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1 Production of exopolymers (EPS) by cyanobacteria: impact on the carbon-to-nutrient ratio of

2 the particulate organic matter

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10

11 **Abstract** Freshwater cyanobacteria can produce large amount of mucilage, particularly during large 12 blooms. The production of these carbon-rich exopolymers (EPS) should influence the carbon-to-13 nutrient ratios of the organic matter (OM), which are regularly used as a proxy for the herbivorous 14 food quality. However, little is known about the consequences of EPS production on the carbon-to-15 nutrient ratio of the OM. Two EPS forms can be distinguished: the free fraction composed of Soluble 16 Extracellular Polymeric Substances (S-EPS) and the particulate fraction corresponding to the 17 Transparent Exopolymer Particles (TEP). The aim of the study was to determine whether the TEP and 18 S-EPS productions by cyanobacteria influence the carbon-to-nutrient ratios of the particulate OM 19 (POM). Five cyanobacteria species were grown in batch culture and characterized in terms of 20 photosynthetic activity, EPS production, and C, N, P contents. The variability in EPS production was 21 compared with the variability in stoichiometry of the POM. Most of cyanobacteria live in association 22 with heterotrophic bacteria (HB) within the mucilage. The effect of the presence/absence of HB on 23 EPS production and the carbon-to-nutrient ratios of the POM was also characterized for the 24 cyanobacteria Microcystis aeruginosa. We showed that TEP production increased the carbon-to-25 nutrient ratios of the POM in the absence of HB, while the stoichiometry did not significantly change 26 when HB were present. The C:N ratio of the POM decreased with production of S- EPS by the five 27 species. Lastly, the three colonial species (Chroococcales) tend to produce more TEP than the two 28 filamentous species (Oscillatoriales), with the two picocyanobacteria being the most productive of 29 both TEP and S-EPS.

31 Keywords Transparent exopolymer particles; mucilage; stoichiometry; *Microcystis aeruginosa*

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34 Introduction

35 Phytoplankton primary production represents one of the basic processes of pelagic ecosystem 36 functioning, with the synthesis of a major source of organic carbon for heterotrophic communities 37 (Cole, Likens & Strayer 1982; Baines & Pace 1991). The carbon-to-nutrient ratio of phytoplankton 38 varies greatly compared with other aquatic heterotrophic organisms, depending on carbon fixation and 39 nutrient uptake (Van de Waal et al. 2010). The nutritional value of the organic matter (OM) is partly 40 controlled by the carbon-to-nutrient ratios (Sterner & Elser 2002; Urabe, Togari & Elser 2003; Van de 41 Waal et al. 2010), with food quality for heterotrophic communities decreasing as stoichiometric ratios 42 increase. Exopolymers (EPS) released by phytoplankton are carbohydrate-rich, and can thus 43 potentially increase the carbon-to-nutrient ratios of the OM. In marine phytoplankton, it was shown 44 that EPS composition can deviate in C:N far from the Redfield ratio, up to 26 (Engel & Passow 2001). 45 EPS production by phytoplankton is highly variable, from 1 to 99.9 % of the net photosynthetically 46 fixed organic carbon, depending on species and environmental conditions (Bertilsson & Jones 2003). 47 The presence of species producing large amount of EPS should control the elemental ratios (C:N, 48 C:P) of the OM in pelagic ecosystems, with potential repercussions on the trophic network. A better 49 characterization of the link between species, EPS production and stoichiometry of the POM is thus 50 needed.

Despite EPS form a size continuum of organic carbon (Verdugo *et al.* 2004), they are commonly divided in two forms, one dissolved and one attached. They are rarely simultaneously characterized, so that little is known about this double production and its variability between and among species. A large portion of exudates corresponds to a dissolved fraction, which is called Soluble Extracellular Polymeric Substances (S-EPS) (Underwood, Paterson & Parkes 1995; Staats *et al.* 1999; Underwood *et al.* 2004). Some phytoplankton species, particularly cyanobacteria, produce large amount of cell-bound EPS, which form a mucilaginous matrix in which cells are embedded. These cell-bounded EPS belong to the widely studied 'Transparent Exopolymer Particles' (TEP) in aquatic ecosystems (Passow & Alldredge 1995). Depending on the form of EPS (dissolved or particulate), the influence of their production on the stoichiometry of the particulate organic matter should differ (POM). Production of TEP should increase the C:N and C:P of the POM, while S-EPS should decrease the stoichiometric ratios, owing to a carbon loss. Studies generally focused on one of the two forms of EPS, and the associated C:N and C:P ratios of the POM are rarely quantified.

64 In freshwater ecosystems, cyanobacteria are known to accumulate in dense blooms, with an 65 increasing frequency and intensity due to global changes (Johnk et al. 2008). These blooms lead to 66 high concentrations of TEP at the water surface (Grossart, Simon & Logan 1997) and one can wonder 67 if such 'TEP events' may induce a change in the carbon-to-nutrient ratios of the particulate OM. These 68 blooms are generally dominated by a few species (Huisman, Matthijs & Visser 2005). This raises the 69 question of the species' role in determining the POM stoichiometry. Colonial species, such as 70 Chroococcales, should produce more TEP compared with other pelagic species, such as single-71 filament species. One can also wonder whether a lower TEP production is counterbalanced by a higher 72 S-EPS production. TEP and S-EPS productions should differ between species depending on their 73 morphological traits, and consequently their impact on the POM stoichiometry.

74 At the species level, it is already known that nutrient limitation is the predominant controlling 75 factor for both TEP (Passow 2002; Reynolds 2007) and S-EPS (Baines & Pace 1991; Myklestad 76 1995). When nitrogen (or phosphorus) become limiting for growth, phytoplankton still accumulate 77 some carbon during photosynthesis, while storage and metabolic uses (proteins production, growth) 78 are limited (Banse 1974; De Philippis & Vincenzini 1998; Engel et al. 2004). The carbon in excess 79 can be either excreted as polysaccharides, through the EPS (overflow) or stored in the cell through the 80 formation of reserve compounds (De Philippis *et al.*, 1996). However, less is known about the 81 influence of heterotrophic bacteria (HB) on EPS production. Indeed, freshwater cyanobacteria are 82 associated with highly diversified and metabolically active HB embedded in their mucilage 83 (Worm & Søndergaard 1998; Casamatta 2000; Berg et al. 2009). TEP constitute suitable habitat for 84 the microorganisms. HB can modulate the magnitude of the effect of nutrient on EPS production,

through mineralization of organic nutrients. HB can also influence directly TEP and S-EPS
concentrations through consumption and/or production of dissolved OM (Azam *et al.* 1994; Gärdes *et al.* 2012). Lastly, nutrients may modulate the magnitude of the effect of HB on EPS production: it has
been demonstrated that nutrient availability influence the type of biological interaction between the
green microalga *Scenedesmus obliquus* and HB (Danger *et al.* 2007).

90 The aim of the study was firstly to characterize the influence of HB and nutrient load on EPS 91 production, and secondly to characterize the impact of EPS production by cyanobacteria (and their 92 associated HB) on the C:N and C:P ratio of the POM. In a first experiment, we characterize the effect 93 of the presence of HB and nutrient load, on the EPS production by Microcystis aeruginosa and the 94 associated C-to-nutrient ratios of the POM. We also test the hypothesis that the variability of the C-to-95 nutrient ratio of the POM may be explained by the species variability in EPS production. In a second 96 experiment, we characterize the C,N,P content and the TEP and S-EPS productions by cyanobacteria, 97 with three colonial (Microcvstis aeruginosa and the picocyanobacteria Aphanothece clathrata and A. 98 minutissima) and two single-filament species (Limnothrix sp. and Planktothrix agardhii).

99

100 Methods

101 Cyanobacteria cultures:

102 Aphanothece clathrata (TCC 4a) and A. minutissima (TCC 323) were provided by the INRA - UMR

103 Carrtel (Thonon Culture Collection), while Oscillatoria sp (LRP 29) and Planktothrix agaardhii are

104 grown in routine in our laboratory. These four strains were all xenic. The axenic strain of *Microcystis*

105 *aeruginosa* (PCC 7806) was provided by the Pasteur Culture collection of Cyanobacteria

106 (http://cyanobacteria.web.pasteur.fr/). The axenic strain was initially checked for bacterial

107 contamination by agar plating, following Briand et al. (2012). *M. aeruginosa* was grown in modified

108 BG11 medium (Rippka 1988), while the four other strains were grown in BG11 medium (Andersen

109 2005).

110 To test for the effect of the presence of HB on the EPS production and the C-to-nutrient ratios of the 111 POM, one of the five species, *M. aeruginosa*, was grown in both xenic (B) and axenic (Ax) conditions, at two nitrates loads (+N and -N). Initial nitrogen concentration was 1.76 mmol N L⁻¹ in 112 the classical N-replete medium (+N) and was $0.178 \text{ mmol N L}^{-1}$ in the N-depleted medium (-N). The 113 114 xenic culture of *M. aeruginosa* was obtained from the axenic one, after adding HB isolated from a 115 French pond (N 48°7'35.465''; W 1°38'14.453''), where *M. aeruginosa* is regularly blooming. 2 mL 116 of water from the pond was filtered on sterile 1 µm Poretics polycarbonate membrane filters, and the 117 filtrate was added to 40 mL of *M. aeruginosa* axenic culture. This xenic culture (B) was grown in 118 batch for two months prior to the experiment, with two inputs of fresh medium (approximately each 119 three weeks). At the beginning of the experiment, the culture reached a total volume of 1.2 L, so that 120 the initial input of pond water represents less than 0.2 % of the total volume. Before and after the 121 experiment, we checked the presence of bacteria in the B culture, and for possible bacterial 122 contamination in the Ax culture, with epifluorescent microscopic observations of 1 to 5 mL sub-123 samples on 0.2 µm Nuclepore membranes after staining with DAPI (4'6-diamidino-2-phenylindole). 124 Even if we cannot totally exclude a possible contamination by small-sized cyanobacteria, neither 125 picocyanobacteria nor other small unidentified cells have been detected by regular microscopic 126 observations.

127 Experiment 1 (xenic versus axenic conditions):

128 *M. aeruginosa* was tested in the presence (B) and in the absence (Ax) of heterotrophic bacteria, at two 129 levels of nitrate availability (+N and -N). Each treatment (Ax-N, Ax+N, B-N, B+N) was run in 130 triplicate in batch culture in climatic chambers at 25 ± 1 °C, 14:10 light:dark cycle with 30 µmol photons m⁻² s⁻¹ irradiance, in 500 mL Erlenmeyers flasks. All the cultures were manually mixed daily. 131 132 Initial cell density of cyanobacteria was 200 000 cells mL⁻¹. Cultures were sampled every two days 133 until the early stationary phase, and characterized in terms of photosynthetic activity and cell density. 134 S-EPS, TEP and the C:N:P molar ratios of the OM were measured initially, during the exponential 135 growth and as soon as cultures reached the early stationary phase.

136 *Experiment 2 (variability among species):*

137 The five species were grown in triplicate in batch culture in climatic chambers at 25 ± 1 °C, 14:10 light:dark cycle with 30 μ mol photons m⁻² s⁻¹ irradiance, in 500mL Erlenmevers flasks. All the 138 139 cultures were manually mixed daily. Initial cell density of cyanobacteria was 200 000 cells mL⁻¹. 140 Cultures were sampled every two days until the early stationary phase, and characterized in terms of 141 photosynthetic activity and cell density, as detailed below. S-EPS, TEP and the C:N:P molar ratios of 142 the OM were measured during the exponential growth and the early stationary phases. To limit 143 cyanobacteria cells lysis and release of EPS, the sampling at the early stationary phase was preferred 144 over the advanced stationary phase. The cultures were assumed to be in early stationary phase when 145 cell density remained stable during two successive sampling dates (four consecutive days) and when a 146 decrease in the dark-adapted photochemical quantum efficiency Fv/Fm was observed.

147 *Cell density and physiological measurements:*

148 Cell density was inferred by the optical density (OD) absorbance following the literature (Svane &

149 Eriksen, 2015; Post et al., 1985; Yepremian et al., 2007; Briand et al., 2008; Rorhlack et al., 2013).

150 The 680 nm wavelength (chlorophyll *a*) was preferred over 750 nm (turbidity), which would include

both bacteria and cyanobacterial cells (Danger et al. 2007). However, OD measured at 750 nm and

152 680 nm were highly correlated both in the presence and absence of HB ($R^2 > 0.997$, N = 105, p <

153 0.001; Fig. S1). The OD at 680 nm was converted into cell density (cells mL⁻¹) based on the highly

154 significant correlations between the two parameters ($R^2 > 0.99$, N = 26, p < 0.001; data not shown).

155 We considered as negligible the intraspecific variability in cell size. The absorbance was measured

156 every two days using a spectrophotometer Uvikon XS (Secomam, France).

157 The maximum growth rate was calculated from the formula:

158 159

= 2- (1) 2- 1

where *I* and *2* correspond to the cell density (cells mL⁻¹) at time t1 and t2 (day⁻¹) respectively.
Some filamentous species tend to form aggregates with time, increasing the daily variability in
biomass measurement. The slope of the time series of the Neperian logarithm of the cell density
(during the exponential growth) was thus preferred over instantaneous growth rate.

164 To characterize the photosynthetic activity and the physiological state of the cyanobacteria, the 165 electron transport rate (ETR) and the photosynthetic yield were measured every two days with a pulse-166 amplitude-modulated fluorescence monitoring system (PhytoPAM, Walz, Germany), following 167 Schreiber (1998) and Zhang et al. (2011). The phytoPAM is equivalent to 4 separate PAM-168 Fluorometers using light-emitting-diodes (LED) with 10 µs light pulses at 4 different excitation 169 wavelengths (470, 520, 645 and 665 nm), with the 645 nm specific to cyanobacteria (due to 170 phycocyanin and allophycocyanin absorption). The phytoPAM was used with only one channel, 171 corresponding to the cyanobacteria. The reference excitation spectrum measured at the factory was 172 used, as it was not significantly different from reference excitation spectra performed on our 173 cyanobacterial cultures. After dark-adaptation for 15 min, fluorescence was measured at low measuring light (0.15 μ mol photons m⁻² s⁻¹) and during saturating light pulses (3000 μ mol photons m⁻² 174 175 s⁻¹ for 0.2 s). Fluorescence was measured at 10 different intensities of actinic light from 1 to 1216 μ mol photons m⁻² s⁻¹, with a 20-s time interval. The initial chlorophyll *a* fluorescence was also 176 177 measured on each sample.

178 During the exponential growth and the stationary phase, the chlorophyll-specific absorption cross-179 section a^* (m² mg chla⁻¹) was measured from *in vivo* absorption spectra of the cyanobacteria between 180 400 and 750 nm and from the chlorophyll *a* concentration, following Shibata et al. (Shibata, Benson & 181 Calvin 1954). The ETR (µmol electron mg chla⁻¹ s⁻¹) was then calculated for each light intensity *I* 182 following Kromkamp and Forster (2003):

183

ETR = $0.5 Y I a^*$

with 0.5 corresponding to the 50 % of photons intercepted by the PSII of the chlorophyll-*a* (Gilbert,
Wilhelm & Richter 2000). *Y* represents the quantum efficiency of the PSII and *I* the light intensity.
The non-linear least squares regression model of Eilers and Peeters (1988) was used to fit the ETR
irradiance curves and to estimate the physiological parameters, such as the light saturated maximum
electron transport rate ETRmax.

189 EPS measurements

To separate cells from the supernatant, centrifugation at 3200 x g for 30 min at 12°C was performed
following Claquin et al. (2008). TEP and S-EPS were then analyzed separately.

192The method of Passow and Alldredge (1995) modified by Claquin et al. (2008) was used to quantify

the TEP fraction in 10 mL of culture. Briefly, two mL of 0.02 % alcian blue in 0.06 % acetic acid were

added to the pellets, and samples were centrifuged at 3200 x g at 4°C for 20 min. Pellets were rinsed

195 with 2 mL of distilled water and centrifuged again until the supernatant remained clear, in order to

evacuate the excess of alcian blue. 4 mL of 80 % sulfuric acid were then added to the pellets.

197 Absorbance was measured at 787 nm after 2h and converted in equivalent xanthan (Passow &

198 Alldredge 1995). A calibration curve was performed using xanthan gum following the same protocol.

199 Xanthan was then converted in equivalent carbon using the factor of 0.75 observed by Engel and

200 Passow (2001).

S-EPS were quantified using the method of Dubois *et al.* (1956). Briefly, 0.5 mL of supernatant was
placed in a glass tube with 1 mL of 5 % phenol solution and 5 mL of 80 % sulfuric acid. After 30 min,
absorption was measured at 485 nm and converted in equivalent glucose, using a standard calibration
of glucose. Glucose was also converted in carbon, using the factor of 0.4 as for hexoses.

205 *C, N, P measurements:*

To separate cells from the medium, centrifugation at 3200 x g for 30 min at 12 °C was performed as for EPS fractionation. Medium and particulate matter were then analyzed separately. To remove the excess of surface-adsorbed C, N and P, pellets were briefly rinsed with distilled water and centrifuged a second time at 3200 x g for 20 min.

Pellets were then resuspended in five mL of deionized water and analyzed for C, N, P content. Total
particulate organic carbon was measured with an high-temperature persulfate oxidation technology
using an OI Analytical carbon analyzer (model 1010 with a 1051 auto-sampler; Bioritech, France)
following the European standard ISO 8245 (1999). Total particulate nitrogen and total particulate

214 phosphorus were measured, after an acidic digestion with potassium persulfate at 120 °C, using a

215 continuous flow Auto-Analyser (Brann and Luebbe, Axflow, France), based on colorimetric methods

according to Aminot and Chaussepied (1983). Molar stoichiometric ratios of the POM (C:N and C:P)
were than calculated, by dividing C content by N and P content respectively.
The supernatant was divided in two samples, with one analyzed for nitrates and phosphates and the
second one for total dissolved nitrogen and total dissolved phosphorus after mineralization through a
potassium persulfate digestion at 120 °C. N and P concentrations were then measured using a
continuous flow Auto-Analyser (Brann and Luebbe, Axflow, France), based on colorimetric methods
according to Aminot and Chaussepied (1983).

223

224 Statistical analysis

All statistical analyses (boxplot, correlation, ordination, linear model) were carried out using R studio
software (R Development Core Team 2011). Wilcoxon rank-sum test followed by a post-hoc Tukey
test were used to detect differences between species and treatments, with significance threshold set at
0.05.

229 Linear models were used to examine the best set of predictor variables affecting the EPS production 230 and the molar stoichiometric ratios of the POM produced by *M. aeruginosa*. One can expect nutrient 231 load to modulate the effect of the presence of HB on EPS production and vice versa. We thus test for 232 statistical interactions between nutrient load and HB in the models. As models for C:N and C:P 233 revealed the same set of explanatory parameters, only the C:N model will be presented here (see 234 supplementary data for the C:P model). Before analysis, data were checked to meet the assumptions of 235 normality and homoscedasticity. A stepwise selection of the variables, which combines backward 236 elimination and forward selection, was used to build the model, using the function "stepAIC" 237 (package MASS version 7.3-31 for R). The 'best' final model showed the lowest Akaike Information 238 Criterion (Sugiura 1978). The significance of the model was tested using an ANOVA, while a 239 Shapiro-Wilk normality test was performed on the residuals of the model. 240 To highlight controlling factors of the EPS production and C:N ratio in the five species of 241 cyanobacteria, multivariate approach has been used on centered and scaled data. Explanatory variables 242 were first reduced using forward selection of constraints with the *forward.sel* function of the 'packfor'

243 library developed by S. Dray, as advised by Blanchet et al. (2008). Monte Carlo permutations tests 244 retained only explanatory variables with probability value lower than 0.05. Redundancy analysis 245 (RDA) was then performed with significant explanatory variables using 'vegan' library (Oksanen 246 2013). This constrained multivariate analysis detects and quantifies the modifications in the biological 247 response (TEP, S-EPS and C:N and N:P ratios of the POM), which can be explained by biological 248 parameters of the species (surface : volume ratio and growth rate) and the availability of the resource 249 (nitrates and phosphates concentration in the medium), through a multiple regression. While the 250 canonical analysis requires a unimodal relationship between the environmental parameters and the 251 biological response (typically environmental gradient analysis), the RDA underlies a linear 252 relationship. The significance of the RDA was tested through a permutation test.

253

254 Results

255 *Experiment 1: the influence of HB.*

256 To highlight the influence of HB and nutrient load on the stoichiometry of the POM, EPS 257 productions and stoichiometric ratios were first measured in *M. aeruginosa* in the presence and in the 258 absence of bacteria, at two nitrates loads. Dissolved inorganic phosphorus (DIP) in the medium was 259 always higher than 96.9 µmol P L⁻¹ throughout the experiment, indicating that phosphorus was never 260 limiting in our experiment. N-NO₃⁻ concentration in the medium remained higher than 570 μ mol N L⁻¹ in nutrient replete condition (+ N), while the concentration was lower than 3.5 μ mol N L⁻¹ during the 261 262 stationary phase in nutrient depleted conditions (-N) (data not shown). Neither the bacterial presence 263 nor the two levels of nitrates availability induced a significant effect on the photosynthetic activity of 264 *M. aeruginosa*, measured through the ETRmax (Fig. 1). Only growth phase changed significantly the 265 ETRmax, in accordance with the decrease in photosynthetic activity when reaching the stationary 266 phase (Fig. 1). TEP and S-EPS productions were also influenced by growth phase (Fig. 2): TEP tend 267 to increase during the stationary phase (Fig. 2A), while S-EPS was at least three times higher during 268 the exponential growth phase than during the stationary phase (Fig. 2B). The productions of TEP and

269 S-EPS showed a similar pattern, in response to nutrient availability and the presence of HB, with a 270 predominating effect of bacteria during the exponential growth and a predominating effect of nutrient 271 during the stationary phase (Fig. 2). Low nitrate availability (-N; nitrate concentrations < 15 µmol N 272 L^{-1} ; data not shown) increased both TEP and S-EPS concentrations, during the stationary phase (Fig. 273 2). The presence of HB increased TEP during the exponential phase when associated with high nitrate 274 availability (Fig. 2A), leading to a significant interaction between HB and nitrates as shown by the 275 linear model (Table 2). The presence of HB (B versus Ax) increased significantly (two fold) the S-276 EPS concentrations during the exponential phase (Fig. 2B).

TEP and S-EPS productions were then compared with modifications of the molar
stoichiometric ratios of the POM. The C:N ratio of the POM was highly correlated with TEP in axenic
conditions (Fig. 3A). There was no significant correlation in the case of the C:P ratio (Fig. 3B). POM
associated with bacteria (+B) showed more variable stoichiometric ratios, with lower values compared
with axenic condition (Fig. 3).

282 Linear models were used to examine the best set of predictor variables for the molar stoichiometric 283 ratios in *M. aeruginosa*. The initial model includes the amount of TEP and S-EPS per cell, the 284 concentration of nitrates and phosphates in the medium, the presence of HB (included as a qualitative 285 factor), and the ETRmax. Interactions between bacteria and TEP and between bacteria and nutrients 286 were also included in the initial model, bacterial activities being able to influence both TEP and 287 nutrients. Regression slopes significantly differed in the presence and in the absence of HB, indicating 288 that the magnitude of the effect of TEP and nitrates on the C:N ratio depends on the presence/absence 289 of bacteria. The best model for the C:N ratio (Table 3), determined by a stepwise selection of the 290 variables using the AIC criterion, selected the amount of TEP per cell, the nitrate concentration in the 291 medium, the presence/absence of HB, and two interactions, both with bacteria. In *M. aeruginosa*, TEP 292 increased C:N, while the presence of bacteria and the availability of nitrate decreased them (Table 3). 293 For both interactions (TEP x HB and nitrates x HB), the presence of bacteria increased the effect's 294 magnitude of the factor (TEP or nitrates) on C:N ratio. In the presence of HB, the C:N increased faster 295 with TEP and decreased faster with nitrate availability, compared with axenic conditions.

297 *Experiment 2:*

298 *Variability among species in EPS production and stoichiometry:*

299 The five species of cyanobacteria were grown in the presence of HB, with initially high nitrates load. 300 Stationary phases were observed after 15 to 24 days, depending on cultures (Fig. 4). The filamentous 301 cyanobacteria (Limnothrix and Planktothrix) were the first cultures reaching the stationary phase, but 302 the maximum cell density was two to three times lower than for the other species (Fig. 4). Their 303 growth rate remained low $(0.08 \pm 0.01 \text{ and } 0.13 \pm 0.01 \text{ day}^{-1} \text{ respectively})$, while the growth rates of 304 the three Chroococcales was higher than 0.25 day⁻¹. A. clathrata showed the highest growth rate, with 305 0.49 ± 0.01 day⁻¹, but this occurred only during the seven first days (Fig. 4). *M. aeruginosa* and *A*. 306 *minutissima* showed similar growth rates, with 0.28 ± 0.03 and 0.30 ± 0.03 day⁻¹ respectively. The 307 ETRmax measured during the exponential growth phase was also higher for the three Chroococcales, 308 compared with the Oscillatoriales, with the highest ETRmax observed for *M. aeruginosa* (Fig. 5). The 309 ETRmax decreased for all species when they reached the stationary phase (Fig. 5), as well as the dark-310 adapted photochemical quantum efficiency (Fv/Fm), indicating the onset of the stationary phase (data 311 not shown).

312 While Chroococcales and Oscillatoriales differed in their growth rate during the exponential phase,

313 they also differed in their TEP production (Fig. 6A), contrary to the S-EPS production and POM's

314 stoichiometry (Fig. 6B, C and D). EPS productions by the five cyanobacteria species, and particularly

315 the picocyanobacteria, showed a high variability among replicates, larger than the variability among

316 species (Fig. 6A and B). However, species producing large amounts of S-EPS (Fig. 6A) tended to

317 produce large quantities of TEP (Fig. 6B). For the three Chroococcales , S-EPS production decreased

318 with growth phase (data not shown), as observed in the previous experiment (Fig. 2B).

319 The five species differ significantly in their molar C:N and C:P ratios, with a low variability among

320 replicates, except for the C:P of *Planktothrix* (Fig. 6C and D). Consequently, variability in the C:N

321 ratio was larger among species than among replicates (Fig. 6C and D). All species together, the C:N

322 ratio was close to the reference value found in the literature (Passow 2002; Thornton 2002; Reynolds

- 323 2006), with on average 6.5 ± 3.0 , a minimum of 1.9 and a maximum of 13.7 (Fig. 6C). The two
- 324 picocyanobacteria showed the same C:N ratio, with values closed to 6, while *M. aeruginosa* had the
- highest C:N ratio with 10.7 ± 2.2 (Fig. 6C). *Limnothrix* had the smallest C:N ratio, with less than 4,
- while *P. agardhii* was closed to 8 (Fig. 6C). The C:P ratio (Fig. 6D) followed the same pattern than
- 327 the C:N ratio (Fig. 6C). While molar C:N and C:P ratios showed the same pattern among the species,
- 328 the TEP and the S-EPS production and stoichiometric ratios varied independently between species
- 329 (Fig. 6). The correlation between TEP production and C:N ratio observed in axenic condition at the
- 330 specific level (Fig. 3A) was not confirmed at the interspecific level.
- 331

332 Influence of EPS production on C, N, P contents and stoichiometry of cyanobacteria:

333 The C, N, P contents of the POM were plotted for the five species (see symbols), for both growth 334 phases (white versus black symbols), and for the three replicates, as a function of the TEP content 335 (Fig. 7). Some species, like the two Aphanothece, showed a great variability in TEP content, 336 associated with their growth phase, while their C, N and P contents changed only little (Fig. 7). All 337 species taken into account, the carbon content of the POM was correlated with the amount of TEP 338 (Fig. 7A), as well as the amount of nitrogen (Fig. 7B) and phosphorus (Fig. 7C). TEP is thus 339 associated with a simultaneous increase of the C,N,P contents of the POM (Fig. 7). On average, 340 increasing TEP content of 1 pg eq. C per cell led to an increase of 4 pg C, 0.4 pg N and 0.07 pg P of 341 the cell (Fig. 7). The POM increase associated with TEP production thus presented a C:N and a C:P 342 ratio of 11.7 and 140 respectively, which correspond to the highest ratios measured during the study 343 (Fig. 6C and D). Lastly, when comparing C:N ratio of the POM with the concentration of S-EPS in the 344 medium, a decrease of the C:N with larger concentration of S-EPS was observed (Fig. 8), indicating a 345 potential loss of the particulate carbon with S-EPS production. Similarly to the TEP content, some 346 species showed a great variability in EPS production, such as A. minutissima and Limnothrix, with 347 only few changes in the C:N ratio of the POM (Fig. 8).

348 A redundancy analysis was performed to explain the C: N and N:P ratios and the EPS productions 349 with species parameters and nutrients availability. The N:P ratio was preferred over the C:P ratio in the RDA analysis, because of the strong correlation between C:N and C:P ($R^2 = 0.77$, p<0.0001). The 350 351 RDA triplot showed that species and growth phases are well separated in the ordination space (Fig. 9). 352 The filamentous cyanobacteria are grouped together on the left part of the triplot, whatever their 353 growth rate, with the picocyanobacteria in stationary phase. The second axis separated *M. aeruginosa* 354 depending on its growth phase from the picocyanobacteria in exponential phase. The first axis (38 % 355 of the total variance) of the ordination was mainly described by TEP, and in a lower extent by S-EPS 356 and C:N. The explanatory variables of the first axis were the phosphates concentration and the cellular 357 surface on volume ratio (S:V ratio), which were opposite to nitrates concentration. Species in the right 358 part of the graph thus presented a higher C:N ratio and higher EPS productions, associated with high 359 phosphates availability and low nitrates one in the medium. These species also presented a higher 360 cellular S:V ratio. The second axis (25 % of the total variance) was mainly described by S-EPS and 361 C:N, with species presenting a high C:N producing low S-EPS. The nitrates concentration and the 362 growth rate explained the second axis. Species with high C:N ratio showed a low growth rate in a 363 nitrate depleted medium.

364

365 Discussion

366 The highest concentrations of TEP in natural environment are regularly observed during and at 367 the end of phytoplankton blooms (Grossart et al. 1997; Passow 2002; Vieira et al. 2008). These POM 368 are rapidly colonized by heterotrophic bacteria (Mari & Kiørboe 1996), forming hotspots with 369 elevated microbial activity and nutrient cycling, particularly as cells become senescent. But, HB also 370 colonize healthy phytoplankton. For instance, numerous specific bacteria are embedded in the colonies 371 of Microcystis (Brunberg 1999; Casamatta 2000). It has been shown that many bloom-associated-372 bacteria can enhance the cyanobacterial growth (Berg et al. 2009). Moreover, there is increasing 373 evidences of mutualistic relationships between phytoplankton and attached bacteria (Passow 2002;

374 Croft et al. 2005). This may be a reason why axenic strains can be more difficult to maintain for long 375 periods in algal culture banks. In our study, the presence of heterotrophic bacteria did not affect 376 significantly the growth of *M. aeruginosa*, neither positively nor negatively. The maximum 377 photosynthetic activity, the mean growth rate and the final cell density of the cyanobacteria were 378 indeed similar in the presence and in the absence of HB. No significant cost neither benefice for the 379 cyanobacteria could be identified from this biotic interaction. However, the presence of HB was 380 associated with a higher EPS production, of both TEP and S-EPS, during the exponential growth 381 phase of *M. aeruginosa*. HB can have produced these additional TEP, even if previous studies showed 382 that HB associated with the mucilage of *M. aeruginosa* produced negligible TEP and S-EPS amounts 383 (Yallop, Paterson & Wellsbury 2000; Shen et al. 2011). Recent studies on HB-phytoplankton 384 interactions also showed that HB can stimulate TEP release by marine diatoms (Bruckner et al. 2008; 385 Gärdes et al. 2012). Increasing TEP production by phytoplankton means higher C-rich organic matter 386 available for heterotrophic bacteria, which can in turn mineralize organic nitrogen and phosphorus. 387 The higher EPS production observed in our experiment occurred under nutrient replete conditions, 388 when mineralization of OM was not essential to support the growth of cyanobacteria. The stimulation 389 is thus not expected here. The additional TEP observed here were thus produced either by HB 390 themselves or by the cyanobacteria after a stimulation of release induced by the HB. The higher S-EPS 391 concentration observed in the medium can be explained by the hydrolytic activity of HB on TEP. The 392 relationship between HB and EPS is complex, as bacteria are involved in both production, 393 modification and degradation of EPS (Passow 2002). As we have no abundance estimates of HB, 394 neither any measure of their diversity and biological activity, we are limited to these hypotheses. 395 Coupling isotopic tracers with imaging mass spectrometry analysis (NanoSIMS) would be a powerful 396 approach to highlight C and N transfers from the cyanobacteria to the heterotrophic bacteria, as 397 performed with earthworms in their burrow-lining (Gicquel et al. 2012) or in N transfer within single 398 filament of cyanobacteria (Ploug et al. 2010).

We observed that the influence of TEP production on the stoichiometry of the POM wasmodulated by the presence of HB (significant statistical interaction). In axenic conditions, the C:N

401 ratio of the POM was increased by TEP production, with a slope of + 6.5 for each added picogram of 402 TEP (in equivalent C) to the cell. TEP, mainly composed of polysaccharides (De Philippis & 403 Vincenzini 1998), are C-enriched compared with living biomass. TEP remaining attached to the POM, 404 their accumulation should increase the C:N ratio of the POM. The C:N ratio of natural TEP from the 405 sea regularly exceeds 20 (Mari et al. 2001). However, we observed that the C:N ratio of the POM in 406 the presence of HB was not influenced by the TEP amount, so that the carbon-to-nutrient ratios of the 407 five species did not increase with TEP production. Hence, we conclude that TEP production increased 408 the C:N ratio of the POM, until colonization of the POM by HB. The presence of HB was indeed 409 associated with an increase of N and P contents of the POM, consequently modulating the effect of 410 TEP production on the stoichiometry of the POM. One can also hypothesize that cells of 411 cyanobacteria themselves influenced the C:N:P ratio of the POM through their storage capacity 412 (Kromkamp 1987; Klausmeier, Litchman & Levin 2004). Indeed, cyanobacteria may accumulate P as 413 polyphosphate, as well as N as cyanophycin, both in granules in the cytoplasm (Kromkamp 1987; 414 Marañón et al. 2013). However, storage would also have occurred in the absence of HB. Heterotrophic 415 bacteria, through their activity and/or biomass, may have led to a N and P enrichment of the POM, 416 resulting to the simultaneous increase of the C, N and P contents of the POM with TEP, as observed 417 here. The C:N of HB, which is also highly variable (Chrzanowski *et al.* 1996), tend to be slightly 418 lower than the Redfield ratio (C:N:P of 106:16:1) (Redfield, Ketchum & Richards 1963), with about 5, 419 while the C:P is twice to five times smaller, with values going from 50 to 19 depending on bacterial 420 growth rate and nutrient availability (Chrzanowski et al. 1996; Fagerbakke, Heldal & Norland 1996). 421 N is mainly associated with proteins and nucleic acids, while P is associated with nucleic acids (20 % 422 of the mass of the cell) and storage through polyphosphate granules (Fagerbakke *et al.* 1996). The C:N 423 and C:P ratios of the POM should thus decrease with bacterial colonization, compensating the increase 424 associated with TEP production. In natural aggregates, HB can represent up to 50% of the total protein 425 of the aggregates (Simon et al. 2002).

The carbon-to-nutrient ratios of the POM are regularly used to estimate the nutritional quality
of the OM for heterotrophic communities (Hessen 1992; Sterner & Elser 2002). Herbivorous

428 zooplankton can become limited by nitrogen or phosphorus if the C-to-nutrient ratio of their food is 429 too high (Boersma & Kreutzer, 2002). Their growth and reproduction is then affected, but not their life 430 span (Jensen & Verschoor 2004). If the C-to-nutrient ratios are too high, or even too low (Boersma & 431 Elser, 2006), heterotrophic grazers must eliminate the molecule in excess, as many organisms are 432 strongly homeostatic in their elemental composition. EPS production associated with the presence of 433 HB did not change here the carbon-to-nutrient ratios of the POM and probably the nutritional quality 434 of the food. This is in accordance with previous studies. For example, a cladocera Ceriodaphnia 435 cornuta fed with TEP released from the cyanobacteria Anabaena spiroides presented a higher growth 436 rate and a higher fitness compared with the cladocera fed on seston at natural concentration (Choueri 437 et al. 2007). TEP were obtained from filtrate of cultures in stationary phase, after evaporation, dialyze 438 and lyophilisation. However, the nutritional quality of the TEP is controversial in the literature, as 439 several studies reported a negative impact of TEP on zooplankton grazing, hypothesizing either an 440 allelochemical activity or an inhibitory effect of the EPS or protection against digestion (Decho & 441 Lopez 1993; Liu & Buskey 2000; Dutz, Breteler & Kramer 2005). Specific allelochemicals might 442 have been produced in association with the EPS, as for instance for the toxic species *Phaeocystis* 443 (Dutz et al. 2005), in response to grazing pressure. While the nutritional quality may not have been 444 affected, its quantity was, as TEP and HB increased the C, N, P contents of the POM. HB attached to 445 aggregates become available as food for larger organisms (Passow & Alldredge 1999). Ling and 446 Alldredge (2003) hypothesized that the consumption of TEP partly shunts organic carbon from the 447 microbial loop to higher trophic levels. A higher size structure of the herbivorous community may be 448 expected in the presence of TEP producing species.

449 Contrary to our expectation, no trade-off between the free fraction and the particulate form has 450 been observed. Species producing more S-EPS also tend to produce more TEP, compared with the 451 other species. Size controlling the S:V ratio and colony formation requesting TEP to embedded cells, a 452 higher TEP production was expecting for small-cell size and colonial species. The smallest species 453 (picocyanobacteria) thus showed the highest production of both forms of EPS, but also the greatest 454 variability. Marine studies, including a greater number of species, revealed no relationship between

455 size and EPS production (López-Sandoval et al. 2013). The most probable hypothesis is that life form 456 is the predominant factor controlling EPS production. The three colonial species Chroococcales (M. 457 aeruginosa and the picocyanobacteria) tend to produce more TEP than our two filamentous 458 cyanobacteria. The large production of TEP may correspond to the functional trait 'colonial 459 mucilaginous species': *M. aeruginosa* aggregates can contain millions of cells, while the 460 picocyanobacteria remained in small aggregates with generally less than 50 cells (Callieri & Stockner 461 2002; Costas et al. 2008), but with a large proportion of mucilage relative to cell volume. Colony 462 forming species may be seen as 'suspended biofilm', with mucilage filling functions of cohesion, 463 protection, retention or exchange (Flemming & Wingender, 2010). EPS production, and particularly 464 TEP, constitutes a functional trait, whose ecological roles are still discussed probably due to a 465 multiplicity of its functions (Reynolds, 2007).

466

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- 672

- 673 Table and Figures legends
- 674

Table 1: origin and morphological characteristics of the five species of cyanobacteria:

Table 2: Parameter estimates for the best model predicting the TEP production in *M. aeruginosa*, as

677 determined by a stepwise selection of the variables using the AIC criterion. The initial model includes

678 the maximum electron transport rate, the concentration of nitrates in the medium, the presence of HB

679 (included as a qualitative factor), and the interaction between nitrates and HB. Result from its

ANOVA is also shown. Significance levels are coded as follow: '***' indicates <0.001, '**' <0.01,

- 681 and '*' <0.05.
- Table 3: Parameter estimates for the best model predicting the C:N ratio in *M. aeruginosa*, as
- determined by a stepwise selection of the variables using the AIC criterion. Result from its ANOVA is
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685

686 Figure 1: Maximum electron transport rate (ETRmax) of *M. aeruginosa*, during exponential growth

- 687 (white area) and stationary phase (grey area), depending on the presence of heterotrophic bacteria and
- 688 nitrate availability: Ax: Axenic, B: associated with bacteria, +N: high level of N availability, -N: low
- 689 level of N availability. Means of replicate value (± standard deviation) are shown. No statistical
- 690 difference based on Wilcoxon rank-sum test and Tukey post hoc test (P > 0.05).

Figure 2: (A) TEP and (B) S-EPS produced by *M. aeruginosa*, during exponential growth (white area)

and stationary phase (grey area), depending on the presence of heterotrophic bacteria and nitrate

693 availability: Ax: Axenic, B: associated with bacteria, +N: high level of N availability, -N: low level of

694 N availability. Means of replicate value (\pm standard deviation) are shown. $a\neq b$ and $c\neq d$ based on

695 Wilcoxon rank-sum test and Tukey post hoc test (P < 0.05).

Figure 3: (A) Molar C:N and (B) C:P ratios of *M. aeruginosa* (axenic condition shown by open

diamonds) and of the cyanobacteria associated with heterotrophic bacteria (filled circles) depending

698 on the amount of TEP per cyanobacterial cell. Correlations in axenic condition and in the presence of

HB (including both +N and -N) are shown.

Figure 4: Time series of the biomass absorbance (means of replicate value), expressed as Chlorophylla concentration, of the cyanobacteria species.

Figure 5: The maximum electron transport rate (ETRmax) measured during exponential growth (white

area) and the early stationary phase (grey area), depending on cyanobacterial species. Means of

- replicate value (\pm standard deviation) are shown. a \neq b and c \neq d based on Wilcoxon rank-sum test and
- Tukey post hoc test (P < 0.05).

- Figure 6: Boxplot of (A) the mean concentration of S-EPS in the culture per unit of cell volume (µg
- 707 ep. C mm³) depending on cyanobacteria, of (B) the TEP per unit of cell volume (μg ep. C mm³), of (C)
- 708 the molar C:N ratio and (D) C:P of the particulate organic matter. C represents the Chroococcales and
- 709 O the Oscillatoriales. $a \neq b \neq c$ based on Wilcoxon rank-sum test and Tukey post hoc test (P < 0.05).
- Figure 7: (a) C content, (b) N content and (c) P content per cyanobacterial cell of the particulate
- 711 organic matter depending on the TEP concentration per cyanobacterial cell, considering the five
- 512 species. Data correspond to the three replicates measured during the exponential phase (open symbols)
- and during the early stationary phase (black symbols). Regressions refer to the entire set of data
- 714 points.
- Figure 8: molar C : N ratio of the particulate organic matter depending on the S-EPS concentration in
- the medium, considering the five species. Data correspond to the three replicates measured during the
- exponential phase (open symbols) and during the early stationary phase (black symbols). Regression
- refers to the entire set of points.
- Figure 9: Redundancy analysis (RDA) triplots for the molar C:N and N:P ratios, the TEP and S-EPS
- per cyanobacterial cell (μ g eq C cell⁻¹) of the five cyanobacteria, explained by the growth rate, the cell
- ratio surface:volume of the species, and the nitrates and the phosphates concentrations in the medium.
- 722 Exponential E growth phase (o) and stationary S phase (\Box) are shown, with the three replicates.
- 723

723 Table 1: origin and morphological characteristics of the five species of cyanobacteria:

Genus	Species	Origin	Form	V (μm ³)	S / V
Microcystis	aeruginosa PCC7806	Pasteur institute	Sphere	33.5	1500
Aphanothece	clathrata (TCC 4a)	INRA UMR	Prolate spheroid	8.4	633
		CARRTEL			
Aphanothece	minutissima (TCC 323)	INRA UMR	Prolate spheroid	8.4	633
		CARRTEL			
Limnothrix	(LRP29)	UMR 6553	Filamentous	8.2	2
Planktothrix	agardhii	UMR 6553	Filamentous	84.8	2

Table 2: Parameter estimates for the best model predicting the TEP production in *M. aeruginosa*, as
determined by a stepwise selection of the variables using the AIC criterion. The initial model includes
the maximum electron transport rate, the concentration of nitrates in the medium, the presence of HB
(included as a qualitative factor), and the interaction between nitrates and HB. Result from its
ANOVA is also shown. Significance levels are coded as follow: '***' indicates <0.001, '**' <0.01,

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and	'*'	< 0.05	
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	Estimate	Std.	Sum of sq	Df	F value	Proba (>F)	
(Intercept)	2.86	0.26					
NO3	-0.01	0.01	0.087	1	0.43	0.52	
bacteria	-0.16	0.24	0.19	1	0.97	0.34	
ETRmax	-0.84	0.30	1.59	1	7.93	0.011	*
interaction NO3 x bacteria	0.042	0.02	0.92	1	4.58	0.045	*
Residuals			3.81	19			

731 Model statistics: AIC = -34.12, Residual standard error: 0.448, DF = 19, R²=0.42, p = 0.027.

- 732 Table 3: Parameter estimates for the best model predicting the C:N ratio in *M. aeruginosa*, as
- determined by a stepwise selection of the variables using the AIC criterion. Result from its ANOVA is
- also shown. Significance levels are coded as follow: '***' indicates <0.001, '**' <0.01, and '*' <0.05.

	Estimate	Std.	Sum of sq	Df	F value	Proba (>F)	
(Intercept)	4.96	2.98					
TEP	5.73	1.23	292	1	55.99	< 0.0001	***
NO3	-0.11	0.07	114	1	21.77	0.0002	***
bacteria	-11.28	4.76	67	1	12.76	0.002	**
interaction TEP x bacteria	4.44	2.04	25	1	4.74	0.043	*
interaction NO3 x bacteria	-0.27	0.11	35	1	6.67	0.019	*
Residuals			94	18			
Model statistics: $AIC = 4$	44.7. Residu	al standa	rd error: 2.28	4 DF	$= 18 R^{2} = 0$	84 $p < 0.0001$	

- 741Figure 1: Maximum electron transport rate (ETRmax) of *M. aeruginosa*, during exponential growth742(white area) and stationary phase (grey area), depending on the presence of heterotrophic bacteria and743nitrate availability: Ax: Axenic, B: associated with bacteria, +N: high level of N availability, -N: low744level of N availability. Means of replicate value (± standard deviation) are shown. No statistical745difference based on Wilcoxon rank-sum test and Tukey post hoc test (P > 0.05).



Figure 2: (A) TEP and (B) S-EPS produced by *M. aeruginosa*, during exponential growth (white area) and stationary phase (grey area), depending on the presence of heterotrophic bacteria and nitrate availability: *Ax:* Axenic, *B:* associated with bacteria, +*N:* high level of N availability, -*N:* low level of N availability. Means of replicate value (± standard deviation) are shown. a≠b and c≠d based on Wilcoxon rank-sum test and Tukey post hoc test (P < 0.05).



Figure 3: (A) Molar C:N and (B) C:P ratios of *M. aeruginosa* (axenic condition shown by open diamonds) and of the cyanobacteria associated with heterotrophic bacteria (filled circles) depending on the amount of TEP per cyanobacterial cell. Correlations in axenic condition and in the presence of HB (including both +N and –N) are shown.



Figure 4: Time series of the biomass absorbance (means of replicate value), expressed as Chlorophyll
a concentration, of the cyanobacteria species.



768Figure 5: The maximum electron transport rate (ETRmax) measured during exponential growth (white769area) and the early stationary phase (grey area), depending on cyanobacterial species. Means of770replicate value (\pm standard deviation) are shown. $a \neq b$ and $c \neq d$ based on Wilcoxon rank-sum test and771Tukey post hoc test (P < 0.05).



Figure 6: Boxplot of (A) the mean concentration of S-EPS in the culture per unit of cell volume (µg ep. C mm³) depending on cyanobacteria, of (B) the TEP per unit of cell volume (µg ep. C mm³), of (C) the molar C:N ratio and (D) C:P of the particulate organic matter. C represents the Chroococcales and O the Oscillatoriales. $a \neq b \neq c$ based on Wilcoxon rank-sum test and Tukey post hoc test (P < 0.05).



Figure 7: (a) C content, (b) N content and (c) P content per cyanobacterial cell of the particulate organic matter depending on the TEP concentration per cyanobacterial cell, considering the five species. Data correspond to the three replicates measured during the exponential phase (open symbols) and during the early stationary phase (black symbols). Regressions refer to the entire set of data









791 Figure 8: molar C : N ratio of the particulate organic matter depending on the S-EPS concentration in 792 the medium, considering the five species. Data correspond to the three replicates measured during the 793 exponential phase (open symbols) and during the early stationary phase (black symbols). Regression 794 refers to the entire set of points.



Figure 9: Redundancy analysis (RDA) triplots for the molar C:N and N:P ratios, the TEP and S-EPS per cyanobacterial cell (μ g eq C cell⁻¹) of the five cyanobacteria, explained by the growth rate, the cell ratio surface:volume of the species, and the nitrates and the phosphates concentrations in the medium. Exponential E growth phase (o) and stationary S phase (\Box) are shown, with the three replicates.



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Supporting Information

- 802 Additional Supporting Information may be found in the online version of this article:
- **Fig. S1.** Absorbance measured at 680 nm as a function of the absorbance measured at 750 nm
- for Microcystis cultures, in axenic condition (blue diamonds) and in the presence ofheterotrophic bacteria (red squares).



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Table S1: Parameter estimates for the best model predicting the C:P ratio in *M. aeruginosa*,
 as determined by a stepwise selection of the variables using the AIC criterion. Result from its
 ANOVA is also shown. Significance levels are coded as follow: '***' indicates <0.001, '**'
 <0.01, and '*' <0.05.

	Estimate	Std.	Sum of sq	Df	F value	Proba (>F)	
(Intercept)	66.79	65.09					
TEP	61.76	22.30	32482	1	27.94	<0.0001	***
NO3	-2.99	1.13	30351	1	26.11	<0.0001	***
bacteria	-176.4	74.1	16159	1	13.90	0.0017	**
interaction tep x bacteria	69.1	31.5	5605	1	4.82	0,042	*
interaction NO3 x bacteria	-4.18	1.67	7262	1	6.25	0.023	*
Residuals			19761	18			