1 **Cyanopeptides Restriction and Degradation Co-mediate** 2 **Microbiota Assembly During a Freshwater Cyanobacterial** 3 **Harmful Algal Bloom (CyanoHAB)**

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21 **Abstract**

22 Cyanobacterial harmful algal blooms (CyanoHABs) are globally intensifying and
 23 exacerbated by climate change and eutrophication. However, microbiota assembly
 24 mechanisms underlying CyanoHABs remain scenario specific and elusive. Especially,
 25 cyanopeptides, as a group of bioactive secondary metabolites of cyanobacteria, could
 26 affect microbiota assembly and ecosystem function. Here, the trajectory of
 27 cyanopeptides were followed and linked to microbiota during *Microcystis*-dominated
 28 CyanoHABs in lake Taihu, China. The most abundant cyanopeptide classes detected
 29 included microginin, spumigin, microcystin, nodularin and cyanopeptolin with total
 30 MC-LR-equivalent concentrations between 0.23 and 2051.54 ppb, of which
 31 cyanotoxins beyond microcystins (e.g., cyanostatin B and nodularin_R etc.) far
 32 exceeded reported organismal IC₅₀ and negatively correlated with microbiota diversity,
 33 exerting potential collective eco-toxicities stronger than microcystins alone. The
 34 microbial communities were differentiated by size fraction and sampling date
 35 throughout CyanoHABs, and surprisingly, their variances were better explained by
 36 cyanopeptides (19-38%) than nutrients (0-16%). Cyanopeptides restriction (e.g.,
 37 inhibition) and degradation are first quantitatively verified as the deterministic drivers
 38 governing community assembly, with stochastic processes being mediated by
 39 interplay between cyanopeptide dynamics and lake microbiota. This study presents an

emerging paradigm in which cyanopeptides restriction and degradation co-mediate lake water microbiota assembly, unveiling new insights about the ecotoxicological significance of CyanoHABs to freshwater ecosystems.

Key words: Cyanobacterial Harmful Algal Blooms, Cyanopeptides, Microbial Community Assembly, High-Throughput Sequencing, Eutrophication, Taihu

1. Introduction

Cyanobacterial harmful algal blooms (CyanoHABs) increasingly threaten marine and freshwater ecosystems worldwide (Huisman et al. 2018, Wang et al. 2021), as cyanobacteria produce diverse bioactive non-ribosomal oligopeptide metabolites known as cyanopeptides (e.g., microcystins, nodularins, spumigins, anabaenopeptins, cyanopeptolins, microginins, microviridins and aerucyclamides) (Janssen 2019). A few recent studies have demonstrated the largely neglected ecological impacts (abbreviated as ‘eco-impacts’, e.g., on nutrients and carbon turnover (Egli et al. 2020)) or life toxicities (e.g., for larval fish (Fernandes et al. 2019)) caused by non-microcystin cyanopeptides, such as microginin, cyanopeptolin A and arucyclamide A (Beverdors et al. 2018, Fernandes et al. 2019, Huang and Zimba 2019). Meanwhile, non-microcystin cyanopeptides have been detected at significant amounts in the surface drinking water sources adversely affected by CyanoHABs, suggesting their ineffective removal by drinking water treatment plants and potential

59 threats to human health (Beverdorf et al. 2018). Still, compared to the microcystins,
60 non-microcystin cyanopeptides, especially for their dynamics in environments and
61 eco-impacts, received much less attention. Exploring cyanopeptides beyond
62 microcystins is critical for elucidating the eco-impacts and health risk posed by
63 CyanoHABs in freshwater ecosystems, particularly for those serving as drinking
64 water reservoirs.

65 Taihu, a shallow, subtropical lake (an average depth of 1.9 m), is the source of
66 drinking and irrigating water for more than 40 million residents around its watershed
67 (Qin et al. 2019). Extensive urbanization and hydrological engineering (e.g. Project of
68 Water Diversion from the Yangtze River to Lake) over the decades have bred the
69 eutrophic and fluctuating status of its water quality, exacerbating the prevalence of
70 CyanoHABs (Huang et al. 2021, Huang et al. 2019, Qin et al. 2019, Qin et al. 2020),
71 of which the causative taxa include prolific cyanopeptide producers like *Microcystis*
72 and *Dolichospermum* (Ma et al. 2016, Osterholm et al. 2020). Current knowledge on
73 cyanopeptide in Taihu is primarily constricted to microcystins (e.g., MC-LR, MC-RR
74 and MC-YR) in water (Sakai et al. 2013, Su et al. 2018, Tang et al. 2018, Xue et al.
75 2020), sediment (Xue et al. 2020), and nearshore soil (Chen et al. 2006). In contrast,
76 non-microcystin components and their dynamics have not been characterized in the
77 changing hydrological and ecological conditions. Untangling the spatiotemporal
78 dynamics and eco-impacts of those CyanoHABs byproducts can provide basis for

79 their health risk assessment, and further inform management of water withdraw or
80 water diversion to prevent their detrimental effects.

81 Microbiota underlying CyanoHABs include cyanopeptide producers (i.e., sources)
82 and degraders (i.e., sinks), and their spatiotemporal assemblages are closely linked
83 with cyanopeptide dynamics. Specifically, taxonomic composition and
84 biogeochemical function of bloom-associated microbiota showed clear succession
85 over the course of toxigenic blooms (Tang et al. 2018, Wan et al. 2019). Size fractions
86 of biomass can form distinct ecological niches within which interactions between
87 cyanobacteria and bloom-associated microbiota are imperative and diverse (Chen et al.
88 2018, Cook et al. 2020, Tang et al. 2017, Xu et al. 2018, Zhu et al. 2021a). For
89 example, microorganisms colonized on cyanobacterial aggregates ($> 100 \mu\text{m}$) are
90 functionally linked with their host in a commensal relationship (Cook et al. 2020).
91 Although taxonomic overlap exists between heterotrophic bacteria from
92 particle-associated ($> 5 \mu\text{m}$) and free-living fractions ($2\text{-}0.2 \mu\text{m}$) (Tang et al. 2017),
93 their community functions and environmental preference are different (Xu et al. 2018).
94 However, the relative contribution of cyanopeptides to the assembly of the
95 niche-differentiated microbiota during CyanoHABs has not been evaluated.
96 Considering the combined effects of their antimicrobial activities (Swain et al. 2017)
97 and nutritional niches during degradation (Li et al. 2017), a much more complicated
98 scenario than previously appreciated may exist regarding niche-based deterministic

99 processes (e.g., differentiation) and their role in mediating assembly processes of
 100 bloom water microbiota. A fundamental but unresolved question arises: How could
 101 dynamics of these bioactive metabolites, as a selective driver, shape cyanobacterial
 102 and non-cyanobacterial communities across size-fractionated bloom water microbiota?
 103 Unravelling ecological rules guiding microbiota assembly during bloom events,
 104 especially the interactions between cyanopeptides and microbiota should provide a
 105 theoretical guidance for microbiome-based control of CyanoHABs byproducts.

106 Using lake Taihu as a model system, this study aims to i) resolve spatiotemporal
 107 dynamics of cyanopeptides and their producers over the course of CyanoHABs; ii)
 108 explore how the degradation of cyanopeptides is related to documented degraders or
 109 microbial setups; and 3) explore how and to what extent can the cyanopeptides affect
 110 microbiota assemblages in different size fractions of bloom-associated community.
 111 Keeping those in mind, we carried out a series of field sampling campaigns in three
 112 lake regions in northeast Taihu and set up in-lab microbial degradation assays. A list
 113 of cyanopeptides was identified through non-targeted screening of cyanobacterial
 114 extracts using high resolution mass spectrometer (HR-MS) based on a recently
 115 published cyanopeptide database (Jones et al. 2021), and then quantified by tandem
 116 mass spectrometer (MS/MS). Moreover, 16S rRNA gene-based absolute abundance
 117 quantification enables a quantitative view on the contrasting changes in the biomass
 118 and diversity of microbiota, and further linking to cyanopeptide dynamics. Examining

the predominant cyanopeptides and associated microbiota would not only advance current understanding of their yet-to-be-recognized eco-impacts on the assembly of lake water microbiota, but also provide guidance for ecotoxicological risk assessment and management policy of CyanoHABs in freshwater ecosystem.

2. Methods

2.1 Sample collection

Water samples were collected between June 9th and September 20th, 2020 on five dates to cover a cyanobacterial bloom process. Eight sites (shown in Fig. 1A) were selected representing three lake regions: the Meiliang Bay (Site 5 and 6), Gonghu Bay (Site 1, 2, and 16) and the open water connecting the two bays (Site 3, 4 and 8). For the surface water (ca. 0-0.4 m of the upper water column), a 48- μ m pore size sieve was used to collect cyanobacterial colonies (scum) from 5-liter of surface water by a 2.5-liter organic glass water sampler (JC-800, Juchuang, China). The scum was backwashed into a 50-ml centrifuge tube with a final volume of 40 ml using Milli-Q water. The filtrate was kept in purified water bottles pre-rinsed with the filtrate. For cyanopeptide extraction, both surface and overlaying water (ca. 0-0.4 m above the sediment) samples were preserved in 50-ml centrifuge tubes. Water temperature, pH, conductivity and dissolved oxygen was determined *in situ* by a portable multimeter (HQ40D, HACH, USA). Additionally, 500 ml of lake water was collected using a

138 polycarbonate bottle for analysis of total nitrogen (TN), total phosphorus (TP),
 139 dissolved total nitrogen (DTN), dissolved total phosphorus (DTP), phosphate, nitrate
 140 and ammonium. And dissolved organic nitrogen (DON) was calculated from
 141 difference between TN and inorganic nitrogen (nitrate and ammonium). All the tubes
 142 and bottles were stored in a cooler (80 L, CONTOOSE, China) with icebags and
 143 delivered back to the lab within 5 hours.

144 **2.2 DNA extraction and 16S rRNA gene analysis**

145 To obtain size-fractionated microbial DNA, 250 to 300 ml pre-sieved lake water was
 146 sequentially vacuum filtered with 2.0 μm and 0.2 μm pore size polycarbonate filters
 147 (Millipore, USA). Then, DNA extraction was performed using DNeasy Powersoil Kit
 148 (Qiagen, Germany) following the manufacture's instruction (see Supporting
 149 Information S1 for details). To enable absolute quantification of the microbial
 150 communities, 16S rRNA gene copy number of each DNA extract was determined by
 151 quantitative PCR assay as described in (Ju et al. 2019) and also detailed in Supporting
 152 Information S1. The V4-V5 regions of prokaryotic 16S rRNA gene were targeted by
 153 the primers F515 (GTGYCAGCMGCCGCGGTAA) and R926
 154 (CCGYCAATTYMTTTRAGTTT). Sequencing libraries were generated by
 155 NEBNext $\text{\textcircled{R}}$ UltraTM $\square$ DNA Library Prep Kit for Illumina $\text{\textcircled{R}}$. Paired-end sequencing

156 was conducted on an Illumina Nova6000 platform with read length set to 250 base
157 pairs (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China).

158 The raw reads were analyzed using the QIIME2 (Bolyen et al. 2019) followed
159 in-house Python scripts for batch processing (see Supporting Information S2 for
160 details). The data that support the findings of this study have been deposited into
161 CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb:
162 www.cngb.org/cnsa) with accession number CNP0001905
163 (<http://db.cngb.org/cnsa/project/CNP0001905/reviewlink/>).

164 **2.3 Extraction, identification and quantification of cyanopeptides**

165 Raw water collected from both the surface and bottom (overlying water) for
166 cyanopeptides quantification were put through two frozen-thaw cycles to release
167 intracellular components. Solid phase extraction (SPE) was used for extraction and
168 purification. A target list of cyanopeptides was first determined by injecting 10 µl
169 concentrated cyanopeptide extracts (collected on July 21) into a high-resolution mass
170 spectrometer (Thermo Orbitrap Fusion Lumos, Thermo, USA). Candidates with at
171 least two daughter ions hit the reference were reserved (Table S1 & Fig. S1), ending
172 up with a list including nine cyanopeptides components. Quantification was then
173 performed on a UPLC-MS/MS system as described in Supporting Information S3.

174 **2.4 Microcystin degradation experiments**

175 For two of the sampling campaigns (on June 30 and July 21), in-lab experiments were
 176 conducted to assess the capability of lake water in degrading microcystins.
 177 Experimental setups were described in Supporting Information S4. To link
 178 degradation rate to documented degrading organisms, a list containing bacterial
 179 genera reported as organisms capable of degrading microcystin was summarized from
 180 published literatures (as summarized in Table S2), which served as a library to
 181 retrieve a subset of potential degrading taxa from the sequencing data.

182 **2.5 Statistical analyses**

183 For each sample, the total copy numbers of 16S rRNA gene measured by qPCR were
 184 multiplied by relative abundances of each ASV. The volume of water filtered or
 185 concentrated during sampling was used to compute the absolute abundance of each
 186 ASV expressed as ‘copy numbers per liter of lake water (copies L⁻¹) and used in
 187 downstream statistical analysis (see Supporting Information S5).

188 To quantify ecological stochasticity of size-fractioned microbial communities,
 189 normalized stochasticity ratio (NST) of cyanobacterial or non-cyanobacterial
 190 communities in three size fractions were calculated using *NST* package in R. The NST
 191 metric is developed as a null-model-based estimation to predict whether community

192 assembly processes are more stochastic or more deterministic (Ning et al. 2019). Fifty
193 percent is considered a boundary where deterministic and stochastic processes are
194 categorized.

195 **3. Results**

196 **3.1 Spatiotemporal distribution of cyanopeptides correlated with cyanobacterial** 197 **communities**

198 Nine major variants belonging to five cyanopeptide classes including one nodularin,
199 three microginins, one spumigin, and one cyanopeptolin were co-identified (Table S1).
200 The average concentrations of all the nine variants reached a “Cyanopeptide Peak” on
201 June 30 (ca. 597 ppb, n = 7) and declined by 50% on July 21 (ca. 300 ppb, n = 8). The
202 cyanopeptides were primarily accumulated on the surface but can also be detected in
203 the overlying water (Fig. 1B). In surface water, cyanostatin B was detected in water
204 column from below limit of quantification to a highest record of 1323.81 ppb
205 (MC-LR-equivalent concentration) on July 21 at Site 4, two orders of magnitude
206 higher than the synchronously detected MC-LR (52.53 ppb) and MC-RR (91.63 ppb).
207 Besides, the contents of microginin (FR6 and KR604) showed ~200% higher
208 concentrations than microcystin components (Fig. 1B). Geographically, the highest
209 total concentrations of cyanopeptides were detected in samples from Site 2 (1859.09

210 ppb) in Gonghu Bay, Site 6 (963.52 ppb) in Meiliang Bay, and Site 4 (2051.54 ppb) in
211 the open water (Fig. 1B).

212 To profile microbiological producers of major cyanopeptides and their
213 spatiotemporal dynamics, 102 size-fractionated biomass DNA samples were
214 quantitatively analyzed using high-throughput quantitative 16S rRNA gene amplicons
215 sequencing. The results showed a sharp temporal change in both bacterial (Fig. S3)
216 and cyanobacterial (Fig. 2A) biomass over the course of blooming, accompanying a
217 contrasting succession in the cyanobacterial community taxonomic composition (Fig.
218 2B) and alpha-diversity (Fig. S4A-4C) across the lake regions and size fractions
219 examined. The total cyanobacterial biomass was significantly and continuously
220 elevated until reaching a Biomass Peak on July 21 (Fig. 2A), whereas the alpha
221 diversity metrics (e.g., observed species, Chao 1 richness and Shannon's H) of
222 cyanobacterial communities significantly decreased (Fig. S4A). This contrasting
223 pattern consisted with the much lower alpha diversity metrics within the colony
224 fractions than PA or FL fractions ($P < 0.01$, Fig. S4C), reflecting the prosperity of
225 bloom-thrived cyanobacterial species at the expense of compressing living conditions
226 of the others. Furthermore, non-metric multidimensional scaling (NMDS) analysis
227 showed that the colony fractions harbored cyanobacterial communities structurally
228 distinct from the FL or PA fractions (Fig. S4). Accordingly, size fraction was more

229 pronounced in explaining community dissimilarities compared to sampling date and
230 lake region (Table 1).

231 Throughout the whole sampling period, *Microcystis* was the most abundant genus
232 (6.9×10^6 to 6.8×10^7 copies L^{-1}), primarily aggregating as colonies (Fig. 2A). Its
233 species largely died off on the August 18 and September 20 (ca. 83.3% reduction in
234 biomass), accompanying a dramatic increase in the average relative abundance of
235 *Cyanobium* and *Dolichospermum* (from 22.5% to 65.8% in total) in the PA and FL
236 fractions of the biomass (Fig. 2B). To resolve cyanobacterial diversity within the
237 cyanobacterial colonies down to the species or population level, a phylogenetic tree
238 was built using cyanobacterial 16S rRNA gene ASVs (Fig. 2C). We then
239 comparatively examine both the 16S-based absolute abundance (copies L^{-1} , bar charts
240 at inner cycle) and relative proportions of each ASV (pies charts) in the colony
241 fraction on four sampling dates (Fig. 2C) and found the co-existence of multiple
242 closely related ASVs within *Microcystis* (5 ASVs) with changing abundances.
243 Similarly, high micro-diversity and population succession dynamics were also
244 observed within genera, e.g., *Cyanobium* spp. (43 ASVs) and *Dolichospermum* spp.
245 (6 ASVs, Fig. 2C).

246 Mantel tests were performed to explore associations between the 162 ASVs
247 assigned to the major cyanobacterial genera (i.e., *Microcystis*, *Cyanobium* and

248 *Dolichospermum*, total relative abundance > 90%) and cyanopeptide profiles (Fig.
249 1C). Generally, microcystins, microginin KR604 and cyanostatin B were correlated
250 significantly (Mantel's $r = 0.155$ to 0.367 , $P < 0.05$) and frequently ($n = 10$ links)
251 with the abundance matrix of *Microcystis* populations (i.e., 15 ASVs), whether for
252 cyanobacterial colony or FL fraction (Fig. 1C), revealing the prominent role of
253 *Microcystis* as a contributor to the cyanopeptide pools. In comparison,
254 *Dolichospermum*-affiliated ASVs were correlated with microginin FR6 and
255 nodularin_R especially in the colony fraction ($n = 2$ links, $P < 0.05$), while
256 *Cyanobium*-affiliated ASVs in cyanobacterial colony or PA fraction ($n = 7$ links, $P <$
257 0.05), reflecting differentiation in their respective interactions with cyanopeptides.
258 Notably, all cyanopeptide components showed significant and positive
259 inter-correlations between themselves (Pearson's r range from 0.29 to 0.99 , 0.68 on
260 average, $P < 0.05$) (Fig. 1C, heatmap), revealing their co-occurrence during
261 CyanoHABs in the lake Taihu.

262 **3.2 Differentiation of non-cyanobacterial communities including microcystin** 263 **degraders**

264 Once cyanopeptides and their producers were identified, we further questioned on the
265 corresponding dynamics of non-cyanobacterial communities and their ecological
266 niches during CyanoHABs. Overall, the alpha diversity was greatly differed by size

fraction with significantly lower diversity in the colony fractions, while the difference between sampling dates and lake regions are less prominent (Fig. S5). For beta-diversity, the non-cyanobacterial communities were also significantly and most differentiated by size fractions, followed by sampling dates (Fig. 3A & 3B). However, unlike cyanobacterial communities, their geographical heterogeneities (lake regions) were not statistically significant in explaining compositional variations (Table 1).

Non-cyanobacterial communities are also known to harbor heterotrophs including cyanopeptides degraders. *Sphingopyxis* sp. was identified as the most prominent degrader in our lake water samples (Fig. S6, A, B). Using microcystins degradation kinetics (Fig. S7 and Fig. 3B), our microcosm experiments showed significant and strong positive correlations ($\rho > 0.74$, $P < 0.01$) between degradation constant (k) and degraders abundance at the Biomass Peak on July 21, but contrastingly not at the Cyanopeptide Peak on June 30. Geographically, the results demonstrated that microbiota in the center lake region (open water, Site 3, 4 and 8) exhibited significantly higher (t-test, $P < 0.05$) degradation capacity compared to the two bay areas (Fig. 3C). Consistently, absolute abundance of *Sphingopyxis* sp. across the three lake regions decreased in the following order: Open water > Meiliang Bay > Gonghu Bay (Fig. S6C, inner bars), making the faster microcystin degradation in the open water than Meiliang and Gonghu Bay plausible.

286 **3.3 Turnover of bloom-associated microbial assemblages ecologically determined** 287 **by cyanopeptides**

288 As cyanopeptides dynamics is shown to linked with the bloom-associated microbiota
289 including their producers and degraders, we next question to which extent such
290 deterministic selective forces contribute to the community assembly compared with
291 stochastic factors (e.g., wind-driven mixing) and processes (i.e., dispersal and drift).
292 Ecological stochasticity of microbial communities benchmarked by normalized
293 stochasticity ratio (NST) showed that deterministic processes generally prevailed over
294 stochastic processes (i.e., $NST < 50\%$) in the colony fraction, particularly for
295 cyanobacterial communities ($NST = 22\%$ to 40% , Fig. 4A), compared with
296 non-cyanobacterial communities ($NST = 46\%$ to 55% , Fig. 4A). Intriguingly,
297 cyanobacterial communities (Fig. 4A, top panel) and non-cyanobacterial communities
298 (Fig. 4A, bottom panel) within PA and FL fractions displayed a distinct temporal
299 dynamics of assembly patterns. For example, stochasticity of cyanobacterial
300 community in PA and FL fraction was decoupled (Fig. 4A, top panel), reaching a
301 peak on July 21 (Biomass Peak, $NST = 98\%$) and Aug. 18 ($NST = 99\%$), respectively.
302 While the assembly of non-cyanobacterial communities was generally parallel,
303 ascending until the Cyanopeptide Peak on June 30 ($NST = 87\%$ for PA and 86% for
304 FL) and then declined (Fig. 4A, bottom panel).

305 To further discern the variation of the bloom-associated microbiota explained by
 306 deterministic factors, variation partitioning analysis (VPA) was employed (Fig. 4B).
 307 The results revealed that the nine cyanopeptides were much better in explaining the
 308 variances of cyanobacterial and non-cyanobacterial communities in all three size
 309 fractions, compared with the eight nutrient parameters (Fig. S2).

310 To quantitatively resolve the extents to which cyanopeptides dynamics in
 311 determining the assembly of bloom-associated microbiota, we sought for significant
 312 correlations between Chao1 richness and the nine cyanopeptide components (Fig. S8)
 313 and further fitted the dissimilarities (UniFrac distance) between bloom-associated
 314 communities against the concentration differences in total cyanopeptides (Fig. 4C).
 315 As expected, significant negative Spearman's rank correlation coefficients (ρ) were
 316 observed between Chao1 richness of non-cyanobacterial communities and
 317 cyanopeptides components (Fig. S8). The trend within cyanobacterial communities
 318 was similar, with negative ρ values ranging from -0.244 to -0.445 for all nine
 319 cyanopeptide components (Fig. S8). On community-level, the influence of total
 320 cyanopeptide differences was the most evident in the colony fraction (Fig. 4C, top
 321 panels). Particularly, within the colony fraction, differences in cyanopeptide contents
 322 were negatively related with both the phylogenetic dissimilarity ($R^2 = 0.33$, $P < 0.01$)
 323 and abundance-weighted dissimilarity ($R^2 = 0.27$, $P < 0.01$) of those
 324 non-cyanobacterial assemblages (Fig. 4C). For communities in the PA and FL

fractions, weighted UniFrac rather than unweighted UniFrac dissimilarities between cyanobacterial assemblages were significantly ($P < 0.05$) related to cyanopeptide variations but on opposite directions (Fig. 4C), revealing that cyanopeptide dynamics created distinct niches for cyanobacterial populations in the two size fractions.

4. Discussion

CyanoHABs across the globe have raised wide and ever-growing concerns on the eco-environmental and human health impacts of bioactive cyanopeptides (Janssen 2019). Here, for the first time, we expand current research scope on cyanopeptides beyond microcystins in Taihu and linked them to the size-fractionated lake water microbiota (i.e., cyanobacterial colony, particle-associated and free-living) over the whole course of a summer cyanobacterial bloom. High-resolution mass spectrometry and high-throughput DNA sequencing approaches provide new and complementary chemical and biological insights into those previously unconsidered but arguably harmful non-microcystin cyanobacterial metabolites, enabling quantitative elucidation of their interplay with microbiota and the key role of cyanopeptides in the microbiota assembly and ecosystem function underlying CyanoHABs.

4.1 Non-microcystin cyanopeptides of emerging ecotoxicological concerns

342 Limited knowledge on the occurrence and eco-impacts of cyanopeptides beyond
 343 microcystins hindered understanding on their toxicology and health risks (Janssen
 344 2019, Natumi and Janssen 2020). In this study, we reported a comprehensive profile
 345 of cyanopeptides in lake Taihu including six major non-microcystin structures from
 346 four classes that were highly prevalent during a cyanobacterial bloom. The presence
 347 of non-microcystin cyanopeptides is not surprising, since their biosynthetic gene
 348 clusters in the dominant taxa have long been recognized (Calteau et al. 2014, Harke et
 349 al. 2016). What noteworthy is their high prevalence and compelling biological
 350 activities that make themselves emerging CyanoHABs-associated byproducts with
 351 deterministic but yet-under-appreciated eco-impacts (Swain et al. 2017).

352 Cyanostatin B, known as a potent inhibitor of leucine aminopeptidases (LAPs,
 353 EC3.4.11.1), was the most abundant cyanopeptide in our investigation (up to 1323.81
 354 $\mu\text{g L}^{-1}$). The concentration is 100 times higher than the reported IC_{50} (12 $\mu\text{g L}^{-1}$) of
 355 LAP – M for microsomal from porcine kidney (Sano et al. 2005), while inhibition on
 356 microbial community are less reported. Considering the critical role of LAPs in
 357 microbial proteolysis and recycling of amino acids from peptides (Matsui et al. 2006),
 358 we speculated that the dominance of cyanostatin B as LAPs inhibitors during the lake
 359 bloom environment could hinder the biodegradation and further remineralization of
 360 cyanopeptides by heterotrophs. Consistent with our new hypothesis, potential
 361 inhibition of the cyanostatin B on the microbial richness were identified, as supported

by the negative correlations with Chao1 richness index (Fig. S8). Likewise, nodularin_R is another putative potent inhibitor of microbial richness, given their negative correlations with both non-cyanobacterial and cyanobacterial communities (Spearman's rank $\rho = -0.200$ and -0.328 , respectively). This cyanotoxin shares similar structure and inhibition potency as microcystins (e.g. IC_{50} of ~ 2 nM or $1.65 \mu\text{g L}^{-1}$ for PP1), therefore may pose a synergistic toxic effect with microcystins (Honkanen et al. 1994, Huang and Zimba 2019). Moreover, although not been statistically detected as an inhibitor of microbial diversity, the concentration level of cyanopeptolin-1020 in the bloom waters (at a peak concentration of $7.1 \mu\text{g L}^{-1}$) is already high enough to exert toxicological effects on trypsin ($IC_{50} = 670$ pM or $0.68 \mu\text{g L}^{-1}$) of freshwater crustacean (Gademann et al. 2010) and zebra fish (Faltermann et al. 2014). Given more than 10 times higher concentrations of total non-microcystin cyanopeptides than the well-described microcystin detected in the lake Taihu, our results suggest that cyanopeptides produced during CyanoHABs should have been exerting hidden eco-impacts (e.g., microbial inhibition as afore-discussed) stronger than previously acknowledged microcystins alone. Therefore, future screening and profiling of cyanotoxins in lake ecosystems need to fully consider non-microcystin cyanopeptides as emerging ecotoxicologically critical components in governing aquatic biodiversity and explore their specific roles in mediating biogeochemical cycling in freshwater ecosystems plagued by CyanoHABs.

4.2 Degradation co-affected by putative cyanopeptide degraders and local microbiological setups

In natural water, the elimination of cyanobacterial secondary metabolites is revealed to mostly attribute to bacterial biodegradation (Li et al. 2017), which in turns involves in turnover of associated bacterial communities (Lezcano et al. 2017). Previous studies have evaluated the microcystin-degrading capacities of *Sphingopyxis* isolates from Taihu (Yan et al. 2012, Yang et al. 2020). Consistently, our study reveals that *Sphingopyxis* spp. are the most predominant microcystin degraders in lake water (Fig. S6). Moreover, we have experimentally verified the dependence of biodegradation capacity on the absolute abundance of the degraders at the Biomass Peak and also across three sampling regions. Interestingly, the positive ‘biomass-degradation’ relationship cannot be inspected at the Cyanopeptide Peak (on June 30) which came earlier than the Biomass Peak. This intriguing temporal disparity is likely due to cyanopeptides restriction on microbial diversity and degradation capacity, implying a dynamic interplay more complex than solely linear correlations between removal of cyanopeptides and biomass of degraders.

Field research has reported an antagonistic relationship between toxic cyanobacteria and microcystin degraders (Lezcano et al. 2018). Consider the inhibition effects of detected cyanopeptides as discussed in 4.1, we would presume

401 that at least partial degrading microbes also be quantitatively restricted to be decoupled
 402 from their respective cyanopeptide substrates, when cyanopeptides (thus their
 403 presumptive toxicities) peaked. Moreover, we can see that the non-linear relationship
 404 at Cyanopeptide Peak is featured by open water samples displaying high k values but
 405 relatively low degrader biomass (grey scatters, left panel), compared with the
 406 Biomass Peak (right panel, Fig. 3B). This result suggests that efficient degraders
 407 beyond we listed (Table S2) might be active when cyanopeptide concentration peaked.
 408 The degradation of cyanopeptides in natural water involves diverse microorganisms
 409 (Li et al. 2017, Zhang et al. 2020) and affected by multiple environmental variables
 410 (Maghsoudi et al. 2016, Moron-Lopez et al. 2017). While our results generally
 411 highlight the nutritional dependency of known degrading bacteria on cyanopeptides
 412 degradation, the role of other unknown degrading processes and cross-feeding of
 413 degrading products (e.g., phenylacetate) cannot be ruled out. For instance, the peaked
 414 cyanopeptides can create a hotspot of organic carbon (Su et al. 2018, Zhang et al.
 415 2020) to stimulate microbial degradation via pathways alternative to those in
 416 *Sphingopyxis* cells (Lezcano et al. 2017, Moron-Lopez et al. 2017), resulting in the
 417 high degradation rates at low biomass of reported degraders as detected in the open
 418 water (grey scatters, left panel, Fig. 3B). In addition, degradation of cyanopeptides
 419 would be favored in indigenous biological setups where pre-adaption (Christoffersen
 420 et al. 2002, Cousins et al. 1996, Moron-Lopez et al. 2017) and quorum sensing system

(Zeng et al. 2020) can both extensively regulate and enhance microbial degradation capacity. Therefore, the lower k values observed for sites within Meiliang bay and Gonghu Bay (Fig. 3C) could result from the premature microbiological setups for cyanopeptide degradation, as the two bay area are more likely be subject to influence of water diversion and inflow than the open water regions (Hu et al. 2021, Zhu et al. 2021b).

Collectively, our results indicate degradation of cyanopeptides in the lake Taihu is co-determined by abundance of putative degraders and local microbiological setups. Geographically, the increased water inflow (by 38.5%, i.e. around 3 billion m³ per year) and accelerated water exchange cycle (from 210 days to 184 days) since 2007 (Zhu et al. 2021b) could also interfere the remediation and restoration (in this case, biodegradation of the cyanopeptide during bloom event) by indigenous populations in Taihu, implying a trade-off between geo-engineering and ecosystem resilience. It should be noted that the above speculation was made by extrapolating degradation kinetics of microcystins to all cyanopeptides, while for a group of cyclic or linear peptides with conserved substructures in each class (Janssen 2019), variances in degradation efficiency are expected and deserve further investigation.

4.3 Cyanopeptides as deterministic factors of bloom-associated microbiota assemblage

Recent studies have suggested that the turnover of microbial communities is tightly associated with CyanoHABs due to prevalent nutrient cycling and signal transduction between bloom-associated microbiota (Wang et al. 2020, Zhu et al. 2021a, Zhu et al. 2019), as well as dynamic of microcystins (Lezcano et al. 2017). Here, we extended the scope to cyanopeptide components beyond microcystin and showed that assembly of bloom-associated microbiota is strongly determined by cyanopeptides, with variances between size-fraction niches and communities.

Cyanobacterial colony represents a unique niche for bloom microbiota where cyanopeptides regulate both cyanobacterial and non-cyanobacterial populations. Sizes of colonies are positively correlated with microcystin concentrations (Gan et al. 2012, Wang et al. 2013), implying an intrinsic connection between cyanopeptides and cyanobacterial community. This is consistent with our observation that deterministic processes mostly contributed to the assembly of cyanobacterial communities within colonies (Fig. 4A). The intriguing co-existence of multiple closely related populations (as proxied by ASVs) and their changing abundances were observed for cyanobacterial genera in the colony (Fig. 2C), reflecting a combined role of cyanopeptide dynamics and environmental filtering in selecting competing populations and driving community turnover. Meanwhile, assembly of non-cyanobacterial communities within the colony was basically controlled by deterministic processes except for that during the Biomass Peak (Fig. 4A, bottom

panel), and the community was also more responsive to cyanopeptides dynamics than nutrients (Fig. 4B). Moreover, their community-level dissimilarities within colonies declined as a function of larger differences in cyanopeptide concentrations (Fig. 4C), indicating a much narrower range of ecological niches (thus lower observed alpha diversity, Fig. S4 & S5) for the colonized microbiota. Similarly, the fit of bacterioplankton communities to neutral model was weakened due to biotic pressure from accompanied cyanobacteria (Wang et al. 2020). Here, our results further indicate that cyanopeptides including those bioactive non-microcystin ones (as afore-discussed in 4.1), could be chemical intermediates of the biotic pressure. Colonies also nurture the most abundant *Bacteroidetes* at the Biomass Peak (Fig. S3A). This phylum members can readily adapt to complex substrates rather than monomeric compounds and therefore play a fundamental role in conversion of high molecular weight planktonic compounds during phytoplankton blooms (Buchan et al. 2014). In this scenario, the increased stochasticity at the Biomass Peak (July 21) could be plausibly co-explained by the rapid degradation of cyanopeptides (from ca. 597 to 300 ppb on average) into labile products (e.g. amino acid) to nourish heterotrophic communities (as discussed in 4.2), and also the relief from the restrictive pressure, as noticed elsewhere (Zhou et al. 2014).

For PA and FL microbiota, the distinct dynamic of assembly patterns for cyanobacterial and non-cyanobacterial communities, varied contributions of

480 cyanopeptides or nutrients in explaining community turnover and the contrasting
 481 community-level responses to cyanopeptides (Fig. 4) together suggest that assembly
 482 of the two communities was dominated by separated processes throughout the bloom
 483 event. For example, the increased in stochasticity for cyanobacteria in PA fraction
 484 from June 09 to July 21 (Fig. 4A, top panel) could originate from the expansion of
 485 *Microcystis* spp. in the PA metacommunity (Fig. 2B, PA fraction), which is more
 486 likely to be taxonomically closer to *Microcystis* populations in the colony (Zhu et al.
 487 2018), leading to a random distribution pattern and in turn, high stochasticity (Ning et
 488 al 2019). Cyanopeptides provide their producers competitive edge over associated
 489 populations (Holland and Kinnear 2013). As the free-living cyanobacteria are
 490 phylogenetically more diverse than communities in the colony and PA fraction, high
 491 cyanopeptide levels may have mediated their stochasticity until the producers were
 492 less prominent in biomass (i.e. on Aug. 18). This may have also explained the
 493 opposite trend of cyanobacterial dissimilarities in PA and FL fraction (Fig. 4C), as
 494 competition exclusion induced by cyanopeptides drove the FL communities more
 495 phylogenetically similar (Chen et al. 2020, Mayfield and Levine 2010). Collectively,
 496 our results highlight that cyanopeptides, through their broad microbial restriction
 497 effects as well as degradation, are the key biotic forces in mediating microbiota
 498 assembly during CyanoHABs.

499 5. Conclusion

Temporal and spatial dynamics of cyanopeptides in the lake Taihu during summer
CyanoHABs were closely followed and linked to the microbiota to elucidate its
assembly mechanisms. Cyanopeptides represent a variety of structural classes and can
exert significant ecotoxicological impacts on bloom-associated microbiota. Biomass
of potential degrading microbes could quantitatively indicate the degradation capacity
of lake water, while the spatiotemporal disparity suggested a complex interplay
between removal of cyanopeptides and local microbiota setup which is subject to
influences of water division and inflow. Furthermore, cyanopeptide was identified as
a selective force (stronger than nutrients) in mediating the assembly and turnover of
bloom-associated microbiota, with variances between size-fraction niches and
communities. Overall, we propose that the prevalence, dynamics and eco-impacts of
cyanopeptides should be considered as a crucial consequence of intractable
CyanoHABs and deserve oriented surveillance and investigation in future to address
their ecological roles in ecosystems like Taihu.

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522 **Competing interests**

523 The authors declare no conflict of interest.

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Figure 1. Spatiotemporal heterogeneity of cyanopeptides and its links with cyanobacterial communities. (A) Sampling sites in Taihu. The purple, grey and blue rectangles represent the lake region Meiliang Bay, Open water and Gonghu Bay, respectively. (B) The log₁₀-transformed concentration (ppb or $\mu\text{g L}^{-1}$) of cyanopeptides detected in eight sampling sites at five sampling dates & (C) Correlations between dominant cyanobacterial genera and cyanopeptide compounds computed by Mantel test. The widths of lines denoted the value of Mantel's r. Colors of edges are distinguished based on P values. Pearson correlation coefficients between cyanopeptide components were visualized in the heatmap. The results showed that cyanopeptides in the lake water was dominated by components from the microginins, microcystins and spumigins class, reaching a cyanopeptide peak on June 30. Significant correlations of cyanopeptide components with those dominant cyanobacterial genera were observed, especially within the colony fraction. Abbreviations used in the figures are, Cyano: Cyanobacteria, Coln: colony, PA: particle-associated, FL: free-living, Mic: *Microcystis*, Doli: *Dolichospermum* and Cybm: *Cyanobium*.

Figure 2. Spatiotemporal dynamics of biomass and composition in three size fractions of the cyanobacterial communities. The analysis was based on 16S rRNA gene sequencing of three fractions of the microbiota defined by water filtration: > 48 μm (colony fraction), 2.0-0.2 μm (particle associated) and < 0.2 μm (free living). (A) 16S-based absolute abundance (copies L^{-1}) or biomass estimates of cyanobacterial genera. (B) Relative abundance (%) of cyanobacterial genera. (C) Phylogenetic tree of all cyanobacterial ASVs and abundance distribution analyses of cyanobacterial populations in the colony fraction. Barplots in the inner cycle represent the log-transformed absolute abundance of each ASV on four sampling dates. Pie plots at the tips of each ASV represent its relative proportions on four sampling dates. Results demonstrated a clear biomass peak of cyanobacterial bloom on July 21 and succession of the dominant genera on Aug. 18 and Sept. 20 in PA and FL fractions with high micro-diversity.

Figure 3. Spatiotemporal dynamics of non-cyanobacterial communities and cyanopeptides degraders in the Taihu Lake. (A) Non-multidimensional scaling (NMDS) analysis based on unweighted and weighted unifracs distances showing the community dissimilarity in terms of phylogenetic membership and abundance profiles, respectively. (B) Spearman's rank correlations coefficients between the 16S-based absolute abundance of potential cyanopeptide degraders (copies L^{-1}) and pseudo-first order degradation constant (k) values were calculated. (C) Boxplot showing degradation constant (k) from samples at separated sites as well as lake regions. Results revealed that the community composition was significantly differentiated by sampling date and size fraction. Abundance of potential degraders was positively correlated with degradation constant at the biomass peak (July 21) rather than cyanopeptide peak (June 30). Water samples from the center lake region (open water, Site 3, 4 and 8) exhibited significantly higher (T-test, $P < 0.05$) degradation capacity compared to the two bay areas.

Figure 4. Cyanopeptides were identified as an important deterministic driver in turnover of both cyanobacterial and non-cyanobacterial communities. Comparison of NST (%) index of cyanobacterial (A, upper panel) and non-cyanobacterial communities (A, lower panel) in the three size-fractions. Colony samples on July 9 were not collected and therefore not shown. (B) Variation partitioning analysis (VPA) results show the relative contribution of cyanopeptides or nutrients to the size-fractioned cyanobacterial and non-cyanobacterial communities. (C) UniFrac distances of cyanobacterial/non-cyanobacterial communities, plotting against differences in total cyanopeptides concentrations. The lines were second-order polynomial regression curves fitted points of two communities denoted by colors. Only regression coefficients with $P < 0.05$ were annotated with a regression coefficient. Each point stands for a sample pair. The results generally showed deterministic effect of cyanopeptides on both cyanobacterial and non-cyanobacterial communities. And cyanopeptide was a factor stronger than nutrient in explaining turnover of bloom-associated microbiota. The community-level dissimilarities declined as a function of greater differences in cyanopeptide concentrations, with variances between size-fraction niches and communities.