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Phosphorus limitation determines the quality of dissolved organic matter released by marine heterotrophic prokaryotes.

Running head: Phosphorus effects on prokaryotic DOM

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Author Contribution Statement: NB, IO and EOR conceived the work. NB and EOR performed the experiments with an active contribution of BM and OC to chemical analyses. NB analyzed data and wrote the paper with significant contributions from IO and EOR.

17 ***Scientific Significance Statement***

18

19 Marine heterotrophic prokaryotes (HP) generate copious amounts of dissolved organic matter (DOM).
20 Some of this DOM is recalcitrant to biological degradation and can persist in the ocean for millennia.
21 This carbon sequestration mechanism is called the microbial carbon pump (MCP). The factors
22 controlling the strength of the MCP are still poorly understood. Phosphorus (P) limitation is a common
23 condition in the ocean that can influence HP growth and metabolism. We addressed here the question
24 of whether P-limitation affects the quantity and quality of DOM released by HP. We found no effect of
25 P-limitation on HP-derived DOM net quantity. However, using fluorescence spectroscopy, we
26 demonstrated that P-limitation altered DOM composition with an increase in humic-like compounds.
27 Our study provides experimental evidence that P availability plays an important role in shaping the
28 chemical composition of HP-derived DOM. Our results have implications for the fate of HP-derived
29 DOM in the ocean, suggesting that P-limitation is a key driver of the MCP.

30

31 ***Data availability statement***

32

33 Data and metadata are available in the Dryad repository at <https://doi.org/10.5061/dryad.xsj3tx9h4>

34

35 The PARAFAC model is available in the OpenFluor Database at <http://openfluor.org>

36

37 ***Abstract***

38

39 We determined phosphorus (P) limitation effect on the quantity and quality of dissolved organic matter
40 (DOM) released by heterotrophic prokaryotes (HP). We grew 2 single bacterial strains from different
41 lifestyles, the copiotrophic *Photobacterium angustum* and the oligotrophic *Sphingopyxis alaskensis*,
42 and natural HP communities collected in fall and spring from the Mediterranean Sea, on glucose under
43 2 treatments: P-replete vs. P-limiting. DOM release by HP comprised up to 30 % of the initial carbon
44 provided for growth. P availability influenced carbon allocation to different cellular processes
45 (respiration vs. growth), but did not significantly affect the net quantity of DOM released by HP.
46 However, using fluorescence spectroscopy, we demonstrated an effect of P-limitation on DOM quality,
47 with a predominance of humic-like compounds under P-limitation but protein-like compounds under
48 P-repletion. Our results suggest that P-limitation could determine the fate of HP-derived DOM in the
49 ocean, thus affecting the microbial carbon pump.

50

51

52

53 ***Keywords***

54

55 Dissolved organic matter, phosphorus limitation, heterotrophic prokaryotes, fluorescent dissolved
56 organic matter.

57 ***Introduction***

58 Dissolved organic matter (DOM) is the dominant pool of reduced carbon in the ocean. DOM covers a
59 continuum of reactivity ranging from labile, with fast turnover, to recalcitrant DOM that persists for
60 millennia being thus a carbon sequestration mechanism (Hansell, 2013). Heterotrophic prokaryotes
61 (HP) are important drivers of DOM biogeochemical cycling in the ocean (Nagata, 2008). They process
62 about half of the carbon fixed by primary producers (Cole, et al., 1988), converting it into CO₂
63 (respiration) and new biomass (production). The fraction of carbon converted into new biomass, defined
64 as the prokaryotic growth efficiency (PGE), is crucial for estimating carbon fluxes in the ocean (del
65 Giorgio & Cole, 1998). But HP also release a myriad of DOM compounds, part of which can be
66 recalcitrant and thus be stored in the ocean (Ogawa, 2001; Gruber et al., 2006; Lønborg et al., 2009;
67 Lechtenfeld et al., 2015; Osterholz et al., 2015). This process, termed the microbial carbon pump (Jiao
68 et al., 2010), may be an important carbon sequestration mechanism.

69 In recent years, different fractions of this HP-derived DOM (HP-DOM) have been characterized, e.g.
70 amino acids concentration (Daoud & Tremblay, 2019), molecular signature (Lechtenfeld et al. 2015;
71 Koch et al. 2014) and fluorescence properties (Shimotori et al., 2009). This last technique has allowed
72 characterizing HP-DOM quality (Rochelle-Newall and Fisher 2002; Kramer and Herndl 2004;
73 Shimotori et al., 2009) and has revealed differences in fluorescent DOM (FDOM) related to the
74 taxonomy of the producing HP (Shimotori et al., 2012; Goto et al., 2020; Ortega-Retuerta et al., 2021).
75 Yet, the reason why FDOM is released and its relation to environmental factors has been scantily studied.
76 HP can release DOM as a direct by-product of their biosynthesis and growth (Kawasaki and Benner
77 2006) or by the imbalance between their anabolism and catabolism (Carlson et al., 2007). Viral lysis
78 and sloppy grazing by protozoans can also release DOM from HP (Middelboe & Jørgensen, 2006;
79 Nagata, 2000) and alter its composition (Gruber et al., 2006; Heinrichs et al., 2022).

80 However, many molecules can also be released for specific needs including competition (antibiotics),
81 communication, nutrient acquisition (siderophores, ectoenzymes) (Kujawinski 2011; Carlson 2002).
82 Therefore, the quantity and chemical nature of HP-DOM could differ as a function of environmental
83 conditions such as nutrient availability. Phosphatase synthesis, for instance, is highly influenced by
84 phosphorus (P) limitation (Romano et al., 2015). Although P-limitation for HP is a dominant condition

85 in the ocean (Wu et al., 2000; Obernosterer et al., 2003; Lazzari et al., 2016), its effect on HP-DOM
86 quantity and chemical composition is unclear (e.g. Thompson & Cotner, 2020). For example, Romano
87 et al. (2014), looking at solid-phase extracted DOM, showed changes in bacterial metabolomes induced
88 by P-limitation, with an increase in phenolic and polyphenolic compounds. Therefore, we hypothesize
89 that P availability would have an effect on DOM quantity and quality.

90 To test this hypothesis, we addressed two main questions: (i) Does P-limitation affect the quantity of
91 DOM released by HP? (ii) Do the fluorescence properties of HP-DOM depend on P availability?

92

93 ***Material and methods***

94

95 *Experimental design*

96

97 To test the effect of P-limitation on HP-DOM, we performed 4 experiments using 2 model bacterial
98 strains and 2 natural HP communities.

99 The two bacterial strains were obtained from the “Laboratoire Arago culture collection”:
100 *Photobacterium angustum* S14, a copiotrophic *Gammaproteobacterium* isolated from coastal waters
101 (Botany Bay, Australia) (Lauro et al., 2009) ; and *Sphingopyxis alaskensis* RB2256, an oligotrophic
102 *Alphaproteobacterium* isolated from North Pacific waters (Eguchi et al., 2001). They were chosen
103 based on their capacity to grow on glucose, the availability of their genome sequenced, and the ample
104 experience culturing them at our laboratory (e.g. Koedooder et al., 2018; Matallana-Surget et al., 2012).
105 Initially preserved in glycerol (33% final concentration) at -80 °C, they were thawed and 2 consecutive
106 acclimation steps (strains inoculated in fresh media, dilution 1:1000) were performed prior to
107 experiments to minimize glycerol presence in the final incubations.

108 The natural HP communities were collected from surface waters of a coastal site located in the NW
109 Mediterranean Sea (SOLA, 42°29'300 N – 03°08'700 E) at 2 different seasons, fall (November 2020)
110 and spring (May 2021), referred to as SOLA-fall and SOLA-spring experiments respectively. Water
111 was prefiltered, immediately after sampling, by 0.8 µm and then cells were concentrated from ~3000
112 mL to ~40 mL by filtering through 0.2 µm filters to allow low inoculum volume, minimizing thus

113 nutrient and DOM supply from seawater to the media. The natural communities were then directly
114 inoculated in the experimental media (dilution 1:1000).

115 Both strains and communities were grown separately on minimum media (Fegatella et al., 1998)
116 consisting of artificial sea water (composition in Table S1) with glucose as a sole carbon (C) source (~
117 200 $\mu\text{mol C L}^{-1}$), nitrogen as NH_4Cl (~ 40 $\mu\text{mol L}^{-1}$), P as NaHPO_4 , trace metals and vitamins. Two
118 experimental treatments were considered, termed “balanced” and “unbalanced”, by changing the initial
119 P concentration in the media. In the “balanced” treatment, P was added at an initial concentration of ~
120 4.4 $\mu\text{mol L}^{-1}$, thus inorganic nutrients were at a C:N:P ratio of 45:9:1, mimicking the internal
121 stoichiometry of HP cells (Goldman et al. 1987). In the “unbalanced” treatment, P was added at an
122 initial concentration of ~ 0.53 $\mu\text{mol L}^{-1}$, so C:N:P ratios were at ~ 374:75:1, similar to the mean C:P
123 ratio of bulk DOM in the surface ocean (Hopkinson & Vallino, 2005).

124 Culture media were inoculated at an initial cell abundance of 0.5 to 9 x10⁴ cells mL⁻¹ for the strains and
125 of 0.4 to 2 x10⁴ cells mL⁻¹ for the natural communities. Two contamination controls per treatment (i.e.
126 culture media with no cell inoculum) were also incubated in parallel. Incubations were set up in
127 combusted (5 hours at 450 °C) 500 mL glass bottles, in triplicate (except for *S. alaskensis*, 4 replicates)
128 at 25 °C in the dark and shaken at 100 rpm.

129 Cell growth was determined by flow cytometry (See Supporting Information) and experiments were
130 conducted until the stationary phase was reached.

131 To quantify and characterize the HP-DOM, samples were taken at the beginning of the experiment (T_0),
132 and in the stationary phase (T_f). At T_0 , samples for HP-DOM were directly taken from the cultures
133 while at T_f , samples were collected after filtering the cultures twice onto pre-rinsed 0.2 μm
134 polycarbonate filters using pre-combusted all-glass vacuum filtration unit (Millipore). Negligible DOM
135 leaching from the polycarbonate filters was previously tested (data not shown). The double filtration
136 procedure ensured minimal cell presence in the filtrates (< 2 % of the abundance before filtration).

137

138 *Chemical analyses*

139

140 Samples for dissolved organic carbon (DOC) were acidified and stored in the dark at room temperature
141 until analysis by temperature catalytic oxidation technique (Benner & Strom, 1993). Glucose was
142 quantified as $\mu\text{mol L}^{-1}$ of carbon equivalent (C-glucose) based on an adapted colorimetric method
143 (Myklestad et al. 1997). Inorganic nutrients (NH_4^+ , NO_3^- , NO_2^- , PO_4^{3-}) were also measured. Detailed
144 chemical analyses are provided in Supporting Information.

145

146 *FDOM measurement*

147

148 FDOM samples were analyzed within few hours upon sampling. Excitation emission matrices (EEMs)
149 were recorded using a JASCO FP-85000 Spectrofluorimeter in a 10 mm quartz cell at scan speed of
150 5000 nm min^{-1} . Excitation scans ranged between 240 to 450 nm at 5 nm intervals and emission scans
151 were recorded between 300 to 560 nm at 2 nm intervals. MilliQ EEM blanks were recorded in every
152 batch of analysis. Fluorescence intensities were reported in Raman units (RU) obtained by dividing the
153 fluorescence units by the MilliQ blank peak area (Raman scatter) excited at 350 nm. EEMs were
154 measured for all replicates except in SOLA-fall experiment where only one EEM per treatment was
155 recorded (See supporting information).

156

157 *Data analyses*

158

159 Growth rates were calculated as the slope of the ln-transformed cell abundances over time. The
160 prokaryotic growth efficiency was determined as the increase of biomass from the lag phase to the
161 maximum growth reached, divided by the decrease of C-glucose between T_0 and T_f following this
162 equation:

$$163 \text{PGE (\%)} = \frac{\Delta \text{biomass } (\mu\text{mol L}^{-1})}{\Delta \text{C-glucose } (\mu\text{mol L}^{-1})} \times 100$$

164 where biomass was obtained using specific cell to biomass conversion factors for each experiment
165 (Table S2).

166 HP-DOM release was quantified as the difference between the concentration of C-glucose remaining
167 at the end of the experiment and bulk DOC concentration (DOC excess) as follows. Our calculation
168 assumes that HP did not release carbon as glucose and other monosaccharides during the incubation.

$$169 \quad \text{HP-DOM } (\mu\text{mol L}^{-1}) = \text{DOC}_{\text{tf}} (\mu\text{mol L}^{-1}) - \text{C-glucose}_{\text{tf}} (\mu\text{mol L}^{-1})$$

170 To better understand FDOM characteristics, EEMs were separated into distinct components using
171 parallel factor analysis (PARAFAC) under the drEEM toolbox in MATLAB (Murphy et al., 2013). A
172 6-component model (Table S3, Fig S2) was validated and associated to identified components in
173 previous studies published in the OpenFluor database (Detailed method in Supporting information).

174 Differences in biological and chemical parameters between the experimental treatments were tested by
175 one-way analysis of variance (ANOVA) and post-hoc Tukey tests.

176 We performed linear regression analysis between the FDOM components and the HP growth rates,
177 pooling data from all experiments. A correspondence analysis (CA) was applied to the identified
178 components and their maximum intensity for each experiment. Statistics were performed using R 3.6.1
179 (R Core Team, 2019) and the FactoMineR (v.2.3; Lê et al., 2008) package.

180

181 **Results**

182

183 *Heterotrophic prokaryotic growth*

184

185 Independent of nutrient regime, *P. angustum* and SOLA-fall grew fast, reaching the stationary phase
186 after 30 h of incubation (Fig. 1a, c), while SOLA-spring only plateaued after 60 h of incubations (Fig.
187 1d). *S. alaskensis* grew slower and reached the stationary phase after about 100 h (Fig. 1b). In the
188 contamination controls, cell numbers remained $< 16 \times 10^4$ cells mL⁻¹. The maximum cell abundance
189 reached was always higher in the balanced than in the unbalanced treatments (*P. angustum*, p-value
190 < 0.05 ; *S. alaskensis*, SOLA-fall and SOLA-spring, p-value < 0.001). P-limitation did not affect the
191 growth rates in any of the incubations (Table 1), but *S. alaskensis* grew significantly slower than *P.*
192 *angustum* and the natural HP communities (p-value < 0.001). In the unbalanced (P-limited) treatments,

193 PGE was always significantly lower than in the balanced treatments (Table 1, *P. angustum*, p-value
194 <0.05; *S. alaskensis* p-value <0.001; SOLA-fall p-value <0.001; SOLA-spring, p-value <0.01).

195

196 *HP-DOM quantity*

197

198 At the end of the incubations, 1.8 to 23.4 $\mu\text{mol L}^{-1}$ of initial C-glucose remained, while DOC
199 concentrations ranged between 18.2-64.6 $\mu\text{mol L}^{-1}$ (Table S4). The single strains released between 50.5
200 to 60.2 $\mu\text{mol C L}^{-1}$, corresponding to 20 - 30 % of initial C-glucose (Table 1). For the natural HP
201 communities, DOC release ranged between 5.3 and 43.2 $\mu\text{mol L}^{-1}$ (3 - 18 % of initial C-glucose), which
202 is significantly lower than the release by the strains (p-values <0.05). P-limitation did not significantly
203 affect the net quantity of HP-DOM (Table 1).

204

205 *HP-DOM fluorescence quality*

206

207 FDOM signals were negligible at the onset of the experiments, but these were markedly noticeable at
208 T_f (Fig S1). Thus, we considered this fluorescence as derived from HP. The fluorescent signal at T_f was
209 decomposed into 6 fluorescence components with distinct properties (Fig S2; Table S3): C_{340} (ex
210 275/em 340, protein-like DOM); C_{460} (ex 270 (385)/em 460, fulvic-like DOM); C_{354} (ex 290/em 354,
211 protein- or phenolic-like DOM); C_{398} (ex 315/em 398, microbial humic-like DOM); C_{440} (ex 340
212 (265)/em 440, humic-like DOM); and C_{424} (ex 250/em 424, humic-like DOM).

213 The DOM released by *P. angustum* and HP communities SOLA-fall and SOLA-spring was enriched in
214 protein-like C_{340} and humic-like C_{424} components, in contrast to *S. alaskensis* that rather released DOM
215 enriched in humic-like C_{460} and C_{398} components, as shown by the CA (Fig. 2).

216 EEMs in the unbalanced treatment showed a more humic-like signature (except SOLA-spring, Fig 3a).

217 DOM released under balanced conditions was significantly enriched in the protein-like component C_{340} ,
218 compared to unbalanced, in all experiments except for *S. alaskensis* (Fig. 3b). Conversely, we detected
219 higher microbial humic-like C_{398} in the unbalanced treatment in both single strains and SOLA-fall, but
220 not in SOLA-spring (Fig. 3d). Humic-like components C_{460} and C_{440} were equally released under both

221 treatments (p-value > 0.05) in all experiments (Table S5). The humic-like C₄₂₄ was not different among
222 treatments in *P. angustum* and SOLA-spring and not detected in the balanced treatments for both *S.*
223 *alaskensis* and SOLA-fall (Table S5).

224 The protein-like C₃₄₀ showed a significant (p-value <0.01) and positive relationship to the growth rates
225 under P-repletion (Fig. 4a) but this relationship was not evidenced under P-limitation (Fig. 4b).
226 Conversely, the humic-like components C₄₆₀ and C₃₉₈ were negatively related to the growth rates in
227 both treatments (p-value <0.01 and p-value <0.05 respectively). The other components were not
228 significantly related to growth rates (Table S6).

229

230 ***Discussion***

231

232 In this study, using 2 marine bacterial strains with contrasting lifestyles and 2 natural HP communities
233 from different seasons, we showed that P-limitation influences the quality of HP-DOM, but not the net
234 quantity released to the media. In addition, under P-replete conditions, the quality of the released FDOM
235 was related to the growth rates.

236 Significant amounts of DOM were released in all experiments regardless of the nutrient condition, in
237 line with previous studies showing that HP can be an important DOM source (Gruber et al., 2006; Koch
238 et al., 2014; Ogawa, 2001; Ortega-Retuerta et al., 2021). Interestingly, DOM released by natural HP
239 communities was lower than DOM released by the 2 single strains. This could be due to trophic
240 cooperation in mixed communities (mainly SOLA-fall), i.e. DOM released by some taxa can be
241 consumed by others within the community (Fritts et al., 2021). Contrary to the single strains, natural
242 communities appeared to be less well adapted to efficiently uptake glucose (Table S4), also suggested
243 by (Lønborg et al., 2019). We cannot exclude that in the mixed communities, viral lysis could contribute
244 to some extent releasing back some glucose and monosaccharides to the media.

245 P-limitation did not induce an increase in the net HP-DOM released during our experiments. However,
246 DOM release per biomass unit tended to be higher (although non-significant) in the unbalanced
247 treatments in single strain experiments (Fig S3). In line with this, a previous study by Thompson &
248 Cotner (2020) showed higher DOM release at high media C:P ratios (P-limitation) for bacteria with

249 strong internal elemental homeostasis. In the case of the mixed natural communities, this trend was not
250 clear especially since the HP-DOM release was much lower.

251 In the present study, growth efficiencies were also lower under P-limitation while the amount of glucose
252 consumed was similar between treatments in all experiments (Table S4). Since no significantly higher
253 DOM was released (although visible in some experiments, Fig S3), this suggests that under P-limitation
254 a higher proportion of the initial C-glucose was respired instead of being incorporated into biomass.
255 This is in line with previous studies showing an enhanced growth efficiency when P is available
256 (Guillemette & del Giorgio, 2012; Smith & Prairie, 2004). Together, these results suggest that the effect
257 of P-limitation on HP-DOM release is complex and could vary among microbial taxa depending on
258 their metabolic strategies or on the presence of cooperation processes in the case of mixed communities.
259 Even though HP-DOM quantity was similar among treatments, the FDOM fingerprints hint towards
260 different HP-DOM chemical composition driven by P (Fig. 3). The microbial humic-like fluorescence
261 (C_{398}), which increased in the P limited treatments of the strains and the natural community from fall,
262 has been used as a marker for microbially derived recalcitrant DOM in the ocean (Catalá et al., 2015;
263 Yamashita & Tanoue, 2008). Our results thus suggest that P-limitation promotes the release of more
264 presumably recalcitrant FDOM by HP. A possible explanation for this is the adaptation strategies of the
265 cells to P depletion. To reduce their P requirements, heterotrophic bacteria can substitute P-containing
266 lipids by P-free lipids in the cellular membrane (Chan et al., 2012; Sebastián et al., 2016). It has also
267 been observed that nutrient limitation, namely P, in the presence of excess glucose, leads to the
268 excretion of “overflow-metabolites”, i.e. intermediary metabolites resulting from alternative metabolic
269 pathways used to reduce the involvement of the limiting nutrient (Krämer, 1994; Neijssel & Tempest,
270 1975). We can speculate that the compounds released through these adaptive metabolic pathways have
271 a distinct fluorescence signature, likely similar to humic-like fluorescence.

272 Interestingly, the natural community from spring did not follow this trend. A different community
273 composition between seasons (Lambert et al., 2019) could explain this different response, since
274 different prokaryotic taxa have contrasting responses to P-limitation (Sebastián and Gasol 2013) .

275 In contrast, the protein-like fluorescence signal was generally higher under balanced growth and
276 decreased with P-limitation. In line with our result, Romano et al. (2014) showed that under P-surplus

277 conditions, peptides were one of the main secreted compounds by *Pseudovibrio* sp. FO-BEG1.
278 However, this was not the case for *S. alaskensis*, showing overall a low protein-like signature that
279 increased under P-limitation. *S. alaskensis*, a model oligotroph (Cavicchioli et al., 2003), presents low
280 growth rates, while *P. angustum* and natural HP communities grew faster. As confirmed in our study,
281 the release of protein-like FDOM under P-repletion is related to growth rates. This emphasizes the role
282 of the different growth strategies between the considered HP, in agreement with (Weissman et al., 2021)
283 who demonstrated that gene families involved in rapid protein production were overrepresented in
284 genomes of fast-growing copiotrophs relative to slow-growing oligotrophs. In the other hand, growth
285 rates did not significantly vary between conditions while protein-like fluorescence changed, indicating
286 that growth rate is not the only predictor of protein-like release. Indeed, P-limitation also affects
287 secondary metabolism of marine bacteria (e.g. Martín, 2004; Romano et al., 2015). This latter study
288 (and references within) detected several proteins potentially involved in oxidative stress response and
289 antibiotic production. It is likely that bacteria invest more in stress coping metabolism than in growth
290 when P starved and this could explain the uncoupling of growth and protein-like FDOM release under
291 P-limitation.

292

293 ***Conclusion***

294

295 We could demonstrate that P-limitation plays an important role in HP-DOM release, leading to a relative
296 increase in the likely recalcitrant (based on previous evidences) humic-like FDOM with a decrease in
297 the presumably labile protein-like. We hypothesize that P availability would thus play an important role
298 shaping the microbial carbon pump. Further work is needed to confirm the influence of P-limitation on
299 the bioavailability of HP-derived DOM.

300

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Tables

Table 1 Growth parameters and DOM quantification in all experiments: Prokaryotic growth efficiency PGE (mean \pm standard deviation), growth rate (mean \pm standard deviation), heterotrophic prokaryotic DOM (HP-DOM) net quantity (mean \pm standard deviation), percentage of initial C-glucose released as HP-DOM. Letters indicate significant differences, n.d. = non-determined.

Figures

Fig. 1 Changes in cell abundance in *P. angustum* (a), *S. alaskensis* (b), SOLA-fall (c), and SOLA-spring (d) experiments. Dots represent the experimental replicates and lines represent mean cell abundances. Note the different scales for x and y axes.

Fig. 2 Biplot of the first 2 axes of correspondence analysis (CA) performed on the PARAFAC components, each dot representing an experimental replicate (See material and methods and Supporting information for details on replicates). C₃₄₀: protein-like; C₄₆₀: fulvic like; C₃₅₄: protein-/phenolic-like; C₃₉₈: microbial humic-like; C₄₄₀: terrestrial humic-like; C₄₂₄: humic-like.

Fig. 3 FDOM characterisation at T_f. EEMs contour plots at T_f (only one out of the replicates is shown) (a). Red labels show modelled component location, only primary excitation peak is considered. Note the difference in colormap scale. FDOM Maximum fluorescence intensities of Protein-like C₃₄₀ component (c) and microbial humic-like C₃₉₈ component (d) at T_f in the different experimental treatments. Dots represent replicates (See material and methods and Supporting information for details on replicates).

Fig. 4 Linear regression between the protein-like component C₃₄₀ and growth rate in (a) balanced treatment and (b) unbalanced treatment. Dots represent replicates (See material and methods and Supporting information for details on replicates).

Tables

Table 2 Growth parameters and DOM quantification in all experiments: Prokaryotic growth efficiency PGE (mean \pm standard deviation), growth rate (mean \pm standard deviation), heterotrophic prokaryotic DOM (HP-DOM) net quantity (mean \pm standard deviation), percentage of initial C-glucose released as HP-DOM. Letters indicate significant differences, n.d. = non-determined.

<i>Experiment-Treatment</i>	<i>Growth rate (h⁻¹)</i>	<i>PGE (%)</i>	<i>HP-DOM ($\mu\text{mol L}^{-1}$)</i>	<i>% to initial C-Glucose</i>
<i>P. angustum</i>				
Balanced	0.480 \pm 0.039 ^a	21.94 \pm 0.27 ^a	50.5 \pm 18.3 ^a	21 \pm 7%
Unbalanced	0.418 \pm 0.042 ^a	16.15 \pm 0.31 ^b	60.2 \pm 44.9 ^a	30 \pm 23%
<i>S. alaskensis</i>				
Balanced	0.093 \pm 0.002 ^a	34.82 \pm 1.64 ^a	52.4 \pm 8.1 ^a	28 \pm 4%
Unbalanced	0.085 \pm 0.003 ^a	12.02 \pm 0.69 ^b	52.1 \pm 13.8 ^a	23 \pm 6%
SOLA-fall				
Balanced	0.500 \pm 0.035 ^a	23.06 \pm 3.53 ^a	18.2 \pm 4.8 ^a	10 \pm 3%
Unbalanced	0.475 \pm 0.028 ^a	9.19 \pm 0.18 ^b	5.3 \pm 5.5 ^a	3 \pm 3%
SOLA-spring				
Balanced	0.221 \pm 0.08 ^a	10.51 \pm 3.22 ^a	43.2 \pm 15.5	18 \pm 7%
Unbalanced	0.179 \pm 0.097 ^a	4.04 \pm 0.20 ^b	n.d.	n.d.

Figures

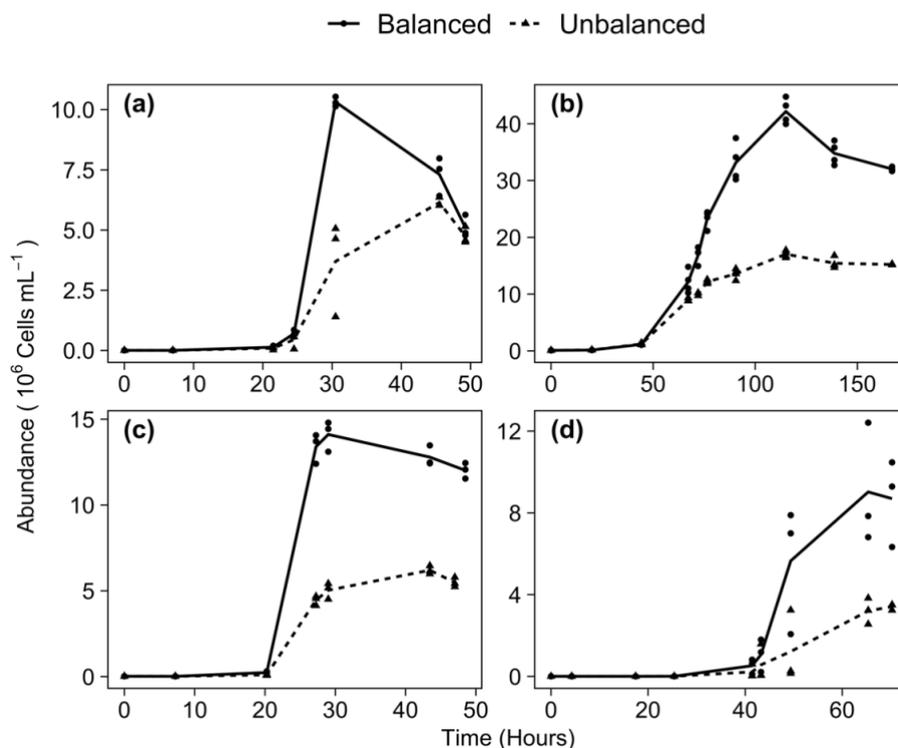


Fig. 5 Changes in cell abundance in *P. angustum* (a), *S. alaskensis* (b), SOLA-fall (c), and SOLA-spring (d) experiments. Dots represent the experimental replicates and lines represent mean cell abundances. Note the different scales for x and y axes.

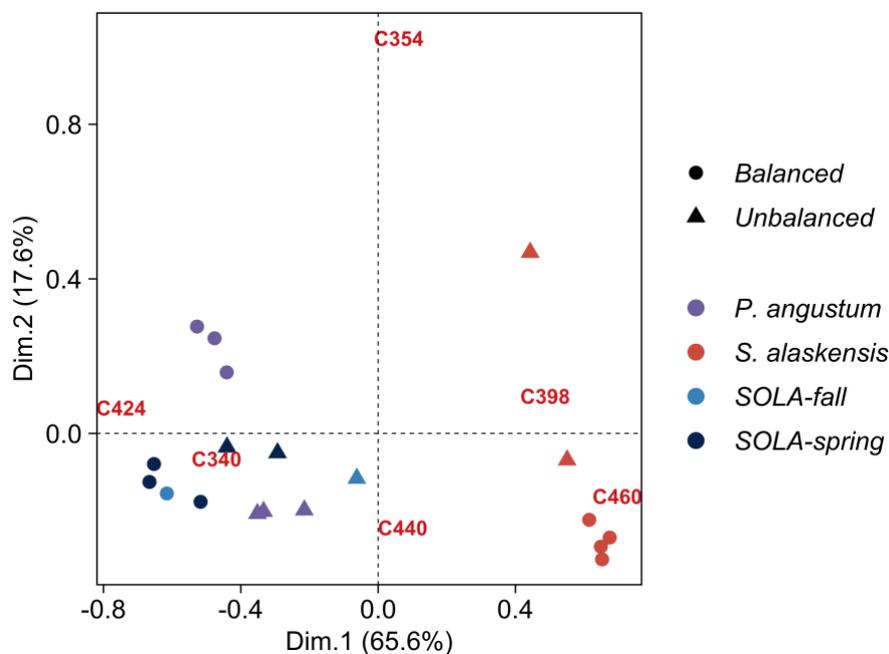


Fig. 6 Biplot of the first 2 axes of correspondence analysis (CA) performed on the PARAFAC components, each dot representing an experimental replicate (See material and methods and Supporting information for details on replicates). C₃₄₀: protein-like; C₄₆₀: fulvic like; C₃₅₄: protein-/phenolic-like; C₃₉₈: microbial humic-like; C₄₄₀: terrestrial humic-like; C₄₂₄: humic-like.

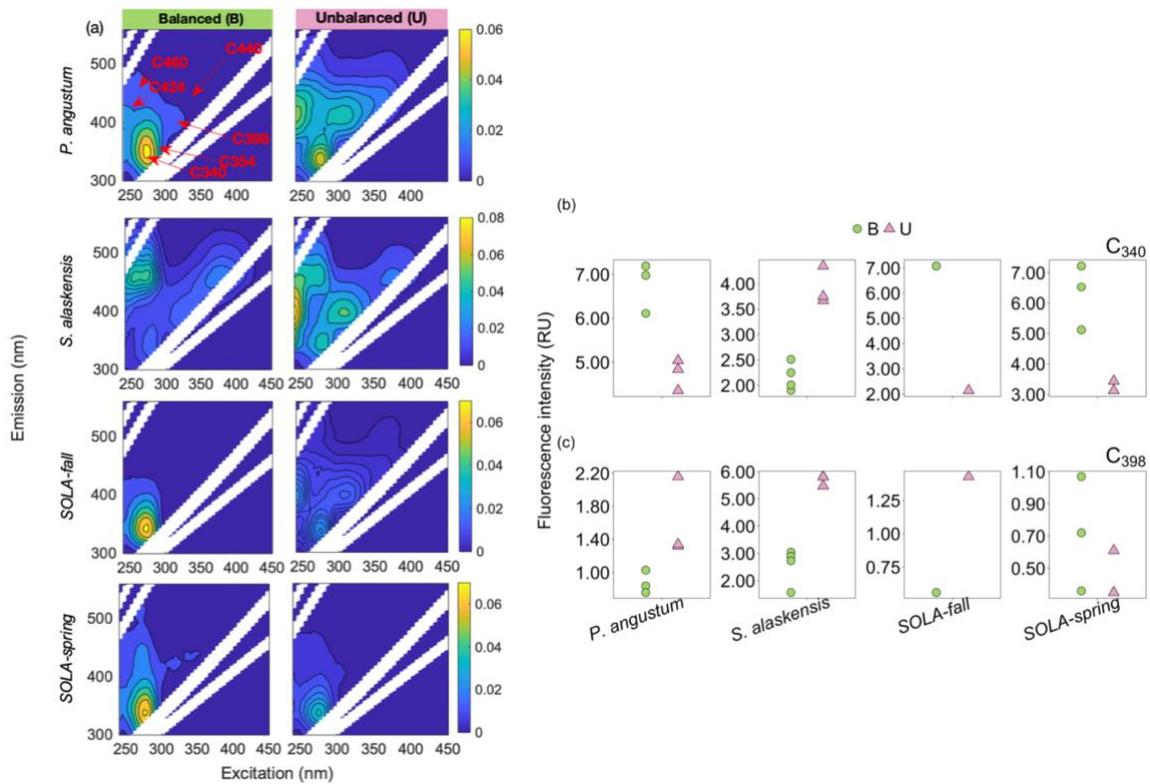


Fig. 7 FDOM characterisation at T_f . EEMs contour plots at T_f (only one out of the replicates is shown) (a). Red labels show modelled component location, only primary excitation peak is considered. Note the difference in colormap scale. FDOM Maximum fluorescence intensities of Protein-like C_{340} component (b) and microbial humic-like C_{398} component (c) at T_f in the different experimental treatments. Dots represent replicates (See material and methods and Supporting information for details on replicates).

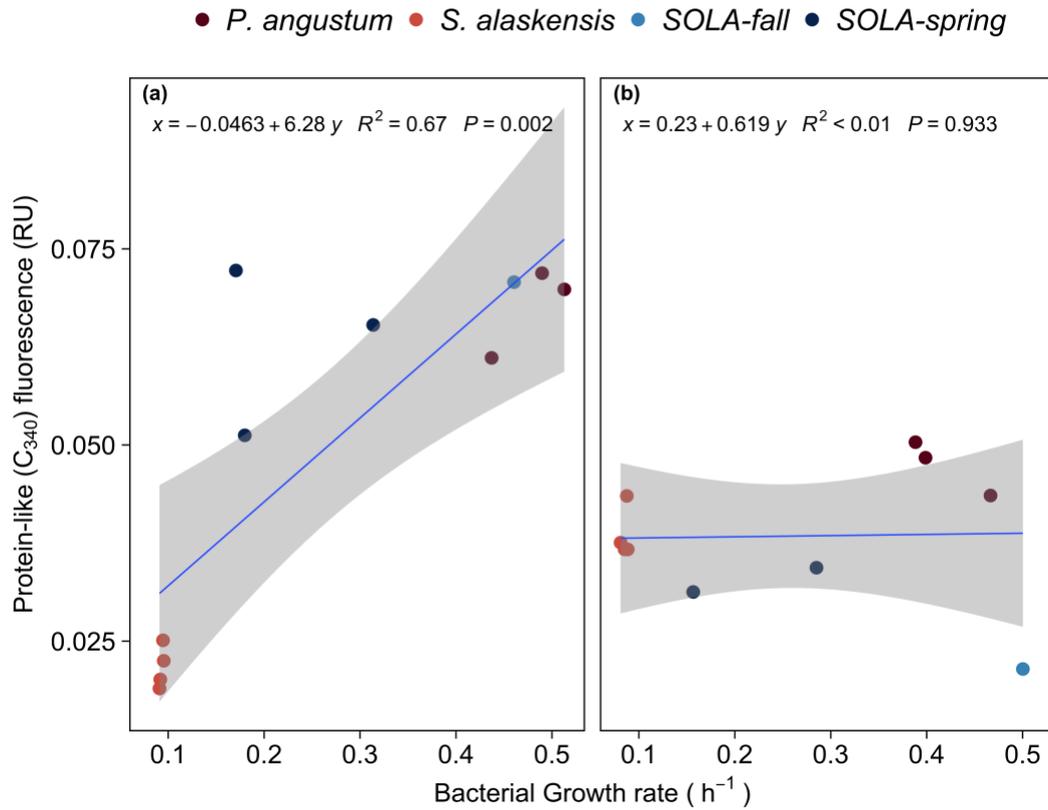


Fig. 8 Linear regression between the protein-like component C_{340} and growth rate in (a) balanced treatment and (b) unbalanced treatment. Dots represent replicates (See material and methods and Supporting information for details on replicates).

Supporting Information

Supplementary material and methods

Heterotrophic prokaryotes abundance

HP growth was followed by flow cytometry using a Beckman CytoFLEX bench instrument. 500 μL samples were fixed with glutaraldehyde (0.5 % final concentration) and preserved at $-80\text{ }^{\circ}\text{C}$ prior to analysis. Just before analysis samples were thawed and stained with SYBR Green (0.025% (v/v) final concentration) and fluorescent beads ($1\mu\text{m}$) were added as an internal standard. Samples were run at medium speed ($30\ \mu\text{L}\cdot\text{min}^{-1}$) for 60 seconds. Prokaryotes were identified in plots of side scatter (SSC) vs. green fluorescence (FITC).

Chemical analyses

Samples for dissolved organic carbon (DOC) concentration were taken into 10 mL pre-combusted glass ampoules, acidified with 85 % H_3PO_4 to $\text{pH}=2$. The ampoules were flame-sealed and stored in the dark at room temperature until analysis. DOC was analyzed using the high temperature catalytic oxidation technique with a Shimadzu TOC-L analyzer (Benner and Strom 1993). Calibration curves were made using an acetanilide solution ($\text{C}_8\text{H}_9\text{NO}$) and deep seawater standards ($44\text{--}45\ \mu\text{mol C L}^{-1}$), provided by the Consensus Reference Material Hansell program (Univ. of Miami), were used to assess the accuracy of the measurements.

Samples ($200\ \mu\text{L}$) for dissolved glucose were stored in pre-combusted glass vials at $-20\text{ }^{\circ}\text{C}$ until analysis. Glucose quantification was done based on an adapted colorimetric protocol from Myklestad et al. (1997). The reagents were calibrated daily using a standard curve made of D-Glucose. MilliQ water and artificial sea water blanks were ran in triplicate before and after each batch of samples. Glucose concentrations were expressed as $\mu\text{mol L}^{-1}$ of carbon equivalent (C-glucose). This approach detects not only glucose but also other dissolved monosaccharides.

Samples for inorganic nutrients ($11\ \text{ml}$, NO_3^- , NO_2^- , and PO_4^{3-}) were stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Quantification was done using segmented flow analyzer (Bran+Luebbe) with colorimetric detection

using methods described in Aminot and K erouel (2007). The accuracy of the method was assessed using reference material (Certipur, Merck). The precisions were in the range of 1–4%, and the detection limits were 0.02 $\mu\text{mol L}^{-1}$ for NO_3^- , 0.01 $\mu\text{mol L}^{-1}$ for NO_2^- and PO_4^{3-} . NH_4^+ (7 mL) samples were collected into pre-rinsed tubes and analyzed freshly by fluorometry using a DeNovix QFX Fluorometer.

FDOM measurements

In addition to the EEMs, 5 discrete excitation emission pairs were measured as described in (Coble, 2007), in all of the experimental replicates. These excitation emission pairs are: 280 nm/350 nm (peak T) and 275 nm/310 nm (peak B) for tryptophan and tyrosine-like substances respectively; 250 nm/435 nm (peak A) for humic substances of different origins; 340 nm/440 nm (peak C) for terrestrial humic-like; 320 nm/410 nm (peak M) for marine humic-like substances. In SOLA-fall experiment, we recorded an EEM in only one randomly chosen replicate per treatment due to time constraints. In this experiment, fluorescence measurements of the 5 discrete excitation and emission pairs showed differences between triplicates from 0.1 to 16 %.

PARAFAC analysis method details

The data set used for PARAFAC modeling consisted of 113 EEMs, including 42 samples from the HP-DOM release experiments, of which 21 were measured during the exponential growth phase (data not shown), and 71 samples from HP-DOM degradation experiments conducted using the same HP-DOM (data not shown). T_0 samples were not included (very low fluorescent signal, Fig S1).

Raman and Rayleigh scattering bands were excised from all the dataset and replaced with missing values. Several models of 2-8 components were then fitted with a non-negative constraint using ‘outliertest’ function (drEEM Toolbox) in order to detect outlier samples and/or wavelengths. At this step, noisy measurements at excitation 240 nm were removed from all EEMs, and one replicate from SOLA-spring experiment (unbalanced treatment) was detected as an outlier and excluded. Based on these preliminary models’ spectral properties, residuals and errors, the 6-component model was determined to be the best fit for this dataset and explained 99.3% of the variance. A final 6-component

model was computed out of 50 random initializations with a convergence criterion of 10^{-8} . The model was then split-half validated by dividing the dataset into 2 randomly selected halves as described by (Murphy et al. 2013). All model's components matched in all the splits with a Tucker Congruence Coefficient (TCC) > 0.95 in the excitation and emission loadings. The final model was compared to previously developed fluorescence DOM models on the OpenFluor database (Murphy et al. 2014). The number of matches was restricted to a threshold of TCC > 0.96 for excitation and TCC > 0.97 for emissions. All the components yielded several matching models allowing to describe their properties (Table S3).

Supplementary tables

Table S3 Minimum media composition

Salt	Final concentration (g L ⁻¹)
NaCl	24
Na ₂ SO ₄	4
KCl	0.68
KBr	0.1
H ₃ BO ₃	0.025
NaF	0.002
MgCl ₂ .H ₂ O	10.8
CaCl ₂	1.5
SrCl ₂	0.024
NaHCO ₃	0.2

Metal	Final concentration (μg L ⁻¹)
CuCl ₂ .H ₂ O	15 x 10 ⁻³
NiCl ₂ .H ₂ O	25 x 10 ⁻³
NaMOO ₄ .2H ₂ O	25 x 10 ⁻³
ZnSO ₄ .7H ₂ O	70 x 10 ⁻³
MnCl ₂ .4H ₂ O	100 x 10 ⁻³
CoCl ₂ .6H ₂ O	120 x 10 ⁻³
FeEDTA	0.068 μM C

Vitamins	Final concentration (nmol C L ⁻¹)
P-aminobenzoic acid	2.6
D-biotin	0.8
Folic Acid	1
D-pantothenic acid	2.5
Pyridoxal	0.9
Pyridoxamine	4.8
Pyridoxine	5.2
Riboflavin	2.4
Thiamine	0.9
D L-6,8-thioctic acid	1
Vitamin B12	2.8
Nicotinamide	3.2

Table S4 Biomass conversion factors used for prokaryotes growth efficiencies calculation.

Conversion factors were chosen to account for the differences in cell size and life styles. For *S. alaskensis* the fgC Cell⁻¹ was obtained by converting fgC μm^{-3} to fgC Cell⁻¹ considering a cell volume of 0.05 μm^3 (Eguchi et al. 2001) and a volume to C content conversion factor of 380 fgC μm^{-3} (Lee and Fuhrman 1987). We chose a higher conversion factor for the copiotrophic *P. angustum* based on a *Vibrio* C content estimate (Fagerbakke et al. 1996). Since our natural communities come from coastal water, we used the conversion factor for coastal bacteria reported in literature (Fukuda et. al. 1998).

Strain	Conversion factor	Reference
<i>P. angustum</i>	62 fgC Cell ⁻¹	(Fagerbakke et al. 1996)
SOLA-fall SOLA-spring	30.2 fgC Cell ⁻¹	(Fukuda et al. 1998)
<i>S. alaskensis</i>	19 fgC Cell ⁻¹	(Lee and Fuhrman 1987; Eguchi et al. 2001)

Table S3 Characteristics of the six components identified by PARAFAC analysis, *ex* = excitation and *em* = emission, examples of matching models for external validation and components classification into known fluorescence types.

Component	Ex	Em	Examples of matching PARAFAC models build in marine or freshwater systems (OpenFluor)		Fluorescence type classification	Classification reference
			Component ex/em	Source and Reference		
C ₃₄₀	275	340	C1 275/342 C6 275/334	Coastal (Brym et al. 2014); Boreal lakes (Lapierre and del Giorgio 2014)	Protein-like (Tryptophan-like)	(Coble 2007; Stedmon and Markager 2005)
C ₄₆₀	270 (385)	460	C2 270(380)/476 C5 260(385)/460	Estuary (Bittar et al. 2016); River (Gonçalves-Araujo et al. 2015)	Fulvic-like and humic-like	(Coble 2007)
C ₃₅₄	290	354	C6 240/350 C4 248/355	Streams (Osburn et al. 2018); Lake (Catalá et al. 2016)	Protein- or phenolic-like related to processed humic-like material	(Catalán et al. 2021)
C ₃₉₈	315	398	C3 240(310)/403	Estuary (Wang et al. 2020);	Marine/Microbial humic-like	(Yamashita et al. 2008; Coble 2007)
C ₄₄₀	340(265)	440	C3 350/428	Waste water* (Murphy et al. 2011)	Terrestrial humic-like (C)	(Coble 2007)
C ₄₂₄	250	424	C1 275/424 C1 250 (295)/412	Boreal lakes (Lapierre and del Giorgio 2014) Lake (Du et al. 2016)	Humic-like from diverse origins (A)	(Coble 2007)

* Only one matching model

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Table S4 Initial and final C-glucose and inorganic nutrient concentrations (mean \pm standard deviation), percentage of C-glucose consumed and final DOC concentrations (mean \pm standard deviation) in the different treatments (B = Balanced, U= Unbalanced), n.d.=not determined.

Experiment-Treatment	C-glucose $\mu\text{mol L}^{-1}$		NH_4^+ $\mu\text{mol L}^{-1}$		PO_4^{3-} $\mu\text{mol L}^{-1}$		C-glucose consumption (%)	DOC $\mu\text{mol L}^{-1}$	
	T ₀	T _f	T ₀	T _f	T ₀	T _f		T ₀	T _f
<i>P. angustum</i>									
B	242.6	2.8 \pm 3.2	35.3	19.8 \pm 0.7	4.51	2.16 \pm 0.03	99.1	218.7	52.6 \pm 14.1
U	197.9	4.3 \pm 1.3	33.4	23.6 \pm 0.4	0.91	0.31 \pm 0.40	97.8	221.9	64.6 \pm 45.0
<i>S. alaskensis</i>									
B	185.1	1.8 \pm 1.7	30.1	8.8 \pm 0.6	4.49	2.84 \pm 0.11	99.0	247.5	53.2 \pm 5.2
U	223.9	9.4 \pm 9.2	32.8	12.0 \pm 0.5	0.68	0.18 \pm 0.21	95.8	271.5	57.7 \pm 6.7
SOLA-fall									
B	174.6	12.5 \pm 18.3	35.2	21.1 \pm 1.0	4.51	1.87 \pm 0.17	92.8	164.9	18.2 \pm 1.0
U	200.4	23.4 \pm 3.0	36.0	24.8 \pm 0.8	0.59	0.06 \pm 0.03	88.3	162	28.6 \pm 1.3
SOLA-spring									
B	245.9	18.2 \pm 5.4	31.6	16.6 \pm 0.9	4.27	2.20 \pm 0.16	92.6	214.8	61.4 \pm 19.0
U	247.5	22.5 \pm 5.3	32.7	21.4 \pm 0.4	0.63	0.06 \pm 0.03	90.9	224.5	n.d.*

* Two out of 3 replicates showed unexpected values ($> 200 \mu\text{mol L}^{-1}$) and suspected to be DOC contaminated and thus SOLA-spring U DOC values were not considered.

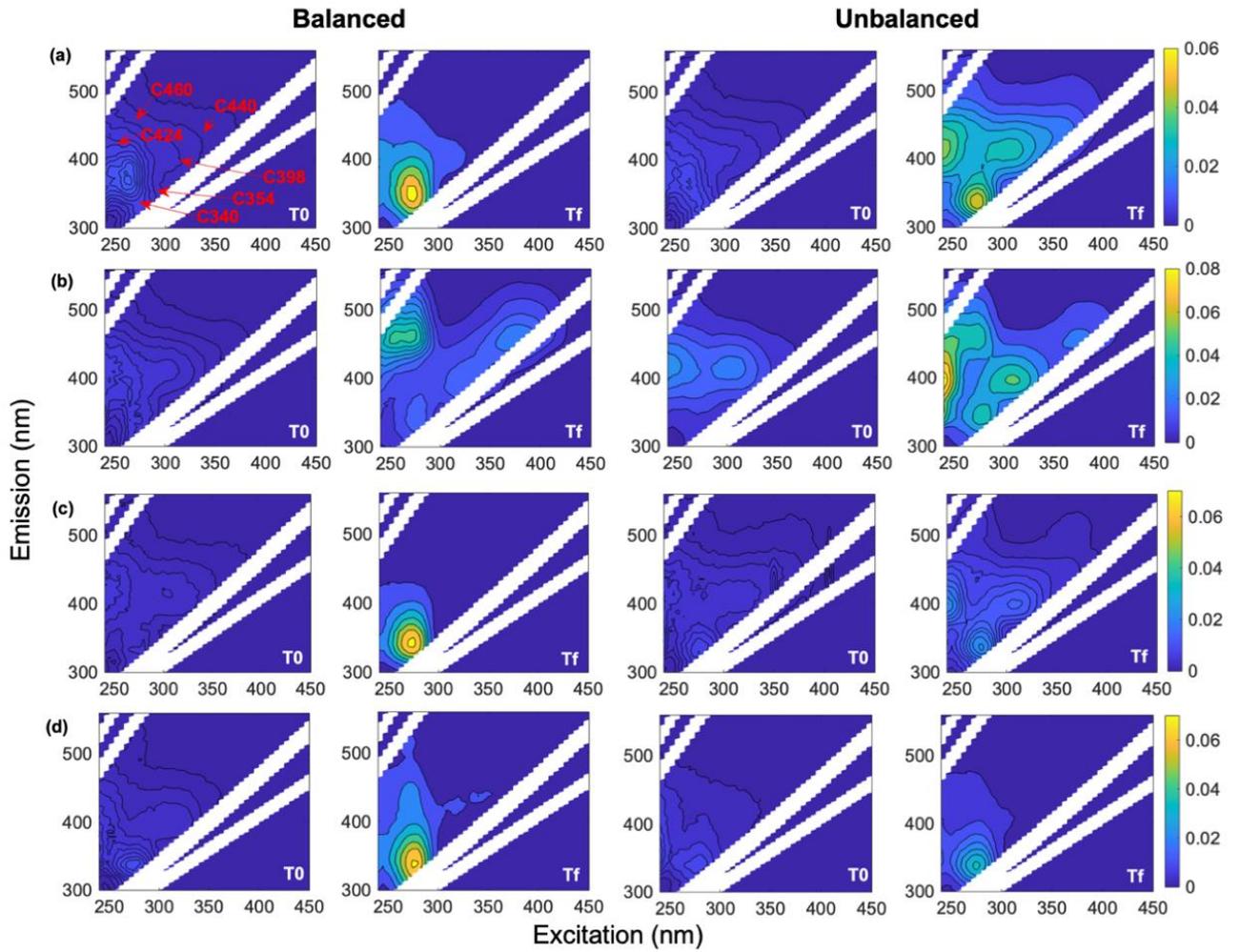
24 **Table S5** Maximum fluorescence intensities of all components at Tf in the different treatments (B =
 25 Balanced, U= Unbalanced). Values are expressed in ($\times 10^{-2}$ RU)

Experiment-Condition	C ₃₄₀	C ₄₆₀	C ₃₅₄	C ₃₉₈	C ₄₄₀	C ₄₂₄
<i>P. angustum</i>						
B	6.76 ± 0.57 ^a	0.86 ^a ± 0.33	1.27 ± 0.14 ^a	0.88 ^a ± 0.14	0.75 ± 0.36 ^a	1.15 ± 0.47 ^a
U	4.74 ± 0.35 ^b	0.89 ^a ± 0.40	0.00 ± 0.00 ^a	1.60 ^a ± 0.48	0.96 ± 0.65 ^a	0.76 ± 0.87 ^a
<i>S. alaskensis</i>						
B	2.17 ± 0.27 ^a	4.50 ^a ± 0.16	0.11 ± 0.08 ^a	2.56 ^a ± 0.67	1.02 ± 0.11 ^a	0.00 ± 0.00 ^a
U	3.86 ± 0.33 ^b	4.24 ^a ± 0.38	10.63 ± 17.49 ^a	5.65 ^b ± 0.20	0.84 ± 0.10 ^a	0.18 ± 0.15 ^a
SOLA-fall						
B	7.08 ± n.d	0.45 ^a ± n.d	0.20 ± n.d	0.56 ± n.d	0.29 ± n.d	0.00 ± n.d
U	2.14 ± n.d	0.53 ^a ± n.d	0.01 ± n.d	1.43 ± n.d	0.37 ± n.d	0.31 ± n.d
SOLA-spring						
B	6.29 ± 1.07 ^a	0.53 ^a ± 0.58	0.19 ± 0.16 ^a	0.72 ^a ± 0.36	0.55 ± 0.28 ^a	0.89 ± 0.34 ^a
U	3.28 ± 0.22 ^b	0.59 ^a ± 0.10	0.28 ± 0.02 ^a	0.48 ^a ± 0.19	0.37 ± 0.07 ^a	0.15 ± 0.03 ^a

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 27 **Table S6** Results of linear regression analysis between maximum fluorescence of the different FDOM
 28 components and growth rate in the different experimental treatments (B = Balanced, U= Unbalanced).

Component	Treatment	Equation	p-value	r ²
C ₄₆₀	B	$y = 0.4 - 6.7 x$	0.009	0.55
	U	$y = 0.4 - 7.6 x$	0.005	0.65
C ₃₅₄	B	$y = 0.2 + 26.6 x$	0.003	0.65
	U	$y = 0.3 - 0.6 x$	0.3	0.16
C ₃₉₈	B	$y = 0.4 - 12.0 x$	0.02	0.46
	U	$y = 0.4 - 5.4 x$	0.02	0.53
C ₄₄₀	B	$y = 0.4 - 23.5 x$	0.2	0.19
	U	$y = 0.2 + 1.6 x$	0.9	0.0013
C ₄₂₄	B	$y = 0.2 + 16.9 x$	0.06	0.33
	U	$y = 0.2 + 17.2 x$	0.1	0.25

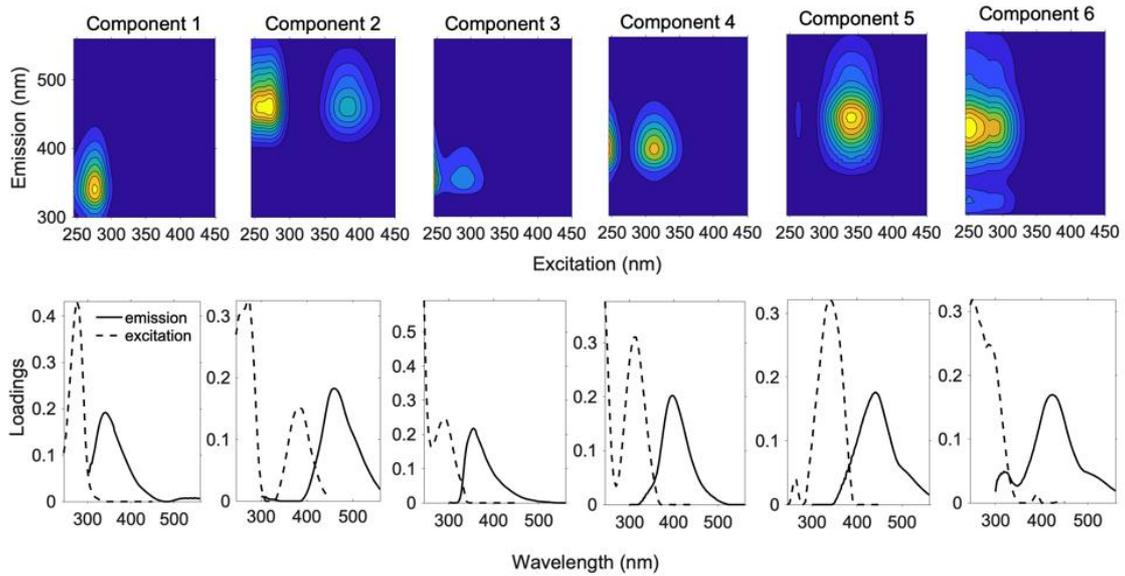
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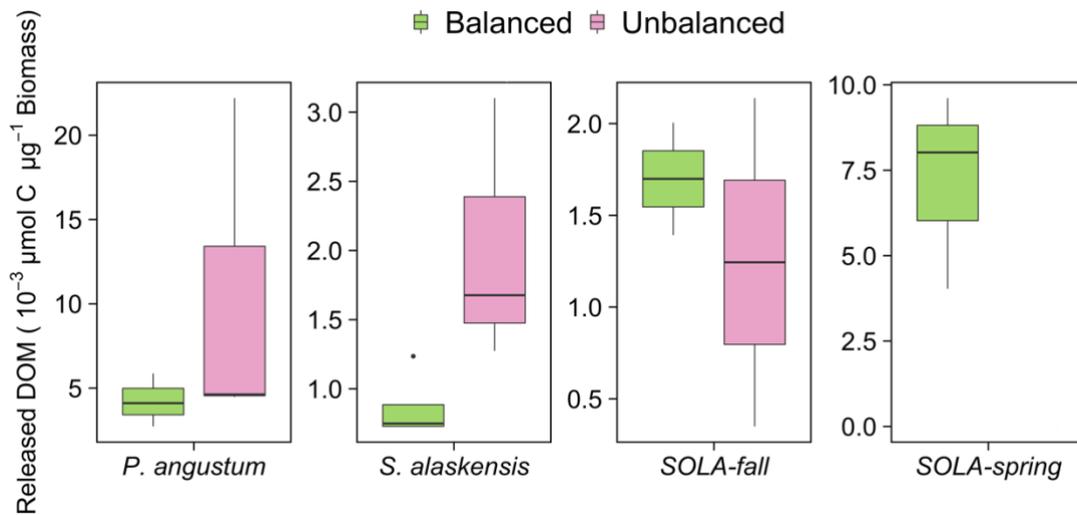
54 *Fig S1 EEMs contour plots at T_0 and T_f (only one out of the replicates is shown): (a) *P. angustum*, (b)*
 55 *S. alaskensis*, (c) SOLA-fall, (d) SOLA-spring. Red labels show modelled component location, only
 56 *primary excitation peak is considered. Note the difference in colormap scale.*

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Fig S2 PARAFAC model results showing fluorescence fingerprint of six components (upper panel) and excitation emission spectra (lower panel)



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*Fig S3 Dissolved organic matter release at the end of the experiment expressed as DOC concentration per unit of integrated biomass (T_0 to T_f) in all experiments. Hinges correspond to median, first and third quartiles. Whiskers extended from hinges to the smallest and largest values (no further than $1.5 * IQR$ from the hinge, where IQR is the inter-quartile range). **No significant difference between balanced and unbalanced over all experiments (ANOVA, p -value >0.1).***

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