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Alexandrine Pannard, Julie Pédrone, Myriam Bormans, Enora Briand, Pascal Claquin, et al.. Production of exopolymers (EPS) by cyanobacteria: impact on the carbon-to-nutrient ratio of the particulate organic matter. *Aquatic Ecology*, 2016, 50 (1), pp.29-44. 10.1007/s10452-015-9550-3 . hal-01219377

HAL Id: hal-01219377

<https://univ-rennes.hal.science/hal-01219377>

Submitted on 22 Oct 2015

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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.

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

Introduction

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

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

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

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

Methods

Cyanobacteria cultures:

Aphanothece clathrata (TCC 4a) and *A. minutissima* (TCC 323) were provided by the INRA - UMR Carrtel (Thonon Culture Collection), while *Oscillatoria sp* (LRP 29) and *Planktothrix agardhii* are grown in routine in our laboratory. These four strains were all xenic. The axenic strain of *Microcystis aeruginosa* (PCC 7806) was provided by the Pasteur Culture collection of Cyanobacteria (<http://cyanobacteria.web.pasteur.fr/>). The axenic strain was initially checked for bacterial contamination by agar plating, following Briand *et al.* (2012). *M. aeruginosa* was grown in modified BG11 medium (Rippka 1988), while the four other strains were grown in BG11 medium (Andersen 2005).

To test for the effect of the presence of HB on the EPS production and the C-to-nutrient ratios of the 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 the classical N-replete medium (+N) and was 0.178 mmol N L⁻¹ in the N-depleted medium (-N). The xenic culture of *M. aeruginosa* was obtained from the axenic one, after adding HB isolated from a French pond (N 48°7'35.465''; W 1°38'14.453''), where *M. aeruginosa* is regularly blooming. 2 mL of water from the pond was filtered on sterile 1 µm Poretics polycarbonate membrane filters, and the filtrate was added to 40 mL of *M. aeruginosa* axenic culture. This xenic culture (B) was grown in batch for two months prior to the experiment, with two inputs of fresh medium (approximately each three weeks). At the beginning of the experiment, the culture reached a total volume of 1.2 L, so that the initial input of pond water represents less than 0.2 % of the total volume. Before and after the experiment, we checked the presence of bacteria in the B culture, and for possible bacterial contamination in the Ax culture, with epifluorescent microscopic observations of 1 to 5 mL subsamples on 0.2 µm Nuclepore membranes after staining with DAPI (4'6-diamidino-2-phenylindole). Even if we cannot totally exclude a possible contamination by small-sized cyanobacteria, neither picocyanobacteria nor other small unidentified cells have been detected by regular microscopic observations.

Experiment 1 (xenic versus axenic conditions):

M. aeruginosa was tested in the presence (B) and in the absence (Ax) of heterotrophic bacteria, at two levels of nitrate availability (+N and -N). Each treatment (Ax-N, Ax+N, B-N, B+N) was run in 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. Initial cell density of cyanobacteria was 200 000 cells mL⁻¹. Cultures were sampled every two days until the early stationary phase, and characterized in terms of photosynthetic activity and cell density. S-EPS, TEP and the C:N:P molar ratios of the OM were measured initially, during the exponential growth and as soon as cultures reached the early stationary phase.

Experiment 2 (variability among species):

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

Cell density and physiological measurements:

Cell density was inferred by the optical density (OD) absorbance following the literature (Svane & Eriksen, 2015 ; Post et al., 1985; Yepremian et al., 2007; Briand et al., 2008; Rorhlack et al., 2013). 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 680 nm were highly correlated both in the presence and absence of HB ($R^2 > 0.997$, $N = 105$, $p < 0.001$; Fig. S1). The OD at 680 nm was converted into cell density (cells mL^{-1}) based on the highly significant correlations between the two parameters ($R^2 > 0.99$, $N = 26$, $p < 0.001$; data not shown). We considered as negligible the intraspecific variability in cell size. The absorbance was measured every two days using a spectrophotometer Uvikon XS (Secomam, France).

The maximum growth rate was calculated from the formula:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

where N_1 and N_2 correspond to the cell density (cells mL^{-1}) at time t_1 and t_2 (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.

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

During the exponential growth and the stationary phase, the chlorophyll-specific absorption cross-section a^* ($\text{m}^2 \text{mg chl}a^{-1}$) was measured from *in vivo* absorption spectra of the cyanobacteria between 400 and 750 nm and from the chlorophyll *a* concentration, following Shibata et al. (Shibata, Benson & Calvin 1954). The ETR ($\mu\text{mol electron mg chl}a^{-1} \text{s}^{-1}$) was then calculated for each light intensity *I* following Kromkamp and Forster (2003):

$$\text{ETR} = 0.5 Y I a^*$$

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 ETR_{max}.

EPS measurements

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

192 The method of Passow and Alldredge (1995) modified by Claquin et al. (2008) was used to quantify
193 the TEP fraction in 10 mL of culture. Briefly, two mL of 0.02 % alcian blue in 0.06 % acetic acid were
194 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
196 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).

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

205 *C, N, P measurements:*

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

210 Pellets were then resuspended in five mL of deionized water and analyzed for C, N, P content. Total
211 particulate organic carbon was measured with an high-temperature persulfate oxidation technology
212 using an OI Analytical carbon analyzer (model 1010 with a 1051 auto-sampler; Bioritech, France)
213 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 then 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).

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.

Linear models were used to examine the best set of predictor variables affecting the EPS production and the molar stoichiometric ratios of the POM produced by *M. aeruginosa*. One can expect nutrient load to modulate the effect of the presence of HB on EPS production and vice versa. We thus test for statistical interactions between nutrient load and HB in the models. As models for C:N and C:P revealed the same set of explanatory parameters, only the C:N model will be presented here (see supplementary data for the C:P model). Before analysis, data were checked to meet the assumptions of normality and homoscedasticity. A stepwise selection of the variables, which combines backward elimination and forward selection, was used to build the model, using the function “stepAIC” (package MASS version 7.3-31 for R). The ‘best’ final model showed the lowest Akaike Information Criterion (Sugiura 1978). The significance of the model was tested using an ANOVA, while a Shapiro-Wilk normality test was performed on the residuals of the model.

To highlight controlling factors of the EPS production and C:N ratio in the five species of cyanobacteria, multivariate approach has been used on centered and scaled data. Explanatory variables were first reduced using forward selection of constraints with the *forward.sel* function of the 'packfor '

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

Results

Experiment 1: the influence of HB.

To highlight the influence of HB and nutrient load on the stoichiometry of the POM, EPS productions and stoichiometric ratios were first measured in *M. aeruginosa* in the presence and in the absence of bacteria, at two nitrates loads. Dissolved inorganic phosphorus (DIP) in the medium was always higher than $96.9 \mu\text{mol P L}^{-1}$ throughout the experiment, indicating that phosphorus was never limiting in our experiment. N-NO_3^- concentration in the medium remained higher than $570 \mu\text{mol N L}^{-1}$ in nutrient replete condition (+ N), while the concentration was lower than $3.5 \mu\text{mol N L}^{-1}$ during the stationary phase in nutrient depleted conditions (-N) (data not shown). Neither the bacterial presence nor the two levels of nitrates availability induced a significant effect on the photosynthetic activity of *M. aeruginosa*, measured through the ETRmax (Fig. 1). Only growth phase changed significantly the ETRmax, in accordance with the decrease in photosynthetic activity when reaching the stationary phase (Fig. 1). TEP and S-EPS productions were also influenced by growth phase (Fig. 2): TEP tend to increase during the stationary phase (Fig. 2A), while S-EPS was at least three times higher during the exponential growth phase than during the stationary phase (Fig. 2B). The productions of TEP and

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

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

296

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 ± 0.01 and $0.13 \pm 0.01 \text{ day}^{-1}$ 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 $0.49 \pm 0.01 \text{ day}^{-1}$, 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 $0.30 \pm 0.03 \text{ day}^{-1}$ respectively. The
307 ETR_{max} measured during the exponential growth phase was also higher for the three Chroococcales,
308 compared with the Oscillatoriales, with the highest ETR_{max} observed for *M. aeruginosa* (Fig. 5). The
309 ETR_{max} decreased for all species when they reached the stationary phase (Fig. 5), as well as the dark-
310 adapted photochemical quantum efficiency (F_v/F_m), 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

ratio was close to the reference value found in the literature (Passow 2002; Thornton 2002; Reynolds 2006), with on average 6.5 ± 3.0 , a minimum of 1.9 and a maximum of 13.7 (Fig. 6C). The two 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 the C:N ratio (Fig. 6C). While molar C:N and C:P ratios showed the same pattern among the species, the TEP and the S-EPS production and stoichiometric ratios varied independently between species (Fig. 6). The correlation between TEP production and C:N ratio observed in axenic condition at the specific level (Fig. 3A) was not confirmed at the interspecific level.

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

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

A redundancy analysis was performed to explain the C: N and N:P ratios and the EPS productions 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 RDA triplot showed that species and growth phases are well separated in the ordination space (Fig. 9). The filamentous cyanobacteria are grouped together on the left part of the triplot, whatever their growth rate, with the picocyanobacteria in stationary phase. The second axis separated *M. aeruginosa* depending on its growth phase from the picocyanobacteria in exponential phase. The first axis (38 % of the total variance) of the ordination was mainly described by TEP, and in a lower extent by S-EPS and C:N. The explanatory variables of the first axis were the phosphates concentration and the cellular surface on volume ratio (S:V ratio), which were opposite to nitrates concentration. Species in the right part of the graph thus presented a higher C:N ratio and higher EPS productions, associated with high phosphates availability and low nitrates one in the medium. These species also presented a higher cellular S:V ratio. The second axis (25 % of the total variance) was mainly described by S-EPS and C:N, with species presenting a high C:N producing low S-EPS. The nitrates concentration and the growth rate explained the second axis. Species with high C:N ratio showed a low growth rate in a nitrate depleted medium.

Discussion

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

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

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

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

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

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

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

Acknowledgements

This research was funded by a grant from CNRS-UMR 6553 Ecobio and research funds from the University of Rennes (« Action incitative, Projets scientifiques émergents 2011 ») and from the french INSU-EC2CO program ('Microflux' 2012). We thank Nathalie Josselin-Le Bris and Marie-Paule Briand for laboratory assistance. Bio-chemical analysis, microscopy and experimental chambers were support by the common technical centers from the UMR Ecobio: the Analysis Biogeochemical center (ABGC), the optical and digital imaging center (COIN) and the Ecology experimental center (ECOLEX). We thank the two anonymous referees for helpful comments on an earlier version of the manuscript.

477 **References**

- 478 ISO 82451999 I.8. (1999) Water quality - Guidelines for the determination of total organic carbon
479 (TOC) and dissolved organic carbon (DOC).
- 480 Aminot A. & Chaussepied M. (1983) *Manuel des analyses en milieu marin*. Brest.
- 481 Andersen R.A. (2005) *Algal Culturing Techniques*. Academic Press.
- 482 Azam F., Smith D.C., Steward G.F. & Hagström A. (1994) Bacteria-organic matter coupling and its
483 significance for oceanic carbon cycling. *Microbial Ecology* **28**, 167–179.
- 484 Baines S.B. & Pace M.L. (1991) The production of dissolved organic matter by phytoplankton and its
485 importance to bacteria: patterns across marine and freshwater systems. *Limnol. Oceanogr* **36**, 1078–
486 1090.
- 487 Banse K. (1974) On the interpretation of data for the carbon-to-nitrogen ratio of phytoplankton.
488 *Limnol. Oceanogr* **19**, 695–699.
- 489 Berg K.A., Lyra C., Sivonen K., Paulin L., Suomalainen S., Tuomi P., et al. (2009) High diversity of
490 cultivable heterotrophic bacteria in association with cyanobacterial water blooms. *The ISME Journal*
491 **3**, 314–325.
- 492 Bertilsson S. & Jones J.B. (2003) *Supply of dissolved organic matter to aquatic ecosystems:*
493 *autochthonous sources*. (Eds F. SEG & S. RL), Academic Press.
- 494 Blanchet F.G., Legendre P. & Borcard D. (2008) Forward selection of explanatory variables. *Ecology*
495 **89**, 2623–2632.
- 496 Boersma, M., & Elser, J. J. (2006). Too much of a good thing: on stoichiometrically balanced diets
497 and maximal growth. *Ecology*, **87**(5), 1325–1330.
- 498 Boersma, M., & Kreutzer, C. (2002). Life at the edge: is food quality really of minor importance at
499 low quantities? *Ecology*, **83**(9), 2552–2561.
- 500 Briand E, Yéprémian C, Humbert J F, Quiblier C (2008) Competition between microcystin- and non-
501 microcystin-producing *Planktothrix agardhii* (Cyanobacteria) strains under different environmental
502 conditions. *Environmental Microbiology*, **10**(12): 3337–3348.
- 503 Briand E., Bormans M., Quiblier C., Salençon M.-J. & Humbert J.-F. (2012) Evidence of the Cost of
504 the Production of Microcystins by *Microcystis aeruginosa* under Differing Light and Nitrate
505 Environmental Conditions. *PLoS ONE* **7**, e29981.
- 506 Bruckner C.G., Bahulikar R., Rahalkar M., Schink B. & Kroth P.G. (2008) Bacteria Associated with
507 Benthic Diatoms from Lake Constance: Phylogeny and Influences on Diatom Growth and Secretion of
508 Extracellular Polymeric Substances. *Applied and Environmental Microbiology* **74**, 7740–7749.
- 509 Brunberg A.K. (1999) Contribution of bacteria in the mucilage of *Microcystis* spp.(Cyanobacteria) to
510 benthic and pelagic bacterial production in a hypereutrophic lake. *FEMS Microbiology Ecology* **29**,
511 13–22.
- 512 Callieri C. & Stockner J.G. (2002) Freshwater autotrophic picoplankton: a review. *Journal of*
513 *Limnology* **61**, 1–14.
- 514 Casamatta D.E.A. (2000) Sensitivity of Two Disjunct Bacterioplankton Communities to Exudates
515 from the Cyanobacterium *Microcystis aeruginosa* Kutzing. *Microbial Ecology*, **41**, 64–73.
- 516 Choueri R.B., Melao M.D.G.G., Lombardi A.T. & Vieira A.A.H. (2007) Effects of cyanobacterium
517 exopolysaccharides on life-history of *Ceriodaphnia cornuta* SARS. *Journal of Plankton Research* **29**,
518 339–345.
- 519 Chrzanowski T.H., Kyle M., Elser J.J. & Sterner R.W. (1996) Element ratios and growth dynamics of
520 bacteria in an oligotrophic Canadian shield lake. *Aquatic Microbial Ecology* **11**, 119–125.

521 Claquin P., Probert I., Lefebvre S. & Véron B. (2008) Effects of temperature on photosynthetic
522 parameters and TEP production in eight species of marine microalgae. *Aquatic Microbial Ecology* **51**,
523 1–11.

524 Cole J.J., Likens G.E. & Strayer D.L. (1982) Photosynthetically produced dissolved organic carbon:
525 an important carbon source for planktonic bacteria [Mirror Lake, New Hampshire, algae]. *Limnol.*
526 *Oceanogr* **27**, 1080–1090.

527 Costas E., López-Rodas V., Toro F.J. & Flores-Moya A. (2008) The number of cells in colonies of the
528 cyanobacterium *Microcystis aeruginosa* satisfies Benford's law. *Aquatic Botany* **89**, 341–343.

529 Croft M.T., Lawrence A.D., Raux-Deery E., Warren M.J. & Smith A.G. (2005) Algae acquire vitamin
530 B12 through a symbiotic relationship with bacteria. *Nature* **438**, 90–93.

531 Danger M., Leflaive J., Oumarou C., Ten-Hage L. & Lacroix G. (2007) Control of
532 phytoplankton?bacteria interactions by stoichiometric constraints. *Oikos* **116**, 1079–1086.

533 Danger M., Oumarou C., Benest D. & Lacroix G. (2007) Bacteria can control stoichiometry and
534 nutrient limitation of phytoplankton. *Functional Ecology* **21**, 202–210.

535 Decho A.W. & Lopez G.R. (1993) Exopolymer microenvironments of microbial flora: multiple and
536 interactive effects on trophic relationships. *Limnol. Oceanogr* **38**, 1633–1645.

537 De Philippis R., Sili C. & Vincenzini M. (1996) Response of an exopolysaccharide-producing
538 heterocystous cyanobacterium to changes in metabolic carbon flux. *Journal of Applied Phycology*. 8 :
539 275–281.

540 De Philippis R. & Vincenzini M. (1998) Exocellular polysaccharides from cyanobacteria and their
541 possible applications. *FEMS Microbiology Reviews* **22**, 151–175.

542 Dubois M., Gilles K., Hamilton J.K., Rebers P.A. & Smith F. (1956) A colorimetric method for the
543 determination of sugars. *Nature*, **28**: 350–356.

544 Dutz J., Klein Breteler W.C.M. & Kramer G. (2005) Inhibition of copepod feeding by exudates and
545 transparent exopolymer particles (TEP) derived from a *Phaeocystis globosa* dominated phytoplankton
546 community. *Harmful Algae* **4**, 929–940.

547 Eilers P. & Peeters J. (1988) A model for the relationship between light intensity and the rate of
548 photosynthesis in phytoplankton. *Ecological Modelling*. **42**, 199–215

549 Engel A. & Passow U. (2001) Carbon and nitrogen content of transparent exopolymer particles (TEP)
550 in relation to their Alcian Blue adsorption. *Marine Ecology Progress Series* **219**, 1–10.

551 Engel A., Delille B., Jacquet S., Riebesell U., Rochelle-Newall E., Terbruggen A., et al. (2004)
552 Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi*
553 exposed to different CO₂ concentrations: a mesocosm experiment. *Aquatic Microbial Ecology* **34**, 93–
554 104.

555 Fagerbakke K.M., Heldal M. & Norland S. (1996) Content of carbon, nitrogen, oxygen, sulfur and
556 phosphorus in native aquatic and cultured bacteria. *Aquatic Microbial Ecology* **10**, 15–27.

557 Flemming H.-C. & Wingender J. (2010) The biofilm matrix. *Nature Reviews, Microbiology*, **8**, 623–
558 633.

559 Gärdes A., Ramaye Y., Grossart H.P., Passow U. & Ullrich M.S. (2012) Effects of *Marinobacter*
560 *adhaerens* HP15 on polymer exudation by *Thalassiosira weissflogii* at different N:P ratios. *Marine*
561 *Ecology Progress Series* **461**, 1–14.

562 Gilbert M., Wilhelm C. & Richter M. (2000) Bio-optical modelling of oxygen evolution using in vivo
563 fluorescence: Comparison of measured and calculated photosynthesis/irradiance (P-I) curves in four
564 representative phytoplankton species. *Journal of Plant Physiology* **157**, 307–314.

565 Grossart H.P., Simon M. & Logan B.E. (1997) Formation of macroscopic organic aggregates (lake
566 snow) in a large lake: The significance of transparent exopolymer particles, phytoplankton, and
567 zooplankton. *Limnol. Oceanogr* **42**, 1651–1659.

568 Hessen D.O. (1992) Nutrient element limitation of zooplankton production, *The American Naturalist*,
569 **140**, 799–814.

570 Hessen D.O. & Anderson T.R. (2008) Excess carbon in aquatic organisms and ecosystems:
571 physiological, ecological, and evolutionary implications. *Limnol. Oceanogr* **53**, 1685–1696.

572 Huisman J., Matthijs H.C.P. & Visser P.M. eds (2005) Harmful cyanobacteria. Springer, Kluwer,
573 Dordrecht.

574 Jensen T.C. & Verschoor A.M. (2004) Effects of food quality on life history of the rotifer *Brachionus*
575 *calyciflorus* Pallas. *Freshwater Biology* **49**, 1138–1151.

576 Johnk K.D., Huisman J., Sharples J., Sommeijer B.P., Visser P.M. & Stroom J.M. (2008) Summer
577 heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology* **14**, 495–512.

578 Klausmeier C.A., Litchman E. & Levin S.A. (2004) Phytoplankton growth and stoichiometry under
579 multiple nutrient limitation. *Limnol. Oceanogr* **49**, 1463–1470.

580 Kromkamp J. (1987) Formation and functional significance of storage products in cyanobacteria. *New*
581 *Zealand Journal of Marine and Freshwater Research* **21**, 457–465.

582 Kromkamp J.C. & Forster R.M. (2003) The use of variable fluorescence measurements in aquatic
583 ecosystems: differences between multiple and single turnover measuring protocols and suggested
584 terminology. *European Journal of Phycology* **38**, 103–112.

585 Ling S.C. & Alldredge A.L. (2003) Does the marine copepod *Calanus pacificus* consume transparent
586 exopolymer particles (TEP)? *Journal of Plankton Research* **25**, 507–515.

587 Liu H. & Buskey E.J. (2000) Hypersalinity enhances the production of extracellular polymeric
588 substance (EPS) in the texas brown tide alga, *Aureocoumbra lagunensis* (Pelagophyceae). *Journal of*
589 *Phycol.*, **36**, 71–77.

590 López-Sandoval D.C., Rodríguez-Ramos T., Cermeno P. & Marañón E. (2013) Exudation of organic
591 carbon by marine phytoplankton: dependence on taxon and cell size. *Marine Ecology Progress Series*
592 **477**, 53–60.

593 Marañón E., Cermeno P., López-Sandoval D.C., Rodríguez-Ramos T., Sobrino C., Huete-Ortega M.,
594 et al. (2013) Unimodal size scaling of phytoplankton growth and the size dependence of nutrient
595 uptake and use. *Ecology Letters* **16**, 371–379.

596 Mari X. & Kiørboe T. (1996) Abundance, size distribution and bacterial colonization of transparent
597 exopolymeric particles (TEP) during spring in the Kattegat. *Journal of Plankton Research*, **18**, 969–
598 986.

599 Mari X., Beauvais S., Lemée R. & Pedrotti M.L. (2001) Non-Redfield C: N ratio of transparent
600 exopolymeric particles in the northwestern Mediterranean Sea. *Limnol. Oceanogr* **46**, 1831–1836.

601 Myklestad S.M. (1995) Release of extracellular products by phytoplankton with special emphasis on
602 polysaccharides. *Science of the Total Environment*, **165**, 155–164.

603 Oksanen J. (2013) Multivariate Analysis of Ecological Communities in R: vegan tutorial. *R package*
604 *version*, 1–43.

605 Passow U. (2002) Transparent exopolymer particles (TEP) in aquatic environments. *Progress in*
606 *Oceanography* **55**, 287–333.

607 Passow U. & Alldredge A.L. (1995) Aggregation of a diatom bloom in a mesocosm: The role of
608 transparent exopolymer particles (TEP). *Deep-Sea Research Part II* **42**, 99–109.

609 Passow U. & Alldredge A.L. (1999) Do transparent exopolymer particles (TEP) inhibit grazing by the
610 euphausiid *Euphausia pacifica*? *Journal of Plankton Research* **21**, 2203–2217.

611 Post AF, deWit R, Mur LR (1985) Interaction between temper- ature and light intensity on growth and
612 photosynthesis of the cyanobacterium *Oscillatoria agardhii*. *J Plankton Res* **7**:487 – 495

613 R Development Core Team R. (2011) R: a language and environment for statistical computing. R
614 Foundation for Statistical Computing, Vienna, Austria.

615 Redfield C.A., Ketchum H.B. & Richards A.F. (1963) The influence of organisms on the composition
616 of sea-water. In: The composition of seawater. Comparative and descriptive oceanography. The sea:
617 ideas and observations on progress in the study of the seas. (Ed. H. NM), pp. 26–77. New York.

618 Reynolds C.S. (2006) The Ecology of Phytoplankton. Ecology, Biodiversity and Conservation.
619 Cambridge.

620 Reynolds C.S. (2007) Variability in the provision and function of mucilage in phytoplankton:
621 facultative responses to the environment. *Hydrobiologia* **578**, 37–45.

622 Reynolds C.S., Huszar V., Kruk C., Naselli-Flores L. & Melo S. (2002) Towards a functional
623 classification of the freshwater phytoplankton. *Journal of Plankton Research* **24**, 417–428.

624 Rippka R. (1988) Isolation and purification of cyanobacteria. *Methods in enzymology* **167**, 3–27.

625 Rohrlack, T., G. Christiansen, and R. Kurmayer. (2013) Putative antiparasite defensive system
626 involving ribosomal and nonribosomal oligopeptides in cyanobacteria of the genus *Planktothrix*. *Appl.*
627 *Environ. Microbiol.* **79**: 2642–2647, doi:10.1128/AEM.03499-12

628 Schreiber U. (1998) Chlorophyll fluorescence: new instruments for special applications.
629 *Photosynthesis: mechanisms and effects* **5**, 4253–4258.

630 Shen H., Niu Y., Xie P., Tao M. & Yang X.I. (2011) Morphological and physiological changes in
631 *Microcystis aeruginosa* as a result of interactions with heterotrophic bacteria. *Freshwater Biology* **56**,
632 1065–1080.

633 Shibata K., Benson A.A. & Calvin M. (1954) The absorption spectra of suspensions of living micro-
634 organisms. *Biochimica et biophysica acta* **15**, 461–470.

635 Simon M., Grossart H.-P., Schweitzer B. & Ploug H. (2002) Microbial ecology of organic aggregates
636 in aquatic ecosystems. *Aquatic Microbial Ecology* **28**, 175–211.

637 Staats N., De Winder B., Stal L. & Mur L. (1999) Isolation and characterization of extracellular
638 polysaccharides from the epipelagic diatoms *Cylindrotheca closterium* and *Navicula salinarum*.
639 *European Journal of Phycology* **34**, 161–169.

640 Sterner R.W. & Elser J.J. (2002) Ecological Stoichiometry: The Biology of Elements from Molecules
641 to the biosphere - Princeton University Press, Oxfordshire.

642 Sugiura N. (1978) Further analysts of the data by akaike' s information criterion and the finite
643 corrections. *Communications in Statistics - Theory and Methods* **7**, 13–26.

644 Svane R and Eriksen NT (2015). Exopolysaccharides are partly growth associated products in
645 *Microcystis flos-aquae*. *J Appl Phycol.* **27** :163-170.

646 Thornton D.C.O. (2002) Diatom aggregation in the sea: mechanisms and ecological implications.
647 *European Journal of Phycology* **37**, 149–161.

648 Underwood G., Paterson D.M. & Parkes R.J. (1995) The measurement of microbial carbohydrate
649 exopolymers from intertidal sediments. *Limnol. Oceanogr* **40**, 1243–1253.

650 Underwood G.J.C., Boulcott M., Raines C.A. & Waldron K. (2004) Environmental effects on
651 exopolymer production by marine benthic diatoms: dynamics, changes in composition and pathways
652 of production. *Journal of Phycology* **40**, 293–304.

653 Urabe J., Togari J. & Elser J.J. (2003) Stoichiometric impacts of increased carbon dioxide on a
654 planktonic herbivore. *Global Change Biology* **9**, 818–825.

655 Van de Waal D.B., Verschoor A.M., Verspagen J.M., Van Donk E. & Huisman J. (2010) Climate-
656 driven changes in the ecological stoichiometry of aquatic ecosystems. *Frontiers in Ecology and the*
657 *Environment* **8**, 145–152.

658 Verdugo P., Alldredge A.L., Azam F., Kirchman D.L., Passow U. & Santschi P.H. (2004) The oceanic
659 gel phase: a bridge in the DOM–POM continuum. *Marine Chemistry* **92**, 67–85.

660 Vieira A.A.H., Ortolano P.I.C., Girolodo D., Oliveira M.J.D., Bittar T.B., Lombardi A.T., et al. (2008)
661 Role of hydrophobic extracellular polysaccharide of *Aulacoseira granulata* (Bacillariophyceae) on
662 aggregate formation in a turbulent and hypereutrophic reservoir. *Limnol. Oceanogr.* **53**, 1887–1899.

663 Worm J. & Søndergaard M. (1998) Alcian blue-stained particles in a eutrophic lake. *Journal of*
664 *Plankton Research* **20**, 179–186.

665 Yallop M.L., Paterson D.M. & Wellsbury P. (2000) Interrelationships between Rates of Microbial
666 Production, Exopolymer Production, Microbial Biomass, and Sediment Stability in Biofilms of
667 Intertidal Sediments. *Microbial Ecology* **39**, 116–127.

668 Yéprémian, C., Gugger, M.F., Briand, E., Catherine, A., Berger, C., Quiblier, C., Bernard, C. (2007)
669 Microcystin ecotypes in a perennial *Planktothrix agardhii* bloom. *Water Res.* **41**, 4446–4456.

670 Zhang M., Shi X., Yu Y. & Kong F. (2011) The acclimative changes in photochemistry after colony
671 formation of the cyanobacteria *Microcystis aeruginosa*. *Journal of Phycology* **47**, 524–532.

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Table and Figures legends

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 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 , 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 also shown. Significance levels are coded as follow: '***' indicates <0.001 , '**' <0.01 , and '*' <0.05 .

Figure 1: Maximum electron transport rate (ETR_{max}) of *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 ($\pm$ standard deviation) are shown. No statistical 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 availability: *Ax*: Axenic, *B*: associated with bacteria, *+N*: high level of N availability, *-N*: low level of N availability. 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 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.

Figure 5: The maximum electron transport rate (ETR_{max}) 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 ($\mu\text{g ep. C mm}^3$) depending on cyanobacteria, of (B) the TEP per unit of cell volume ($\mu\text{g ep. C mm}^3$), 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 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 ($\mu\text{g eq C cell}^{-1}$) 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 ($\square$) are shown, with the three replicates.

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

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

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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, and ‘*’ <0.05.

	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			

Model statistics: AIC = -34.12, Residual standard error: 0.448, DF = 19, $R^2=0.42$, $p = 0.027$.

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 = 44.7, Residual standard error: 2.284, DF = 18, $R^2=0.84$, $p < 0.0001$.

Figure 1: Maximum electron transport rate (ETRmax) of *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 ($\pm$ standard deviation) are shown. No statistical difference based on Wilcoxon rank-sum test and Tukey post hoc test ($P > 0.05$).

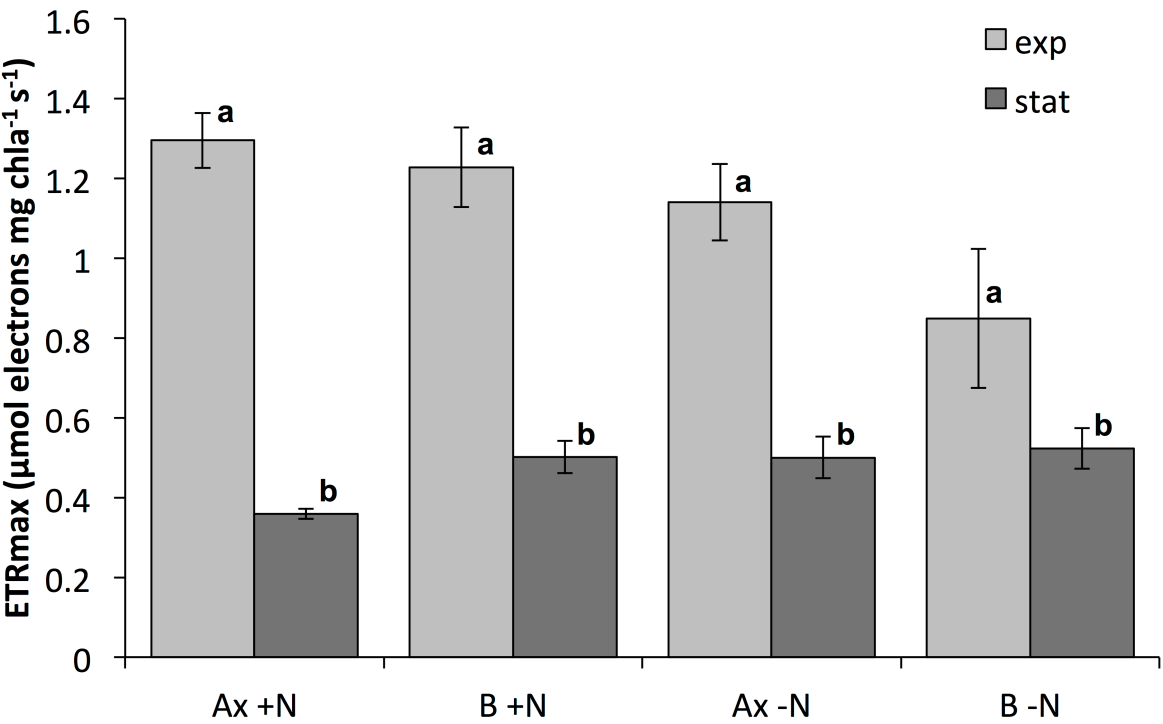


Figure 2: (A) TEP and (B) S-EPS produced by *M. aeruginosa*, during exponential growth (white area) and stationary phase (grey area), depending on the presence of heterotrophic bacteria and nitrate availability: Ax: Axenic, B: associated with bacteria, +N: high level of N availability, -N: low level of N availability. 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 ($P < 0.05$).

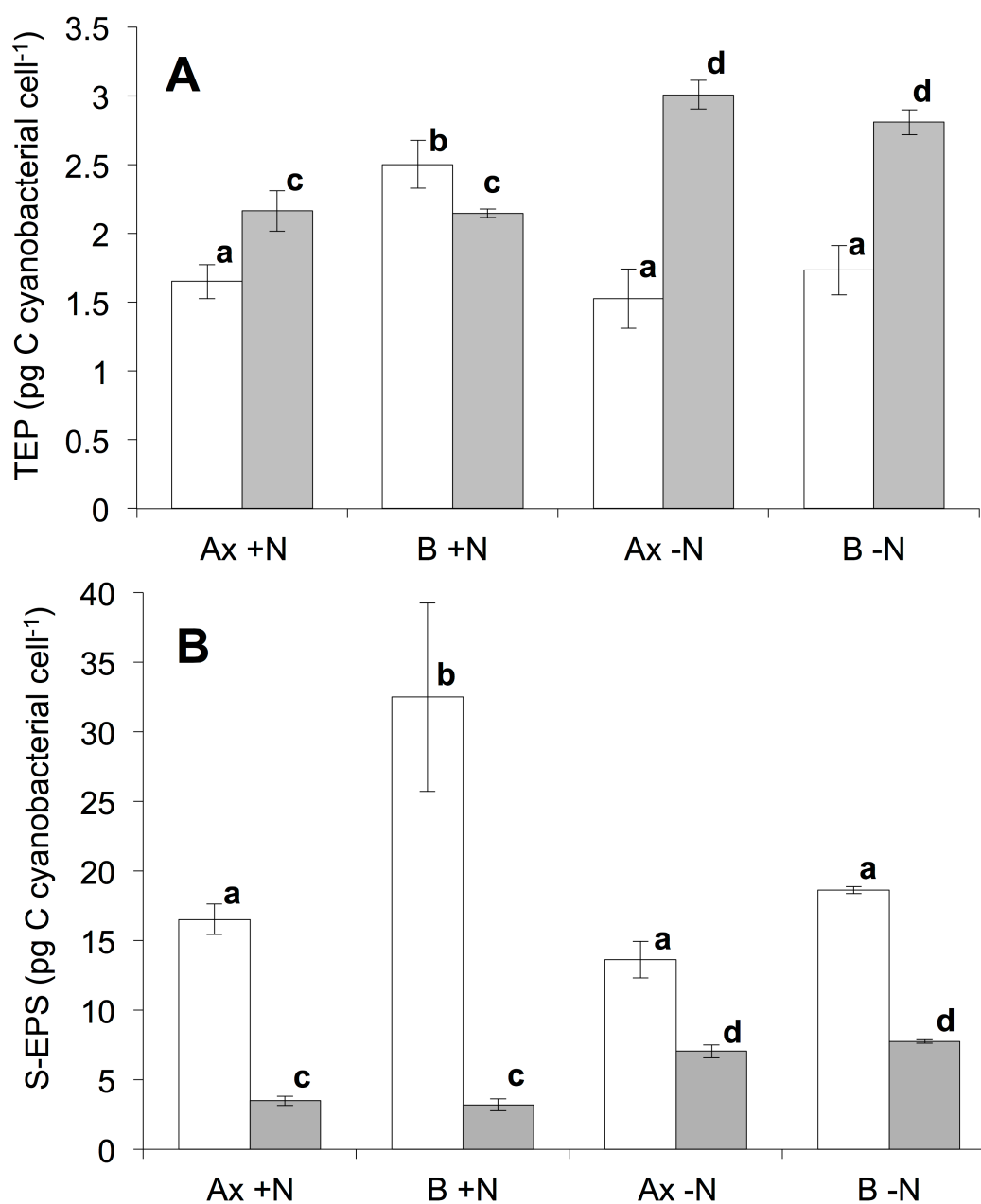


Figure 3: (A) Molar C:N and (B) C:P ratios of *M. aeruginosa* (axenic condition shown by open diamonds) and of the cyanobacteria associated with heterotrophic bacteria (filled circles) depending on the amount of TEP per cyanobacterial cell. Correlations in axenic condition and in the presence of HB (including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

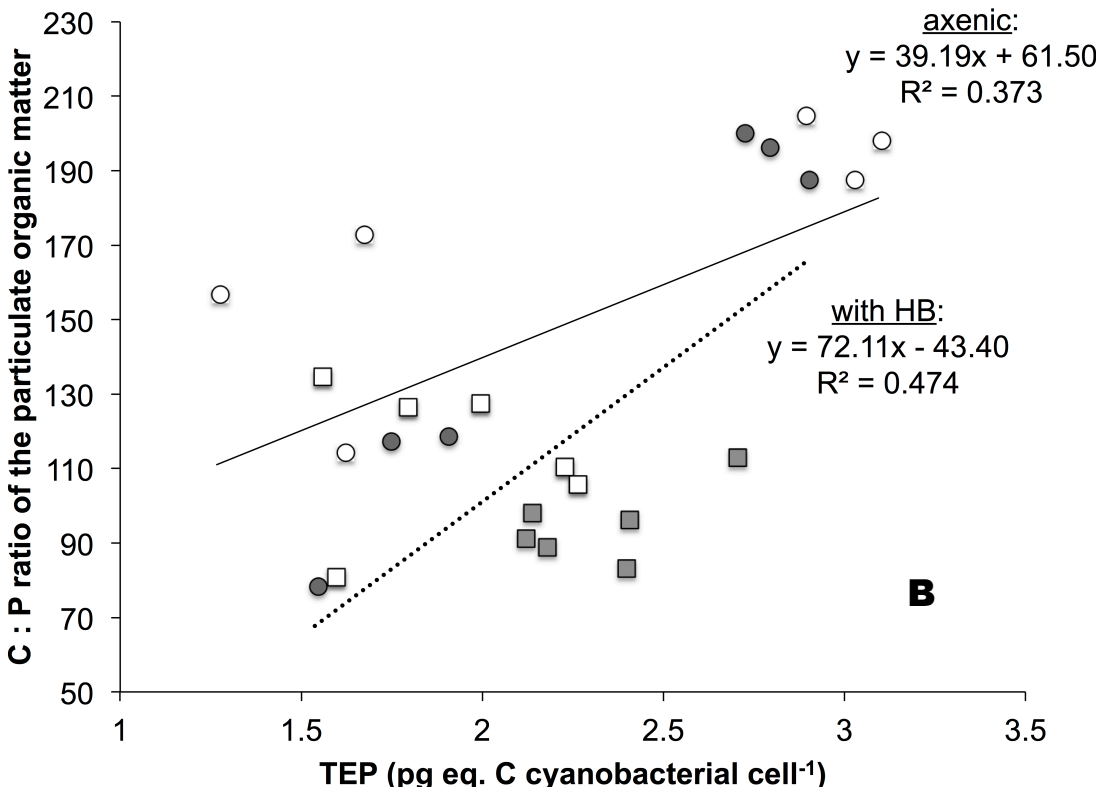
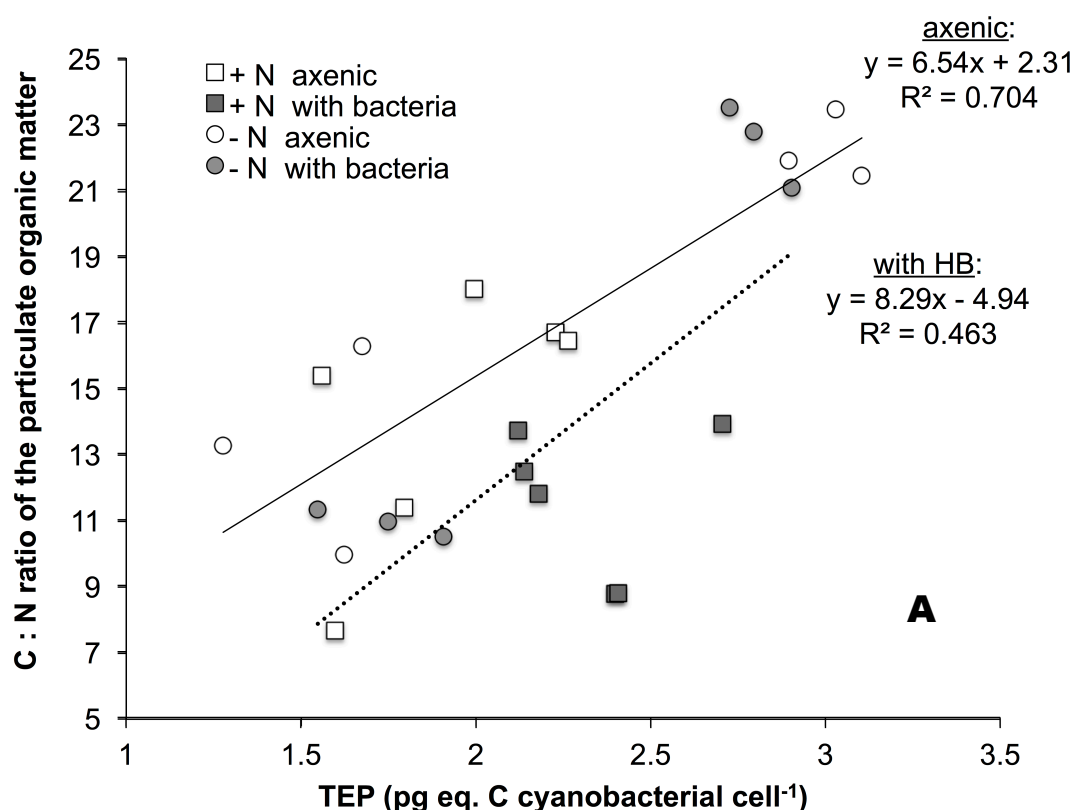


Figure 4: Time series of the biomass absorbance (means of replicate value), expressed as Chlorophyll a concentration, of the cyanobacteria species.

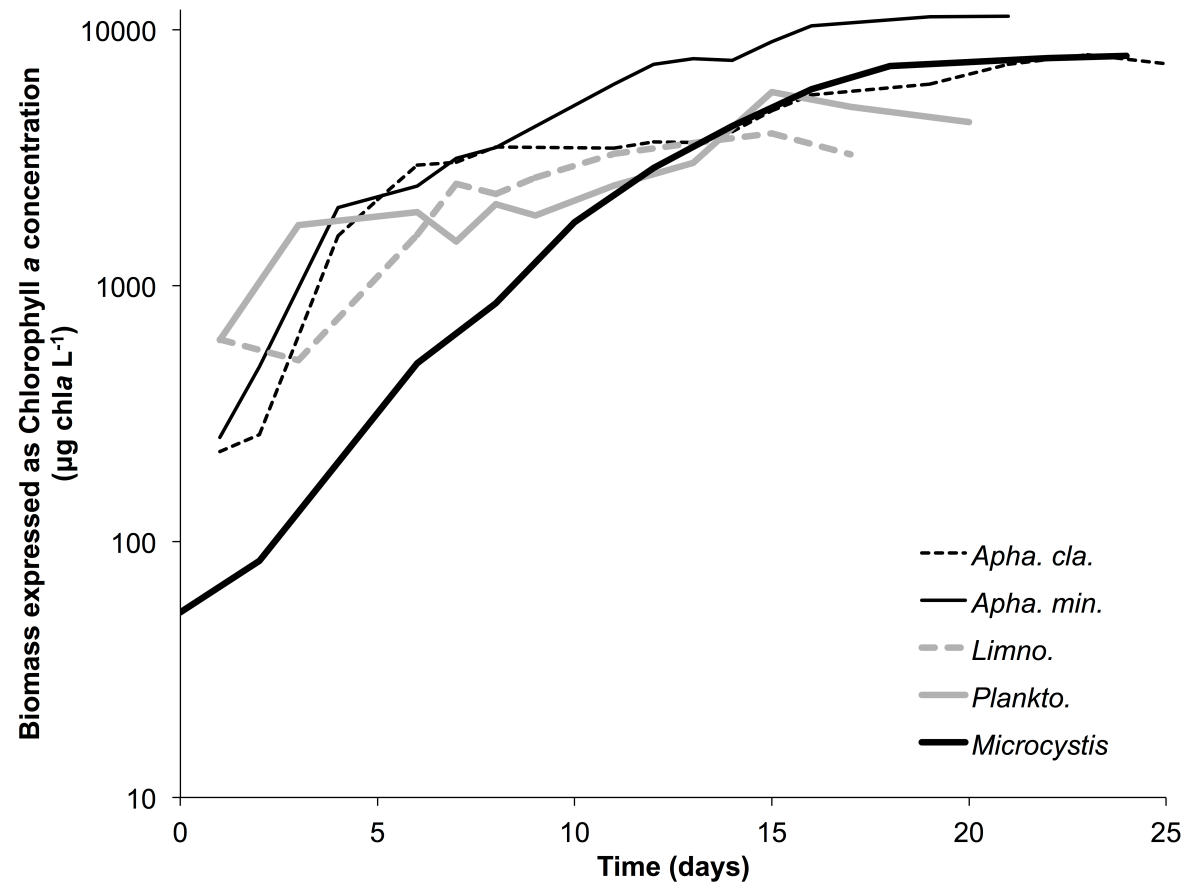


Figure 5: The maximum electron transport rate (ETRmax) measured during exponential growth (white area) and the early stationary phase (grey area), depending on cyanobacterial species. Means of replicate value ($\pm$ standard deviation) are shown. $a \neq b$ and $c \neq d$ based on Wilcoxon rank-sum test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

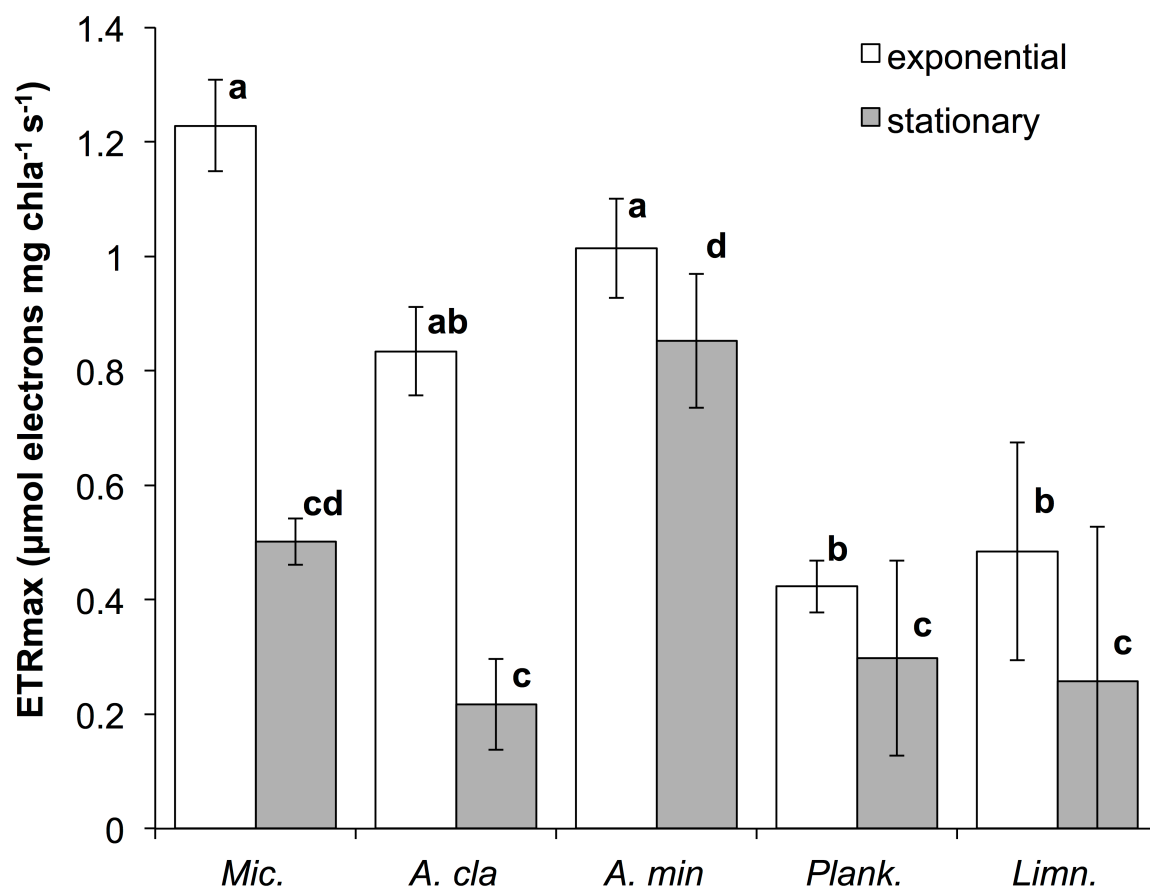


Figure 6: Boxplot of (A) the mean concentration of S-EPS in the culture per unit of cell volume ($\mu\text{g ep. C mm}^{-3}$) depending on cyanobacteria, of (B) the TEP per unit of cell volume ($\mu\text{g ep. C mm}^{-3}$), of (C) the molar C:N ratio and (D) C:P of the particulate organic matter. C represents the Chroococcales and O the Oscillatoriales. $a \neq b \neq c$ based on Wilcoxon rank-sum test and Tukey post hoc test ($P < 0.05$).

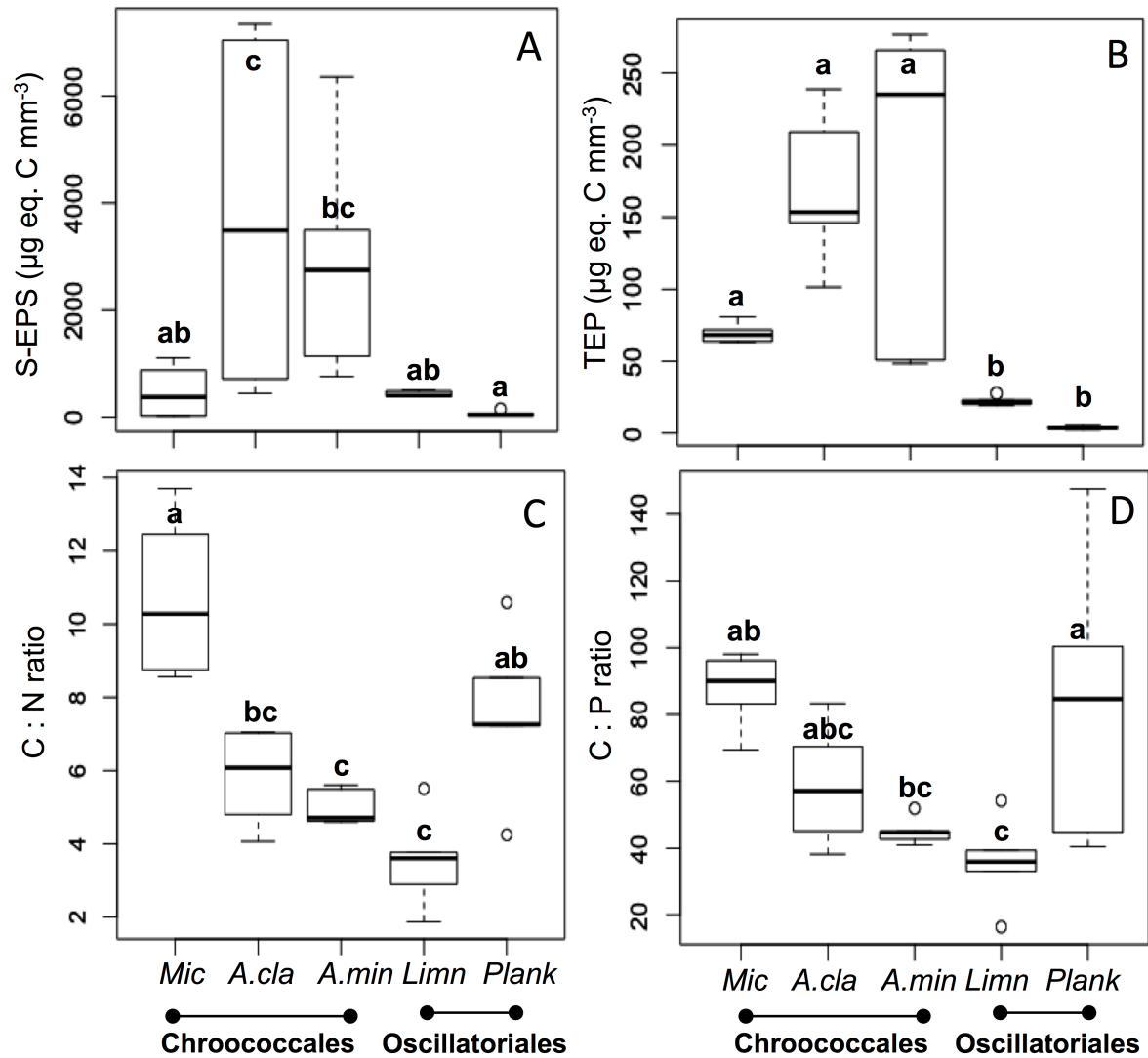


Figure 7: (a) C content, (b) N content and (c) P content per cyanobacterial cell of the particulate organic matter depending on the TEP concentration per cyanobacterial cell, considering the five species. Data correspond to the three replicates measured during the exponential phase (open symbols) and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

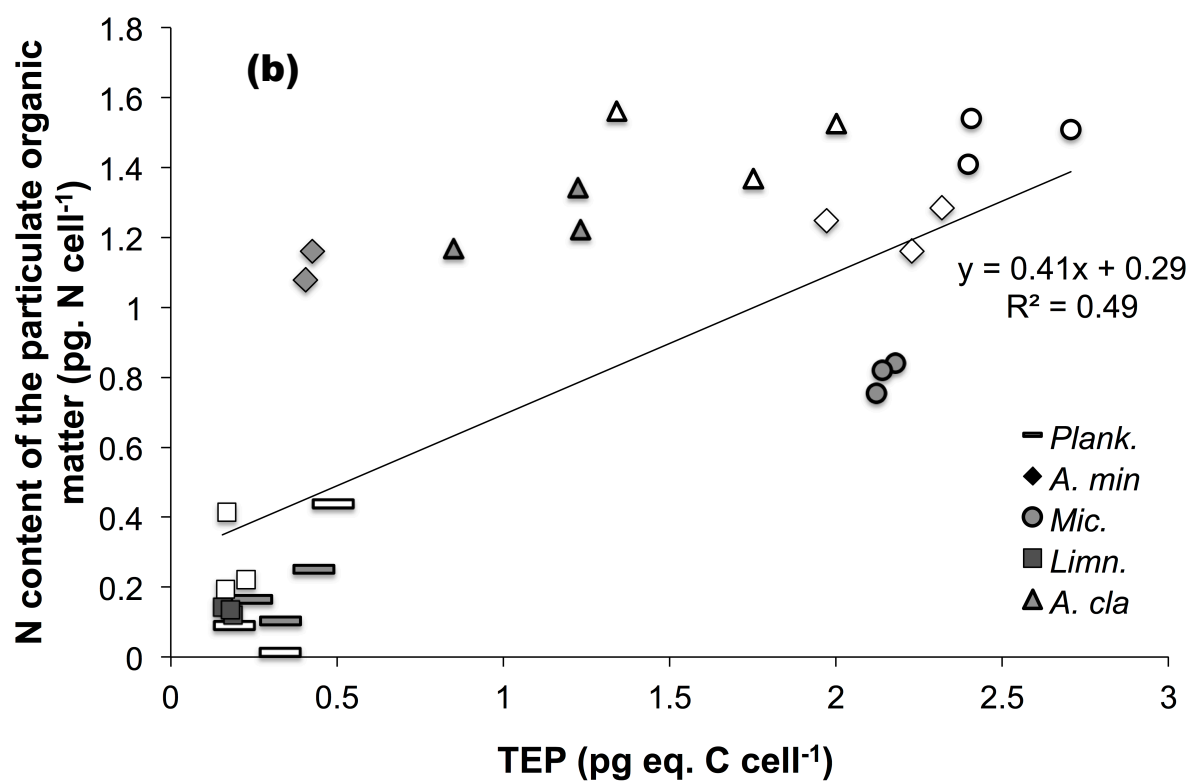
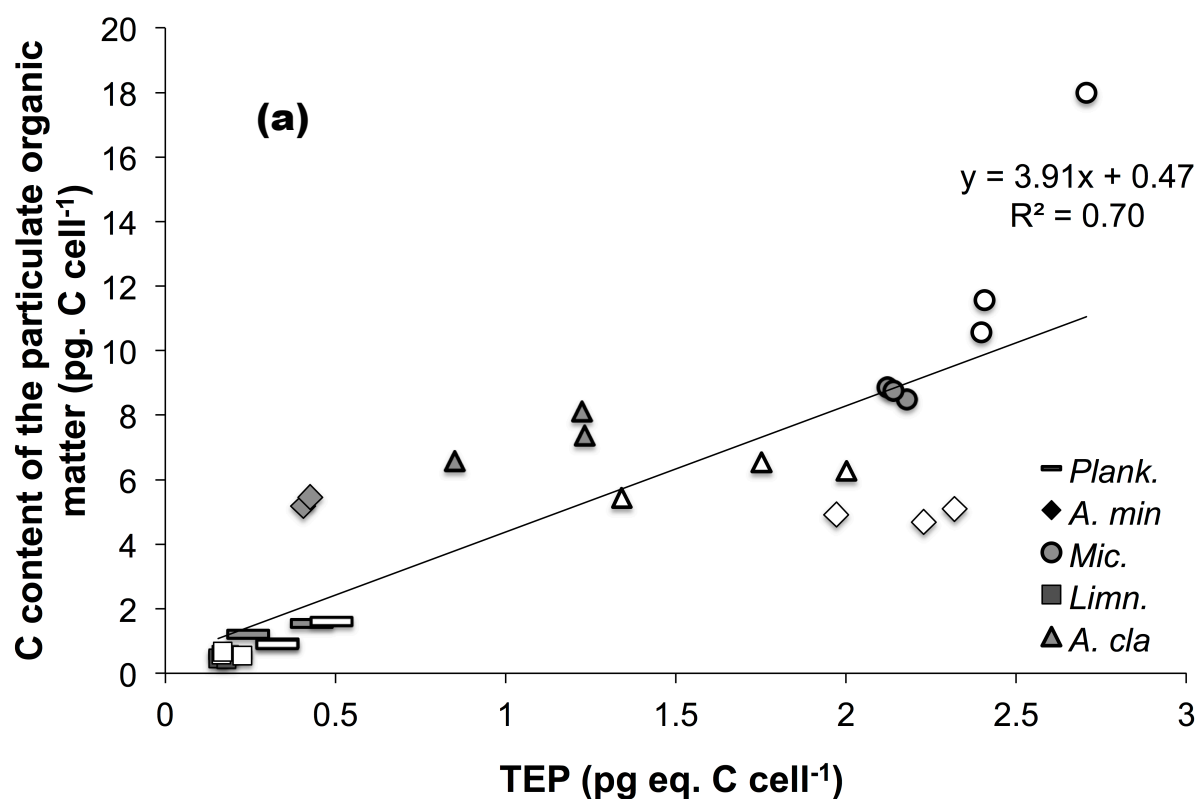


Figure 8: molar C : N ratio of the particulate organic matter depending on the S-EPS concentration in the medium, considering the five species. Data correspond to the three replicates measured during the exponential phase (open symbols) and during the early stationary phase (black symbols). Regression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

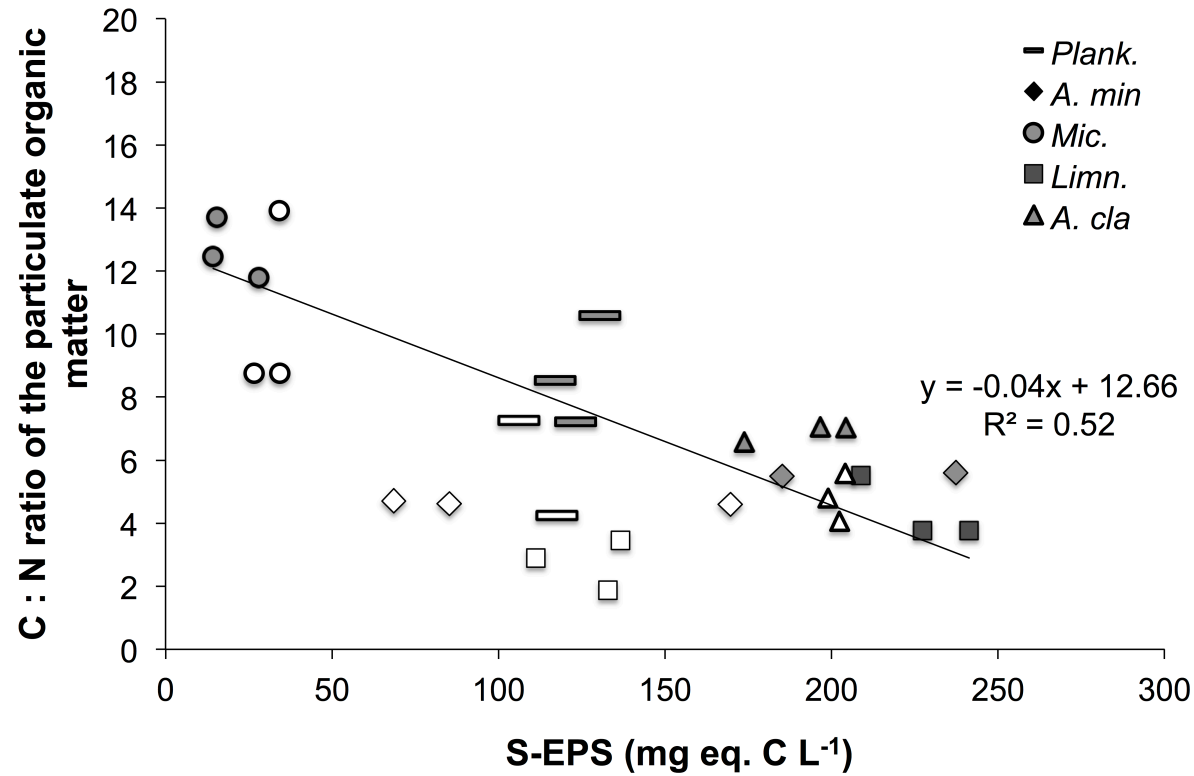
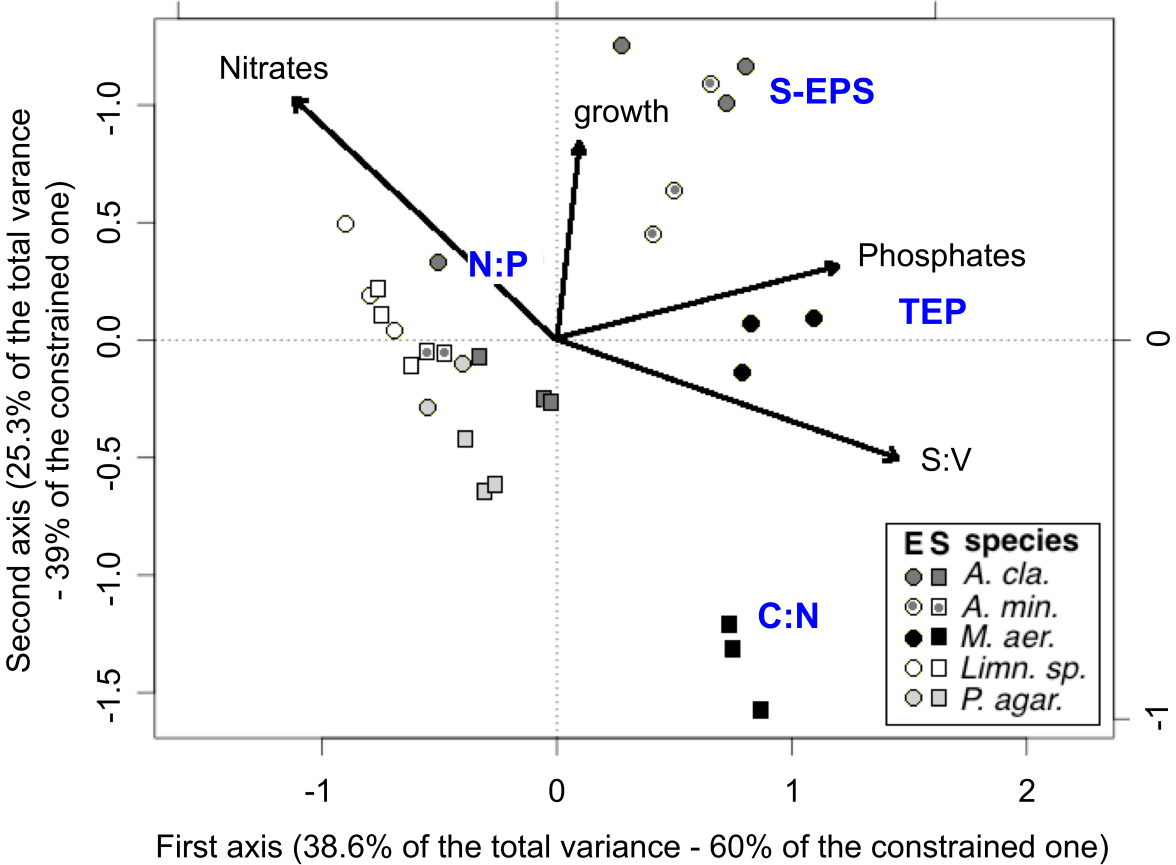
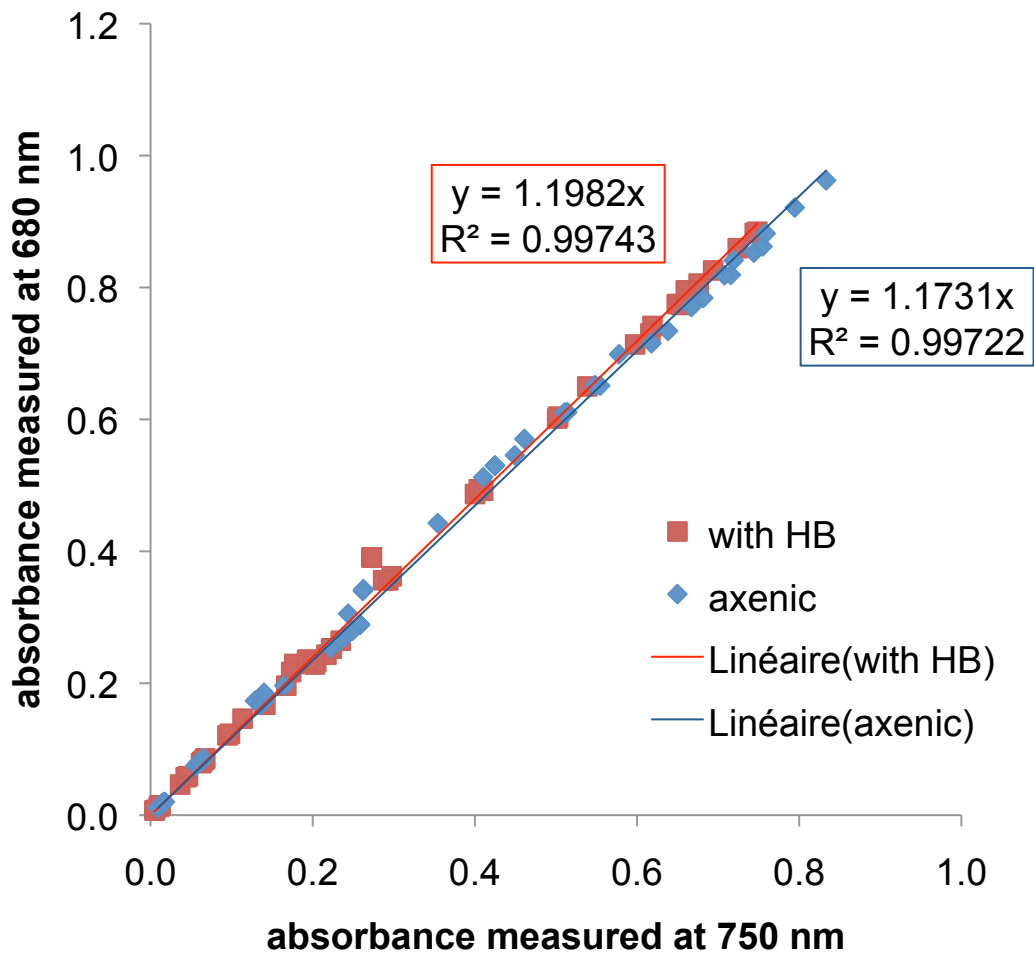


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802 Additional Supporting Information may be found in the online version of this article:

803 **Fig. S1.** Absorbance measured at 680 nm as a function of the absorbance measured at 750 nm
804 for *Microcystis* cultures, in axenic condition (blue diamonds) and in the presence of
805 heterotrophic bacteria (red squares).



806

807 **Table S1:** Parameter estimates for the best model predicting the C:P ratio in *M. aeruginosa*,
808 as determined by a stepwise selection of the variables using the AIC criterion. Result from its
809 ANOVA is also shown. Significance levels are coded as follow: ‘***’ indicates <0.001, ‘**’
810 <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			

