

1 **Influence of potential grazers on picocyanobacterial abundance in**
2 **Lake Biwa revealed with empirical dynamic modeling**

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Influence of potential grazers on picocyanobacterial abundance in Lake Biwa revealed with empirical dynamic modeling

Picocyanobacteria in lakes generally occur as single cells (single-celled picocyanobacteria; SPcy) or colonies (colonial picocyanobacteria; CPcy), and the latter form has been considered an adaption to grazing pressure. In addition to direct effects of grazing, grazers may also have important indirect effects on picocyanobacteria, such as those from nutrient regeneration and trophic cascades. Interactions between picocyanobacteria and their grazers in lakes can thus be complex and difficult to predict. In the present study, we aimed to evaluate the influence of various grazers on SPcy and CPcy in Lake Biwa, Japan. We followed seasonal changes in the abundances of SPcy, CPcy, and their potential grazers biweekly over two years. The data collected were analyzed using empirical dynamic modeling (EDM), a model-free, nonlinear time-series method. We found that heterotrophic nanoflagellates (HNF), rotifers (*Keratella*, *Polyarthra*, and *Trichocerca*), cladocerans, and copepods played important and differing roles in controlling the abundances of SPcy and CPcy. Notably, HNF had an apparent positive influence on SPcy abundance, despite being considered major consumers of SPcy. This result suggested that the enhancement of SPcy growth due to nutrient regeneration by HNF might exceed losses from mortality due to grazing by HNF. EDM also suggested that colony formation by picocyanobacteria may be unidirectional, with SPcy tending to form CPcy. Our findings show that the seasonal dynamics of SPcy and CPcy in Lake Biwa are influenced by a variety of grazers, which may play differing ecological roles in the aquatic food web.

Keywords: empirical dynamic modeling, grazers, heterotrophic nanoflagellates, indirect effects, picocyanobacteria

Introduction

Picocyanobacteria, a diverse group of cyanobacteria defined by cell sizes of less than 2 μm , are numerous and ubiquitous in freshwater and marine ecosystems (Stockner and Antia 1986; Stockner 1988). Despite their small size, these photoautotrophic organisms

41 contribute largely to phytoplankton biomass and primary production, and play important
42 roles in aquatic ecosystems (Weisse 1993). In freshwaters, single-celled
43 picocyanobacteria (SPcy) dominate in oligotrophic environments and are mainly
44 represented by the genera *Synechococcus* and *Cyanobium* (Fogg 1995; Sanchez-
45 Baracaldo et al. 2005). Colonial picocyanobacteria (CPcy) are also common and often
46 abundant in meso-eutrophic environments (Stockner 1991; Stockner et al. 2000). They
47 consist of colonial species (e.g., *Aphanothece*, *Aphanocapsa*, *Cyanodictyon*) and
48 microcolonies formed by SPcy (Passoni and Callieri 2000; Callieri et al. 2012).

49 Understanding how the abundances of picocyanobacterial populations are
50 controlled by grazers is essential to elucidating their ecology. Grazing has been
51 considered a key top-down control process affecting SPcy abundance (Horn and Horn
52 2008). Small protists such as heterotrophic nanoflagellates (HNF) and ciliates are
53 considered the major consumers of SPcy (Stockner and Antia 1986; Šimek et al. 1995
54 and 1997; Sanders et al. 2000). An uptake of nearly 80% of the carbon produced by
55 SPcy was estimated for HNF and ciliates in an oligotrophic lake (Callieri et al. 2002).
56 Metazoan zooplankton are also important grazers of SPcy. Filter feeders such as
57 planktonic rotifers (e.g., *Keratella*, *Polyarthra*) and cladocerans (e.g., *Daphnia*,
58 *Bosmina*) can feed on pico-sized particles and thus consume SPcy (Gophen and Geller
59 1984; Weisse 1993; Ronneberger 1998; Callieri et al. 2012). Copepods are also efficient
60 SPcy grazers, even when alternative foods are available (Motwani and Gorokhova
61 2013). By contrast, CPcy appear to be resistant to grazing (Blomqvist 1996). It has been
62 suggested that colony formation by picocyanobacteria may act as an anti-grazing
63 strategy (Callieri et al. 2012). Grazing experiments have shown that some strains of
64 *Synechococcus* could form microcolonies when co-cultivated with HNF (Callieri et al.

2016). Huber et al. (2017) demonstrated that grazing by *Bosmina* favored the dominance of *Cyanodictyon* over SPcy. However, zooplankton such as *Daphnia* have the ability to ingest particles up to tens of micrometers in size (Ronneberger 1998), and thus small colonies of CPcy may be edible to such large grazers (Van Donk et al. 2011).

Grazers also play other important roles in controlling the abundances of picocyanobacteria based on indirect interactions such as nutrient regeneration and trophic cascades. Heterotrophic protists and metazoan zooplankton can excrete nitrogen and phosphorus and thereby support the growth of phytoplankton (Johannes 1965; Moegenburg and Vanni 1991; Nakano 1994a, b, c). Indeed, Callieri et al. (2004) reported a significant increase in picocyanobacterial photosynthetic efficiency in the presence of *Daphnia* grazing. In addition, predator-prey relationships exist among HNF, ciliates, rotifers, cladocerans, and copepods (Arndt 1993; Sanders et al. 1994; Suzuki et al. 1999; Nakano et al. 2001; Christoffersen and Gonzalez 2003; Brandl 2005), and therefore the abundances of picocyanobacteria may be affected indirectly through trophic cascades of these grazers (Wickham 1995; Sundt-Hansen et al. 2006). Taking the direct effects of grazing into consideration, interactions between picocyanobacteria and grazers in nature can thus be highly complex and difficult to predict.

To elucidate the influence of potential grazers on picocyanobacteria, we carried out a two-year study in Lake Biwa, Japan, focusing on the differing ecological properties of SPcy and CPcy. We collected samples biweekly and followed seasonal changes in abundances of picocyanobacteria (SPcy and CPcy) and their potential grazers (HNF, ciliates, rotifers, cladocerans, and copepods). Finally, we applied empirical dynamic modeling (EDM), which is a model-free, nonlinear time-series analysis method, to the time-series data collected. EDM was developed to specifically

analyze the dynamics of nonlinear systems such as ecosystems, where traditional linear tools are not applicable. Thus, this method is suitable for the analysis of the nonlinear, state-dependent behaviors of picocyanobacteria observed in our study system. The main objectives of the present study are two-fold: 1) identification of potential grazers that affect SPcy and CPcy abundances under natural conditions, and 2) quantification of the overall effects of various grazers on SPcy and CPcy abundances using EDM.

Methods

Sample collection and measurement of environmental variables

Sample collection was conducted biweekly at observation site Ie-1 (35°12'58"N; 135°59'55"E; maximum depth, 73 m) in the north basin of Lake Biwa (Mukherjee et al. 2017) from July 2015 to June 2017. Vertical profiles of water temperature, chlorophyll *a* (Chl-*a*) concentration and photosynthetically active radiation (PAR) throughout the water column were obtained using a CTD profiler (SBE-911 plus; Sea Bird Electronics, Sea-logger, WA, USA). In addition, samples for analysis of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) were collected monthly at depths of 0, 5, 10 and 20 m from August 2015 to July 2016. NH₄-N concentrations were measured using a sensitive fluorometric assay (Holmes et al. 1999). Concentrations of NO₂-N, NO₃-N and DIP were analyzed using an AACS-II autoanalyzer (BRAN+LUEBBE).

Samples of picocyanobacteria, protists and metazoan zooplankton were collected from depths of 0, 5, 10, 15 and 20 m with a 5 L Niskin sampler (General Oceanics, Miami, USA). All samples were collected at around the same time of day (10:00 to 12:00 h). For picocyanobacteria, unfiltered water samples were collected. For

112 HNF, water samples were prefiltered using a plankton net with a mesh size of 20 μm ,
113 and the filtrate was collected. After collection, 100 ml of each water sample was fixed
114 with glutaraldehyde at a final concentration of 1% for enumeration of picocyanobacteria
115 and HNF, and the fixed samples were then stored in the dark at 4 °C. For other
116 zooplankton (ciliates, rotifers, cladocerans, and copepods), 10 L of lake water was
117 concentrated to 100 ml using a plankton net with mesh size 20 μm , then fixed with 5%
118 acid Lugol's solution and stored in the dark.

119 ***Sample treatment and plankton enumeration***

120 For the enumeration of picocyanobacteria, fixed samples of 1–10 ml were filtered
121 through 0.2 μm polycarbonate membrane filters to retain cells. Duplicate filters were
122 prepared for each sample. An epifluorescence microscope (Olympus BX53, 1000x) was
123 used to enumerate SPcy and CPcy cells under green excitation (530–550 nm). At least
124 100 cells or 50 fields were counted from each filter. For the enumeration of HNF, fixed
125 samples of 30–50 ml were filtered through 0.8 μm polycarbonate membrane filters.
126 HNF cells on the filter were stained with primulin according to methods in Caron
127 (1983) and enumerated under ultraviolet excitation. Nanoflagellates that exhibited no
128 apparent red chlorophyll fluorescence under green excitation were identified as HNF.

129 To count metazoan zooplankton, fixed samples were poured into 100 ml
130 cylinders and concentrated through natural sedimentation for at least 48 h. One ml of
131 the concentrated sample was then loaded onto a Sedgewick-Rafter counting chamber
132 (Pyser-SGI Limited, British) and checked under an optical microscope (Olympus BX51,
133 100x). Each sample was counted twice.

134 *Time series and state space reconstruction (SSR)*

135 Time series can be defined as any set of sequential observations of the system state, and
136 the dynamic behaviors can be delineated as a trajectory of a state over time in a
137 multidimensional state space by plotting time series. Time series taken from ecosystems
138 can be used to trace out trajectories of the system, which provide information on
139 ecosystem dynamics. For example, if one has performed sequential observations on a
140 three-species ecological system, e.g., grasses (primary producer), rabbits (consumer)
141 and foxes (predator), then the dynamics of the three-species system can be reconstructed
142 by plotting time series of grasses, rabbits, and foxes along the x , y , and z axis,
143 respectively, in a three-dimensional state space. The motion of the three-dimensional
144 vectors can be understood as the system behavior.

145 In a natural ecosystem, however, it is usually impossible to collect time series of
146 all potentially important variables involved in a target system. Fortunately, Takens
147 (1981) offered a theoretical basis to solve this problem: a mathematical theorem,
148 Takens' embedding theorem, demonstrated that a shadow version of the attractor can be
149 reconstructed by a single observed time series. In other words, delineation of
150 trajectories, originally constructed using multivariables, can be possible even if a time
151 series is available only for a single variable (Sauer et al. 1981; Takens 1981). To embed
152 such a single time series, vectors in the putative phase space are formed from time-
153 delayed values of the time series, $\{x(t), x(t-\tau), x(t-2\tau), \dots, X(t-[E-1]\tau)\}$, where E is the
154 embedding dimension, and τ is the time lag. This procedure, the reconstruction of the
155 original dynamics, is known as State Space Reconstruction (SSR).

156 ***Empirical dynamics modeling (EDM)***

157 EDM, a time-series analytical framework rooted in SSR and designed specifically for
158 the analysis of nonlinear dynamics such as ecosystem processes (Sugihara et al. 2012;
159 Ye et al. 2015; Deyle et al. 2016), was applied to our time-series data. Because EDM
160 recovers dynamics directly from time series using SSR, it does not assume any set of
161 equations governing the system, and thus is suitable for analyzing complex systems, for
162 which it is often difficult to make reasonable *a priori* assumptions about their
163 underlying mechanisms. EDM provides tools for various purposes, including the
164 identification of causal factors and quantification of interaction strengths in nonlinear
165 systems where traditional linear statistical tools are not applicable, and has been
166 recently proven effective for analyzing the dynamics of natural complex ecosystems
167 (Ye et al. 2015; Ushio et al. 2018). The analysis workflow in the present study was as
168 follows: first, causal links between picocyanobacteria and other variables (e.g., water
169 temperature, Chl-*a*, or HNF) were identified using convergent cross mapping (CCM;
170 Sugihara et al. 2012); second, when causal links were identified, the interaction
171 strengths between variables were quantified using the multivariate S-map method
172 (Deyle et al. 2016). Detailed descriptions of CCM and the multivariate S-map are
173 available in previous studies (Sugihara et al. 2012; Deyle et al. 2016; Chang et al. 2017;
174 Ushio et al. 2019). Considering the thermal stratification and vertical migration of the
175 plankton community, we used the average of time-series data collected from 0 to 20 m
176 in our analyses. Data were normalized to a zero mean and unit variance prior to EDM
177 analysis. The library size (i.e., the length of time-series data) of most variables was 48,
178 whereas that of CPcy was 38.

179 First, CCM was applied to detect causal links. Briefly, if two variables are
180 causally related in a dynamic system, they should share the same attractor, making it
181 possible to predict the values of the causal variable by using the reconstructed state
182 space of the effect variable (Sugihara et al. 2012). CCM quantifies how well an effect
183 variable predicts the values of a putative causal variable, and the forecasting accuracy
184 (i.e., cross map skill) and its convergence against the library size are important criteria
185 for determining causality (for more details, see Sugihara et al. 2012). An essential
186 parameter of CCM, the optimal embedding dimension (E), was determined using
187 simplex projection (Sugihara and May 1990). Simplex projection can be used to find the
188 optimal value of E by identifying which E maximizes the forecasting accuracy of a
189 given time series. According to simplex projection, the optimal E for the time series of
190 SPcy and CPcy were 2 and 3, respectively. Another important parameter, time lag (τ) in
191 the lagged coordinate embedding, was set to 1 following a previous study (Chang et al.
192 2017), which corresponds to 2 weeks in our time series. Due to the time-lagged causal
193 relationship, preliminary CCM was conducted to find the optimal cross-map lag (t_p ,
194 time to prediction) for each causal variable. The optimal t_p value which maximized the
195 forecasting accuracy within the range of 0 to -6 (i.e., between now and three months
196 ago) was chosen. Then, CCM was performed to calculate cross map skill and thus
197 identify causal variables that affect SPcy and CPcy. Fisher's z-test and surrogate test
198 were successively applied to determine whether the cross map skill was statistically
199 significant (Chang et al. 2017). Fisher's z-test examines whether the cross map skill
200 obtained using the maximal library length is significantly higher than that obtained
201 using the minimal library length (i.e., convergence). Surrogate test examines whether

the cross map skill is significantly different from the null model expectation generated using surrogate time series.

Second, based on the set of significant causal variables identified through CCM, the multivariate S-map was built to quantify the influence of each variable on SPcy or CPcy, which was approximated using partial derivatives of the causal variables. Time-series data of the effect variable (SPcy or CPcy) and significant causal variables with lag equal to the optimal t_p were used to reconstruct the state space. For example, if the variables Y and Z influence variable X with t_p of -2 and -3 , respectively, the state space is reconstructed as follows: $\{X(t), X(t-1), \dots, X(t-[E-1]), Y(t-1), Z(t-2)\}$, and $X(t+1)$ is predicted using the multivariate S-map. In the multivariate S-map analysis, the nonlinear parameter (θ) that minimizes the forecasting error was chosen according to previous studies (Deyle et al. 2016; Ushio et al. 2018).

Lastly, Spearman's correlation analysis was conducted to infer potential relationships between the abundances of picocyanobacteria and concentrations of nutrients (DIN and DIP). This method was used because the lengths of time series of nutrients ($N = 13$) were not sufficient for EDM. All analyses described above were carried out using R v3.4.3 (R Development Core Team, 2018). EDM was performed using the "rEDM" package (version 0.7.2, Ye et al. 2015), and the step-by-step tutorial is available at <https://ha0ye.github.io/rEDM/index.html>.

Results

Seasonal profiles of water temperature, Chl-a, and nutrients

In Lake Biwa, thermal stratification was pronounced from June to October (the stratification period) with a thermocline between 15 and 30 m (Fig. 1a). In August and

225 September, water temperature in the epilimnion reached as high as 29.8 °C. Water
226 column started mixing in November and circulated totally from February to March (the
227 mixing period). During the mixing period, the average ($\pm$ standard deviation) water
228 temperature was 8.4 ± 0.5 °C.

229 The annual mean concentration of Chl-*a* in the euphotic zone (from 0 to 20 m,
230 calculated by PAR, data not shown) was 2.65 ± 1.85 $\mu\text{g L}^{-1}$ (Fig. 1b). Elevated Chl-*a*
231 concentrations (>5 $\mu\text{g L}^{-1}$) were recorded several times: from April to May 2016, from
232 November 2016 to January 2017 and from May to June 2017. The mean concentration
233 of DIN was 1.64 ± 1.26 $\mu\text{mol L}^{-1}$. DIN was depleted in the epilimnion during the
234 stratification period but relatively high at other times (Fig. S1a). DIP concentrations
235 were low and remained nearly constant across depths and seasons, with an average of
236 0.0045 ± 0.0025 $\mu\text{mol L}^{-1}$ (Fig. S1b).

237 ***Seasonal dynamics of picocyanobacteria and potential grazers***

238 The annual average SPcy abundance in the water column above 20 m was $5.64 \pm 9.00 \times$
239 10^4 cells mL^{-1} during the study period (Fig. 2a). High cell densities (up to 4.50×10^5
240 cells mL^{-1}) were recorded in June and August 2016. Generally, SPcy were highly
241 abundant ($>10^5$ cells mL^{-1}) throughout the stratification period and were mainly
242 distributed in the epilimnion. During the mixing period, SPcy density decreased to
243 around 10^2 cells mL^{-1} and the cells were almost homogeneously distributed throughout
244 the water column.

245 The annual average CPcy abundance was $3.22 \pm 8.45 \times 10^4$ cells mL^{-1} (Fig. 2b;
246 calculated using data collected from July 2016 to June 2017, as data from 2015 were
247 incomplete). CPcy density increased dramatically from 10^3 to $>3 \times 10^5$ cells mL^{-1} in the
248 epilimnion at the beginning of July. The majority of CPcy were observed near the

thermocline after 2 weeks and the greatest cell density (up to 4.54×10^5 cells mL⁻¹) was recorded at a depth of 15 m in mid-July 2016. From September onward, CPcy density declined rapidly to $<10^3$ cells mL⁻¹ and became undetectable during the mixing period.

HNF were observed throughout the year in the water column, with an average cell density of $7.86 \pm 6.44 \times 10^2$ cells mL⁻¹ (Fig. S2a). Generally, HNF were abundant ($>10^3$ cells mL⁻¹) in the epilimnion during stratification periods and remained scarce ($>10^2$ cells mL⁻¹) during mixing periods. By contrast, ciliates were not a major protistan group in Lake Biwa (Fig. S2b). At most times, ciliates were at low abundance (<10 cells L⁻¹) or even below the detection limit, with an annual average cell density of 51.7 ± 153.8 cells L⁻¹. Nevertheless, the genera *Epistylis* and *Codonella* sometimes formed transient blooms in summer or autumn with densities greater than 10^3 cells L⁻¹.

We observed no clear seasonal or vertical trends in the abundances of rotifers, cladocerans, and copepods. These grazers could be abundant in spring, summer or autumn at different depths (Fig. S2c–e). The annual average density of rotifers was 111.6 ± 124.5 individuals L⁻¹. Rotifers in Lake Biwa were highly diverse, so we also recorded seasonal changes in their genus-level composition (Fig. S3). The dominant genera during the study period were *Polyarthra*, *Keratella* and *Trichocerca*, accounting for 55.1%, 11.5%, and 10.8% of total rotifer abundance, respectively. Average densities of cladocerans and copepods were 13.2 ± 15.6 and 58.8 ± 59.1 individuals L⁻¹, respectively. The dominant genera of cladocera were *Daphnia* and sometimes *Bosmina*, whereas copepods were dominated by *Eodiaptomus japonicus*.

Results of EDM and correlation analysis

According to CCM, temperature, HNF, cladocera, copepod, and *Keratella* were identified as causal variables that affected seasonal changes in the abundance of both

SPcy and CPcy, whereas *Polyarthra* affected only SPcy and *Trichocerca* affected only CPcy (Table 1; see details in Fig. S4 and S5). In addition, we also found causal links between SPcy and CPcy. No significant causal link was found between SPcy and other variables such as Chl-*a* concentration, ciliates or non-dominant rotifers (e.g., *Pleosoma*, *Pompholyx*), or between CPcy and those variables (data not shown).

The influences of causal variables on SPcy and CPcy were then quantified using the multivariate S-map (Fig. 3; see Table S1 for optimal parameters of the multivariate S-map). Positive and negative values of interaction strengths can be interpreted as an effect variable tending to increase and decrease, respectively, in response to the increase in a causal variable (Deyle et al. 2016). Although the multivariate S-map method enables the calculation of time-varying interaction strengths, we used time-averaged values of interaction strengths to evaluate the overall effects of causal variables on picocyanobacterial abundance for convenience and simplicity (Table 1; Fig. 4).

Therefore, temperature, HNF, and *Polyarthra* had positive effects on SPcy, whereas CPcy, *Keratella*, and cladocera negatively affected SPcy abundance. And copepods had a moderating effect on SPcy. On the other hand, SPcy, HNF, *Trichocerca*, and copepod had positive effects on CPcy abundance, whereas temperature, *Keratella*, and cladocera had negative effects on CPcy.

Lastly, potential relationships among SPcy, CPcy, DIN, and DIP were examined using Spearman's correlation analysis. The results showed that DIN was negatively correlated with SPcy and CPcy ($r_s = -0.668$, $p < 0.001$; $r_s = -0.734$, $p < 0.001$, respectively). DIP was negatively correlated with CPcy ($r_s = -0.528$, $p < 0.001$), but not significantly correlated with SPcy ($r_s = 0.092$, $p = 0.516$).

296 **Discussion**

297 In the present study, we applied EDM for exploring the environmental variables and
298 organisms that are potential drivers of the seasonal dynamics of picocyanobacteria.
299 Interaction strengths estimated using the multivariate S-map revealed how these
300 variables affect picocyanobacterial abundance. In many cases, both positive and
301 negative values were obtained simultaneously, suggesting complex relationships
302 between the causal variable and SPcy or CPcy. The influence by one causal variable on
303 SPcy or CPcy abundance (i.e., the average of interaction strengths) should be regarded
304 as a “net” interaction strength. In other words, the time-averaged interaction strength
305 calculated in the present study indicates whether positive “bottom-up” effects were
306 larger than negative “top-down” effects or not (Deyle et al. 2016).

307 ***Effects of environmental variables on picocyanobacteria***

308 The positive influence of temperature on SPcy as revealed through EDM (Fig. 3a)
309 indicates that temperature played an important role in increasing the abundance of
310 picocyanobacteria (Vörös et al. 2009; Jodłowska and Śliwińska 2014). By contrast,
311 CPcy was negatively affected by temperature (Fig. 3b), despite being reported to
312 increase dramatically in the warm summer months (Callieri et al. 2012). This result may
313 be caused by sinking due to the large colony sizes of CPcy (Deng et al. 2016). After
314 forming transient blooms near the water surface, CPcy immediately sank to the
315 metalimnion (15–20 m), where water temperature was relatively low during the
316 stratification period (Fig. 1a and 2b). On the other hand, we did not find a relationship
317 between Chl-*a* concentration and picocyanobacterial abundance. Although
318 picocyanobacteria could be dominant (45% of total Chl-*a*) during the stratification

period, they were not a major group in the phytoplankton community during other parts of the year in Lake Biwa (< 5% of total Chl-*a* in other months; Nagata 1986). Lastly, the negative correlations found between DIN or DIP and picocyanobacterial abundance suggested that low nutrient availability facilitated the dominance of SPcy (Nagata 1986; Schallenberg and Burns 2001; Callieri 2008), and that nutrient limitation could be one of the factors inducing colony formation (Callieri et al. 2012).

Relationships between single cells and colonies

Some strains of SPcy are known to form microcolonies under certain conditions such as ultraviolet radiation or grazing pressure (Jezberová and Komárková 2007; Callieri et al. 2011; Callieri et al. 2016). Conversely, some CPcy genera have single-cell stages in their life histories (Komárková and Šimek 2003) and many genera in the order *Synechococcales* that were originally described as colonial lose their mucilaginous envelopes in cultivation (Komárek et al. 2014). Therefore, transformation between single cells and colonies may occur frequently in lakes. Fortunately, we found significant causal links between SPcy and CPcy, suggesting that they were affected by each other. Furthermore, the multivariate S-map results suggested that SPcy could have enhanced the abundance of CPcy, whereas CPcy decreased that of SPcy (Fig. 4). Therefore, the transformation of the morphology of picocyanobacteria is likely unidirectional in Lake Biwa, with SPcy tending to form CPcy.

Effects of protists on picocyanobacteria

HNF have been regarded as key grazers of SPcy that contribute strongly to the latter's mortality losses (Nagata 1988; Callieri et al. 2002), and thus HNF are expected to have negative effects on SPcy abundance. However, the opposite result was obtained from

EDM, with HNF increasing SPcy abundance rather than reducing them (Fig. 4). A possible explanation for this is the enhancement of SPcy growth due to nutrient regeneration by HNF exceeding mortality losses due to grazing by HNF. This is consistent with the results in previous studies (Ferrier-Pages and Rassoulzadegan 1994; Selph et al. 2003), though it is impossible to quantify the amount of nutrients excreted respectively by HNF or other grazers from nutrient samples we collected.

The other possible explanation is the presence of trophic cascade, as HNF fall within the food size ranges of a variety of predators and are vulnerable to predation in aquatic environments (Pace et al. 1998; Nakano et al. 2001). We investigated the influence of putative predators on HNF using EDM, and found that *Polyarthra* and cladocera showed negative influences on HNF abundance (Table S2), suggesting the presence of top-down controls on HNF by these predators (Pourriot 1977; Stemberger and Gilbert 1985; Jürgens et al. 1996). So it is likely that grazing pressure of HNF on picocyanobacteria can be suppressed by predation of *Polyarthra* and cladocerans. Furthermore, HNF were enumerated at the community level in the present study, and grazing on SPcy by HNF is species-specific (Callieri et al. 2012). Therefore, the HNF species that prey on SPcy could be minor in our samples, which could result in the moderate influence of SPcy on HNF (Table S2).

On the other hand, the positive effect on CPcy abundance by HNF (Fig. 4) suggested that HNF play an important role in stimulating colony formation by picocyanobacteria, possibly through grazing on single cells, as previously reported (Callieri et al. 2016). Indeed, microcolony-forming bacteria generally cannot be consumed by HNF due to their large size (Hahn et al. 2000), and we found an apparent

negative effect of CPcy on HNF (Table S2), which suggests that HNF are unlikely to graze on CPcy.

Ciliates are also important grazers of picocyanobacteria (Šimek et al. 1995 and 1997), but EDM did not show a significant causal link between their abundance and that of SPcy or CPcy. Ciliates may not be involved in controlling picocyanobacterial abundance, due to their low abundance in the north basin of Lake Biwa (Yoshida et al. 2001b).

Effects of metazoan zooplankton on picocyanobacteria

The relationships between metazoan zooplankton and picocyanobacteria can be more complex because they are both potential grazers of picocyanobacteria and predators of HNF and small zooplankton. For example, rotifers feed significantly on nanoflagellates and small ciliates (Arndt 1993), whereas cladocerans prey upon a wide range of food particle sizes (1–50 μm) that includes small protists (Gophen and Geller 1984; Stockner and Porter 1988; Sanders et al. 1994). Copepods use a variety of hunting and feeding techniques, enabling them to prey on diverse planktonic animals (Suzuki et al. 1999; Brandl 2005). The influence of rotifers, cladocerans, and copepods on SPcy or CPcy can thus be interpreted as the synergistic effects of grazing, nutrient regeneration, and trophic cascades. In addition, we also conducted EDM analyses to investigate whether and how these grazers could be affected by other variables (Table S2). However, potential causal variables of metazoan zooplankton, such as abundances of bacteria, phytoplankton and their predators, are not available. The reconstructed dynamics of metazoan zooplankton might not be reasonably resolved, and thus the interaction strengths calculated by the multivariate S-map might be counter-intuitive and difficult to explain. Therefore, detailed discussion on influences of causal variables on metazoan

389 zooplankton can be speculative and should be avoided as possible.

390 Rotifers that have been previously reported to prey on picocyanobacteria are
391 *Keratella* and *Polyarthra* (Callieri et al. 2012), both of which were dominant genera in
392 Lake Biwa (Yoshida et al. 2001b). *Keratella* showed apparent top-down control on
393 picocyanobacterial abundance (Fig. 4), indicating that they may be effective grazers of
394 both SPcy and CPcy (Pourriot 1977; Callieri et al. 2012; Table S2, positive influence of
395 CPcy on *Keratella*). The positive effect of *Polyarthra* on SPcy (Fig. 4) suggested that
396 *Polyarthra* may enhance SPcy abundance through preying on HNF, as discussed
397 previously. The bacterivorous rotifer *Trichocerca* enhanced the abundance of CPcy
398 (Fig. 4), and the negative influence of CPcy on *Trichocerca* may suggest that CPcy
399 were not grazed by *Trichocerca* (Table S2). However, it is unclear whether grazing
400 pressure from *Trichocerca* plays a role in inducing colony formation, as they had no
401 effect on SPcy (Table 1). Overall, rotifers have seldom been investigated as grazers of
402 picocyanobacteria, and therefore further research is needed to clarify the food chain
403 between rotifers and picocyanobacteria.

404 Cladocerans are well-known grazers of SPcy (Callieri et al. 2012), and they had
405 an apparent negative influence on SPcy abundance (Fig. 4). Similar to the rotifer
406 *Keratella*, cladocerans (mainly *Daphnia*) induced the decrease in CPcy abundance (Fig.
407 4), suggesting that they graze on CPcy. Although the CPcy found during the present
408 study generally had large colony sizes (up to several hundred μm), microcolonies
409 ranging from several to tens of micrometers can be eaten by cladocerans (Ronneberger
410 1998; Table S2, positive influence of CPcy on cladocera).

411 The effect of copepods on SPcy abundance was nearly moderate, despite their
412 ability to ingest SPcy effectively (Fig. 4; Motwani and Gorokhova 2013; Table S2,

413 positive influence of SPcy on copepod). A possible reason for this finding is that the
414 negative effects of grazing were offset by positive indirect effects, especially through
415 trophic cascades, as copepods represent the highest trophic level among grazers of
416 picocyanobacteria. A trophic cascade involving copepods may also be a major
417 contributor to their positive influence on CPcy (Fig. 4). Copepods increased the
418 abundance of CPcy, possibly by preying on microzooplankton such as *Keratella*
419 (Yoshida et al. 2001a) that have negative effects on CPcy abundance.

420 **Conclusions**

421 Increasing picocyanobacterial abundance along with climate change in future have been
422 indicated in oceans (Flombaum et al. 2013). In lakes, growing blooms of
423 picocyanobacteria also have been reported in recent years, and some species of
424 picocyanobacteria can produce harmful toxins and secondary metabolites thus causing
425 problems to public health (Jakubowska and Szeląg-Wasielewska 2015; Jasser and
426 Callieri 2016; Śliwińska-Wilczewska et al. 2018). Despite the increasing impacts of
427 picocyanobacteria on aquatic ecosystems, however, ecology of them remain largely
428 unclear. So far, few studies have been reported discussing the comprehensive impacts of
429 grazers on picocyanobacteria.

430 In the present study, we found that HNF, *Keratella*, *Polyarthra*, *Trichocerca*,
431 cladocerans and copepods had important impacts on SPcy and CPcy, and played various
432 roles in controlling their abundances (Fig. 4). Notably, we found that HNF might
433 stimulate the growth of SPcy through indirect positive effects such as nutrient
434 regeneration in excess of direct negative effects such as grazing, which is a novel result.
435 We also found that single cells of picocyanobacteria tended to form colonies, possibly
436 due to the positive effects of HNF, *Trichocerca* and copepods on CPcy. Our findings

clearly show that natural seasonal dynamics of picocyanobacteria in Lake Biwa are influenced by a variety of grazers, and that the influences of grazers in complex natural food webs are often counter-intuitive. Furthermore, because SPcy and CPcy are influenced by different grazers, they may thus play differing ecological roles in the aquatic food web. It should be noticed that we did not conduct any *in situ* or laboratory experiments to validate the results of the present study. Further research, especially *in situ* manipulative experiments, is needed to elucidate the detailed interspecific interactions among picocyanobacteria and their grazers.

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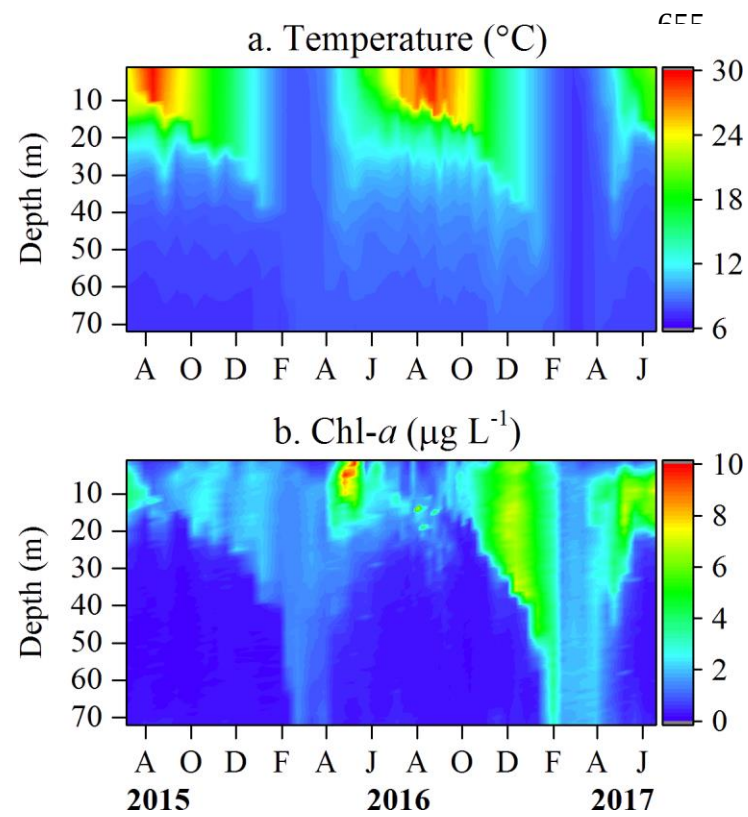
645

Table 1. Significant causal variables affecting the abundances of single-celled picocyanobacteria (SPcy) and colonial picocyanobacteria (CPcy) identified by CCM, and according time-averaged interaction strengths calculated by the multivariate S-map.

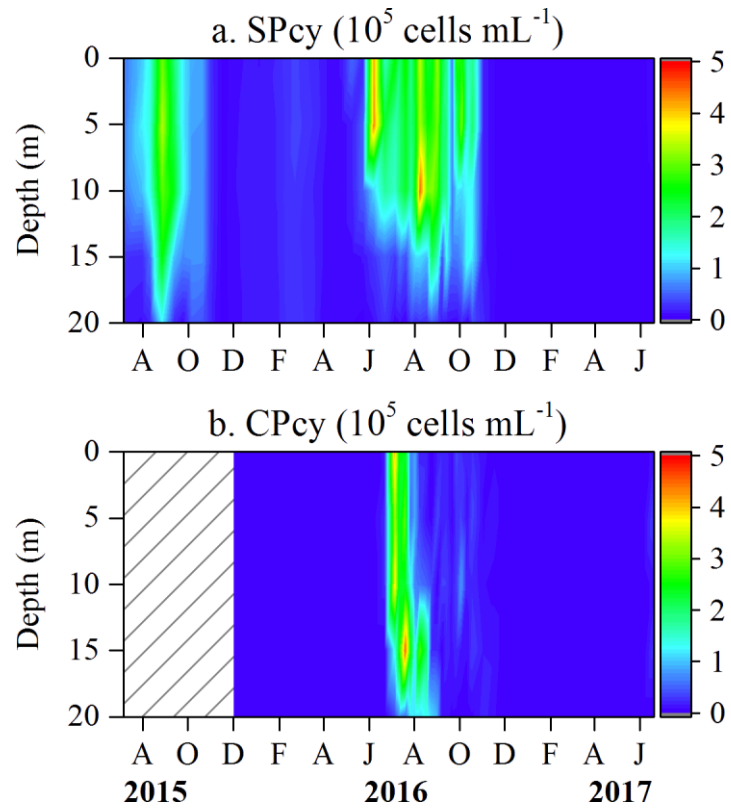
Effect variable	Causal variable	t_p	$\rho_{\max}$	$\Delta\rho$	P_z	P_s	Time-averaged interaction strength
SPcy	Temperature	0	0.75	0.42	0.000	0.048	0.164
	CPcy	-2	0.57	0.46	0.000	0.004	-0.467
	HNF	-1	0.58	0.31	0.007	0.006	0.324
	<i>Keratella</i>	-4	0.42	0.44	0.001	0.024	-0.213
	<i>Polyarthra</i>	-5	0.51	0.47	0.000	0.003	0.133
	<i>Trichocerca</i>	0	0.26	0.13	0.342*	0.081*	
	Cladocera	0	0.62	0.54	0.000	0.001	-0.300
	Copepod	-3	0.54	0.41	0.001	0.001	0.008
CPcy	Temperature	-2	0.91	0.59	0.000	0.001	-0.299
	SPcy	-2	0.82	0.57	0.000	0.001	0.125
	HNF	-3	0.81	0.63	0.000	0.001	0.212
	<i>Keratella</i>	-4	0.49	0.47	0.000	0.011	-0.059
	<i>Polyarthra</i>	0	0.12	0.16	0.267*	0.309*	
	<i>Trichocerca</i>	-1	0.47	0.30	0.019	0.004	0.262
	Cladocera	-1	0.29	0.29	0.040	0.044	-0.081
	Copepod	-6	0.55	0.45	0.000	0.002	0.139

t_p : cross-map lag; $\rho_{\max}$: ρ at maximal library size; $\Delta\rho$: ρ at maximum library size minus ρ at minimum library size; P_z : P value of Fishfer's z test; P_s : P value of surrogate test; *: not statistically significant at the 0.05 level.

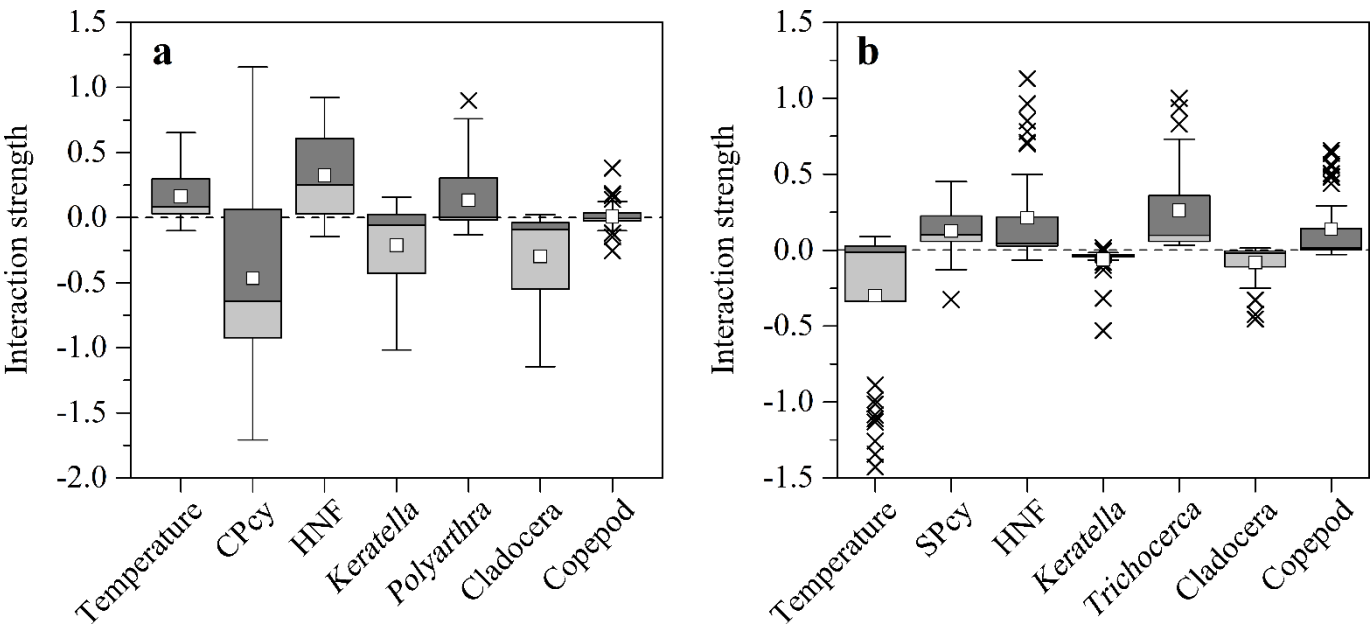
653 Figure 1. Seasonal changes in vertical profiles of (a) water temperature and (b)
654 chlorophyll-*a* concentration.



662 Figure 2. Seasonal changes in vertical abundances of (a) single-celled picocyanobacteria
663 (SPcy) and (b) colonial picocyanobacteria (CPcy).
664

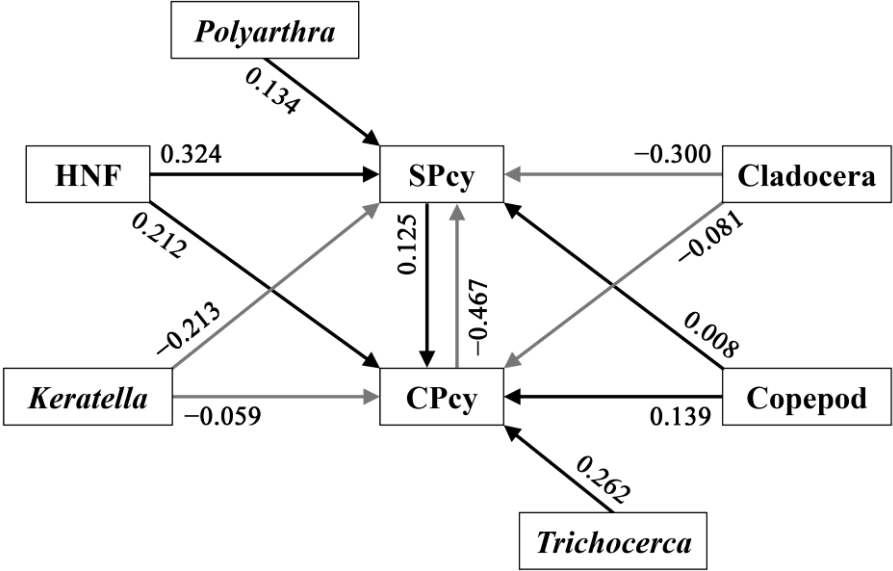


665 Figure 3. The effects of causal variables on picocyanobacterial abundance determined
 666 using the multivariate S-map. The boxplots show the interaction strengths of causal
 667 variables on the abundances of (a) SPcy and (b) CPcy. The bottom and top of each box
 668 show the lower (25%) and upper (75%) quartiles, respectively; the band and square
 669 within each box represent the median and the mean, respectively; whiskers indicate the
 670 minimum and maximum; and crosses represent outliers. Note that original time series
 671 were standardized, and thus the interaction strengths from different variables can be
 672 compared directly to discuss the relative importance of each variable.



673
 674

675 Figure 4. The effects of grazers on picocyanobacterial abundance, and the relationship
 676 between SPcy and CPcy. Numbers beside the arrows represent time-averaged values of
 677 interaction strengths.



678
 679
 680

681 Table S1. Optimal parameters of the multivariate S-map.

Variable to predict	θ	Number of predictions	ρ	MAE	RMSE	<i>P</i> value
SPcy	3.75	36	0.90	0.28	0.46	0.000
CPcy	1.45	35	0.65	0.43	0.86	0.000

682 θ : the nonlinear parameter; ρ : the forecasting accuracy; MAE: mean absolute error;
 683 RMSE: root mean square error; *P* value: *P* value that ρ is significantly greater than zero
 684 using Fishfer's z-transformation.

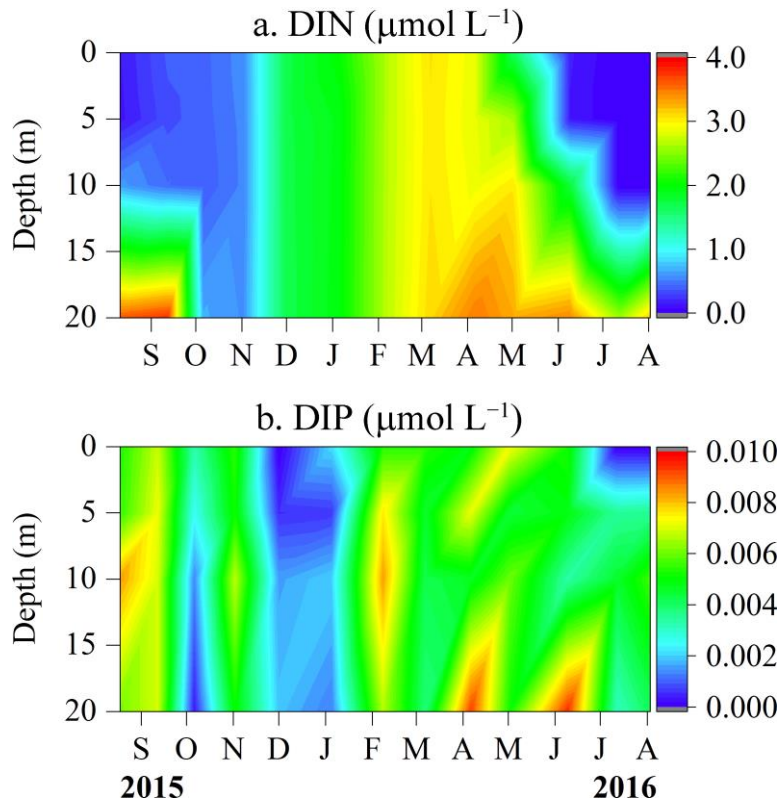
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Table S2. Significant causal variables affecting the abundances of HNF and metazoan zooplankton identified by CCM, and according time-averaged interaction strengths calculated by the multivariate S-map.

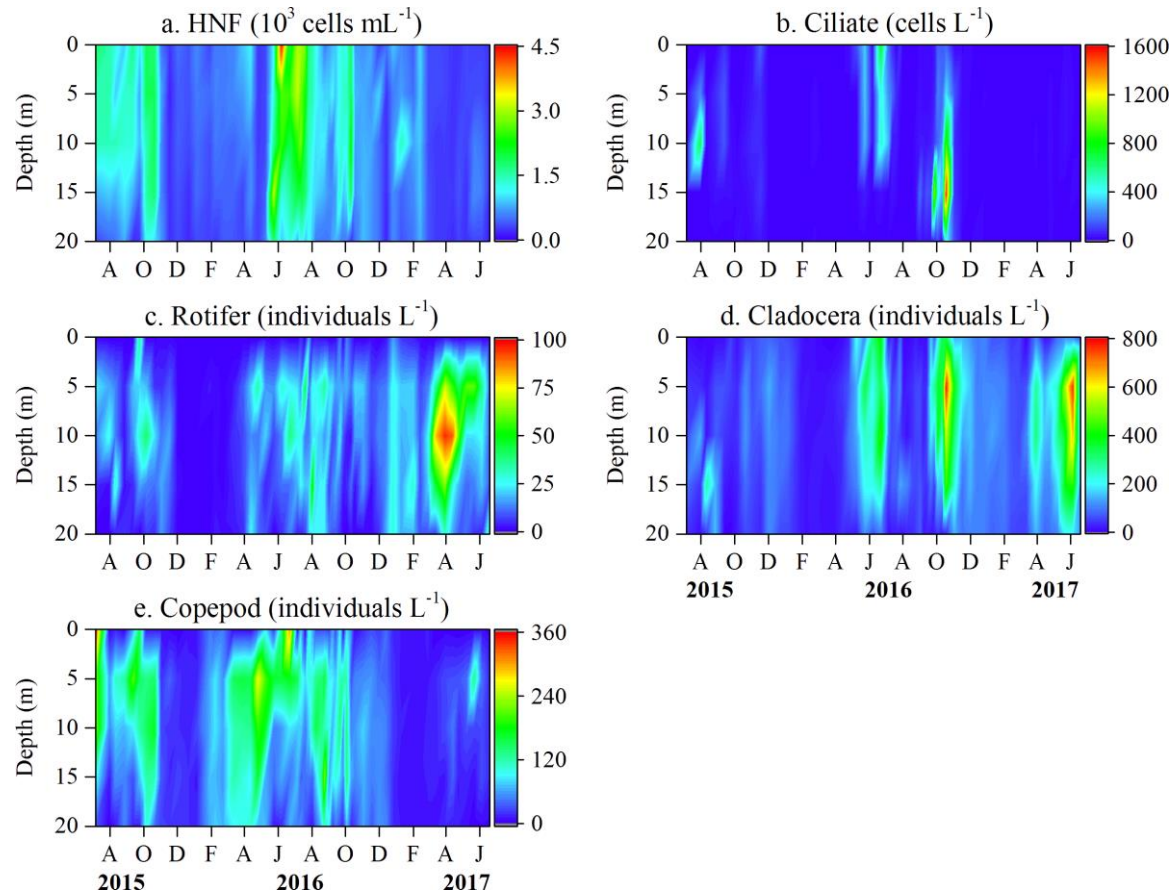
Effect variable	Causal variable	t_p	$\rho_{\max}$	$\Delta\rho$	P_z	P_s	Time-averaged interaction strength
HNF	SPcy	0	0.63	0.30	0.007	0.045	-0.002
	CPcy	-5	0.55	0.53	0.000	0.013	-0.119
	<i>Polyarthra</i>	-3	0.27	0.30	0.033	0.046	-0.297
	Cladocera	-1	0.51	0.53	0.000	0.004	-0.058
Keratella	CPcy	-6	0.48	0.42	0.001	0.017	0.483
	HNF	0	0.30	0.26	0.047	0.036	0.072
	<i>Polyarthra</i>	-5	0.27	0.32	0.023	0.046	-0.142
	Cladocera	-2	0.26	0.32	0.023	0.027	0.024
<i>Polyarthra</i>	HNF	-3	0.40	0.36	0.007	0.030	-0.035
	Cladocera	-5	0.77	0.60	0.000	0.001	0.331
Trichocerca	CPcy	0	0.51	0.49	0.000	0.009	-0.091
	HNF	-5	0.51	0.52	0.000	0.028	0.417
	Cladocera	-5	0.63	0.59	0.000	0.001	-0.006
	Copepod	-2	0.56	0.58	0.000	0.045	0.083
	<i>Polyarthra</i>	0	0.30	0.33	0.017	0.042	0.436
Cladocera	CPcy	-6	0.53	0.51	0.000	0.017	0.170
	HNF	-2	0.39	0.34	0.011	0.049	0.273
	<i>Polyarthra</i>	-6	0.55	0.37	0.002	0.006	0.400
	Trichocerca	-1	0.46	0.25	0.046	0.004	-0.154
Copepod	SPcy	0	0.56	0.39	0.001	0.003	0.321
	Cladocera	0	0.34	0.36	0.009	0.009	0.062
	Trichocerca	-2	0.44	0.36	0.006	0.003	-0.317

t_p : cross-map lag; $\rho_{\max}$: ρ at maximal library size; $\Delta\rho$: ρ at maximum library size minus ρ at minimum library size; P_z : P value of Fishfer's z test; P_s : P value of surrogate test.

693 Figure S1. Seasonal changes in vertical distributions of (a) dissolved inorganic nitrogen
694 (DIN) and (b) dissolved inorganic phosphorus (DIP) concentrations.
695



697 Figure S2. Seasonal changes in vertical abundances of picocyanobacteria grazers: (a)
 698 HNF, (b) ciliate, (c) rotifer, (d) cladocera, and (e) copepod.
 699



700 Figure S3. Seasonal changes in the genus-level composition of rotifers.

701

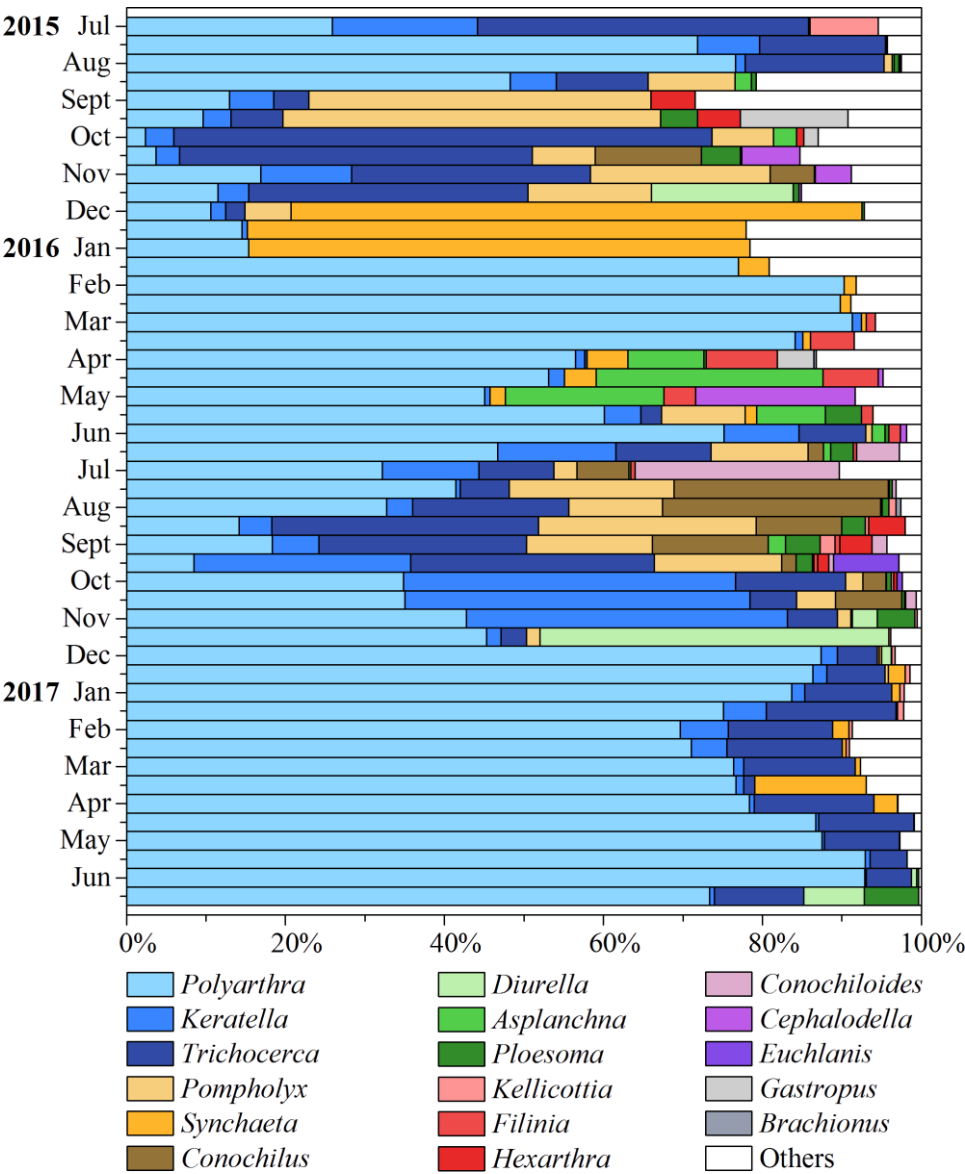
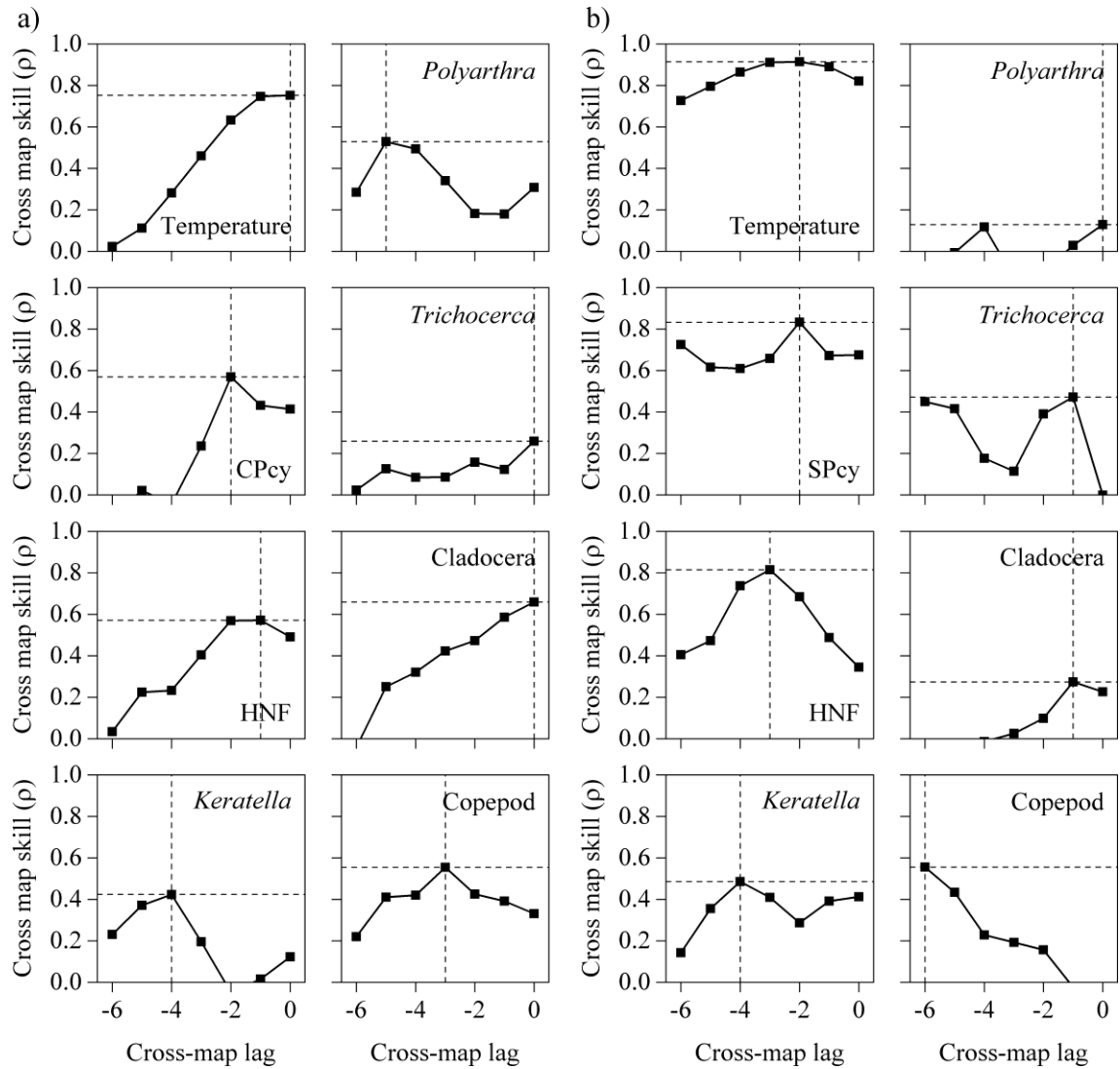


Figure S4. Time-delayed convergent cross mapping (CCM). (a) SPcy and (b) CPcy cross-mapping causal variables. Crossed dash lines indicate the optimal cross-map lag (t_p), which maximizes cross map skill (ρ).



710 Figure S5. CCM at the optimal cross-map lag. (a) SPcy and (b) CPcy cross-mapping
 711 causal variables
 712

