

1 **Nanoplastics promote microcystin synthesis and release from cyanobacterial**  
2 ***Microcystis aeruginosa***

3

4 Li-Juan Feng<sup>a</sup>, Jia-Qi Liu<sup>a</sup>, Lin-Lin Zhou<sup>a</sup>, Fan-Ping Zhu<sup>a</sup>, Xiao-Dong Sun<sup>a</sup>, Xiao-Yu  
5 Liu<sup>a</sup>, Shu-Guang Wang<sup>a</sup>, Tamara Susan Galloway<sup>b\*</sup>, Xian-Zheng Yuan<sup>a\*</sup>

6

7

8 a Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School  
9 of Environmental Science and Engineering, Shandong University, Qingdao,  
10 Shandong 266237, P. R. China

11

12 b College of Life and Environmental Sciences, University of Exeter, Exeter EX4  
13 4QD, Devon, United Kingdom

14

15

16 Corresponding authors:

17 Xian-Zheng Yuan

18 School of Environmental Science and Engineering, Shandong University

19 72 Rd. Binhai, Qingdao, Shandong 266237, P. R. China

20 E-mail: xzyuan@sdu.edu.cn

21

22 Tamara Susan Galloway

23 College of Life and Environmental Sciences, University of Exeter

24 Exeter EX4 4QD, Devon, United Kingdom

25 Email: t.s.galloway@exeter.ac.uk

26 Although the fate of nanoplastics (< 100 nm in size) in freshwater systems is  
27 increasingly well studied, much less is known about its potential threats to  
28 cyanobacterial blooms, as the ultimate phenomenon of eutrophication occurring  
29 world-wide. A handful of studies has evaluated the consequences of nanoplastics  
30 increasing the membrane permeability of microbes, but there is no direct  
31 evidence for interactions between nanoplastics and microcystin; intracellular  
32 hepato-toxins produced by some genera of cyanobacteria. Here we show that  
33 amino-modified polystyrene nanoplastics (PS-NH<sub>2</sub>) promote microcystin  
34 synthesis and release from *Microcystis aeruginosa*, a dominant species causing  
35 cyanobacterial blooms. We demonstrate that PS-NH<sub>2</sub> inhibits photosystem II  
36 efficiency, reduces organic substance synthesis, and induces oxidative stress,  
37 enhancing the synthesis of microcystin. Furthermore, PS-NH<sub>2</sub> promotes the  
38 extracellular release of microcystin from *M. aeruginosa* via transporter protein  
39 up-regulation and impaired cell membrane integrity. Our findings propose that  
40 the presence of nanoplastics in freshwater ecosystems might enhance the threat  
41 of eutrophication to aquatic ecology and human health.

42

43

44

45

46

47

48

49

50

51 **MAIN TEXT**

52 Plastic debris are increasingly considered a global concern due to their negative social  
53 and ecological impacts<sup>1,2,3,4</sup>. The discarded plastic can be degraded into microplastics  
54 (0.1-5 mm in size), and even nanoplastics (< 100 nm) by abiotic and biotic factors<sup>5</sup>. In  
55 addition, nanoplastics are directly derived from personal care and cosmetic products  
56 and industrial processes where nanoplastics are used or formed<sup>6,7</sup>. Although the fate  
57 and effect of nanoplastics in marine environments are increasingly well studied, much  
58 less is known about the ecological effects of nanoplastics in freshwater environments,  
59 which have been considered a major source of nanoplastics. In freshwater systems,  
60 the cyanobacteria, the extensive occurrence of which is regarded as eutrophication<sup>8</sup>,  
61 have repeatedly been detected as a main component attached to the plastic surface.  
62 Furthermore, a number of cyanobacterial species can produce microcystin, with  
63 *Microcystis* as the predominant producer in freshwater systems<sup>9,10</sup>. Microcystin have  
64 been associated with liver cancer and fatality, for example with the deaths of 60  
65 patients after renal dialysis with microcystin-contaminated water in Brazil<sup>11,12</sup>. Hence,  
66 the interactions between cyanobacteria and nanoplastics in freshwater potentially  
67 affects the formation and persistence of cyanobacteria blooms as well as microcystin.

68

69 We cultured *M. aeruginosa* in standard blue-green (BG-11) medium containing  
70 polystyrene nanoplastics with differential surface charge, to simulate changes caused  
71 by weathering of plastics and adsorbing of natural organic matter<sup>13</sup>. We found that the  
72 negative-charged sulfonic acid-modified polystyrene nanoplastics (PS-SO<sub>3</sub>H) showed  
73 no obvious inhibitory effect even when the exposure concentration was 100 µg/mL  
74 (Supplementary Figure 1A). Due to electrostatic repulsion, positively charged  
75 nanoparticles interact more easily than negatively charged nanoparticles with negative

76 membrane residuals. Hence, we found positive-charged amino-modified polystyrene  
77 nanoplastics (PS-NH<sub>2</sub>) had a greater influence on *M. aeruginosa* than negatively  
78 charged plastics (Supplementary Figure 1B). During the acute 48-h exposure, growth  
79 inhibition of *M. aeruginosa* by low (3.40 µg/mL) and high (6.80 µg/mL)  
80 concentrations of PS-NH<sub>2</sub> was 23.57% and 46.10%, respectively, with the normally  
81 green *M. aeruginosa* turning yellow (Fig. 1A and B), in parallel with a significant  
82 reduction in the chlorophyll-a content (Supplementary Figure 1C). This inhibition was  
83 significantly reduced upon long-term exposure (10 days), with *M. aeruginosa*  
84 regaining its green coloration (Fig. 1A), indicating that the interaction of nanoplastics  
85 and cyanobacteria was dynamic and without persistence within the experimental  
86 period. However, the synthesis of microcystin increased significantly both under acute  
87 and long-term exposure, compared to the control group (Fig. 1C). And microcystin-  
88 leucine-arginine (MC-LR), the most common and harmful microcystin in freshwater  
89 environments, also increased significantly (Supplementary Figure 2A). Furthermore,  
90 both exposures of PS-NH<sub>2</sub> significantly stimulated the extracellular release of  
91 microcystin and MC-LR from *M. aeruginosa* (Fig. 1D; Supplementary Figure 2B).

92

93 To identify the molecular mechanisms responsible for the effects described above, the  
94 proteomics of *M. aeruginosa* under PS-NH<sub>2</sub>-induced stress were performed. The  
95 proteomics results revealed that PS-NH<sub>2</sub> had a substantial influence on cyanobacterial  
96 growth (Supplementary Figure 3). The proteins that were significantly down-  
97 regulated in both low- and high-concentration treatments suggested that PS-NH<sub>2</sub> may  
98 inhibit the photosynthetic activity, weaken the photosynthetic electron transport chain,  
99 and reduce carbohydrate metabolism (Supplementary Figure 4; Supplementary Table  
100 1). In addition, the acute exposure of low-concentration PS-NH<sub>2</sub> only influenced the

101 light reaction of photosynthesis comparing with both the light and dark reactions of  
102 photosynthesis impaired by high-concentration PS-NH<sub>2</sub> treatment (Supplementary  
103 Figure. 5A). The main proteins (*psbB*, *psbC*, and *psbD*) involved in photosystem II  
104 were all down-regulated (Supplementary Table 1), indicating that the photosynthetic  
105 efficiency of photosystem II was significantly inhibited by PS-NH<sub>2</sub>. The weakening  
106 of the photosynthetic electron transport chain, alleviated gradually by detoxification  
107 enzymes for low-concentration exposure and continuing in the later period under  
108 high-concentration exposure, lead to the accumulation of surplus electrons and  
109 inducedoxidative stress (Supplementary Figure 5B; Supplementary Table 1). The  
110 reduction of carbohydrate metabolism was consistent with decreased growth after  
111 treatment with PS-NH<sub>2</sub> (Supplementary Figure 5C). Furthermore, the down-  
112 regulation of lipopolysaccharide biosynthetic process-related proteins (Supplementary  
113 Table 1), involved in synthesizing integral components of the membrane, indicated  
114 damage to cell membrane integrity after PS-NH<sub>2</sub> exposure. The exposure to both  
115 concentrations of PS-NH<sub>2</sub> led to the up-regulation of proteins involved in the  
116 biological transport process (Supplementary Figure 5D; Supplementary Table 1), such  
117 as ABC transporters, which are transmembrane complexes that span both the plasma  
118 membrane and outer membrane of *M. aeruginosa* and actively export substrates, such  
119 as macrolide antibiotics, peptides, virulence factors, and cell envelope precursors<sup>14</sup>.

120

121 To verify the above proteomics results, we performed a qRT-PCR assay on *psbD*, a  
122 key gene associated with photosynthesis II. Pearson's correlation coefficient between  
123 the qRT-PCR data (Fig. 1E) and the proteomics data was 0.80, which indicates the  
124 accuracy of the proteomics data. The down-regulation of *psbD* is known to interfere  
125 with electron transport, leading to the accumulation of surplus electrons and oxidative

126 stress<sup>15, 16</sup>. Upon acute exposure of PS-NH<sub>2</sub>, we observed a significant increase in the  
127 levels of reactive oxygen species (ROS) (Fig. 1G) and superoxide dismutase (SOD)  
128 (Supplementary Figure 6A), a part of the cell's antioxidant defense system. The  
129 induction of oxidative stress was consistent with the proteomics data. The level of  
130 reduced glutathione (GSH) decreased significantly comparing with the untreated  
131 control, to eliminate the oxidative stress (Supplementary Figure 6B). For validation of  
132 proteomics analysis for cyanobacterial membrane transport, we used green-  
133 fluorescent PS-NH<sub>2</sub> to determine whether PS-NH<sub>2</sub> entered and accumulated in *M.*  
134 *aeruginosa*. The behavior of the fluorescently labelled PS-NH<sub>2</sub> was similar to that of  
135 PS-NH<sub>2</sub>, both in the culture medium and deionized water (Supplementary Figure 7).  
136 The fluorescently labelled PS-NH<sub>2</sub> penetrated the cell membrane and accumulated  
137 inside the cells (Fig. 1G). The process of the nanoparticles entering the cell appeared  
138 to rupture the cell membrane, an effect seen both under acute and long-term exposure  
139 to PS-NH<sub>2</sub> (Fig. 1H). Such a membrane rupture could enhance the release of  
140 intracellular materials such as microcystin.

141

142 Given that the up-regulation for most of the proteins involved in the synthesis of  
143 microcystin<sup>17</sup> was not significant (Supplementary Table 2), we built co-expression  
144 modules from our proteomic data using weighted gene correlation network analysis  
145 (WGCNA). We identified 18 co-expression modules from the data regarding the 2413  
146 proteins from the 18 samples (Fig. 2A and B), and effectively identified three groups  
147 of hub proteins (Fig. 2C, D, and E and Supplementary Fig. 8), which were placed in  
148 the middle of the protein-protein interaction (PPI) networks (Fig. 2F). The top hub  
149 proteins marked with yellow in the brown module, with a negative correlation to the  
150 synthesis of intracellular microcystin, are tryptophan synthase C (involved in organic

151 substance metabolic pathways). This indicates that the decrease in synthesis of  
152 organic substances stimulates the microcystin synthesis, which protects *M.*  
153 *aeruginosa* from oxidative damage.<sup>18</sup> The top hub proteins in the turquoise module  
154 (pyrG) and blue module (apcB1 and N44\_03141) are positively and negatively  
155 correlated with extracellular microcystin, respectively (Supplementary Table 3). The  
156 proteins of pyrG and apcB1 are related to the regulation of phospholipid synthesis<sup>19</sup>  
157 and thylakoid membrane by module GO enrichment, respectively. Additionally,  
158 N44\_03141 is an alkaline phosphatase-like protein that is associated with the integral  
159 components of the membrane. The up-regulation of pyrG protein may be a defense  
160 response against cell membranes damage. Down-regulation of apcB1 and N44\_03141  
161 was in accordance with the self-protection of thylakoid membrane and damage of cell  
162 membrane integrity, respectively. Hence, the increased synthesis of microcystin was  
163 a defense response to protect cells from oxidative damage and enhance the fitness of  
164 *M. aeruginosa* to the stresses caused by nanoplastics<sup>18,20</sup>, which was observed under  
165 exposure to antibiotic<sup>21</sup>, iron-limiting conditions<sup>18</sup> and herbicide<sup>22</sup>. The damage to  
166 membrane integrity and the up-regulation of biological-transport proteins were the  
167 main explanation for the stimulated release of microcystin.

168

169 Our study provides a better understanding of the fate, distribution, and molecular  
170 basis of nanoplastics in aquatic primary producers, such as cyanobacteria. The extent  
171 to which the environment is contaminated with nanoplastics remains to be quantified,  
172 given the technical challenge of detecting such small and carbon-based particles in  
173 complex natural matrices. However, in the controlled laboratory, 0.3% (w/w) of a  
174 polymeric latex film formed nanoparticles with average diameter of 196.52 nm ( $\pm$   
175 89.48) after 200-days exposure into the freshwater environment. Based on the limited

176 reports on microplastics abundance in Three Gorges Reservoir (1597 to 12,611  
177 items/m<sup>3</sup>) and midstream of the Los Angeles River (12,000 items/m)<sup>23</sup>, the  
178 concentration of nanoplastics in freshwater systems might be in the level of µg/mL,  
179 not to mention the meso- or macro-plastics. The exposure to nanoplastics in this  
180 concentration in the current study promoted microcystin synthesis and release from  
181 *Microcystis aeruginosa*, even without the change of coloration. Cyanobacterial  
182 blooms have negative consequences for both human health and aquatic ecology.  
183 Cyanobacteria form the base of many food chains; furthermore, the accumulation of  
184 nanoplastics in cyanobacteria might have effects on other trophic levels, which could  
185 pose a potential risk to food safety.

186

## 187 **METHODS**

### 188 **Characterization of Nanoplastics**

189 The PS-NH<sub>2</sub> (50 nm) particles and green fluorescently labelled 50 nm PS-NH<sub>2</sub>  
190 particles (excitation wavelength, 475 nm; emission wavelength, 510 nm) were  
191 purchased from Bangs Laboratory (USA) and micromod Partikeltechnologie GmbH  
192 (Germany), respectively. Sulfonic acid-modified polystyrene nanoplastics (PS-SO<sub>3</sub>H)  
193 were synthesized in the laboratory through nitrogen-protected emulsion  
194 polymerization with styrene as a monomer<sup>24, 25</sup>. Before the experiment, the  
195 nanoparticles were transferred to a dialysis bag (1 kDa) for 3 days to remove  
196 redundant monomers or initiators<sup>26</sup>. The diameter and morphologies of PS-NH<sub>2</sub> were  
197 characterized by a scanning electron microscope (SEM; S-5000, Hitachi, Japan). The  
198 size (Z-average) and ζ-potential (mV) were determined using dynamic light scattering  
199 (DLS; Zetasizer Nano ZS, Malvern, UK). The structure and composition of  
200 nanoplastics (Supplementary Figure 9) were determined via a Fourier transform



201 infrared (FTIR) spectrometer (Aratar, Thermo NicoLet, USA) at wavenumbers from  
202 4000 to 400  $\text{cm}^{-1}$ . Ultraviolet-visible (UV-Vis) spectra (190 - 450 nm) of PS-NH<sub>2</sub> in  
203 the aqueous phase were collected using an ultraviolet-visible (UV-Vis)  
204 spectrophotometer (UV-6100, Metash, China), and the concentration of the  
205 nanoplastics in the aqueous phase was determined by measuring the UV absorbance at  
206 220 nm (Supplementary Figure 9).

207

### 208 **Exposure of *M. aeruginosa* to nanoplastics**

209 *M. aeruginosa* (FACHB 905), purchased from the institute of hydrobiology, Chinese  
210 Academy of Sciences (Wuhan, China), was cultured in autoclaved standard blue-  
211 green (BG-11) medium at a pH of around 7.2. PS-NH<sub>2</sub> was added on the 10<sup>th</sup> day of  
212 cyanobacterial growth. The systems without PS-NH<sub>2</sub>, and those with PS-NH<sub>2</sub> at  
213 concentrations of 3.40 and 6.80  $\mu\text{g}/\text{mL}$  were set as the control, low-concentration, and  
214 high-concentration exposure treatments, respectively. All the experiments were  
215 performed in six replicates. After acute (2 days) and long-term (10 days) exposure to  
216 PS-NH<sub>2</sub>, the responses of *M. aeruginosa* by PS-NH<sub>2</sub> were investigated.

217

### 218 **Analysis of microcystin, chlorophyll-a, and cell membrane integrity**

219 *M. aeruginosa* samples were centrifuged at 10000 rpm and 4 °C for 5 min. The  
220 supernatant was used to analysis the extracellular microcystin. The residue was re-  
221 suspended in an original volume of ultra-pure water, and then frozen in liquid  
222 nitrogen and thawed at room temperature thrice. Then, the solution was centrifuged at  
223 10000 rpm and 4 °C for 5 min. The supernatant was filtered through 0.22- $\mu\text{m}$  acetate  
224 cellulose membranes for the analysis of intracellular microcystin. The extracellular  
225 and intracellular microcystin concentrations were detected using microcystin enzyme-

226 linked immunosorbent assay kits (Runyu Biotechnology CO., China). The  
227 chlorophyll-a analysis was performed based on the previous research.<sup>27</sup> The cell  
228 membrane integrity was evaluated according to the protocol<sup>28,29</sup>. The PS-NH<sub>2</sub>  
229 distribution in *M. aeruginosa* was observed through confocal microscopy. Confocal  
230 imaging was performed using a laser-scanning confocal microscope (LSM-700,  
231 ZEISS, Japan).

232

### 233 **Analysis of antioxidant responses**

234 *M. aeruginosa* samples were centrifuged at 10000 rpm and 4 °C for 5 min. The  
235 supernatant was discarded, and the residue was re-suspended in 300 µL of ultra-pure  
236 water. Then, the samples were frozen in liquid nitrogen and thawed at room  
237 temperature thrice. After centrifugation at 10000 rpm and 4 °C for 5 min, the  
238 supernatant was filtered through 0.22-µm acetate cellulose membranes for the  
239 analysis of the antioxidant responses of *M. aeruginosa*. Commercially SOD and GSH  
240 assay kits (Nanjing Jiancheng, China) were used to determine the activities of  
241 antioxidant enzymes, using a programmable microplate reader (Infinite F50, Tecan,  
242 Switzerland). The concentrations were normalized to the cell numbers before  
243 statistical analysis.

244

### 245 **Analysis of Gene expression**

246 Total RNA was extracted according to the procedures of Rnaprotect<sup>®</sup> Bacteria  
247 Reagent and Spin Column Bacteria Total RNA Purification Kit. The RNA  
248 concentration and purity were quantified by a nucleic acid analyzer. Before reverse  
249 transcription, a Primescript<sup>™</sup> RT reagent kit with gDNA Eraser was utilized to  
250 remove genomic DNA contamination in RNA samples. The cDNA was then

251 synthesized through a process of reverse transcription polymerase chain reaction,  
252 which were stored at -20 °C until real-time qPCR analysis<sup>30</sup>. The primers of the 16S  
253 rRNA gene, which was used as the housekeeping gene, and *psbD* are shown in  
254 Supplementary Table 4<sup>31</sup>.

255

## 256 **Analysis of proteomic responses**

257 *M. aeruginosa* cells were sampled in three biological replicates for the control and  
258 experimental groups during two phases: acute exposure (2 days) and long-term  
259 exposure (10 days). Protein extraction was performed via the trichloroacetic  
260 acid/acetone precipitation and the SDTLysis procedure<sup>32</sup>. After quantification, the  
261 proteins extracts were digested according to the filter-aided sample preparation  
262 (FASP) protocol procedure<sup>33</sup>. The iTRAQ labelled peptides were fractionated by  
263 strong cation exchange chromatography using the AKTA Purifier system. The  
264 collected fractions were desalted on C18 Cartridges and injected for nano-LC-MS/MS  
265 analysis. LC-MS/MS analysis was performed on a Q-Exactive mass spectrometer  
266 coupled to an Easy nLC. Protein identification was performed using the MASCOT  
267 engine embedded into Proteome Discoverer 1.4. To reduce the probability of false  
268 peptide and protein identification, the cutoff global false discovery rate was set to  
269 0.01. Differentially expressed proteins were defined based on fold changes of > 1.2 or  
270 < 0.83 and a p value of < 0.05 in all three replicates. WGCNA was performed  
271 according to the R package of the WGCNA<sup>34</sup>. The PPI information of differentially  
272 expressed proteins was retrieved from the IntAct molecular interaction database using  
273 the STRING software, and the results were visualized via Cytoscape5 software  
274 (version 3.2.1). Furthermore, the degree of each protein was calculated to evaluate the  
275 importance of the protein in the PPI network.

276

277 **Statistical analysis**

278 All the experiments were run at least six independent experiments unless stated  
279 otherwise. For cell density evaluation, microcystin assay, gene expression, and  
280 antioxidant responses, one-way analysis of variance (ANOVA) with an unpaired *t* test  
281 were performed using Graphpad Prism. The differences were considered significant at  
282  $p < 0.05$  and, are referred to as \* $p < 0.05$ , \*\* $p < 0.01$ .

283

284 **Data availability**

285 The mass spectrometry proteomics data have been deposited to the  
286 ProteomeXchange Consortium via the PRIDE partner repository with the dataset  
287 identifier PXD011664. All other data are available from the corresponding author on  
288 reasonable request.

289

290

291

292

293

294

295

296

297

298

299

300

301

302

303

304

305 **References**

- 306 1. Wilcox C, Van Sebille E, Hardesty BD. Threat of plastic pollution to seabirds  
307 is global, pervasive, and increasing. *Proceedings of the National Academy of*  
308 *Sciences* **112**, 11899-11904 (2015).
- 309 2. Galloway TS, Lewis CN. Marine microplastics spell big problems for future  
310 generations. *Proceedings of the National Academy of Sciences* **113**, 2331-2333  
311 (2016).
- 312 3. Nizzetto L, Langaas S, Futter M. Do microplastics spill on to farm soils?  
313 *Nature* **537**, 488 (2016).
- 314 4. Bornscheuer UT. Feeding on plastic. *Science* **351**, 1154-1155 (2016).
- 315 5. Rochman CM. Microplastics research—from sink to source. *Science* **360**, 28-  
316 29 (2018).
- 317 6. Lu Y, Mei Y, Walker R, Ballauff M, Drechsler M. 'Nano-tree' - type spherical  
318 polymer brush particles as templates for metallic nanoparticles. *Polymer* **47**,  
319 4985-4995 (2006).
- 320 7. Dekkers S, *et al.* Presence and risks of nanosilica in food products.  
321 *Nanotoxicology* **5**, 393-405 (2011).
- 322 8. Pace ML, *et al.* Reversal of a cyanobacterial bloom in response to early  
323 warnings. *Proceedings of the National Academy of Sciences* **114**, 352-357  
324 (2017).
- 325 9. Paerl HW, Otten TG. Blooms bite the hand that feeds them. *Science* **342**, 433-

- 326 434 (2013).
- 327 10. Michalak AM, *et al.* Record-setting algal bloom in Lake Erie caused by  
328 agricultural and meteorological trends consistent with expected future  
329 conditions. *Proceedings of the National Academy of Sciences*, 201216006  
330 (2013).
- 331 11. Carmichael WW, *et al.* Human fatalities from cyanobacteria: chemical and  
332 biological evidence for cyanotoxins. *Environmental health perspectives* **109**,  
333 663 (2001).
- 334 12. Jochimsen EM, *et al.* Liver failure and death after exposure to microcystins at  
335 a hemodialysis center in Brazil. *New England Journal of Medicine* **338**, 873-  
336 878 (1998).
- 337 13. Della Torre C, *et al.* Accumulation and embryotoxicity of polystyrene  
338 nanoparticles at early stage of development of sea urchin embryos  
339 *Paracentrotus lividus*. *Environmental science & technology* **48**, 12302-12311  
340 (2014).
- 341 14. Okada U, Yamashita E, Neuberger A, Morimoto M, van Veen HW, Murakami  
342 S. Crystal structure of tripartite-type ABC transporter MacB from  
343 *Acinetobacter baumannii*. *Nature Communications* **8**, 1336 (2017).
- 344 15. Latifi A, Ruiz M, Zhang CC. Oxidative stress in cyanobacteria. *FEMS*  
345 *microbiology reviews* **33**, 258-278 (2009).
- 346 16. Zhang C, Yi Y-L, Hao K, Liu G-L, Wang G-X. Algicidal activity of *Salvia*  
347 *miltiorrhiza* Bung on *Microcystis aeruginosa*—Towards identification of  
348 algicidal substance and determination of inhibition mechanism. *Chemosphere*  
349 **93**, 997-1004 (2013).
- 350 17. Pearson LA, Hisbergues M, Börner T, Dittmann E, Neilan BA. Inactivation of

- 351 an ABC transporter gene, *mcyH*, results in loss of microcystin production in  
352 the cyanobacterium *Microcystis aeruginosa* PCC 7806. *Applied and*  
353 *environmental microbiology* **70**, 6370-6378 (2004).
- 354 18. Yeung AC, *et al.* Physiological and Proteomic Responses of Continuous  
355 Cultures of *Microcystis aeruginosa* PCC 7806 to Changes in Iron  
356 Bioavailability and Growth Rate. *Applied and environmental microbiology* **82**,  
357 5918-5929 (2016).
- 358 19. Carman GM, Kersting MC. Phospholipid synthesis in yeast: regulation by  
359 phosphorylation. *Biochemistry and cell biology* **82**, 62-70 (2004).
- 360 20. Pimentel JS, Giani A. Microcystin production and regulation under nutrient  
361 stress conditions in toxic *Microcystis* strains. *Applied and environmental*  
362 *microbiology*, AEM. 01009-01014 (2014).
- 363 21. Liu Y, Chen S, Zhang J, Gao B. Growth, microcystin-production and  
364 proteomic responses of *Microcystis aeruginosa* under long-term exposure to  
365 amoxicillin. *Water research* **93**, 141-152 (2016).
- 366 22. Berenice E. Exposure to the herbicide 2, 4-D produces different toxic effects  
367 in two different phytoplankters: A green microalga (*Ankistrodesmus falcatus*)  
368 and a toxigenic cyanobacterium (*Microcystis aeruginosa*). *Science of the total*  
369 *environment*, (2017).
- 370 23. Moore CJ, Lattin G, Zellers A. Working our way upstream: a snapshot of land  
371 based contributions of plastic and other trash to coastal waters and beaches of  
372 Southern California. In: *Proceedings of the Plastic Debris Rivers to Sea*  
373 *Conference, Algalita Marine Research Foundation, Long Beach, CA* (ed^(eds)  
374 (2005).
- 375 24. Feng L-J, Wang J-J, Liu S-C, Sun X-D, Yuan X-Z, Wang S-G. Role of

- 376 extracellular polymeric substances in the acute inhibition of activated sludge  
377 by polystyrene nanoparticles. *Environmental Pollution* **238**, 859-865 (2018).
- 378 25. Besseling E, Wang B, Lürling M, Koelmans AA. Nanoplastic affects growth  
379 of *S. obliquus* and reproduction of *D. magna*. *Environmental science &*  
380 *technology* **48**, 12336-12343 (2014).
- 381 26. Pikuda O, Xu EG, Berk D, Tufenkji N. Toxicity Assessments of Micro-and  
382 Nanoplastics Can Be Confounded by Preservatives in Commercial  
383 Formulations. *Environmental Science & Technology Letters*, (2018).
- 384 27. Zhu X, Kong H, Gao Y, Wu M, Kong F. Low concentrations of polycyclic  
385 aromatic hydrocarbons promote the growth of *Microcystis aeruginosa*. *Journal*  
386 *of hazardous materials* **237**, 371-375 (2012).
- 387 28. Huo X, Chang DW, Tseng JH, Burch MD, Lin TF. Exposure of *Microcystis*  
388 *aeruginosa* to Hydrogen Peroxide under Light: Kinetic Modeling of Cell  
389 Rupture and Simultaneous Microcystin Degradation. *Environmental science &*  
390 *technology* **49**, 5502-5510 (2015).
- 391 29. Rasmussen RE, Erstad SM, Ramos-Martinez EM, Fimognari L, Porcellinis  
392 AJ, Sakuragi Y. An easy and efficient permeabilization protocol for in vivo  
393 enzyme activity assays in cyanobacteria. *Microbial cell factories* **15**, 186  
394 (2016).
- 395 30. Guo N, *et al.* Effect of bio-electrochemical system on the fate and proliferation  
396 of chloramphenicol resistance genes during the treatment of chloramphenicol  
397 wastewater. *Water research* **117**, 95-101 (2017).
- 398 31. Qian H, Yu S, Sun Z, Xie X, Liu W, Fu Z. Effects of copper sulfate, hydrogen  
399 peroxide and N-phenyl-2-naphthylamine on oxidative stress and the  
400 expression of genes involved photosynthesis and microcystin disposition in



401           Microcystis aeruginosa. *Aquatic Toxicology* **99**, 405-412 (2010).

402   32.   Wu L, *et al.* Comparative proteomic analysis of the maize responses to early  
403           leaf senescence induced by preventing pollination. *Journal of proteomics* **177**,  
404           75-87 (2018).

405   33.   Wiśniewski JR, Zougman A, Nagaraj N, Mann M. Universal sample  
406           preparation method for proteome analysis. *Nature methods* **6**, 359 (2009).

407   34.   Voineagu I, *et al.* Transcriptomic analysis of autistic brain reveals convergent  
408           molecular pathology. *Nature* **474**, 380 (2011).

409

410

411

412

413

414

415

416

417

418

419

420

421

422

423

424

425

426

427

428

429

430

431

432 **Acknowledgments**

433 This work was supported by the National Science Foundation of China (No.  
434 51478453, No. 21776163), the Qilu Youth Talent Programme of Shandong University  
435 and the Fundamental Research Funds of Shandong University (No. 2017JC021).

436

437 **Authors information**

438 **Affiliations**

439 Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of  
440 Environmental Science and Engineering, Shandong University, Qingdao, Shandong  
441 Province 266237, P. R. China

442 Li-Juan Feng, Jia-Qi Liu, Lin-Lin Zhou, Fan-Ping Zhu, Xiao-Dong Sun, Xiao-Yu Liu,  
443 Shu-Guang Wang, Xian-Zheng Yuan

444

445 College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD,  
446 Devon, United Kingdom

447 Tamara S. Galloway

448

449 **Contributions**

450 X-Z. Yuan and L-J. Feng designed the experiments. X-Z. Yuan, L-J. Feng, T-S.

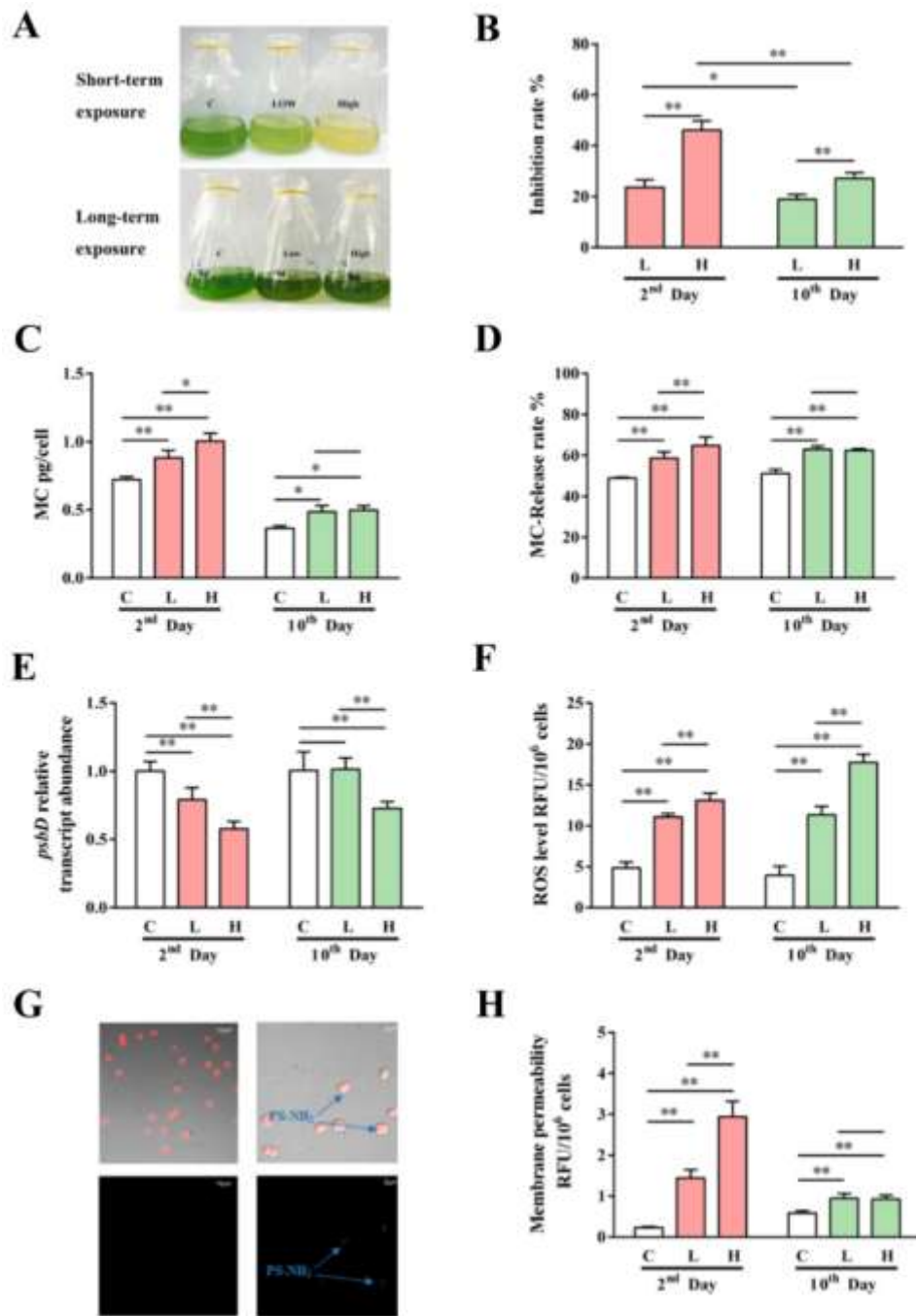
451 Galloway, and S-G. Wang wrote the manuscript. L-J. Feng, J-Q. Liu, L-L. Zhou, and  
452 X-Y. Liu performed the experiments. X-Z. Yuan, L-J. Feng, X-D. Sun, F-P. Zhu, and  
453 T-S. Galloway analyzed the results.

454

455 **Competing interests**

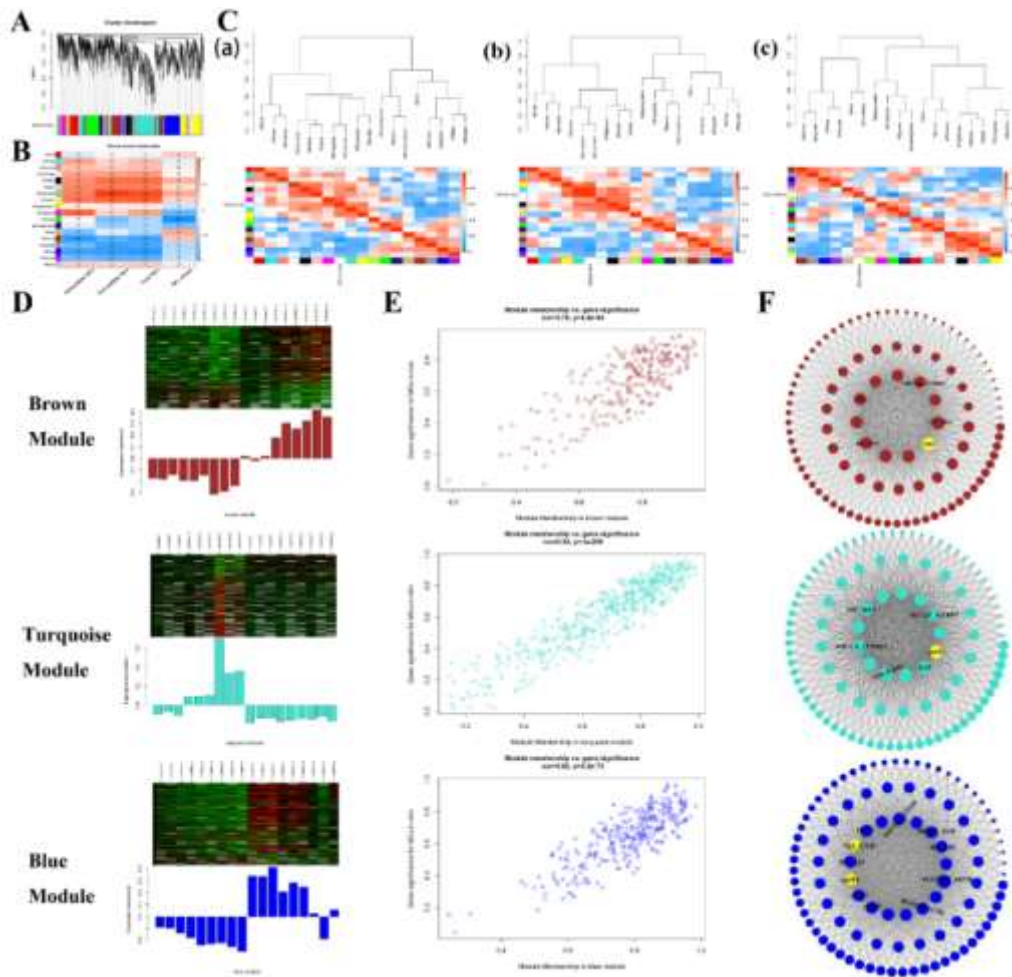
456 The authors declare no competing interests.

457 **FIGURES**



458

459 **Fig. 1** The response of *Microcystis aeruginosa* to acute and long-term exposure of  
 460 PS-NH<sub>2</sub>. Effects of PS-NH<sub>2</sub> after different exposure times on phenotype change (A),  
 461 growth inhibition rate (B), synthesis of total microcystin (C), release rate of  
 462 microcystin (D), transcriptional level of photosynthesis genes (E), ROS concentration  
 463 (F), location of PS-NH<sub>2</sub> in the cells (G) and cell membrane permeability (H). The  
 464 statistical significance was estimated by the two-tailed t-test and differences were  
 465 considered significant at  $p < 0.05$ , and are referred to as \* $p < 0.05$ , \*\* $p < 0.01$ .



466

467 **Fig. 2 The top hub proteins identified by WGCNA.** (A) Clustering dendrograms of  
 468 proteins, with dissimilarity based on topological overlap, together with assigned  
 469 module colors. (B) Identification of protein modules associated with microcystin  
 470 phenotypic traits. Each row corresponds to a module eigengene, while each column  
 471 corresponds to a trait. Each cell contains the corresponding correlation and p value.  
 472 The table is color-coded by correlation according to the color legend. (C) The  
 473 eigengene dendrogram and heatmap identify groups of correlated eigengenes. The  
 474 dendrogram (C, a) indicates that the magenta modules are highly related to  
 475 intracellular microcystin. The dendrogram (C, b) indicates that the turquoise modules  
 476 are highly related to extracellular microcystin. The dendrogram (C, c) indicates that  
 477 no modules are highly related to microcystin release. (D) Heatmap of proteins in the  
 478 module and eigengene expression in 18 samples. (E) A scatterplot of Gene  
 479 Significance for different traits vs. Module Membership in the brown, turquoise, and  
 480 blue modules. (F) The visualization of modules in the brown, turquoise, and blue  
 481 module. The top hub proteins in the modules have been indicated in bold with a

482 yellow color.