   (https://github.com/almlab/SmileTrain/wiki/; Supplementary Methods) that combined reads

156   quality filtering, chimera filtering, paired-end joining and, de-replication using USEARCH

157   (version 7.0.1090, http://www.drive5.com/usearch/) (Edgar, 2010), Mothur (version 1.33.3)

158   (Schloss *et al*., 2009), Biopython (version 2.7) and custom scripts. SmileTrain also incorporates a

159   *de novo* distribution-based clustering: dbOTUcaller algorithm (Preheim *et al*., 2013)

160   (https://github.com/spacocha/dbOTUcaller, version 2.0), which was performed to cluster

161   sequences into Operational Taxonomic Units (OTUs) by taking into account the sequence

162   distribution across samples. The OTU table generated was then filtered using

163   filter_otus_from_otu_table.py QIIME scripts (Caporaso *et al*., 2010) (version 1.8,

164   http://qiime.org/) to remove OTUs observed less than 10 times, minimizing false-positive OTUs

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165   (Table S1). Fifteen samples with less than 1000 sequences were removed from the OTU table

166   using filter_samples_from_otu_table.py QIIME script, yielding a final dataset of 135 samples.

167   Taxonomy was assigned post-clustering using a two different approaches: (i) the latest 97%

168   reference OTU collection of the GreenGenes database (release 13_8, August 2013,

169   ftp://greengenes.microbio.me/greengenes_release/gg_13_5/gg_13_8_otus.tar.gz;

170   http://greengenes.lbl.gov), using assign_taxonomy.py QIIME script (default parameters), and (ii)

171   a combination of GreenGenes and a freshwater-specific database (Freshwater database 2016

172   August 18 release; Newton *et al*., 2011), using the TaxAss method

173   (https://github.com/McMahonLab/TaxAss, access date: September 13th 2016). Taxonomy

174   information was then added to the OTU table using the biom add-metadata scripts (http://biom-

175   format.org/). We removed OTUs that were not prokaryotes but still present in the database

176   (Cryptophyta, Streptophyta, Chlorophyta and Stramenopiles orders). A total of 7,321,195

177   sequences were obtained from our 135 lake samples, ranging from 1,392 to 218,387 reads per

178   sample, with a median of 47,072. This dataset was clustered into 4061 OTUs. Of these OTUs,

179   4053 were observed in littoral samples and 4042 in pelagic samples, with 4034 in common to

180   both sites, 19 unique to littoral and 8 to pelagic.

181   To evaluate the quality of the SmileTrain OTU picking pipeline used and estimate the

182   potential false positive OTUs generated by the approach used, we compared the number and

183   identity of OTUs obtained for two different mock communities that were generated from

184   plasmids containing 16S rRNA sequences from a clone library as described on Preheim *et al.*

185   (2013). SmileTrain (using the dbOTUcaller algorithm) recovered 100% of the expected OTUs in

186   the mock community, *i.e* we found a perfect match between 16S sequences from the library and

187   the OTU representative sequences generated post-clustering. However we also found some false

188   positives (Table S1). We removed OTUs represented by fewer than 10 sequences in total to

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189   minimize false positives using filter_otus_from_otu_table.py QIIME script. (Table S1). After this

190   filtering, we still recovered 97% for Mock10 and 100% for Mock11. Details of the post-

191   sequencing computational pipeline are described in Supplementary Methods, and R scripts (for

192   analyses described here and below) are in Supplementary File 2 (File_S2_R_scripts.txt).

193

194   **Diversity analysis**

195   To calculate the alpha diversity, indexes known for their robustness to sequencing depth

196   variation were used: Shannon diversity (Shannon and Weaver, 1949), evenness (the equitability

197   metric calculated in QIIME as: Shannon diversity / log2(number of observed OTUs)), and

198   Balance-Weighted-abundance Phylogenetic Diversity (BWPD) (McCoy and Matsen IV, 2013).

199   To assess the impact of variable sequencing depth on these diversity measures, rarefaction curves

200   were made with multiple rarefactions from the lowest to the deepest sequencing depth, at

201   intervals of 3000 sequences, with replacement and 100 iterations (Fig S1) using

202   parallel_multiple_rarefactions.py, parallel_alpha_diversity.py and collate_alpha.py QIIME

203   scripts. Alpha diversity metrics were then calculated using the mean of the 100 iterations of the

204   deepest sequencing depth for each sample (McMurdie and Holmes, 2014). This approach was

205   used to avoid losing data, and to estimate alpha diversity as accurately as possible. The Shannon

206   index (OTU richness and evenness), and Equitability (evenness) were calculated using QIIME

207   scripts as described above. The BWPD index that captures both the phylogeny (summed branch

208   length) and the relative abundance of species was calculated using the guppy script with *fpd*

209   subcommand   (http://matsen.github.io/pplacer/generated_rst/guppy_fpd.html).   Boxplots   and

210   statistical analyses were performed with IBM SPSS version 22.

211   To calculate the beta diversity between groups of samples (*e.g.* months or seasons), we

212   used a non-rarefied OTU table to calculate two metrics that are robust to sequencing depth

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213 variation: weighted Unifrac (Lozupone et al,. 2007) and Jensen-Shannon divergence (JSD)

214 (Fuglede and Topsoe 2004; Preheim *et al*., 2013). We used the phyloseq R package (version

215 1.19.1) (McMurdie and Holmes, 2013) (https://joey711.github.io/phyloseq/) to first transform the

216 OTU table into relative abundance (defined here as the counts of each OTU within a sample,

217 divided by the total counts of all OTUs in that sample) then to calculate the square root of each

218 metric (JSD or weighted UniFrac), and finally to perform principal coordinates analysis (PCoA)

219 (Gower, 1966). As we observed potential arch effects with sqrt(JSD), we decided to use

220 Nonmetric multidimensional scaling (NMDS, from the phyloseq package that incorporates the

221 *metaMDS*() function from the R vegan package, Oksanen, J. *et al*., 2010. R package version 2.4-

222 1) (Shepard, 1962; Kruskal, 1964) plots. A square root transformation is necessary here to

223 transform weighted Unifrac (non Euclidean metric) and JSD (semi-metric) into Euclidean metrics

224 (Legendre and Gallagher, 2001). Differences in community structure between groups (*e.g.* bloom

225 vs. non-bloom samples) were tested using: (i) analysis of similarity (Clarke, 1993) using the

226 *anosim*() function. The non-parametric Analysis of Similarity (ANOSIM; Clarke, 1993) has been

227 used to test if the similarity among group sample is greater than within-group sample. If the

228 *anosim*() function returns an R value of 1, this indicates that the groups do not share any

229 members of the bacterial community. (ii) Differences in community structure between groups

230 was also tested using permutational multivariate analysis of variance (PERMANOVA; Anderson,

231 2001) with the *adonis*() function. Both ANOSIM and PERMANOVA tests can be sensitive to

232 dispersion, so we first tested for dispersion in the data by performing an analysis of multivariate

233 homogeneity (PERMDISP, Anderson, 2006) with the permuted *betadisper*() function. In our

234 analysis, we observed a significant dispersion effect when cyanobacterial sequences were

235 included. The dispersion effect makes the PERMANOVA and ANOSIM results difficult to

236 interpret. Dispersion mostly disappeared when we removed the cyanobacterial sequences,

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237   meaning that cyanobacteria were in part responsible for the differences in dispersion between

238   groups. PERMANOVA, PERMDISP and ANOSIM were performed using the R vegan package

239   (Oksanen, J. *et al*., 2010. R package version 2.4-1), with 999 permutations. Beta diversity

240   analyses were also performed using a rarefied OTU table (rarefied to 10,000 reads per sample)

241   and similar results were observed (data not shown). Phylogenetic trees used for phylogenetic

242   analysis were built using FastTree (version 2.1.8, Price *et al*., 2009)

243   (http://meta.microbesonline.org/fasttree/). Three other tree inference methods were tested,

244   yielding similar results to FastTree (Supplementary Methods).

245

246   **Bloom definition and K-means partitioning**

247   Only a small subset of our samples were associated with estimates of cyanobacterial cell

248   counts. We therefore estimated the relative abundance of cyanobacteria based on 16S rRNA gene

249   amplicon data, which was significantly (but imperfectly) correlated with *in situ* cyanobacterial

250   cell counts from a limited number of samples (Figure S6, adjusted R^2=0.336; $F_{1,50}$=27.46,

251   P<0.001). The reason for the imperfect correlation is that, even when their absolute numbers are

252   low, cyanobacteria can still dominate the community in relative terms.

253   To define cyanobacterial blooms, we followed the biological pulse disturbance definition

254   described in Shade *et al*. (2012). Specifically, we defined a critical threshold of cyanobacterial

255   relative abundance above which the Shannon diversity of the community begins to decline

256   sharply, consistent with a major ecological disturbance (Figure S2). The decline in diversity is

257   most pronounced when cyanobacteria make up 20% or more of the community, so we defined

258   samples with 20% cyanobacteria or more as "bloom samples" (Table S7).

259   As an alternative and completely independent way of binning samples, we used the K-means

260   partitioning algorithm (MacQueen, 1967), implemented with the function *cascadaKM*() from the

261   vegan package in R, with 999 permutations. The OTU table was first transformed by Hellinger

262   transformation (Rao, 1995) as advised in Legendre and Legendre (1998) by using the

263   *decostand*(x, method="hellinger") function from R vegan package. OTU tables are generally

264   composed of many zeros (as is the case for our data), which is inappropriate for the calculation of

265   Euclidean distance. Hellinger transformation is a method to avoid this problem by down-

266   weighting low-abundance OTUs (Legendre and Gallagher, 2001). We tested the partitioning of

267   the 135 samples into 2 to 10 groups, based on the microbial community composition. The

268   Calinski-Harabasz index (Caliński & Harabasz, 1974) was used to determine that our samples

269   naturally clustered into two groups (Figure S3), and bloom samples (defined as above) were all

270   found in a single K-means group (Figure S5). This suggests that the lake samples are naturally

271   divided into two groups, and that cyanobacteria are a major distinguishing feature between

272   groups.

273

274   **Changes in community composition over time**

275   In order to investigate microbial community variation over time, we first analysed the

276   change in Bray-Curtis dissimilarity over years. We performed separated analyses for littoral and

277   pelagic OTU tables, after filtering out singleton OTUs only observed in one sample. This yielded

278   3491 OTUs for littoral samples and 3371 OTUs for pelagic samples. These two OTU tables were

279   transformed to relative abundances prior to analysis. We calculated the Bray-Curtis dissimilarity

280   between all pairs of samples using the QIIME script beta-diversity.py. We verified that

281   distribution of Bray-Curtis dissimilarity across samples was approximately normal. Then, we

282   used a custom script (see Supplementary file: "File_S2_R_scripts.txt") to group the samples

283   based on the amount of time (years) separating them, and to plot the mean dissimilarity of

284   samples against their separation in time. Error bars were determined by calculating the standard

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285   error of the mean.

286

287   In a second approach, we used multivariate regression tree analyses (Breiman *et al*. 1984;

288   De'ath 2002) with different time scales: year, season, month, week and day of the year. The goal

289   here is to identify the temporal variables that best explain the variation in microbial community

290   composition. An analysis was performed for each temporal variable (year, season, month or

291   DoY) using the function *mvpart*() and *rpart.pca*() from the R mvpart package (Therneau and

292   Atkinson, 1997; De'ath, 2007). Prior to analysis, the OTU table was Hellinger transformed (Rao,

293   1995) as advised in Ouellette *et al*., (2012). This approach is particularly useful to investigate

294   both linear and non-linear relationships between community composition and a set of explanatory

295   variables without requiring residual normality (Ouellette *et al*., 2012). After 100 cross-validations

296   (Breiman *et al*. 1984), we plotted and pruned the tree using the 1-SE rule (Legendre & Legendre

297   2012) to select the least complex model, avoiding over-fitting. We then used the function

298   *rpart.pca*() from mvpart package to plot a PCA of the MRT.

299

300   **Taxa-environment relationships**

301   To investigate taxa-environment relationships, we performed a redundancy analysis

302   (RDA; Rao, 1964) that searches for the linear combination of explanatory variables (the matrix of

303   abiotic environmental data) that best explains the variation in a response matrix (the OTU table).

304   The OTU table was transformed by Hellinger transformation (Rao, 1995) as advised in Legendre

305   and Legendre (1998). The explanatory (environmental) matrix was first log-transformed then z-

306   score standardized using the function *decostand*(x, method="standardize") because different

307   environmental parameters are in different units. The environmental matrix variables included:

308   total phosphorus in μg/L (TP), total nitrogen in mg/L (TN), particulate phosphorus in μg/L (PP,

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309   the difference between TP and DP), particulate nitrogen in mg/L (PN, the difference between TN

310   and DN), soluble reactive phosphorus in μg/L (DP), dissolved nitrogen in mg/L (DN), 1-week-

311   cumulative precipitation in mm, 1-week-average air temperature in Celsius and microcystin

312   concentration in μg/L. The functions *corvif*(x) (Zuur *et al*., 2009) and *cor*(x, method="pearson")

313   (the Pearson correlation; Bravais, 1846; Pearson, 1896) from the R stats package were applied to

314   assess colinearity among explanatory variables (Table S2). Based on these correlation tests, we

315   concluded that TP and TN were highly correlated with PP and PN, respectively, so TP and TN

316   were removed. RDA was performed using the *rda*(scaling=2) function from the R vegan

317   package. To determine the significance of constraints, we used the *anova.cca*() function from the

318   R vegan package (Table S4A). Finally, we performed another RDA with all possible interactions

319   between variable (except for Microcystin that is more a consequence of the bloom) to test if

320   interactions between environmental variables could better explain the cyanobacterial bloom. The

321   significance of the interactions is shown table S4B. Both RDAs were performed on a reduced

322   dataset (a subset of 74 samples for which environmental data were available; see Supplementary

323   file: File_S1_Environmental_Table.txt).

324

325   **Differential OTU abundance analysis**

326   To identify genera and OTUs associated with blooms, we used the ALDEx2 R package

327   (version: 1.5.0 (Fernandes *et al*., 2014). We used the aldex() function to perform a differential

328   analysis with Welsh's t-test and 128 Monte Carlo samples. ALDEx2 uses the centred log-ratio

329   transformation to avoid compositionally issue. Taxa (OTUs or genera) with a Q-value below 0.05

330   after Benjamini-Hochberg correction were considered biomarkers. The top 25 biomarkers (with

331   the highest differential scores) are listed in Table S8.

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Bloom classification

To classify bloom and non-bloom samples (Table S7), we used the Bayesian inference of microbial communities (BIOMICO) model described by Shafiei *et al*., (2015). This supervised machine learning approach infers how OTUs are combined into assemblages, and how combinations of these assemblages differ between bloom and non-bloom samples. An assemblage here is defined as a set of co-occurring OTUs. We defined bloom samples as described above, and trained the model with two different approaches: (i) with 2/3 of the total data, selected at random, and (ii) with two distinctive years: 2007, a year with only a short-lived fall bloom, and 2009, a year in which Fortin *et al*., (2015) observed a high biomass of cyanobacteria during the summer. In the training stage, BIOMICO learns how OTU assemblages contribute to community structure, and what assemblages tend to be present during blooms. In the testing stage, the model classifies the rest of the data (not used during training), and we assess accuracy as the percentage of correctly classified samples. To assess the performance of BIOMICO relative to a random classifier, we approximated a random classifier using a binomial distribution with correct classification probability of 0.5.

Bloom prediction

We attempted to predict the timing of blooms using sequence or environmental data. As many OTUs or genera may have such low abundances that they might be missed in some samples, and might also increase the probability of finding spurious correlations, we pre-filtered the OTU table by removing taxa with summed relative abundances (over the 135 samples) lower than an arbitrary threshold of 0.1. Our goal was to predict the timing of the next bloom, using sequencing and/or environmental data from samples taken before a bloom event. Samples taken during a bloom were not used in these analyses. Thus, we used 21 samples with full

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357 environmental information when the analysis included these variables, and 54 samples when the

358 analysis did not require the environmental variable. We defined the time (in days) from each non-

359 bloom sample to the next bloom sample of the year as the dependent variable. In these analyses,

360 we used either OTUs, genera, or environmental data, as predictor variables. We also calculated

361 the trend in all predictor variables from one sample to the next by subtracting the latter values

362 from the former and dividing by the number of days that separated the two sample dates. In this

363 way, we obtained a trend value for each predictor variable.

364   Genetic programming, in the form of symbolic regression (SR) (Koza, 1992), is a

365 particular derivation of genetic algorithms that searches the space of mathematical equations

366 without any constraints on their form, hence providing the flexibility to represent complex

367 systems, such as lake microbial communities. Contrary to traditional statistical techniques,

368 symbolic regression searches for both the formal structure of equations and the fitted parameters

369 simultaneously (Schmidt and Lipson 2009). There are however some caveats associated with SR.

370 First, as with any other regression technique, overfitting may occur and measures that correct for

371 model complexity, such as the Akaike information criterion (AIC,) should be used to compare

372 equations. Second, contrary to standard regression techniques, there are no standard ways to

373 interpret SR equations. Finally, SR suffers from the same limitations of evolutionary algorithms

374 in general. In many cases the algorithm may get stuck in local minima of the search space,

375 requiring time (or even a restart with different parameters) to find the global minimum. We used

376 the software *Eureqa* (http://www.nutonian.com/products/eureqa/, version 1.24.0) to implement

377 SR, using 75% of the data for model training and 25% for testing. As building blocks of the

378 equations we used all predictor variables (including trends), random constants, algebraic

379 operators ($+$, $-$, $\div$, $\times$) and analytic function types (exponential, log and power). As no *a priori*

380 assumptions regarding relationships between terms could be made, the search was fully

16

381   unbounded. Given the inherent stochasticity of the process, ten replicate runs were conducted for

382   each analysis. All runs were stopped when the percentage of convergence was 100, meaning that

383   the formulas being tested were similar and were no longer evolving. Each run produces multiple

384   formulas along a Pareto front (see Cardoso *et al*. 2015.). For each formula, we calculated the

385   Akaike information criterion (AIC) and the corrected AIC (Burnham and Anderson, 2002) for

386   small sample sizes. Based on *Eureqa* complexity (number of parameter) and Eureqa fit score

387   (model accuracy), multiple formulae were selected from each of the ten runs (see Supplementary

388   File : File_S3_SR_table.xlsx). The formula with the lowest AICc for each analysis was retained

389   and considered the "best" formula (Table 2).

390

391

392   **Results**

393   *Defining and characterizing blooms*

394   To survey microbial diversity over time, we analysed 135 lake samples sequenced to an

395   average depth of 54,231 reads per sample (minimum of 1000 reads per sample), and clustered the

396   sequences into 4,061 operational taxonomic units (OTUs). Rarefaction curves showed that this

397   depth of sequencing provided a thorough estimate of community diversity (Figure S1). To assess

398   the repeatability and predictability of cyanobacterial blooms, we first needed to define bloom

399   events. Instead of defining blooms based on cyanobacterial cell counts, we used a definition

400   based on the extent to which the bloom disturbs the community. Above 20% cyanobacteria,

401   Shannon diversity begins to decline sharply (Figure S2). We therefore used a 20% cutoff to bin

402   our samples into "bloom" or "non-bloom" (Table S7).

403   Based on our definition, bloom samples necessarily have lower Shannon diversity than

404   non-bloom samples (Figure 1). More surprisingly, bloom samples had significantly (Mann-

405   Whitney test, U= 814, P<0.001) higher phylogenetic diversity (BWPD) compared to non-bloom

406   samples (Figure 1A). These result suggests that cyanobacterial blooms lead to (i) an increase in

407   phylogenetic diversity by adding additional, relatively long cyanobacterial branches to the

408   phylogeny, and (ii) a decrease of taxonomic evenness due to the dominance of cyanobacteria.

409   However, when we repeated the same analysis after removing all cyanobacterial OTUs, we found

410   that blooms did not alter the diversity of the remaining (non-cyanobacterial) community (Figure

411   1 D, E, F). These exploratory alpha diversity analyses prompted us to investigate how community

412   composition changed between bloom and non-bloom samples, and over time.

413   Despite their limited impact on the diversity of the non-cyanobacterial community, we

414   found that blooms clearly alter the community composition of the lake. Using weighted UniFrac

415   distances to assess differences in community composition, we observed a separate grouping of

416   bloom and non-bloom samples (Figure 2A). However, the difference in community composition

417   could not be assessed with PERMANOVA statistics because bloom and non-bloom samples were

418   differently dispersed (Table S6. When we removed the Cyanobacteria counts and re-normalized

419   the OTU table (Figure 2B), we still observed a significant, but less pronounced difference

420   between bloom and non-bloom samples (PERMANOVA, R^2=0.035; P<0.001; ANOSIM

421   R=0.211; P<0.01; PERMDISP P = 0.084; Table S6). We observed the same trend using another

422   beta diversity metric, JSD (Table S6 and Figure S7). These results suggest that even excluding

423   Cyanobacteria (the bloom-defining feature), the bloom community still differs to some extent

424   from the non-bloom community.

425

426   *Abiotic factors associated with blooms*

18

427   A subset of our samples was associated with environmental measurements that might

428   explain bloom events. We performed an RDA to identify environmental variables that could

429   explain how bloom and non-bloom samples are grouped, and found particulate nitrogen (PN),

430   particulate phosphorus (PP), microcystin concentration, and to a lesser extent soluble reactive

431   phosphorus (DP), to be most explanatory of the bloom (Figure S8; adjusted R^2=0.273; ANOVA,

432     $F_{7,66}$=4.919, P<0.001). DN and temperature explain less variation and act in opposing directions

433   (Pearson correlation = -0.18), perhaps because higher temperatures favour the growth of

434   microbes that rapidly consume dissolved nitrogen (Hong *et al.*, 2014). Together, these

435   environmental variables explain ~25% of the microbial community variation (axis 1: 18.5%; axis

436   2: 6.9%) suggesting that unmeasured biotic or abiotic factors are needed to explain the remaining

437   ~75% of the variation. We also explored the ability of interactions among environmental

438   variables to explain variation, but despite the modest increase in R^2 to 0.34 (to be expected given

439   the added variables) we did not observe any significant interactions (Supplementary Table 4B).

440

441   *Community dynamics vary more within than between years*

442   We next asked how the lake microbial community varied over time, at scales ranging

443   from days to years. As described above, samples can be partially separated according to season

444   (spring, summer or fall) based on weighted UniFrac distances (Figure 2). However, seasons

445   differed significantly in their dispersion (with summer samples visibly more dispersed in Figure

446   2), violating an assumption of PERMANOVA and ANOSIM tests, and preventing us from

447   determining whether samples varied more by months, seasons or years (Table S6). However, it is

448   visually clear from Figure 2 that bloom samples explain much of the variation in summer

449   community composition.

450   To more clearly track changes in community composition over time (temporal beta

451   diversity), we calculated the Bray-Curtis dissimilarity between pairs of samples separated by

452   increasing numbers of years. We did not observe any tendency for the community to become

453   more dissimilar over time, suggesting a long-term stability of the bacterial community on the

454   time scale of years in both the littoral (linear regression, $F_{(1,1999)}$=1.171, P>0.05) and pelagic

455   sampling sites (linear regression, $F_{(1,2078)}$=0.8467, P>0.05; Figure S4). Consistently, even though

456   years differed significantly in their dispersion (PERMDISP $P < 0.05$), community composition

457   remained relatively similar from year to year. (Weighted Unifrac: ANOSIM R<0.1, P<0.010;

458   PERMANOVA R^2=0.011, P=0.098).

459   To further explore temporal signals in the data, we used a multivariate regression tree

460   (MRT) approach to determine how community structure varies over time scales of days to years.

461   Consistent with the stable Bray-Curtis similarity over years (Figure S4), we found that year-to-

462   year variation explains very little of the variation in community structure (R^2=0.027; Table S5).

463   Week of the year explained the most the community variation (R^2=0.274; Figure 3, Table S5),

464   followed closely by day (R^2=0.254; Table S5) and month (R^2=0.216; Table S5). Even though

465   weeks explained the most variation, much of this variation is captured at longer time scale of

466   months. Figure 3 shows how the regression tree roughly divides samples by season: Split 1 (red)

467   corresponds to samples taken before May 12 (early spring), split 2 (green) to samples taken

468   between May 12 and June 23 (late spring), split 3 (yellow) to samples taken after October 6 (fall),

469   split 4 (blue) to samples taken between June 23 and July 14 (early summer), split 5 (cyan) to

470   samples taken between July 14 and August 11 (mid-summer), and split 6 (purple) to samples

471   taken between August 11 and October 6 (late summer). The PCA ordination based on MRT

472   (Figure 3B) shows that community dynamics appear to be somewhat cyclical, returning to

473   roughly the same composition each year. Different times of year are characterized by different

474   sets of OTUs, for example AcI-B1 and PnecB in early summer and *Microcystis* and

475   *Dolichospermum* in mid-summer.

476   To determine if the variation observed during summer (Figures 2 and 3) could be driven

477   by cyanobacterial bloom events, we repeated the MRT analyses after removing all cyanobacterial

478   sequences. Similar MRT results were obtained after removing cyanobacteria, suggesting that the

479   entire bacterial community, not just cyanobacteria, are responsible for temporal variation (Table

480   S5). Together, these results show how bacterial community dynamics follow an annually

481   repeating, cyclical pattern, and that both cyanobacteria and other bacteria contribute to the

482   dynamics.

483

484   *Blooms are repeatably dominated by Microcystis and Dolichospermum*

485   To explore potential biological factors involved in bloom formation, we attempted to

486   identify taxonomic biomarkers of bloom or non-bloom samples, at the genus and OTU levels. To

487   do so, we performed a differential analysis using ALDEx2 to identify the genera or OTUs that are

488   most enriched in bloom samples. We found several significant biomarkers and as expected, the

489   strongest bloom biomarkers belonged to the phylum Cyanobacteria (Table S8). The two strongest

490   OTU- and genus-level biomarkers were *Microcystis* (Microcystacae) and *Dolichospermum*

491   (Nostocaceae, previously named *Anabaena*), both genera of Cyanobacteria.

492

493   *Blooms can be accurately classified based on non-cyanobacterial sequence data*

494   Given the observation that bloom samples have distinct cyanobacterial and non-

495   cyanobacterial communities (Figure 2), we hypothesized that blooms could be classified based on

496   their bacterial community composition. We trained a machine-learning model (BIOMICO) on a

497   portion of the samples, and tested its accuracy in classifying the remaining samples (Methods).

21

498   BIOMICO was able to correctly classify samples with ~92% accuracy (Table 1). Such high

499   accuracy is expected because blooms are defined as having >20% cyanobacteria, so the model

500   should be able to easily classify samples based on cyanobacterial abundance.

501   In a more challenging classification task, BIOMICO was able to classify samples with 83-

502   86% accuracy after excluding cyanobacterial sequences. This result supports the existence of a

503   characteristic non-cyanobacterial community repeatably associated with the bloom. Two different

504   training approaches (Methods) yielded similar classification accuracy, both significantly better

505   than random (Table 1), but found different bloom-associated assemblages. When we compared

506   the best assemblages obtained with the two different trainings, focusing only on the 50 best OTU

507   scores, only 11 OTUs were found in both trainings (Table S9). This result suggests that data can

508   be classified into bloom or non-bloom samples, but different assemblages (containing different

509   sets of OTUs) can be found with similarly high classification accuracy (Table S9). This is

510   consistent with a general lack of repeatability at the level of individual OTUs, but that there exist

511   combinations of OTUs (Table S8) that are characteristic of blooms.

512

513   *Blooms can be predicted by sequence data*

514   The existence of microbial taxa and assemblages characteristic of blooms suggests that

515   blooms could, in principle, be predicted based on amplicon sequence data. We therefore used

516   symbolic regression (SR) to model the response variable "days until bloom" as a function of

517   OTU- or genus-level relative abundances, their interactions, and their trends over time (Methods).

518   To achieve true prediction, not simply classification, we used only samples collected prior to

519   each bloom event in order to predict the number of days until a bloom sample (*i.e.* bloom

520   samples themselves were not used). We based our analysis on 54 samples, ranging from 7 to 112

521   days before a bloom sample. Due to limitations in the resolution of sampling (approximately

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522   weekly), we cannot know the exact start date of a bloom, only the first date sampled. Using

523   OTUs or genera, we were able to predict the timing of the next bloom event with 80.5% or 78.2%

524   accuracy on tested data, respectively (Table 2). Using a subset of 21 samples with a full

525   complement of environmental data, we were able to compare the predictive power of sequence

526   data (OTU or genus level) versus environmental data. Predictions based on genus-level sequence

527   data clearly outperformed predictions based on environmental data. Predictions based on OTU-

528   level sequence data explained less variance than predictions based on genera, consistent with

529   OTUs being more variable and less reliable bloom predictors than higher taxonomic units.

530   All models tend to overshoot when based on samples taken closer to the bloom (*i.e.*

531   negative residuals), and tend to predict bloom events too soon when based on samples farther

532   from the bloom (Figure S9). One taxon – a member of the order Burkholderiales in the family

533   Oxalobacteraceae (unknown genus; Greengenes taxonomy) was consistently found in every

534   predictive formula (Table 2). At the OTU level, seq413 (Table 2) is assigned to Oxalobacteraceae

535   by Greengenes (with 67% confidence) but to *Polynucleobacter* C-subcluster (with 99%

536   confidence) based on TaxAss, a freshwater-specific database (Table S10). While *Microcystis* and

537   *Dolichospermum* are dominant closer to bloom events, seq413 showed the opposite pattern,

538   decreasing in relative abundance as the bloom approaches (Figure 4). The fact that seq413, but

539   not *Microcystis* or *Dolichospermum*, appears in the predictive equations suggests that the decline

540   in Oxalobacteraceae/seq413 is detectable before the increase in Cyanobacteria. Indeed, seq413

541   appear to decline before *Microcystis* or *Dolichospermum* increase (Figure 4). However, the

542   predictive analyses were done at the OTU or genus level, such that Cyanobacteria were not

543   treated as one entity (*i.e.* one variable in predictive equations). It is therefore possible that the

544   decline in seq413 was driven by a total increase in the sum of all Cyanobacteria, none of which

545   could be detected individually. To test this possibility, we repeated the SR analysis after merging

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546 Cyanobacteria into a single variable, and found that Cyanobacteria were never found in any

547 predictive equation. This is consistent with Oxalobacteraceae/PnecC declining before

548 Cyanobacteria increase. Hence, changes in the microbial community provide information about

549 impending blooms before they occur.

550

Discussion

552 We used a deep 16S rRNA amplicon sequencing approach to profile the bacterial

553 community in Lake Champlain over eight years, spanning multiple cyanobacterial blooms. We

554 sequenced with sufficient depth that bacterial diversity estimates reached a plateau (Figure S1),

555 and proposed a bloom definition based upon cyanobacterial relative abundance in 16S data.

556 Although there is no consensus bloom definition, the World Health Organization has proposed

557 guidelines, based on cyanobacterial cell density, to connect blooms to potential health risks

558 (WHO, Guidelines for safe recreational water environments, 2003). We found that, while

559 cyanobacterial relative abundance in 16S data is significantly correlated with cyanobacterial cell

560 density, the correlation is imperfect (Figure S6) because cyanobacteria can have high relative

561 abundance without achieving a high absolute cell density. Our bloom definition, based on

562 relative, not absolute abundance is therefore more a measure of how cyanobacteria impact their

563 surrounding bacterial community than a direct measure of human health risks.

564 Our results should be interpreted in light of four methodological caveats. First, the OTU

565 data are compositional, such that only the relative OTU abundances are meaningful, and the

566 relative abundances are non-independent (Gloor and Reid, 2016). As a result, removing certain

567 OTUs or taxa (*e.g.* Cyanobacteria, as discussed in the paragraph below) does not remove their

568 influence on the rest of the data. For some purposes, corrections for compositionality can be

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569 performed (*e.g.* ALDEx performs a centered log transform before inferring differentially

570 abundant OTUs). BioMico might identify OTUs that are not truly associated with blooms, but

571 that are falsely correlated with OTUs that are truly associated. However, this is not a major

572 problem because the goal of BioMico is bloom classification, not identification of bloom-

573 associated OTUs. A similar logic applies to prediction with SR: if the goal is pragmatic

574 prediction, whether the predictive taxa are biologically meaningful (or mere artefacts of

575 compositionality) is irrelevant. In reality, the fact that SR repeatably converged on equations with

576 the same taxa (Table 2) suggests that these taxa are indeed biologically meaningful. The second

577 caveat is that the same data was used to define blooms and also to classify/predict blooms, which

578 could be considered circular reasoning. However, the bloom definition was based on a univariate

579 summary of the data (Shannon diversity), while BioMico classification uses the multivariate data

580 (the relative abundance of each OTU across samples). Therefore, circularity is limited because

581 blooms were defined based on one feature of the data (a decline in Shannon diversity), and

582 classification was based on a different feature (OTU identities). For the prediction task,

583 circularity was limited because only non-bloom samples were used to predict the timing of a

584 bloom event. The third caveat is that phylogenetic measures of alpha and beta diversity (BWPD

585 and UniFrac, respectively) rely on a phylogenetic tree, which may be inaccurate. However, trees

586 inferred using FastTree, ML or neighbour-joining gave very similar results (Supplementary

587 Methods), so we expect tree errors to have a limited impact on our conclusions. The fourth caveat

588 is that the choice of OTU calling will influence the number and identify of OTUs. We used a

589 distribution-based OTU caller (Preheim *et al.*, 2013), which uses the distribution of OTUs across

590 samples to reduce the number of false-positive OTUs (*e.g.* due to sequence errors). Other

591 methods, such as DADA2 (Callahan *et al.*, 2016), oligotyping or minimum entropy

592 decomposition (Eren *et al.*, 2013; 2015), are similarly able to de-noise 16S data, while calling

593   OTUs at fine taxonomic resolution (*e.g.* 99% rather than 97% identity). In the future, these

594   methods could be used to analyze bloom dynamics at finer taxonomic resolution than the 97%

595   cutoff used here.

596   Our results suggest that blooms decrease community diversity because of an increase in

597   the relative abundance of cyanobacteria, not due to a reduction in the diversity of other bacteria.

598   This result is based on an analysis of three diversity measures, before and after removing

599   cyanobacterial sequences (Figure 1). Before removing Cyanobacteria, bloom samples clearly

600   have lower Shannon diversity and evenness compared to non-bloom samples (this is true by

601   definition, based on the nature of our bloom definition). After removing Cyanobacteria, there is

602   no apparent difference in diversity or evenness. Removing cyanobacterial reads does not remove

603   their influence on other OTUs, because of the dependence structure of compositional data (Gloor

604   and Reid, 2016; Morton *et al.*, 2017). However, even if removing Cyanobacteria creates a bias in

605   the rest of the data, the same bias is introduced in both bloom and non-bloom samples alike, so

606   the comparison should remain valid. The removal of cyanobacterial reads is analogous to the

607   common practice of first removing eukaryotic reads from 16S data, and continuing all subsequent

608   analyses on bacterial reads only. The dataset as a whole is biased by the removal of eukaryotes

609   (*i.e.* the data becomes a 'subcomposition') but all samples have the same bias, so it is still possible

610   to compare among samples. Regardless, these diversity comparisons (Figure 1) were exploratory

611   in nature, and served as an entry point for more detailed beta diversity analyses, classification,

612   and prediction.

613   Consistent with our current knowledge of temperate lakes (Shade *et al.*, 2007; Crump *et*

614   *al.*, 2005), we found that community structure varied more within years than between years

615   (Figures 2, 3, and S4; Tables S5 and S6). In agreement with previous observations in eutrophic

616   lakes (Shade *et al.*, 2007), Lake Champlain appears to return to a steady-state (Figure S4, Table

26

617   S5), despite the biological disturbance induced by dramatic bloom events. Various studies have

618   already shown temporal patterns in microbial community structure (Hofle *et al*., 1999; Lindstrom

619   *et al*., 2000; Crump et al., 2003; Shade *et al*., 2007; Kara et al., 2013; Fuhrman *et al*., 2015), but

620   ours does so in the context of cyanobacterial blooms.

621   The RDA results (Figure S8) are consistent with many previous studies describing the

622   environmental factors responsible for blooms (Owens and Esaias, 1976; Hecky and Kilham,

623   1988). For example, cyanobacterial growth is optimal at higher temperatures, between 15 and

624   30°C (Konoka and Brock, 1978). We confirmed that cyanobacterial blooms are correlated with,

625   and likely respond to nutrient concentrations, as previously described (Fogg, 1969; Jacoby *et al*.,

626   2000; Paerl and Huisman, 2008; Paerl and Huisman, 2009, Fortin *et al* 2015, Isles *et al*., 2015).

627   Dissolved nitrogen and temperature were negatively correlated, which could be explained by the

628   fact that the lake becomes enriched in nitrates during spring, when temperatures are lower, and

629   rain and drainage bring nutrients into the lake (Shade *et al*., 2007; Fortin *et al*., 2015). Another

630   explanation would be that in the spring, before most of the bloom events occur, the majority of

631   the nitrogen is dissolved, but when cyanobacteria and other phytoplankton increase in abundance

632   over the summer, nitrogen becomes concentrated in particulate forms within cells. We found that

633   measured abiotic variables explained only a part (~25%) of the variation between bloom and non-

634   bloom samples. Including interactions between variables in the model increased the adjusted R^2

635   to ~35%; however no significant interactions were found (Table S4B). The rest of the variation

636   could be explained by unmeasured variables, such as different nitrogen species, water column

637   stability and mixing (although Missisquoi Bay is shallow [~2-5m] and likely never stratified), or

638   time-lagged variables. More variance might also be explained with a larger dataset containing

639   more samples.

640   In addition to environmental variables, we showed that biological variables, in the form of

641   bacterial OTUs or genera, also characterize bloom events. Differential analysis using ALDEx2

642   identified *Microcystis* and *Dolichospermum* as the top bloom biomarkers (Table S8). These two

643   bloom-forming genera are associated with lake eutrophication (O'Neil *et al*., 2012) and are also

644   known to produce cyanotoxins (Gorham and Carmichael *et al*., 1979; Carmichael, 1981). We

645   found additional bloom biomarkers in the genus *Pseudanabaena* and the family Cytophagacaea,

646   previously found to be associated with cyanobacterial blooms (Rashidan and Bird, 2001; O'Neil

647   *et al*., 2012). The order Chthoniobacterales (in the phylum Verrucomicrobia) was also found as a

648   bloom biomarker, consistent with previous studies that observed this taxon in association with

649   *Anabaena* blooms (Louati *et al*., 2015). Other studies have reported specific association between

650   Verrucomicrobia and Cyanobacteria, suggesting that members of this phylum might assimilate

651   cyanobacterial metabolites (Parveen *et al*., 2013; Louati *et al*., 2015). We also found N_2 –fixing

652   members of *Rhizobiales* order as bloom biomarkers. These taxa might be associated with the

653   non-N_2-fixing cyanobacteria *Microcystis*, potentially supporting its growth.

654   Using machine learning, we were able to classify bloom samples with high accuracy

655   based on microbial assemblages, confirming that there is a specific microbial community

656   associated with blooms. Consistent with the ALDEx2 results, *Microcystis* and *Dolichospermum*

657   were present in all bloom assemblages (Table S9). Cyanobacterial blooms have been previously

658   suggested to alter the local environment and the surrounding microbial community (Louati *et al*.,

659   2015). As a result, these assemblages may include bacteria that are reliant on cyanobacterial

660   metabolites and biomass. For example, we found that bloom assemblages included potential

661   cyanobacterial predators from the order Cytophagales and the genus Flavobacterium (Table S9),

662   both associated with bloom termination (Rashidan and Bird, 2001; Kirchman, 2002) but also taxa

663   such as Methylophilaceae, acI, and acIV that have been previously associated with cyanobacterial

664   blooms (Li *et al*., 2015; Woodhouse *et al*., 2016). We found that acI was abundant in early

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665   summer, just before the *Microcystis* and *Dolichospermum* blooms of mid-summer (Figure 3B).

666   While acI might help "set the stage" for a bloom, acIV might have the capacity to use metabolites

667   from cyanobacterial decomposition, and Methylophilaceae is a potential microcystin degrader

668   (Bogard *et al.*, 2014; Ghylin *et al.*, 2014, Mou *et al.*, 2013).

669   Finally, we show the potential for bloom events to be predicted based on amplicon

670   sequence data. We acknowledge that long-term environmental processes such as global

671   warming, and punctual seasonal events such as floods and droughts, are major determinants of

672   whether a bloom will occur in a given year (Paerl and Huisman, 2008; Paerl and Paul, 2012).

673   For example, no bloom occurred in 2007, likely due to a spring drought which dramatically

674   reduced nutrient run-off into the lake. However, sequence data might be useful to predict

675   bloom dynamics on shorter time scales of days, weeks or months. We demonstrated that it is

676   possible to use pre-bloom sequence data to predict the number of days until a bloom event,

677   with errors on the order of weeks (Figure S9) – the best that could be expected, given that

678   sampling density was also on the order of weeks. Sequence data appears to be a strong

679   predictor, similar or better than prediction with environmental variables (Table 2). These

680   results are consistent with a recent study suggesting that abiotic environmental factors could

681   be crucial to initiate blooms, but that biotic interactions might also be important in the exact

682   timing and dominant members of the bloom (Needham and Fuhrman, 2016). Similarly,

683   environmental variables explained relatively little variation in freshwater bacterial

684   composition, while biotic variables (*i.e.* phytoplankton) explained more (Kent *et al*. 2004). It

685   is possible that measuring more environmental variables, or using more complex time-lagged

686   environmental variables (beyond the simple trends used in SR equations) could provide better

687   predictions. However, microbial variables (OTUs) can be measured nearly exhaustively in a

688   single sequencing run, whereas it is hard to know which environmental variables to measure

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689   (*e.g.* temperature, pH, nitrogen, etc. seem relevant but what about Fe, As, Mg, etc.?) and hard

690   to measure them all in high-throughput. However, SR models might be prone to overfitting,

691   which might explain why better predictive accuracy is achieved with fewer samples (Table 2).

692   Our samples were rarely taken more often than weekly, explaining why prediction error is on

693   the order of weeks (Figure S9). We expect that more samples taken over shorter time periods

694   will reduce both overfitting and prediction error. We also note that the "best" predictive

695   equations found by SR are not necessarily global optima, because the space of possible

696   equations is not explored exhaustively.

697   Surprisingly, we never found Cyanobacteria as a bloom predictor in any of the predictive

698   models (Table 2). This means that the models are not simply tracking a positive trend in

699   cyanobacterial abundance, possibly because bloom events are "spiky" (Figure 4) and hence

700   difficult to predict with weekly sampling. Instead, predictive equations always included a

701   member of the order Burkholderiales, classified as Oxalobacteraceae with 67% confidence by

702   Greengenes, or *Polynucleobacter* C (PnecC) with 99% confidence by TaxAss. We acknowledge

703   this taxonomic uncertainty, but give preference to the higher-confidence PnecC assignment. PneC

704   tends to be relatively abundant further ahead of bloom events (Figure 4). This observation could

705   be explained by an ecological succession between PnecC and *Microcystis*/*Dolichospermum*. The

706   fact that PnecC was chosen as a better predictor than Cyanobacteria suggests that PnecC begins

707   to decline before any detectable increase in Cyanobacteria, providing a potential early warning

708   sign. Šimek *et al*., (2011) showed that some PnecC taxa grow poorly in co-culture with algae,

709   suggesting that negative interactions could also occur with cyanobacteria.

710   We have shown that cyanobacterial blooms contain highly (but not exactly) repeatable

711   communities of Cyanobacteria and other bacteria. It appears that the community begins to change

712   before a full-blown bloom, suggesting that sequence-based surveys could provide useful early

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713   warning signals. While the predictions of our models are fairly coarse-grained (*e.g.* prediction

714   error on the order of weeks), they suggest that more accurate prediction might be enabled with

715   increased sampling frequency. It remains to be seen to what extent bloom and pre-bloom

716   communities – which show repeatable dynamics within one lake – are also repeatable across

717   different lakes, and to what extent predictors could be universal or lake-specific. To improve

718   predictions going forward, we suggest sampling additional lakes with dense time-courses, paired

719   with 16S or metagenomic sequencing. In order to predict not just blooms but also the toxicity of

720   blooms, sequencing should be paired with detailed toxin analyses.

721
722   **Data availability**
723
724   Raw sequence data have been deposited NCBI GenBank under BioProject number
725   PRJNA353865.
726

727   **Author information**

728   The authors declare no competing financial interest.

729   **Acknowledgments**

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1028

1029

1030 **Table legends**

1031

1032 **Table 1. Bloom classification results.** We used a supervised machine learning approach

1033 (BioMico) to determine if samples can be classified into bloom bins based on microbial

1034 assemblages (Methods). Accuracy was calculated as the percentage of correctly classified

1035 samples (true positives + true negatives) relative to the total number of samples in the testing set.

1036 The 95% confidence intervals of a random classifier (Methods) and the *P*-values (that the real

1037 classifier differs from random) are also shown.

1038

1039 **Table 2. Predicting bloom timing with symbolic regression (SR).** The best formula found by

1040 SR is shown for each category of predictor variables. SR was performed on two datasets. First,

1041 OTUs and genera were used as predictor variables, using the maximum number of non-bloom

1042 samples ($N = 54$). Second, in order to determine the impact of including environmental data as

1043 predictor variables, we used only samples with a full set of metadata ($N = 21$). (*/** indicate

1044 OTUs/genera found multiple times in SR formulas).

1045

1046

1047 **Figure legends**

1048

1049 **Figure 1. Comparison of alpha diversity between bloom and non-bloom states.** Three alpha

1050 diversity metrics were employed: (A) BWPD, (B) the Shannon index, and (C) the Shannon

1051 evenness (equitability) to compare alpha diversity between bloom (black) and non-bloom (grey)

1052 samples. We repeated the same analysis after removing Cyanobacteria. Comparisons were

1053 performed using a Mann-Whitney test (* P < 0.05, ** P < 0.01, *** P < 0.001).

1054

1055 **Figure 2. Changes in community composition across seasons and bloom events.** Each point

1056 in the PCoA plot represents a sample, with distances between samples calculated using weighted

1057 UniFrac as a measure of community composition. Non-bloom samples are shown in black, bloom

1058 samples in grey. Different shapes describe the different seasons: circle for Spring, triangle for

1059 Summer and star for Fall. (A) Samples with all OTUs included. (B) Samples excluding OTUs

1060 from the phylum Cyanobacteria.

1061

1062 **Figure 3. Cyclical community composition dynamics.** Multivariate regression tree (MRT)

1063 analysis was used to estimate the impact of time on bacterial community structure. (A) The most

1064 parsimonious tree shows how the community is partitioned by MRT using week of the year as a

1065 temporal variable. Six different leaves (large coloured circles) were defined based on microbial

1066 abundance and composition. (B) The community composition within leaves is represented in a

1067 PCA plot, where small points represent individual samples and large points represent the group

1068 mean (within the leaf). The grey barplot in the background indicates OTUs whose differential

1069 abundance explains variation in the PCA plot.

1070

1071 **Figure 4. Oxalobacteraceae and seq413 decline while *Microcystis* and *Dolichospermum***

1072 **increase as a bloom event approaches**. We plotted the relative abundance of relevant taxa from

1073 112 to 7 days before a bloom sample. Oxalobacteraceae (genus unclassified) and the OTU seq413

1074 (Oxalobacteraceae, genus unclassified or *Polynucleobacter* PnecC) are relatively abundant long

1075 before a bloom event, and gradually decline as bloom events approach. *Microcystis* and

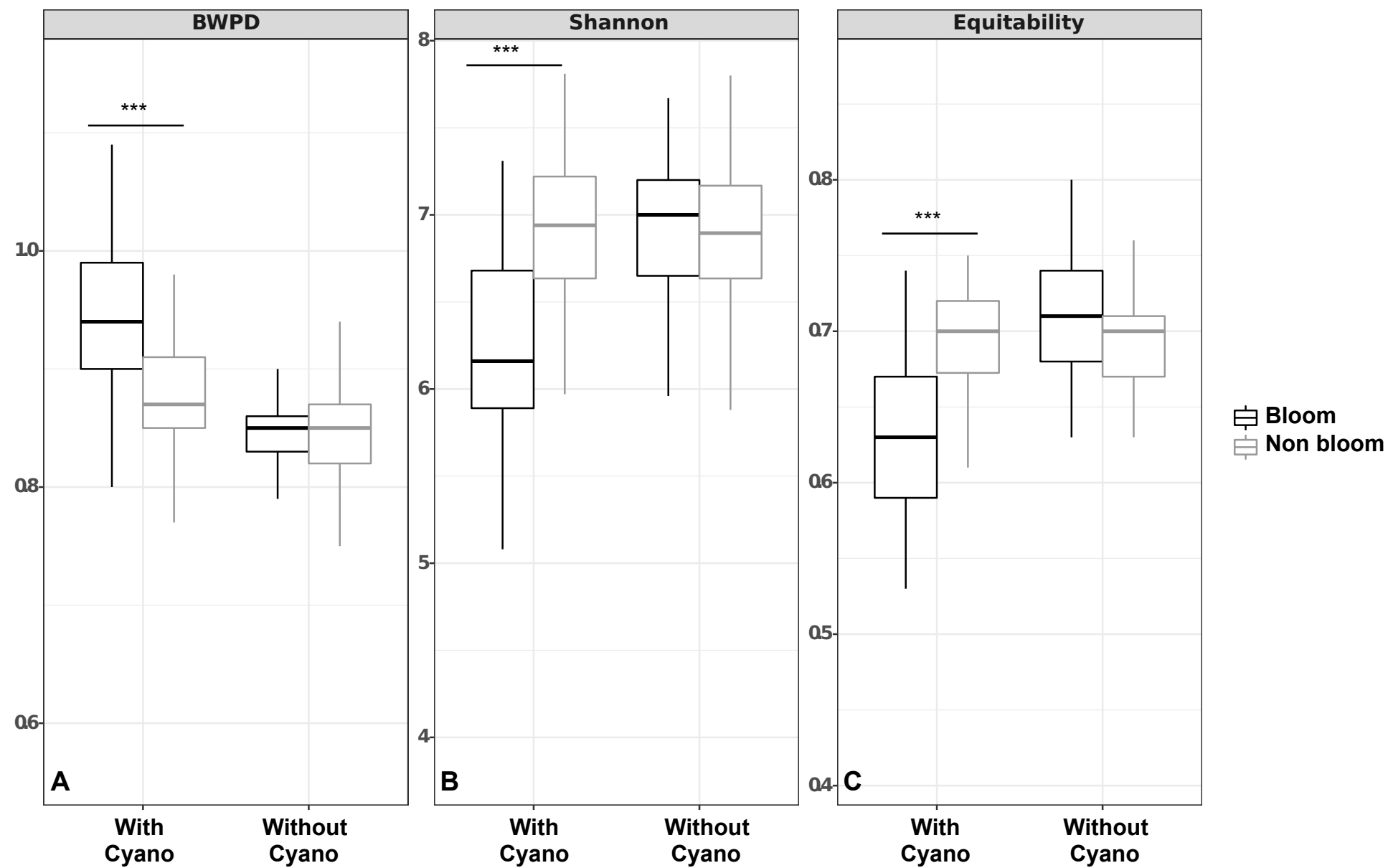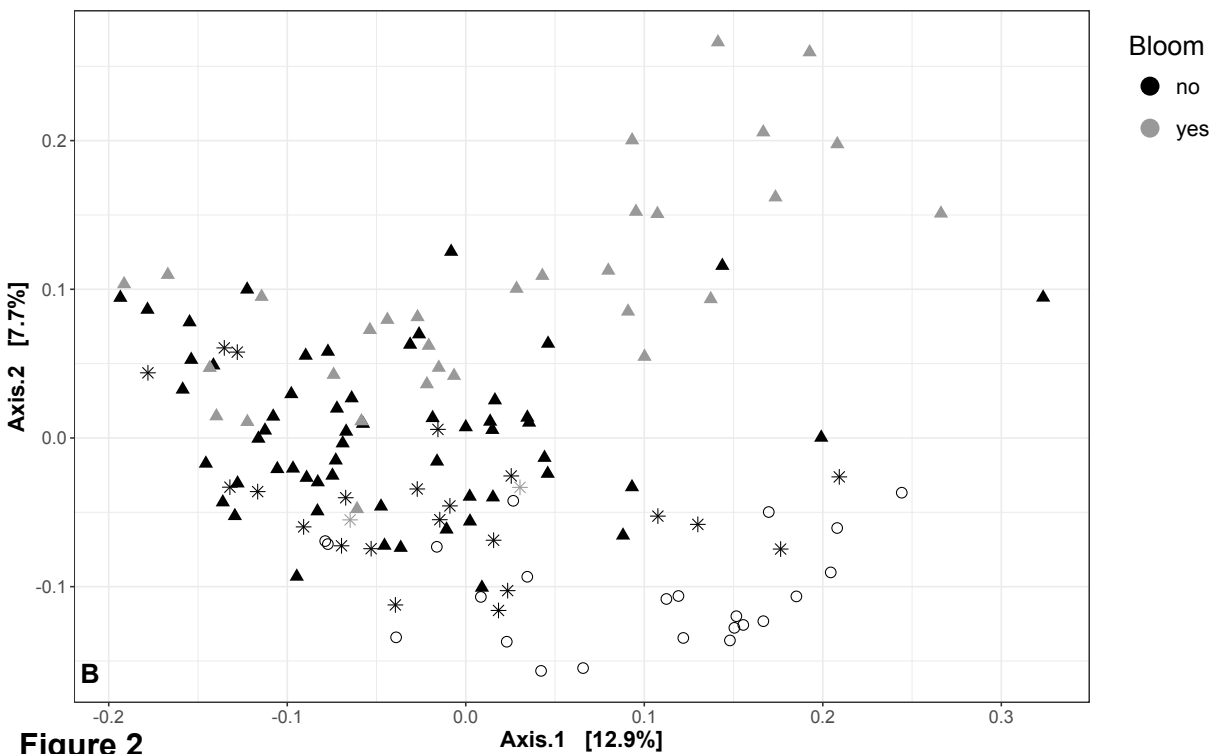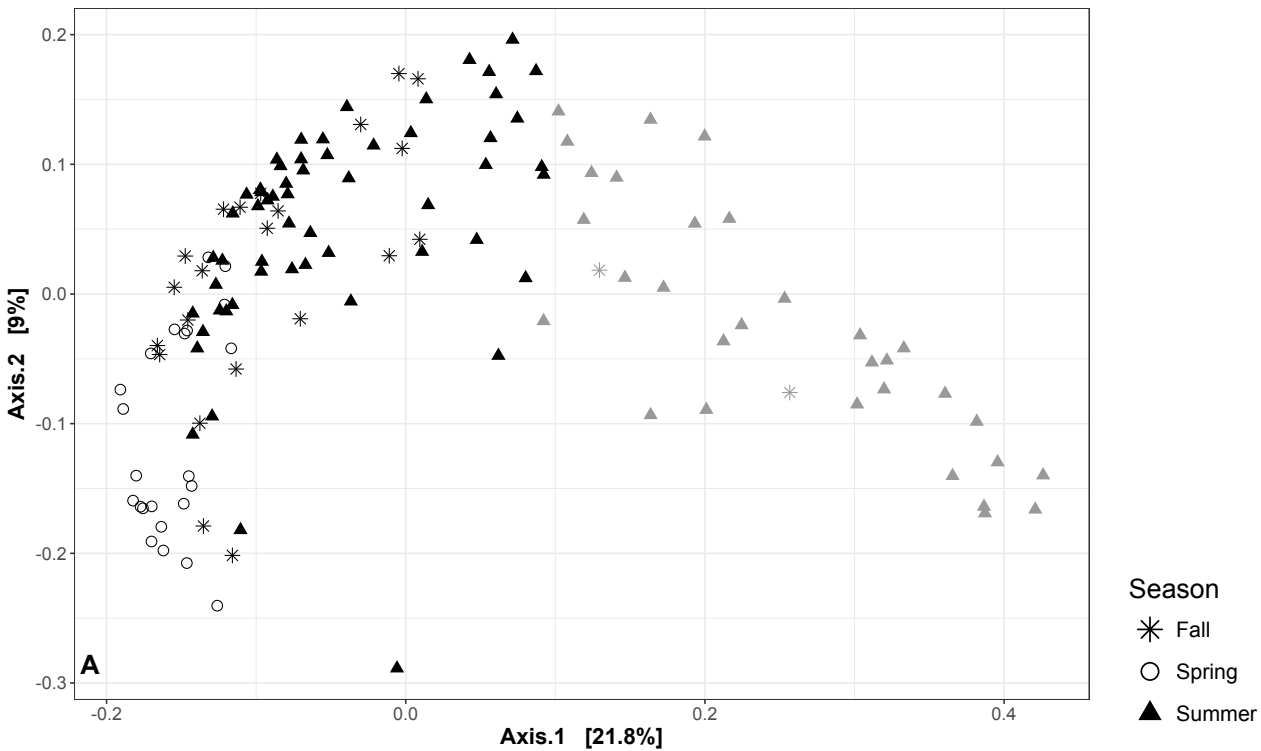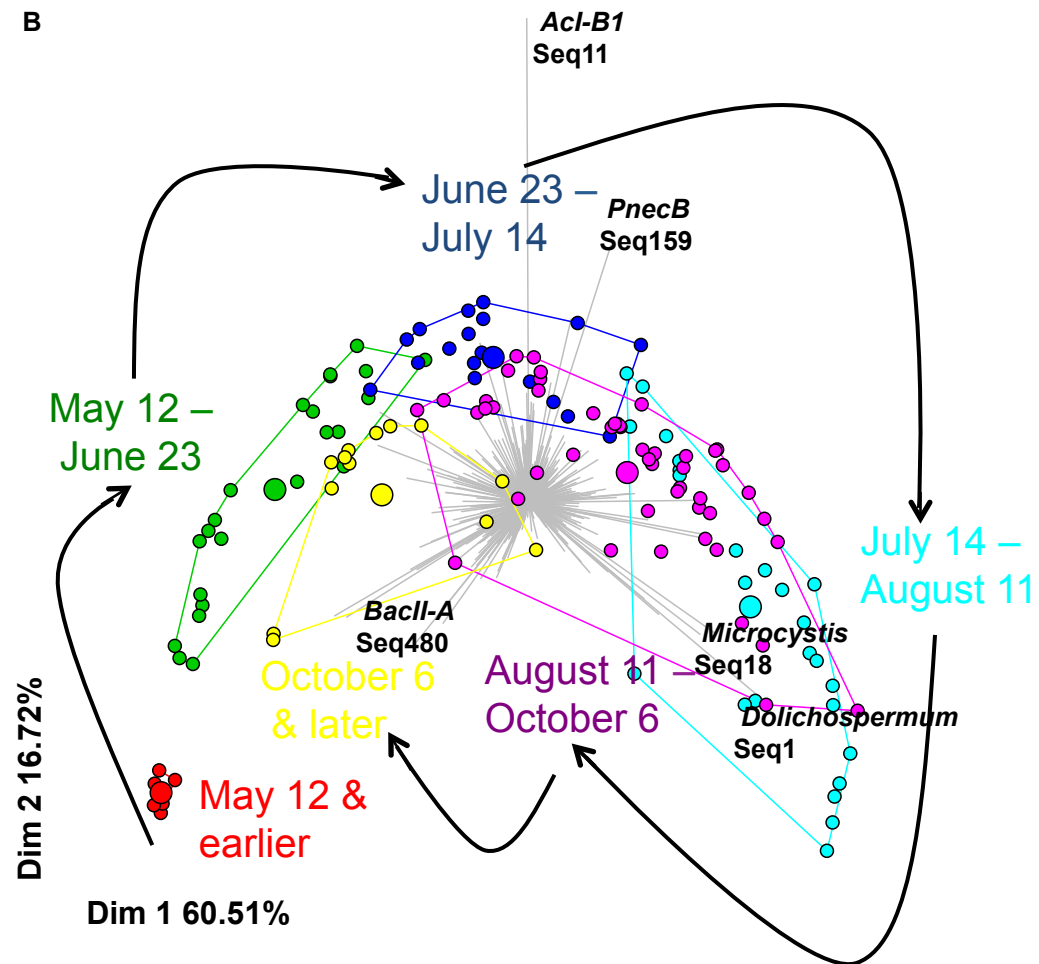1076 *Dolichospermum* are the two most dominant bloom-forming cyanobacteria.

Figure 1

Figure 2

A

Week< 25.5 (June 23)   Week >= 25.5

Week< 19.5 (May 12)   Week>=19.5

Week< 40.5 (October 6)   Week >= 40.5

n=6   n=23

Week< 28.5 (July 14)   Week >= 28.5

n=13

n=18

Week< 32.5 (August 11)   Week >= 32.5

n=26   n=49

R-squared= 27.4%

B

AcI-B1
Seq11

June 23 –
July 14

PnecB
Seq159

May 12 –
June 23

July 14 –
August 11

Dim 2 16.72%

BacII-A
Seq480

October 6
& later

August 11 –
October 6

Microcystis
Seq18

Dolichospermum
Seq1

May 12 &
earlier

Dim 1 60.51%
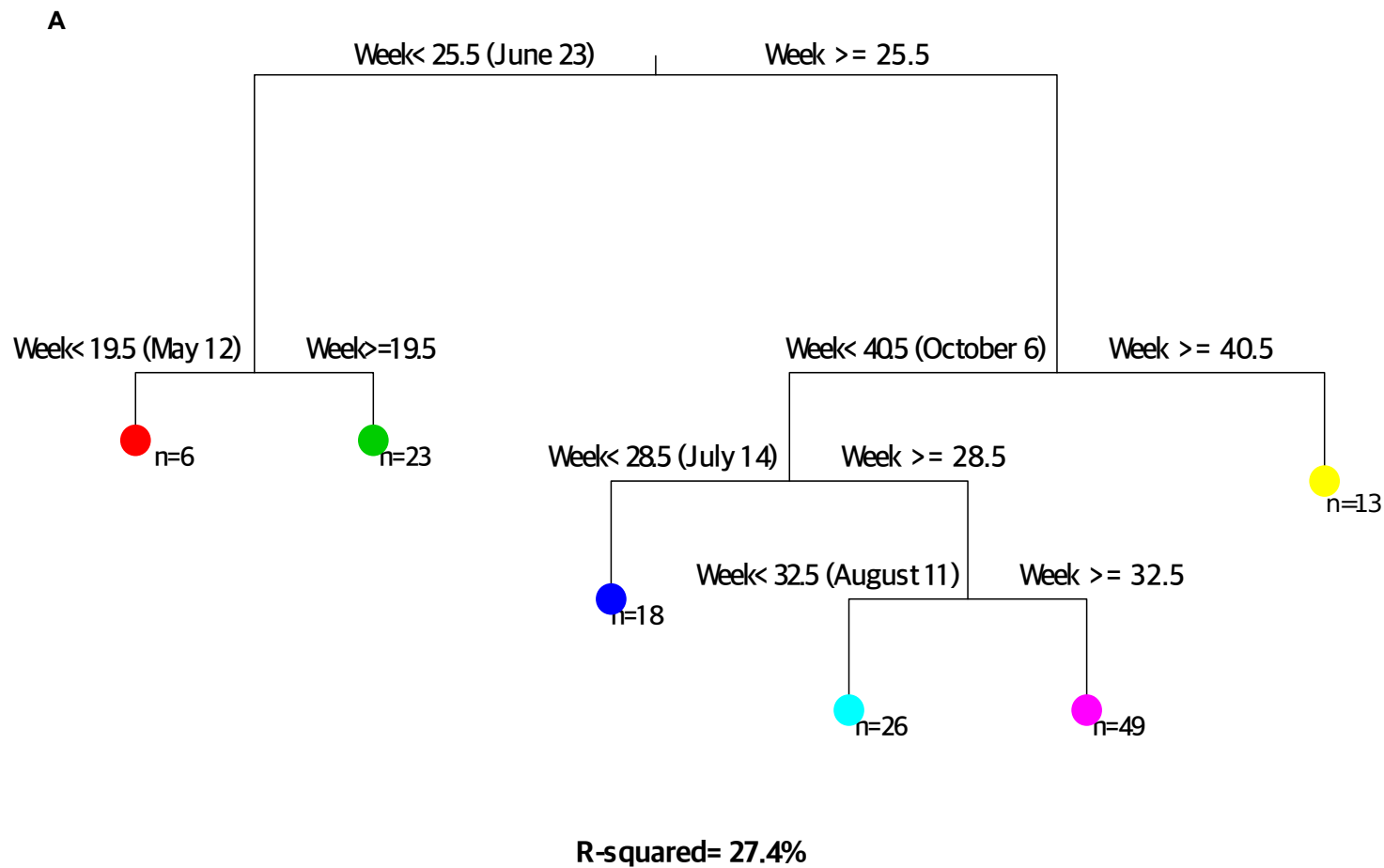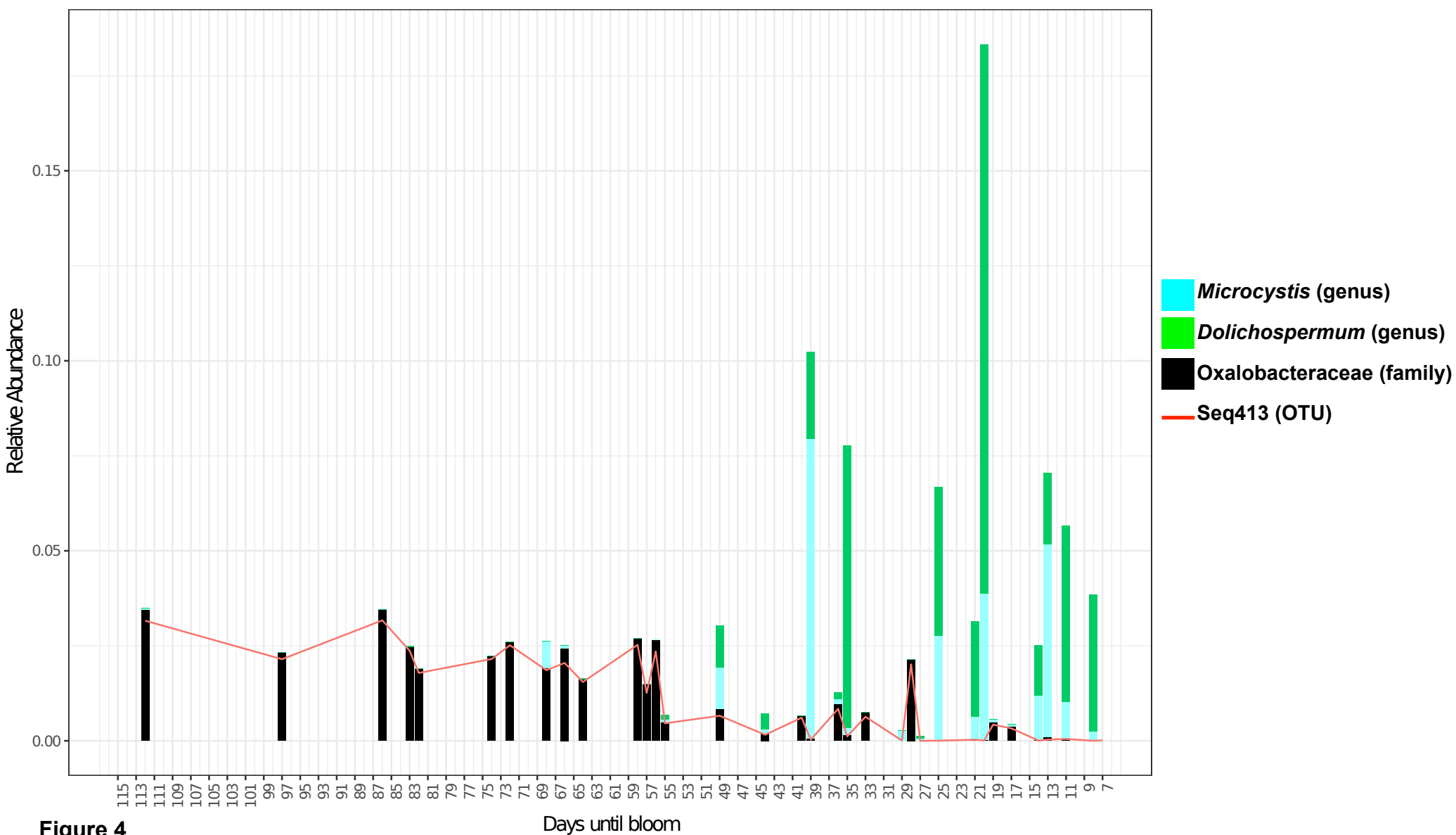
Figure 3

Figure 4

Table 1.

Training set	Testing set	Classification Accuracy	False positives	False negatives	True negatives	True positives	95% confidence interval of random classifier	P-value (real classifier differs from random)
2/3 of all samples	1/3 of all samples	91.84 %	4	0	33	12	36-64%	8.225×10^{-10}
2007 & 2009 samples	All other samples	92.52%	8	0	73	26	40-60%	$< 2.2 \times 10^{-16}$
2/3 of all samples, without cyanobacteria	1/3 of all samples, without cyanobacteria	85.71%	6	1	31	11	36-64%	3.625×10^{-07}
2007 & 2009 samples, without cyanobacteria	All other samples, without cyanobacteria	83.18%	9	9	72	17	40-60%	1.781×10^{-12}

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_g_unclassified_seq436 + 2007.048 × f__Oxalobacteraceae_ g_unclassified **_seq413****	0.805	4	54	117.540	265.406	266.222
Genera	19.780 + 2057.652 × **f_Oxalobacteraceae_ g_unclassified*** + 703.606 × f_Armatimonadaceae_ g_unclassified - 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_ g_unclassified _seq548) + 2511.838 × f__Oxalobacteraceae_ g_unclassified **_seq413****	0.826	4	21	83.845	101.008	103.508
Genera	21.185 + 2646.333 × **f_Oxalobacteraceae_ g_unclassified*** - 13323.212 × trend(genus_Flavobacterium) - 16288.058 × o_Ellin329_ g_unclassified	0.914	5	21	31.776	82.633	86.633
Environmental data	114.017 + 192.663 × trend(MeanT) + 137.168 × DN - 0.413 × PP - 6.915 × MeanT - 223.712 × DN × trend(MeanT) - 51.424 × DN^2	0.828	8	21	63.493	103.170	115.170
OTU + Environmental data	15.941 + 49774.285 × trend(f_Cerasicoccaceae_ g_unclassified _seq548) + 2511.838 × f__Oxalobacteraceae_	0.826	4	21	83.845	101.008	103.508

	g_unclassified _seq413**						
Genera + Environmental data	$23.353 + 2389.349 \times$ **f_Oxalobacteraceae|_g_unclassified*** - $13323.212 \times$ trend(genus_Flavobacterium) - $16288.057 \times$ o_Ellin329_g_unclassified	0.923	5	21	28.375	80.256	84.256