

1 **Summer dynamics drive the microbial response to DOC additions**  
2 **in a high-altitude lake**

3 *Running head:* DOC additions on planktonic interactions

4 Flavia Dory<sup>a\*</sup>, Laurent Cavalli<sup>a</sup>, Evelyne Franquet<sup>a</sup>, Magalie Claeys-Bruno<sup>a</sup>, Benjamin  
5 Misson<sup>b</sup>, Thierry Tatoni<sup>a</sup>, Céline Bertrand<sup>a</sup>

6 <sup>a</sup> Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France

7 <sup>b</sup> Université de Toulon, Aix Marseille Univ, CNRS, IRD, MIO, Toulon, France

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9 **Contact Information**

10 **\*Corresponding author: Flavia Dory**, Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE,  
11 Marseille, France

12 **E-mail address:** [flavia.dory@imbe.fr](mailto:flavia.dory@imbe.fr) ; Phone: 04 13 94 49 85

13 Orcid ID: <https://orcid.org/0000-0002-5083-0737>

14

15 [laurent.cavalli@imbe.fr](mailto:laurent.cavalli@imbe.fr) ; <https://orcid.org/0000-0001-9151-3247>

16 [evelyne.franquet@imbe.fr](mailto:evelyne.franquet@imbe.fr) ; <https://orcid.org/0000-0002-7779-772X>

17 [magalie.claeys-bruno@imbe.fr](mailto:magalie.claeys-bruno@imbe.fr) ; <https://orcid.org/0000-0001-7168-2190>

18 [benjamin.misson@mio.osupytheas.fr](mailto:benjamin.misson@mio.osupytheas.fr) ; <https://orcid.org/0000-0002-4107-0916>

19 [thierry.tatoni@imbe.fr](mailto:thierry.tatoni@imbe.fr) ; <https://orcid.org/0000-0002-2557-4444>

20 [celine.bertrand@imbe.fr](mailto:celine.bertrand@imbe.fr) ; <https://orcid.org/0000-0003-3085-3234>

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23 summer phytoplankton dynamics, co-limitation, mixotrophy

24

25 **Abstract**

26

27 1. The predicted increase in allochthonous dissolved organic carbon (DOC) in high-altitude  
28 lakes is expected to alter the phytoplankton-bacterioplankton relationship. However, few  
29 studies address the influence of summer phytoplankton dynamics on planktonic responses  
30 to DOC additions.

31 2. We sampled natural plankton assemblages during two contrasting periods of summer in a  
32 high-altitude lake in the French Alps and subjected them to DOC inputs and nutrient  
33 enrichments under two light conditions (dark or light) and two temperature conditions  
34 (10°C or 18°C).

35 3. Our results indicate that DOC use by bacteria differs over the summer, depending on the  
36 availability of autochthonous DOC and the nutrient limitation. DOC was consumed by  
37 bacteria more in early summer but was assimilated into biomass more in late summer.  
38 This pattern likely arose from the greater availability of phytoplankton-derived DOC in  
39 late summer, reducing the need for alternative carbon sources in late summer, when  
40 phytoplankton biomass was high. We found greater competition between bacteria and  
41 phytoplankton in late summer after DOC addition, linked to the summer nutrient  
42 limitation pattern. Mixotrophic taxa were stimulated after DOC additions both in early  
43 summer and in late summer. Our study thus highlights a differential response depending  
44 on the timing of summer DOC inputs.

45 4. The global changes forecast for the French Alps should increase heterotrophic and  
46 mixotrophic processes in planktonic communities of shallow high-altitude lakes with  
47 vegetated catchments. This experimental study provides insights that will be useful in  
48 predicting ecological trajectories and in refining predictions of sentinel lakes' responses to  
49 global changes.

50

51

## 1. Introduction

52       Dissolved organic carbon (DOC) inputs associated with terrestrial dissolved organic  
53 matter (DOM) have substantially increased in recent decades in lake ecosystems, and will  
54 likely continue to evolve (Solomon et al., 2015). DOC acts as a major regulator of lakes'  
55 functioning (Williamson et al., 1999). DOC concentration and quality have been shown to  
56 alter water transparency and thermal stratification (Solomon et al., 2015), modify pH and  
57 alkalinity (Evans et al., 2005) and attenuate harmful ultraviolet radiation in lakes (Warner et  
58 al., 2020). By changing the lakes' pool of available nutrients, DOC acts as an important driver  
59 of bacterioplankton biomass (Schulhof et al., 2020), phytoplankton biomass (Hazukova et al.,  
60 2021; Isles et al., 2021) and their relationships in lake ecosystems. Allochthonous DOC inputs  
61 are consequently directly related to the degree of net heterotrophy in lakes (Jansson et al.,  
62 2008). Through its effects on planktonic processes, allochthonous DOC regulates energy  
63 transfer to higher trophic levels (Jansson et al., 2000), food web dynamics and lake  
64 metabolism (Mitrovic & Baldwin, 2016). However, its influence depends on the trophic  
65 nature of lake ecosystems (Dorado-García et al., 2014).

66       In oligotrophic ecosystems, bacteria have an inherent advantage over phytoplankton in  
67 assimilating inorganic nutrients (Currie & Kalff, 1984; Jansson et al., 2006) but their growth  
68 is usually limited by C (Fouilland et al., 2014). Inputs of allochthonous DOC can increase  
69 bacterioplankton biomass and lead to more competitive dynamics between bacteria and  
70 phytoplankton for inorganic nutrients (Carney et al., 2016a; Hitchcock & Mitrovic, 2013).  
71 These changes benefit mixotrophs, who acquire organic carbon and/or mineral nutrients from  
72 their bacterial prey (Caron, 1994), allowing them to eliminate their main competitors for  
73 inorganic nutrients (Thingstad et al., 1996). As different mixotrophic taxa have different  
74 ingestion rates (Flynn et al., 2018), the types of mixotrophs that constitute the communities

75 and their proportions could directly modify predation pressure on bacteria after allochthonous  
76 DOC inputs.

77 However, it has also been observed in temperate lakes that autochthonous DOC derived  
78 from phytoplankton is preferentially consumed and incorporated into biomass over  
79 allochthonous DOC, owing to its greater accessibility and nutritional quality (Guillemette et  
80 al., 2013). The quantity of phytoplankton biomass and primary production, which together  
81 regulate the bioavailability of autochthonous organic carbon, condition the C-limitation of  
82 bacteria and thus bacterial abundance and production in lakes (Ejarque et al., 2018; Gasol &  
83 Duarte, 2000; Kritzberg et al., 2005; Morán et al., 2010). As an illustration, in oligotrophic  
84 ecosystems where phytoplankton-derived organic carbon was sufficient to support bacterial  
85 demands, bacteria did not experimentally respond to C additions like sucrose because of  
86 primary limitation by inorganic nutrients (Dorado-García et al., 2014). The quantity of  
87 phytoplankton biomass, by regulating the quantity of autochthonous DOC bioavailable for  
88 bacteria, could therefore modify the microbial response to allochthonous DOC inputs. In  
89 addition, evidence suggests that the nature of the molecules liberated in the water differs  
90 among phytoplankton species (Romera-Castillo et al., 2010; Sarmiento & Gasol, 2012) and the  
91 uptake of autochthonous DOC by bacteria varies according to the phytoplankton species  
92 composing the bulk of the community (Sarmiento et al., 2013). Thus, phytoplankton biomass  
93 and composition, by exercising a dual bottom-up and top-down control over bacteria, could  
94 impact the microbial response to allochthonous DOC inputs in oligotrophic lakes. However,  
95 little is known about the mechanisms through which phytoplankton biomass and composition  
96 regulate the microbial response to DOC additions in oligotrophic ecosystems.

97 In high-altitude lakes, DOC concentrations are generally low ( $< 1$  mg C/L, see Laurion  
98 et al., 2000), and these ecosystems could respond significantly to increased flux from the  
99 surrounding catchments. In mountain lakes, most DOC is of autochthonous origin during

100 summer (Reche et al., 2001; Sadro & Melack, 2012). However, the observed upward shift in  
101 the treeline (Elliott & Kipfmueller, 2011; Harsch et al., 2009) and the predicted increase in  
102 extreme precipitation events (IPCC, 2013) are likely to increase allochthonous DOC fluxes to  
103 high-altitude lakes. Recent studies highlight the sensitivity of mountain lakes to climate  
104 changes (Moser et al., 2019; Sadro et al., 2019; Smits et al., 2020). Higher DOC values  
105 associated with wetter periods were observed between 2015 and 2017 in subalpine (Ejarque et  
106 al., 2018) and mountain lakes (Moser et al., 2019). This increased carbon flux may cause  
107 dramatic changes in mountain lakes' metabolism (Sadro & Melack, 2012).

108 In high-altitude lakes, rapid environmental changes usually induce rapid modifications of  
109 phytoplankton community composition over a short period (Tiberti et al., 2013; Ventura et al.,  
110 2000). For example, Jacquemin et al. (2018) observed changes in the structure and  
111 composition of phytoplankton communities, associated with an evolution of limitation  
112 patterns, during summer in alpine lakes. Due to poor light conditions, mixotrophic species  
113 dominate autotrophic ones during the ice-covered period (Leppäranta, 2015) and are still  
114 dominant in phytoplankton assemblages in early summer (Waibel et al., 2019). Rapid changes  
115 in chemical parameters are also conditioning biomass dynamics in high-altitude lakes  
116 (Bergström et al., 2020; Oleksy et al., 2020). A bimodal pattern in phytoplankton biomass  
117 dynamics has been observed, with a first peak immediately after the snowmelt and a second  
118 one occurring at the end of the summer period (Tiberti et al., 2013). Since phytoplankton is an  
119 important regulator of bacteria in ecosystems receiving few allochthonous inputs,  
120 phytoplankton communities' seasonal dynamics in terms of biomass and composition should  
121 regulate the impact of DOC inputs on PBR in high-altitude lakes. However, few studies  
122 address the influence of summer phytoplankton dynamics on PBR in response to DOC  
123 additions in high-altitude lakes.

124 This study examines how shifts in phytoplankton community structure and composition  
125 impact the microbial response to DOC additions. We sampled natural plankton assemblages  
126 during two contrasting periods of summer in a high-altitude lake in the French Alps. These  
127 samples received DOC inputs in the form of glucose and nutrient enrichments (N and P)  
128 under controlled laboratory conditions in microcosms. To characterize the influence of  
129 phytoplankton on bacteria, the assemblages were subjected to two light conditions: a dark:  
130 light cycle condition (LIGHT) and a total darkness condition (DARK). The planktonic  
131 organisms were maintained at two temperatures, one reproducing the average temperature of  
132 high-altitude lakes during summer (10°C) and the other that accelerates metabolic processes  
133 (18°C). Firstly, we hypothesised that the effect of DOC additions on planktonic communities  
134 depends on the initial phytoplankton biomass. This means that DOC additions should  
135 stimulate bacteria more in early summer when the initial phytoplankton biomass is expected  
136 to be low. Secondly, we assumed that the communities' response to DOC additions depends  
137 on the initial phytoplankton composition. This means that mixotrophs should benefit less  
138 from DOC additions in late summer, when they are expected to represent a lower proportion  
139 of the initial community than autotrophs.

## 140 **2. Methods**

### 141 **2.1. Study area and sampling**

142

143 Water for experiments was sampled in Lake Cordes, an oligotrophic high-mountain  
144 lake located in the French Alps, adjacent to the Queyras regional natural park. The lake is  
145 located at an elevation of 2446 m.a.s.l. It is shallow (maximum depth = 9 m) and small (1.8  
146 ha) but has a large catchment area (140 ha) with developed soil and meadows (approximately  
147 60% alpine grass cover). Water sampling campaigns were conducted twice over the summer,

148 on 7<sup>th</sup> July 2019 (early summer) and on 28<sup>th</sup> August 2019 (late summer). The campaigns were  
149 characterized by highly contrasting turbidity. Turbidity was high in early summer (average  
150 turbidity = 7 NTU), related to frequent previous storms, but low in late summer (average  
151 turbidity = 0.5 NTU). Water samples totalling 42 L were collected using a Niskin Bottle every  
152 metre to sample the entire water column and were filtered through a 50 µm mesh to exclude  
153 any larger grazers on food web components. Filtered water was stored in 8 L HDPE  
154 containers and transported in a cool box to the laboratory.

## 155 **2.2. Enrichment experiments under controlled conditions**

### 156 **2.2.1. Experimental design**

157 Microbial community was acclimatized overnight in 10 L sterilised transparent  
158 polycarbonate plastic bottles (Nalgene®) at 10°C. Physico-chemical and biological variables  
159 were measured in each bottle to define initial conditions before the start of the experiments.  
160 Then water from each bottle was manually homogenised and transferred to 96 microcosms.  
161 Transparent sterilised polycarbonate plastic bottles (Nalgene®) were filled with 350 ml of  
162 sampled water. In C treatments, glucose was added to the microcosms as an available DOC  
163 source at four different concentrations: no addition [0C], + 2 mg/l [2C], + 4 mg/l [4C] and + 6  
164 mg/l [6C]. In NP treatments, planktonic communities were either not enriched [0NP] or  
165 enriched with N and P. N was added as NH<sub>4</sub>NO<sub>3</sub> sufficient to increase N concentrations to  
166 335 µg/L and P was added as KH<sub>2</sub>PO<sub>4</sub> sufficient to increase P concentrations to 35 µg/L. In  
167 LIGHT conditions, the microcosms were placed under a circadian cycle (Light: dark cycle =  
168 14:10), at a fixed irradiance ( $45 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). In DARK conditions, the microcosms  
169 were placed in the dark in growth chambers, surrounded by aluminium, to target heterotrophic  
170 processes while minimising the influence of autotrophs. Finally, the microcosms were  
171 subjected to temperatures of either 10°C, the average summer temperature, or 18°C, sufficient

172 to accelerate the metabolic processes of organisms without representing a threat to the  
173 planktonic organisms (Jacquemin et al., 2018). Thus, the experimental set-up consisted of a 4  
174 x 2 x 2 x 2 factorial design (32 treatments in triplicate) implemented for 10 days, as in our  
175 previous study (Dory et al., 2021), allowing the estimation of the coefficients of the  
176 interaction effects.

### 177 **2.2.2. Physico-chemical analysis**

178 Average in situ temperatures were calculated from the mean temperatures measured  
179 every metre on the water column during the sampling dates (hourly observations). Sub-  
180 samples for dissolved inorganic nitrogen ( $\text{DIN} = \text{NH}_4^+ + \text{NO}_2^+ + \text{NO}_3^{2-}$ ), soluble reactive  
181 phosphorus ( $\text{SRP} = \text{PO}_4^{2-}$ ), and dissolved organic carbon (DOC) concentrations were filtered  
182 through pre-combusted 25 mm glass filters (Whatman GF/F, 0.7 mm) prior to launching the  
183 experiments. The dissolved fraction for DIN and SRP was stored in a 125 ml HDPE bottle,  
184 and frozen ( $-18^\circ\text{C}$ ) until analysis. DIN and SRP were determined by ionic chromatography  
185 (Metrohm, 930 Compact IC Flex combined with 863 Compact Autosampler). Dissolved  
186 organic carbon was measured under initial conditions and at the end of the experiments.  
187 Subsamples stored in 24 ml pre-combusted (4 hr at  $450^\circ\text{C}$ ) glass tubes (Wheaton equipped  
188 with Teflon/silicone septa) and preserved in 30  $\mu\text{l}$  of sodium azide solution (1 M  $\text{NaN}_3$ ) at  
189  $+4^\circ\text{C}$ . DOC concentration was determined using a TOC-VCSH analyzer (Shimadzu, TOC-V).  
190 Consumed DOC is the difference between the initial DOC concentrations after carbon  
191 additions and the final DOC concentrations measured at the end of the experiments.

### 192 **2.2.3. Biological variables**

#### 193 *Phytoplankton*

194 Phytoplankton were enumerated and identified via microscopy. Phytoplankton  
195 communities were analysed under initial conditions and at the end of the experiments. The  
196 samples destined for phytoplankton composition analysis were fixed with alkaline Lugol



197 solution (0.5%) and stored in 150 ml HDPE bottles at +4°C. Phytoplankton counts were  
198 performed according to the Utermöhl (1958) method, at 40-fold magnification under an  
199 inverted microscope (Olympus IX 70). Phytoplankton samples were identified at genus level  
200 and species level when possible, using appropriate taxonomic guides.

201 We checked for any development of predators in our microcosms by estimating ciliate  
202 abundances via the Utermöhl method, under initial conditions and at the end of the  
203 experiments.

#### 204 *Picoplankton*

205 Sub-samples of 1.5 ml were taken under initial conditions and thereafter every 2  
206 experimental days in the microcosms for picoplankton analyses by flow cytometry.  
207 Subsamples were fixed with glutaraldehyde (0.25% final concentration) and stored at -80°C  
208 until flow cytometry analysis. Picoplankton was characterised and enumerated using an  
209 Accuri C6 flow cytometer equipped with a blue laser (488 nm) and using BD Accuri CFlow  
210 Plus Analysis software (BD-Biosciences). For picophytoplankton, 500 µl of sample were run  
211 at fast speed (66 µl/min). Picophytoplankton were identified according to size (FSC),  
212 complexity (SSC) and fluorescence (emissions in the orange and red wavelength ranges,  
213 respectively  $585 \pm 20$  and  $>670$  nm). Non-fluorescent polystyrene microspheres (Flow  
214 Cytometry Size Calibration Kit, Thermo Fisher Scientific) were used as a size standard. Flow  
215 cytometer analysis distinguished between two picophytoplanktonic groups. Small cells ( $<2$   
216 µm) with high orange fluorescence and low complexity were classified as phycoerythrin-rich  
217 picocyanobacteria (picocyanobacteria). A picoeukaryote group was identified by its high red  
218 and low orange fluorescence, small size ( $<2$  µm) but higher complexity than the  
219 picocyanobacteria. For heterotrophic prokaryotic plankton (HPP), samples were stained with  
220 1:10,000 (vol/vol) SYBR® Green II and incubated 20 min in darkness. 50 µl of stained  
221 samples were run at medium speed (35 µl/min). We checked for any double-counting in

222 cytometry and microscopy by calculating the average size of each picophytoplankton and  
223 phytoplankton group, as described in Dory et al. (2021). HPP were identified by their small  
224 size (low FSC), low complexity (low SSC), high green fluorescence ( $530 \pm 15$  nm), and lack  
225 of red ( $> 670$  nm) fluorescence. Picoplankton data were acquired and analysed using BD  
226 Accuri CFlow Plus Analysis software (BD-Biosciences).

#### 227 *Cellular carbon biomass calculation*

228 Abundances were transformed into carbon content based on the literature. Cell  
229 abundance (cells/ml) of HPP was converted to biomass ( $\mu\text{g C/L}$ ) using  $20$  fg C/cell as  
230 constant conversion factor (Ducklow, 2000; Linacre et al., 2015). A conversion factor of  $237$   
231 fg C/cell was used for picocyanobacteria (Gerea et al., 2019) and a conversion factor of  $183$   
232 fg C/ $\mu\text{m}^3$  for picoeukaryotes (Lagaria et al., 2017). For phytoplankton, biovolume was  
233 estimated by shape assimilation to defined geometric forms and direct measurement of the  
234 main cell dimensions. Mean biovolume was calculated for each class of phytoplankton and  
235 then converted into biomass using the particular carbon content defined for each class by  
236 Wetzel & Likens (2000).

### 237 **2.2.4. Data analysis**

#### 238 *Quantitative analysis*

239 For initial conditions, all biological and physico-chemical variables were checked for  
240 normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Mean values were compared  
241 between early summer and late summer using Student or Welch t-tests. To test the DOC  
242 addition effect on all response variables (phytoplankton biomass, HPP biomass, consumed  
243 DOC and mixotrophs/autotrophs ratio) at the end of the experiments, and to determine  
244 whether DOC additions interacted with the other experimental factors (nutrients, temperature,  
245 light and campaign), Design of Experiments (DOE) was used. Design of Experiments  
246 combines mathematical and statistical techniques that optimise the relevance of the data

247 obtained by quantifying the relationship between output and input variables (Box et al., 1978;  
248 Montgomery, 2017). Contrary to a one-factor-at-a-time approach, DOE involves simultaneous  
249 variation of all the factors so that their effects can be estimated by considering the interactions  
250 between them. We considered the result of each experiment to be a linear combination of the  
251 main effects and interaction effects among the five factors (interaction order = 4,  $b_{ijklm}$ ),  
252 considering two levels for each experimental factor. Thus, the linear models were performed  
253 three times for each combination of two glucose additions. This model could be written as  
254 follows:

$$255 \quad Y = b_0 + b_i X_i + b_{ij} X_i X_j + b_{ijk} X_i X_j X_k + b_{ijkl} X_i X_j X_k X_l + b_{ijklm} X_i X_j X_k X_l X_m$$

$$256 \quad i = 1 \text{ to } 5$$

$$257 \quad j = 1 \text{ to } 5, j > i$$

$$258 \quad k = 1 \text{ to } 5, k > j$$

$$259 \quad l = 1 \text{ to } 5, l > k$$

$$260 \quad m = 1 \text{ to } 5, m > l$$

261 With 1 = glucose, 2 = nutrients, 3 = light, 4 = temperature, 5 = campaign

262 Quantitative analyses were performed with R (v. 3.6.3), and Azurad (version *Expert*,

263 AZURAD SAS, Marseille, France, 2019) software.

#### 264 *Multivariate analysis*

265 We performed multivariate analyses on the phytoplankton biomass to detect any  
266 changes in phytoplankton community structure between early summer and late summer under  
267 initial conditions, and between treatments at the end of the experiments. Multivariate analyses  
268 were performed with the vegan package on R software (v. 3.6.3). Permutational multivariate  
269 analysis of variance (PERMANOVA) with 999 permutations ( $p = 0.05$ ) on the Bray–Curtis

270 dissimilarity matrix (ADONIS function in R) was used to test the hypothesis that the  
271 campaign (under initial conditions) or DOC additions (at the end of the experiments)  
272 structured the distribution of phytoplankton communities.

273

### 3. Results

274

#### 3.1. Initial conditions

275

Initial concentrations of dissolved organic carbon (DOC), soluble reactive phosphorus

276

(SRP) and dissolved inorganic nitrogen (DIN) were low in both campaigns (**Table 1**).

277

Average DOC concentrations were significantly lower in early summer ( $0.61 \pm 0.02$  mg/L)

278

than in late summer ( $1.04 \pm 0.09$  mg/L) ( $t = -9.4$ ,  $df = 3.53$ ,  $p = 0.001$ ). Average SRP

279

concentrations were low (below  $10 \mu\text{g/L}$ ) and were not significantly different between

280

campaigns ( $t = -0.44$ ,  $df = 7.9$ ,  $p = 0.67$ ). Average DIN concentrations were significantly

281

higher in early summer ( $0.60 \pm 0.06$  mg/L) than in late summer ( $0.15 \pm 0.04$  mg/L) ( $t = 16.22$ ,

282

$df = 10.42$ ,  $p < 0.001$ ). Water temperature was significantly lower in early summer ( $8.9^\circ\text{C}$ )

283

than in late summer ( $14.6^\circ\text{C}$ ) ( $t = -99.56$ ,  $df = 15.62$ ,  $p < 0.001$ ).

284

Concerning biotic parameters, the initial HPP biomass did not significantly differ

285

between early summer and late summer ( $t = 1.61$ ,  $df = 7.96$ ,  $p = 0.14$ ), being close to  $17 \mu\text{g}$

286

C/L in both campaigns. Initial phytoplankton biomass was almost 7-fold greater in late

287

summer than in early summer ( $t = -17.45$ ,  $df = 6.19$ ,  $p < 0.001$ ). The initial phytoplankton

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community differed significantly between campaigns (permanova,  $F_{1,9} = 214$ ,  $p = 0.004$ ). In

289

early summer, diatoms dominated the community (37%), followed by chrysophytes (23%),

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cryptophytes (17%), dinoflagellates (15%) and picocyanobacteria (6%). In late summer,

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diatoms were even more predominant (47%), followed by picocyanobacteria (22%),

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chrysophytes (16%) and dinoflagellates (13%). The mixotrophs/autotrophs (M/A) ratio was

293

higher in early summer ( $1.13 \pm 0.44$ ) than in late summer ( $0.45 \pm 0.05$ ) ( $t = 3.04$ ,  $df = 3$ ,  $p =$

294

$0.05$ ). Finally, ciliates were almost thirty-fold more abundant in late summer than in early

295

summer ( $t = -3.00$ ,  $df = 11.19$ ,  $p = 0.01$ ).

296

297 **Table 1** : Biotic and abiotic parameters measured under initial conditions before each experiment.  
 298 Mean  $\pm$  standard error. DOC: Dissolved organic carbon; SRP: Soluble reactive phosphorus; DIN:  
 299 Dissolved inorganic nitrogen; HPP: Heterotrophic prokaryotic plankton. Significant P-values:  
 300  $<0.001^{***}$ ;  $<0.01^{**}$ ;  $<0.05^{*}$ .

	Early summer	Late summer
DOC (mg/L)	0,61 $\pm$ 0,02	1,04 $\pm$ 0,09
SRP ( $\mu$ g/L)	5 $\pm$ 0,14	3 $\pm$ 0,03
DIN (mg/L)	0,60 $\pm$ 0,06	0,15 $\pm$ 0,04 <sup>***</sup>
Temperature ( $^{\circ}$ C)	8,98 $\pm$ 0,08	14,60 $\pm$ 0,18 <sup>***</sup>
Phytoplankton biomass ( $\mu$ g C/L)	16,25 $\pm$ 1,41	111,94 $\pm$ 14,55 <sup>**</sup>
HPP biomass ( $\mu$ g C/L)	17,51 $\pm$ 1,39	16,56 $\pm$ 0,64
Mixotrophs/autotrophs ratio	1,13 $\pm$ 0,44	0,45 $\pm$ 0,05 <sup>**</sup>
Ciliate abundance (cell/ml)	0,12 $\pm$ 0,41	3,98 $\pm$ 4,43 <sup>***</sup>

301

### 302 **3.2. Plankton responses to experiments in microcosms**

303 Based on the linear model, the response of all biotic variables (phytoplankton biomass,  
 304 HPP biomass, consumed DOC and M/A ratio) to experiments varied between early summer  
 305 and late summer (CAMP effect, **Table S1**). Moreover, CAMP effect interacted with at least  
 306 three other factors for each variable, which means that the effects of DOC, nutrients, light,  
 307 and temperature on planktonic communities varied between early summer and late summer.

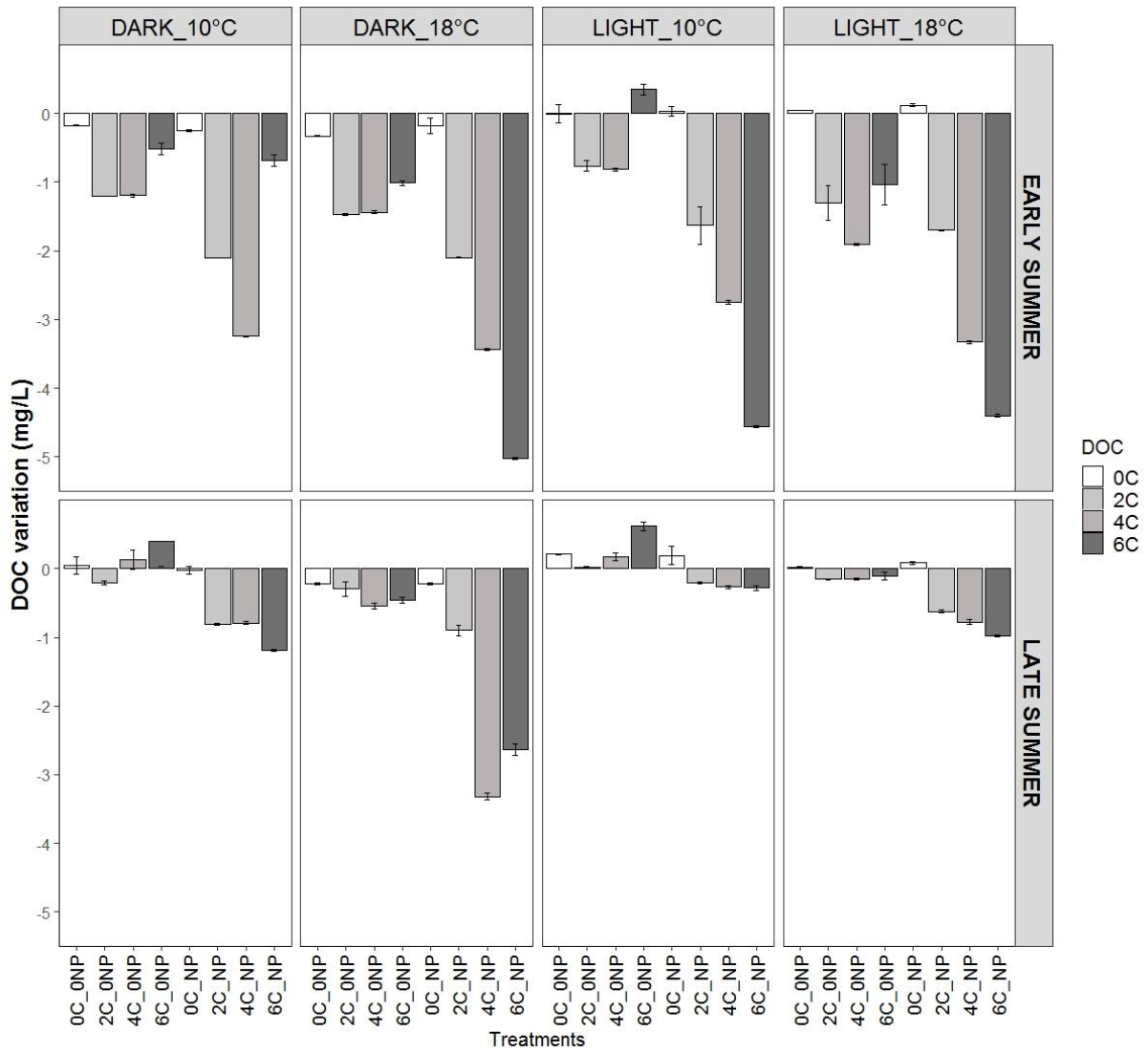
#### 308 **3.2.1. Consumed DOC**

309 At the end of the experiments, DOC was consumed more in the GLUCOSE treatments  
 310 (2C, 4C, 6C) than in the C-control treatments (0C), in both early (**Figure 1**) and late summer  
 311 (**Figure 1**). The exceptions were treatment 6C0NP at 10 $^{\circ}$ C\_LIGHT in early summer, and  
 312 treatments 4C0NP and 6C0NP at 10 $^{\circ}$ C (DARK and LIGHT) in late summer. DOC was  
 313 consumed more in early summer than in late summer with glucose added at 2 mg/L, 4 mg/L  
 314 and 6 mg/L (DOC\*CAMP interaction, **Table S1**), the average consumed DOC in all  
 315 GLUCOSE treatments reaching 49.2 % in early summer and 13.9 % in late summer. For  
 316 example, in the 6CNP treatment, the average consumed DOC reached 5.03 mg/L in early

317 summer but only 2.63 mg/L in late summer. In both campaigns, the maximum values for  
318 consumed DOC were reached at 18°C\_DARK.

319 DOC was consumed more in NP treatments than in ONP treatments when glucose was  
320 added at 2 mg/L, 4 mg/L, and 6 mg/L (DOC\*NUT interaction, **Table S1**). DOC was  
321 consumed more at 18°C than at 10°C when glucose was added at 4 mg/L and 6 mg/L  
322 (DOC\*T interaction, **Table S1**).

323 Finally, DOC was consumed more in DARK conditions than in LIGHT conditions,  
324 when glucose was added at 4 mg/L and 6 mg/L (DOC\*LIGHT interaction, **Table S1**). This  
325 effect was higher in late summer than in early summer (DOC\*LIGHT\*CAMP interaction,  
326 **Table S1**).



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Figure 1 : DOC variation in the microcosms between initial conditions and end of experiments, in early summer and late summer. Concentrations are shown for each DOC\*nutrient treatment under the four temperature\*light conditions. Error-bars represent standard error (n = 3).

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332 **3.2.2. HPP biomass**

333 A fivefold interaction was detected on HPP biomass (COD\*NUT\*LU\*T°\*CAMP,  
334 **Table S1**), particularly on the 8<sup>th</sup> day for all glucose concentrations.

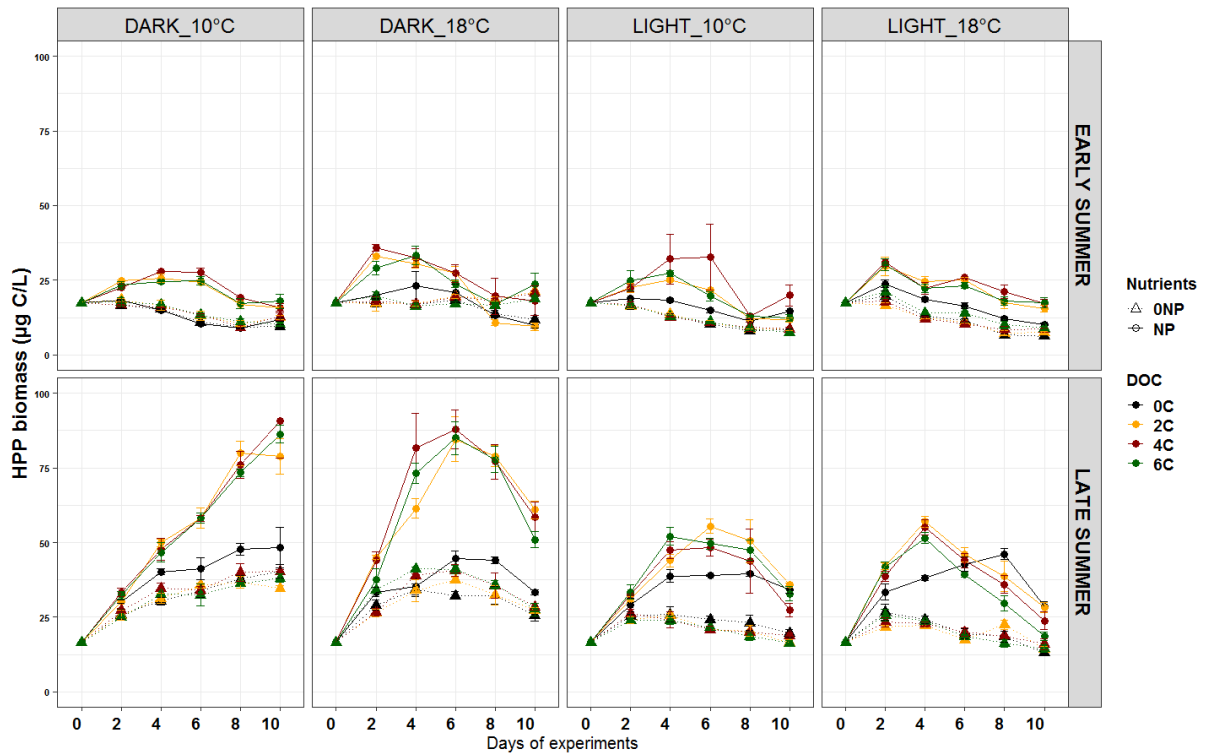
335 HPP biomass dynamic differed between early and late summer: in early summer, HPP  
336 biomass rapidly decreased in almost all treatments except the CNP treatments (Figure 2). In  
337 late summer however, HPP biomass increased in almost all treatments, including controls  
338 (0CNP and 0C0NP) especially in the DARK conditions. A smaller and briefer increase in  
339 HPP biomass in the controls was observed in the LIGHT conditions (Figure 2).

340 Glucose additions stimulated HPP biomass more in late summer than in early summer  
341 (COD\*CAMP, **Table S1**) from the 4<sup>th</sup> experimental day until the end of the experiments.

342 In early summer, under 10°C\_DARK, 18°C\_DARK and 10°C\_LIGHT conditions,  
343 HPP biomass increased in the CNP treatments until the 4<sup>th</sup> or 6<sup>th</sup> day compared to 0CNP, then  
344 declined until the end of the experiment (**Figure 2**). At 18°C\_LIGHT, HPP biomass increased  
345 in all the CNP treatments until the 2<sup>nd</sup> day and then declined; however, biomass remained  
346 higher in the CNP treatments than in the 0CNP treatments.

347 In late summer, at 10°C\_DARK, HPP biomass increased more in all the CNP  
348 treatments than in the 0CNP treatment and continued to increase until the end of the  
349 experiment (**Figure 2**). Under 18°C\_DARK, 10°C\_LIGHT and 18°C\_LIGHT conditions,  
350 HPP biomass increased more in the CNP treatments than in the 0CNP treatment, but only  
351 until the 4<sup>th</sup> or 6<sup>th</sup> day, then declined until the end of the experiments. In 0NP treatments,  
352 glucose additions stimulated HPP biomass only at 18°C\_DARK.

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Figure 2: Heterotrophic prokaryotic plankton (HPP) biomass per experimental day, in early summer and late summer. Biomass is shown for each DOC\*nutrient treatment under the four temperature\*light conditions. Error-bars represent standard error (n = 3).

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### 3.2.3. Shifts in phytoplankton community biomass, structure and composition

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Total phytoplankton biomass differed strongly between campaigns (CAMP effect,

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**Table S1**), and was higher in late summer than in early summer in all conditions and

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treatments (Figure 3). Nutrient addition significantly stimulated phytoplankton biomass in

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both campaigns (NUT effect, **Table S1**). A quadruple interaction was detected on

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phytoplankton biomass at the end of the experiments (NUT\*LU\*T°\*CAMP, **Table S1**).

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However, DOC additions had almost no effect on phytoplankton biomass, neither alone nor in

365

interaction.

366 At the end of the experiments, DOC additions significantly modified community  
367 composition in early summer, but DOC addition effect was weak (permanova,  $F_{1,94} = 5.19$ ,  $p$   
368  $= 0.004$ ). DOC additions significantly didn't modified community composition in late  
369 summer (permanova,  $F_{1,94} = 0.94$ ,  $p = 0.41$ ). However, phytoplankton community  
370 composition differed significantly among conditions and treatments, both in early summer  
371 (permanova,  $F_{31,64} = 10.83$ ,  $p < 0.001$ ) and in late summer (permanova,  $F_{31,64} = 17.02$ ,  $p <$   
372  $0.001$ ) (**Figure 3**).

373 Concerning autotrophic taxa, diatoms mainly consisted of *Fragilaria nanana* in both  
374 campaigns. Chlorophytes were mainly composed of *Tetraedron minimum* and  
375 *Monoraphidium minutum* in both campaigns, but *Dictyococcus* sp. was also present in late  
376 summer. Cyanobacteria mainly consisted of *Synechococcus elongatus* in both campaigns.  
377 Concerning mixotrophic taxa, dinoflagellates mainly consisted of *Gymnodinium* sp. and  
378 *Peridinium* sp. in both campaigns. Chrysophytes were mainly composed of *Dinobryon*  
379 *divergens* and *Ochromonas* sp. in both campaigns. In early summer, *Chrysolykos* sp. and  
380 *Hyalobryon* sp. were also present. In late summer, *Dinobryon crenulatum*, *Chrysococcus*  
381 *minutus*, *Bitrichia ollula* and *Bitrichia chodatii* were also present. Cryptophytes were mainly  
382 composed of *Cryptomonas* sp. and *Goniomonas truncata* in both campaigns, but *Chilomonas*  
383 sp. was also present in late summer. Finally, choanoflagellates mainly consisted of  
384 *Salpingoeca* sp. in both campaigns, but *Protospongia* sp. was also present in late summer.

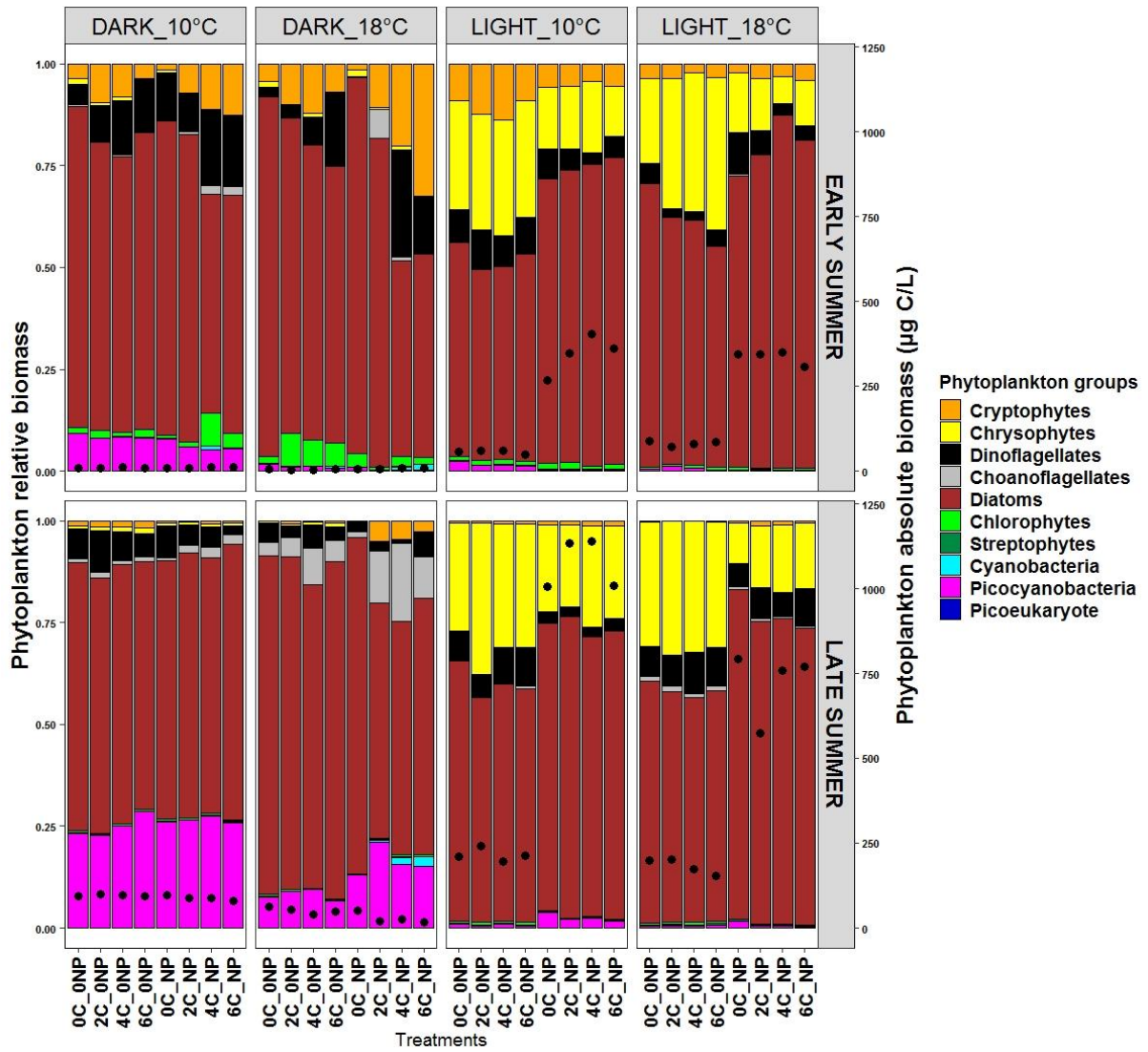
385 Based on the linear model, a quadruple interaction was detected on the  
386 mixotrophs/autotrophs (M/A) ratio at the end of the experiments (DOC\*NUT\*LU\*CAMP,  
387 **Table S1**), when glucose was added at 4 mg/L and 6 mg/L.

388 In early summer, the relative and absolute biomass of cryptophytes and dinoflagellates  
389 increased with DOC additions in DARK conditions, both in ONP and in NP treatments  
390 (**Figure 3** and **Figure S2**). Consequently, the proportion of mixotrophs increased in DARK

391 conditions after DOC additions, with an accentuated effect in NP treatments where DOC was  
392 added at 4 and 6 mg/L, at 10°C and 18°C (**Figure S1**). In LIGHT conditions, the relative  
393 biomass of cryptophytes, chrysophytes and dinoflagellates increased with DOC additions in  
394 the ONP treatments at 10°C, and the relative and absolute biomass of chrysophytes increased  
395 with DOC additions in the ONP treatments at 18°C (**Figure S3**). Consequently, the proportion  
396 of mixotrophs increased with DOC additions in LIGHT conditions in the ONP treatments  
397 (**Figure S1**).

398 In late summer, the relative and absolute biomass of choanoflagellates increased with  
399 DOC additions in DARK conditions at 18°C, mainly in the NP treatments (**Figure 3** and  
400 **Figure S4**). The relative and absolute biomass of cryptophytes increased with DOC additions  
401 in NP treatments at 18°C\_DARK. Thus, the proportion of mixotrophs increased after DOC  
402 additions in DARK conditions at 18°C in NP treatments (**Figure S1**). In LIGHT conditions,  
403 the relative biomass of chrysophytes increased with DOC additions in ONP treatments, and  
404 their absolute and relative biomass increased with DOC additions at 18°C in NP treatments  
405 (**Figure S5**). The relative and absolute biomass of dinoflagellates increased with DOC  
406 additions at 18°C in NP treatments. Thus, the proportion of mixotrophs increased in LIGHT  
407 conditions with DOC additions, at 10°C in ONP treatments and at 18°C in NP treatments.  
408 (**Figure S1**).

409 Finally, the biomass of autotrophs decreased with DOC in one condition in early  
410 summer (average decrease of 14.4 µg C/l compared to 0C treatments, data not shown) and in  
411 two conditions in late summer (average decrease of 45.8 µg C/l compared to 0C). The  
412 biomass of mixotrophs increased with DOC in three conditions in both campaigns, but the  
413 average increase compared to 0C treatments was higher in late summer (31.6 µg C/l) than in  
414 early summer (7.8 µg C/l).



415

416 Figure 3 : Phytoplankton groups' relative biomass and total absolute biomass (black points) at the end  
 417 of experiments, in early summer and late summer. Biomass is shown for each DOC\*nutrient treatment  
 418 under the four temperature\*light conditions.

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420                    **3.2.4. Ciliate abundance**

421                    In early summer, ciliate abundance increased in the 4CNP and 6CNP treatments at  
422 10°C\_DARK, and in all the CNP treatments at 18°C\_DARK (**Figure S6**). In late summer,  
423 ciliate abundance increased in the 4CNP treatments at 10°C\_LIGHT, and in the 6CNP  
424 treatments at 18°C\_LIGHT (**Figure S6**). There was no difference in DOC-stimulated increase  
425 in ciliate abundance between early summer and late summer, except in the dark, where  
426 ciliates increased more in early summer than in late summer in the 4CNP ( $p = 0.018$ ) and  
427 6CNP treatments ( $p = 0.02$ ).

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## Discussion

Dissolved organic carbon (DOC) acts as a major control of high-altitude lakes' functioning (Moser et al., 2019). Allochthonous DOC can be assimilated by microbial organisms, changing interactions between them. However, the summer dynamics of phytoplankton biomass and composition could also impact planktonic responses to DOC inputs. Our study aimed to assess how changes in the phytoplankton community between early summer and late summer modify these microbial responses.

### 1. Differential prokaryotic assimilation of DOC over the summer

In our microcosms, bacterial response was higher when glucose and nutrients were added jointly than after glucose addition alone. Moreover, HPP were able to develop only when provided with inorganic nutrients, especially in late summer. These two results suggest limitation primarily by nutrients and co-limitation by C of bacteria. This pattern of co-limitation has already been observed in high-latitude lakes (Dorado-García et al., 2014) and in Lake Cordes in the ice-covered period (Dory et al., 2021). Both the present study and the previous one found evidence that bacterioplankton in Lake Cordes were co-limited by nutrients and C from April to August.

We observed that glucose was consumed more in early summer than in late summer (Figure 4), whatever the conditions of temperature and light and the nutrient treatments. Initial DOC concentrations were lower in early summer (0.61 mg/l) than in late summer (1.04 mg/l). As the magnitude of plankton response to nutrient additions depends on the nutrient limitation (O'Donnell et al., 2017), it could be that the greater glucose consumption observed in early summer resulted in greater C limitation of bacteria. Initial DOC concentrations measured in both campaigns varied with phytoplankton biomass, DOC concentrations being higher when the phytoplankton biomass was higher in late summer. The contrasting glucose consumption

455 between campaigns could also be related to changes in DOC lability over the summer.  
456 Phytoplankton biomass was far lower in early summer (16.25  $\mu\text{g/l}$ ) than in late summer  
457 (111.94  $\mu\text{g/l}$ ), and the increased phytoplankton biomass could result from greater availability  
458 of autochthonous DOC in late summer (Ejarque et al., 2018). This interpretation of the  
459 phytoplankton biomass influence is also supported by the finding of greater differences in  
460 DOC consumed in DARK versus LIGHT conditions in late summer than in early summer.  
461 Our findings are consistent with the conclusions of Kritzberg et al. (2006), namely that the  
462 bacterial growth efficiency and production were higher with amendments of algae leachates  
463 than with more refractory substrates in oligotrophic lakes. In addition, it has been established  
464 that phytoplankton-derived DOC is more quickly and efficiently consumed by bacteria than  
465 allochthonous DOC (Guillemette et al., 2013). To conclude, in early summer, HPP actively  
466 consumed the glucose added to the microcosms likely because phytoplankton-derived DOC  
467 was low. In late summer, phytoplankton-derived DOC was more available and HPP probably  
468 needed less glucose, which resulted in lower glucose consumption.

469         Based on our linear model, glucose in interaction with nutrient additions was  
470 consumed more in early summer than in late summer, but glucose additions stimulated HPP  
471 biomass more in late summer than in early summer (Figure 4). The possibility that the  
472 discrepancies regarding glucose consumption and HPP biomass production are related to a  
473 top-down regulation of bacteria by predators in our microcosms cannot be excluded. In fact,  
474 ciliates and mixotrophs developed in the microcosms after glucose additions, and a previous  
475 study under the same experimental conditions demonstrated the strong influence of predator  
476 development on the bacterial response to glucose (Dory et al., 2021). However, biomasses  
477 measurements on the 4<sup>th</sup> experimental day confirm that DOC was assimilated by HPP more in  
478 late summer. Seasonal modifications in bacterial communities' composition have previously  
479 been described in oligotrophic lakes, linked to qualitative and quantitative changes in



480 allochthonous or autochthonous organic substrates (Kritzberg et al., 2006). In our study, lake  
481 conditions differed strongly between early summer and late summer, as demonstrated by the  
482 campaign effect detected by the linear model. In early summer, frequent storms associated  
483 with high turbidity likely constituted stressful environmental conditions for heterotrophic  
484 bacteria. Such environmental conditions have been linked to increased respiration of  
485 dissolved organic carbon by heterotrophic bacteria in high-altitude lakes (Sadro & Melack,  
486 2012). Thus, in our experiments, the bacterial metabolism probably changed over summer,  
487 related to the increased stability of environmental conditions and the more available  
488 autochthonous DOC in late summer. Unfortunately, our results do not permit us to determine  
489 whether the observed change was due to a taxonomic modification of the bacterial  
490 community, a functional modification of the bacterial community, or both.

491           In summary, our results indicate that DOC was consumed by bacteria more in  
492 early summer than in late summer but was assimilated more in late summer. In agreement  
493 with our first hypothesis, these seasonal changes in bacterial metabolism could be linked to  
494 environmental changes, especially phytoplankton biomass dynamics over the summer.

## 495       **2. Changes in competition and predation after glucose addition over the** 496       **summer**

497           Our results indicate more competitive processes for inorganic nutrients between  
498 bacteria and phytoplankton in late summer in the GLUCOSE treatments. This competition  
499 resulted in a greater decrease in autotroph biomass and a greater increase in mixotroph  
500 biomass in late summer after glucose additions. The latter was likely related to the decline in  
501 HPP biomass observed from the 4th experimental day. In addition, we observed a decrease in  
502 initial DIN concentrations over the summer (Figure 4), consistent with the findings of  
503 Jacquemin et al. (2018) on the evolving nutrient limitation pattern in Lake Cordes during  
504 summer 2016. Our results support the findings of Carney et al. (2016), who observed

505 increased competition between bacteria and phytoplankton depending on limitation by  
506 inorganic nutrients in mesocosms. In our microcosms, it is therefore possible that the more  
507 pronounced increase in HPP biomass in the DARK conditions in late summer than in early  
508 summer can also be explained by reduced competition with phytoplankton for nutrients in the  
509 DARK conditions.

510         Based on the linear model, our results indicate that top-down predatory control by  
511 ciliates and mixotrophs increased after glucose additions, both in early summer and in late  
512 summer. In phytoplankton communities, we observed differences in the stimulation of the  
513 different mixotrophs, depending on light and temperature conditions, between early summer  
514 and late summer. We were able to identify common trends in the relative increase in  
515 mixotrophic groups between early and late summer: chrysophytes were consistently  
516 stimulated more than autotrophs after glucose additions only in the LIGHT treatments, while  
517 choanoflagellates were consistently stimulated more by glucose additions in the DARK  
518 treatments. Rottberger et al. (2013) highlighted the inability of the chrysophyte species  
519 *Dinobryon divergens* to develop in the dark through prey ingestion alone. However, this  
520 earlier study also pointed out major differences in general nutritional preferences between  
521 different mixotrophs. In our microcosms, all species of chrysophytes were unable to develop  
522 in the dark through prey ingestion alone, whatever the campaign. Our results therefore  
523 indicate that the nutritional strategy of chrysophytes in Lake Cordes remains relatively stable  
524 over the summer.

525         In our microcosms, the biomass of cryptophytes increased mainly in early summer in  
526 the DARK conditions after glucose additions. By contrast, in late summer cryptophytes  
527 increased only in relative biomass. It is therefore possible that a change in the nutritional  
528 preferences of cryptophytes occurs between early summer and late summer. Light availability  
529 has been shown to be a crucial factor in the proportions of mixotrophs in high-altitude lakes.

530 In these ecosystems, the highest proportion of mixotrophs is usually observed after snowmelt,  
531 because the mixotrophic strategy is advantageous under ice-cover, when nutrients and light  
532 are limiting factors (Waibel et al., 2019). A recent microcosm study on Lake Cordes  
533 demonstrated that under-ice species of cryptophytes were able to develop in the dark by  
534 ingesting bacterial prey (Dory et al., 2021). Conversely, other studies positioned cryptophytes  
535 towards the autotrophic end of an increasing trophic state gradient (Saad et al., 2016). These  
536 conclusions highlight the controversy surrounding the nutritional strategies of cryptophytes,  
537 which may differ in their degree of heterotrophy due to specific environmental limitations like  
538 light, nutrients, prey availability or a combination of these (Bergström et al., 2003). In  
539 addition, proportions of choanoflagellates in DARK conditions were higher in late summer  
540 than in early summer. The observed differential response of cryptophytes to glucose additions  
541 in the dark could also result from evolving competition with other groups for prey. To sum  
542 up, in our study cryptophytes were only able to develop through prey ingestion in early  
543 summer, not in late summer, probably due to changes in environmental conditions or  
544 competition mechanisms.

545 Overall, we clearly observed a stimulation of mixotrophic taxa after glucose additions  
546 in our experiments. Contrary to our hypothesis, however, no difference in the magnitude of  
547 mixotroph stimulation was observed between campaigns. This might be because there was  
548 little difference in the mixotrophic assemblage's composition between early summer and late  
549 summer, with a relatively similar mixotrophic potential in the communities over the season.

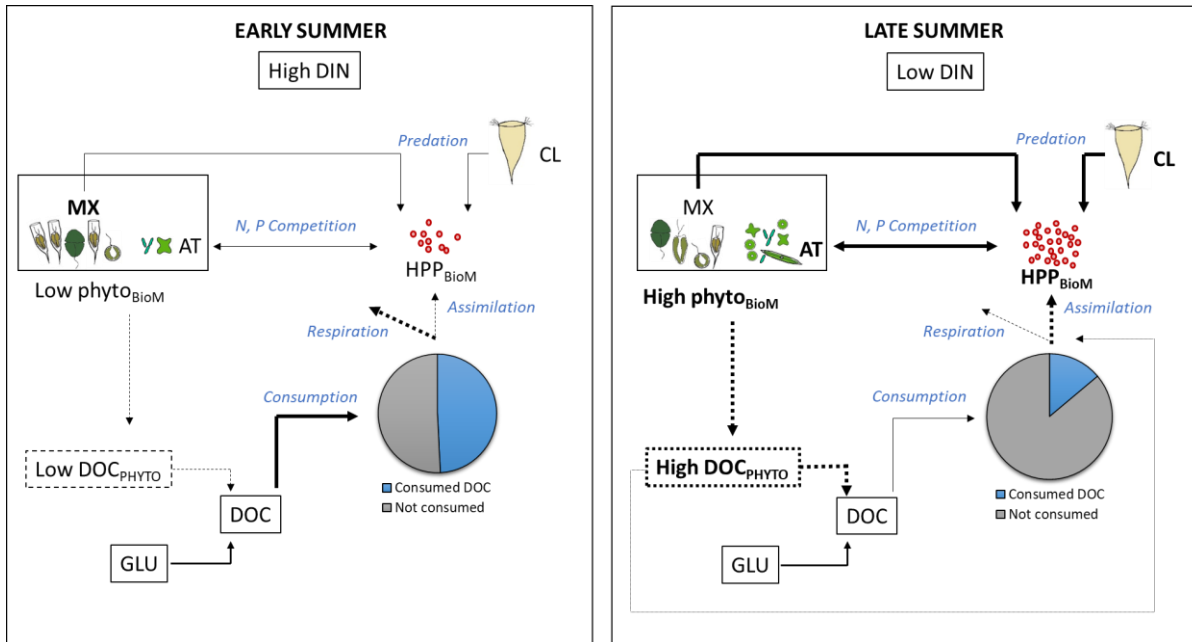
### 550 **3. Conclusion**

551 High-altitude lakes are particularly vulnerable to the combined action of DOC and  
552 nutrients. In our experimental study, DOC in combination with inorganic nutrients rapidly  
553 modified the relationship between bacterioplankton and phytoplankton. We show here that

554 DOC was incorporated differently into the food web in early summer and in late summer,  
555 depending on the availability of autochthonous DOC and the nutrient limitation. In general,  
556 DOC additions promoted heterotrophic and mixotrophic pathways in the planktonic food web.  
557 Thus, our findings contribute information that can help predict the ecological trajectories of  
558 shallow high-altitude lakes with large, vegetated catchments: more heterotrophic and  
559 mixotrophic communities can be expected in the future under the environmental changes  
560 forecast for these ecosystems.

561         Moreover, differences in the assimilation of DOC by bacteria between early summer  
562 and late summer underline how the timing of DOC flux affects the role high-latitude lakes  
563 play in the global carbon cycle. The predicted increase in DOC inputs to high-altitude lakes is  
564 expected to impact planktonic communities differently depending on the timing of DOC flux  
565 and the community characteristics (biomass, composition, and physiological state). Our  
566 results highlight the importance of thoroughly investigating the proportion of DOC  
567 assimilated by heterotrophic bacteria for biomass versus respiration, to better understand  
568 high-altitude lakes' role as carbon source and sink. The response of microbial organisms to  
569 glucose additions is a good starting point to better assess the influence of DOC variations in  
570 high-altitude lakes induced by global change. While phytoplankton-derived DOC seemed to  
571 be a more bio-available C source than glucose for bacteria in our experiments, other carbon  
572 sources like terrestrial DOC can modify the bacterioplankton-phytoplankton relationships in  
573 natural lakes. Further experiments could contribute to our understanding of how complex  
574 terrestrial carbon sources may modify phytoplankton-bacterioplankton relationships in high-  
575 altitude lakes.

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578 Figure 4: Scheme of the phytoplankton regulation of bacterial response to glucose addition in early  
 579 summer and late summer. Black lines represent processes that are reinforced (heavy line) or weakened  
 580 (light line). Dashed lines represent variables or processes not measured. GLU = glucose; DOC =  
 581 dissolved organic carbon; CL = ciliates; MX = mixotrophs; AT = autotrophs; HPP<sub>BioM</sub> = Heterotrophic  
 582 prokaryotic plankton biomass; Phyto<sub>BioM</sub> = phytoplankton biomass; DOC<sub>PHYTO</sub> = phytoplankton-  
 583 derived DOC.

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585

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594 paper is part of her Ph.D.

595 **Conflict of interest**

596 The authors declare no conflicts of interest.

597 **Data availability statement**

598 The datasets generated and analysed during the current study are available from the  
599 corresponding author on reasonable request.

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801 **Supplementary information**

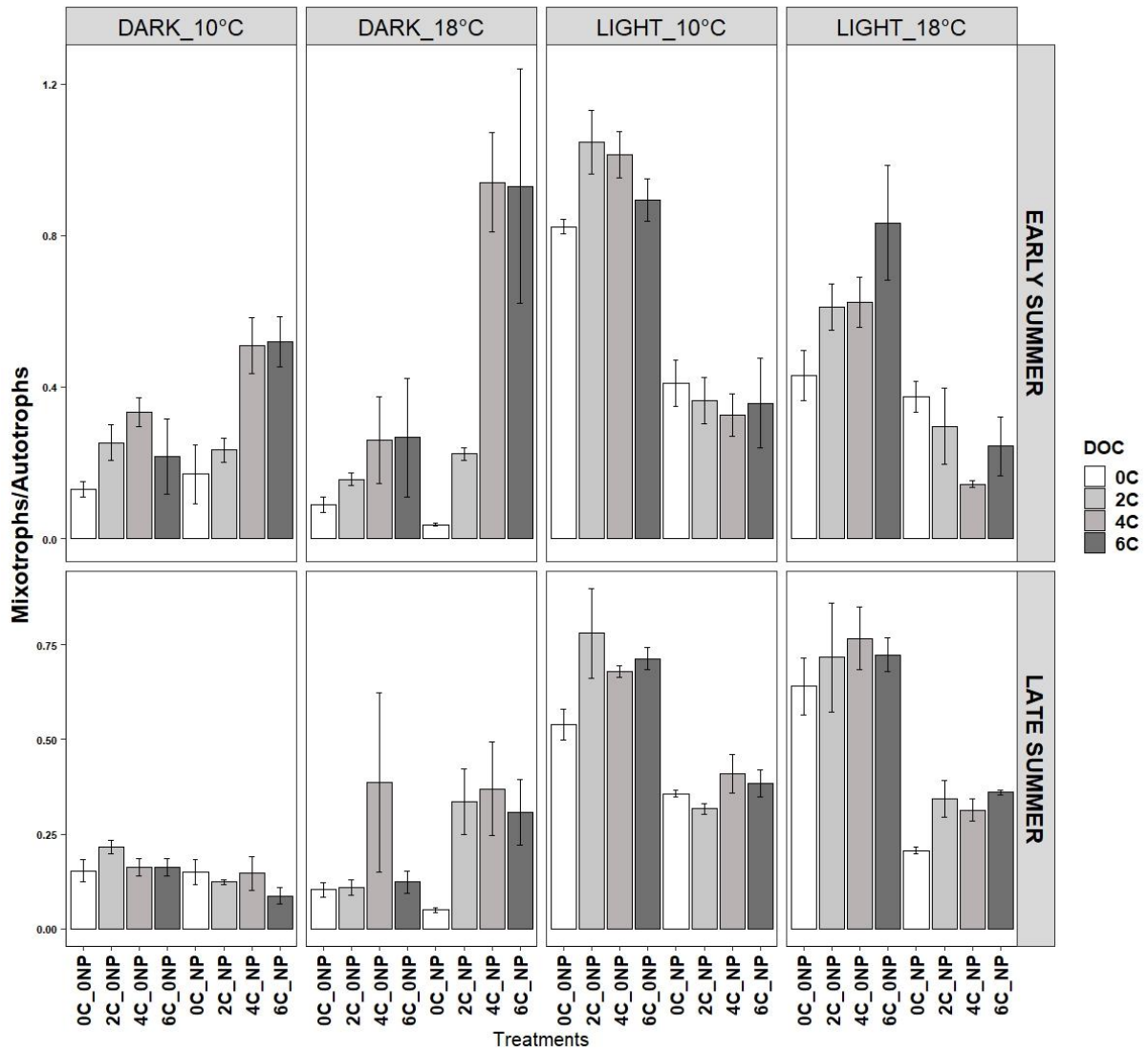
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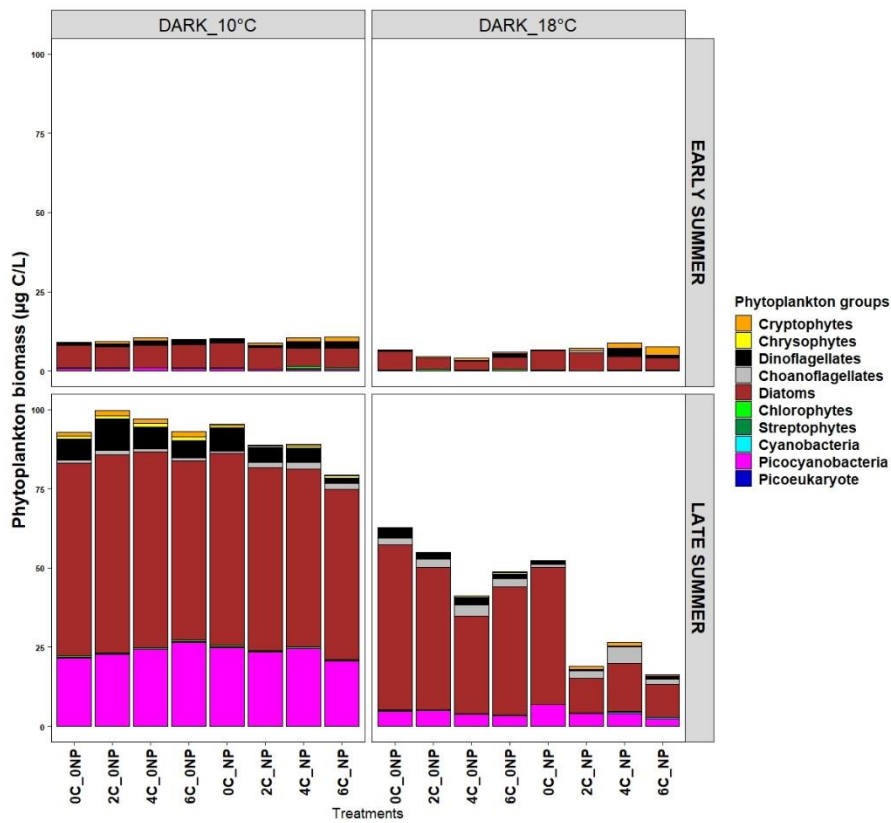
804 Table S1: Simple effects and interaction effects detected with the linear model from Design of Experiments on abiotic and biotic variables. Phyto\_Total: total phytoplankton  
 805 biomass on the 10<sup>th</sup> day; HPP<sub>2...10</sub>: Biomass of heterotrophic prokaryotic plankton on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day; Consumed\_DOC: DOC consumed at the end of the  
 806 experiment; M/A: mixotrophic / autotrophic ratio. DOC: dissolved organic carbon; NUT: inorganic nutrients; LU: light; T: temperature; CAMP: campaign. Significant P-  
 807 values: <0.001 \*\*\*; <0.01 \*\*; <0.05\*.

	PHYTO_TOTAL			HPP <sub>2</sub>			HPP <sub>4</sub>			HPP <sub>6</sub>			HPP <sub>8</sub>			HPP <sub>10</sub>			CONSUMED_DOC			M/A		
	0-2	0-4	0-6	0-2	0-4	0-6	0-2	0-4	0-6	0-2	0-4	0-6	0-2	0-4	0-6	0-2	0-4	0-6	0-2	0-4	0-6	0-2	0-4	0-6
<b>[GLUCOSE]</b>																								
<b>DOC</b>	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
<b>NUT</b>	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
<b>LU</b>	***	***	***	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
<b>T</b>	***	***	***	***	***	***	**	*	***	***	*	***	ns	ns	ns	***	***	***	***	***	***	***	ns	ns
<b>CAMP</b>	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	ns	**	***
<b>DOC*NUT</b>	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***	***	***	ns	ns	ns
<b>DOC*LU</b>	ns	ns	ns	*	*	ns	*	**	***	***	***	***	***	***	***	***	***	***	ns	***	*	ns	***	*
<b>DOC*T</b>	**	*	ns	ns	ns	*	*	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	***	ns	*	*
<b>DOC*CAMP</b>	ns	ns	ns	ns	ns	ns	*	*	***	**	ns	**	***	ns	ns	*	ns	ns	***	***	***	ns	ns	*
<b>NUT*LU</b>	***	***	***	ns	ns	*	**	ns	**	*	ns	ns	ns	ns	ns	ns	ns	*	ns	***	*	***	***	***
<b>NUT*T</b>	***	***	**	***	***	ns	**	ns	ns	**	ns	*	ns	ns	ns	*	ns	***	ns	**	***	**	ns	ns
<b>NUT*CAMP</b>	***	***	***	*	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns	ns
<b>LU*T</b>	**	*	ns	ns	ns	ns	*	**	***	***	***	***	ns	ns	*	*	*	**	ns	***	***	*	***	*
<b>LU*CAMP</b>	***	***	***	ns	ns	ns	ns	*	**	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns	ns
<b>T*CAMP</b>	***	***	***	ns	ns	ns	ns	*	ns	ns	ns	ns	**	*	***	***	***	***	ns	***	*	**	*	ns
<b>DOC*NUT*CAMP</b>	ns	ns	ns	ns	ns	ns	**	ns	**	***	ns	***	***	*	***	***	*	***	*	***	***	ns	ns	ns
<b>DOC*LU*CAMP</b>	ns	ns	ns	ns	ns	ns	ns	ns	*	**	**	***	***	***	***	***	***	***	ns	***	***	ns	**	**
<b>DOC*T*CAMP</b>	ns	ns	ns	ns	ns	ns	**	***	***	ns	**	**	ns	ns	ns	ns	ns	***	ns	***	***	ns	ns	ns
<b>NUT*LU*CAMP</b>	***	***	***	ns	ns	ns	ns	ns	*	ns	ns	ns	***	ns	**	***	***	***	ns	***	***	ns	**	*
<b>NUT*T*CAMP</b>	***	***	*	ns	ns	ns	ns	ns	ns	*	**	*	ns	ns	ns	ns	ns	**	*	***	**	ns	***	ns
<b>LU*T*CAMP</b>	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	***	***	***	ns	***	***	ns	*	ns
<b>DOC*NUT*LU*CAMP</b>	ns	ns	*	ns	ns	ns	ns	ns		*	*	**	***	**	***	***	***	***	ns	***	***	ns	***	**
<b>DOC*NUT*T*CAMP</b>	ns	ns	ns	ns	ns	ns	**	**	**	ns	ns	ns	ns	ns	*	ns	ns	***	ns	***	**	ns	ns	ns
<b>DOC*LU*T*CAMP</b>	ns	ns	ns	ns	ns	ns	ns	ns	**	***	ns	***	*	ns	***	ns	ns	ns	ns	***	***	ns	ns	ns
<b>NUT*LU*T*CAMP</b>	***	***	*	ns	ns	ns	*	ns	ns	*	ns	*	*	ns	*	ns	ns	ns	ns	***	***	***	ns	ns
<b>DOC*NUT*LU*T*CAMP</b>	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	***	*	***	ns	ns	ns	ns	**	***	ns	ns	ns



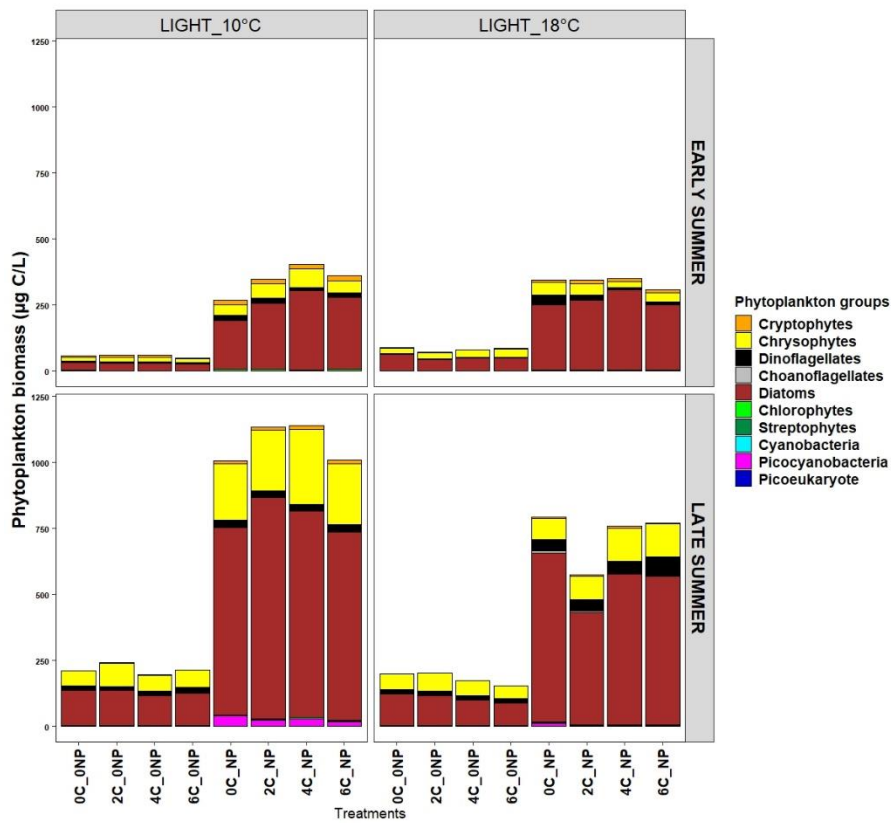
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Figure S1 : Mixotrophs / autotrophs ratio and phytoplankton absolute biomass (black points) at the end of the experiments, in early summer and late summer. Ratio and biomass are shown for each DOC\*nutrient treatment under the four temperature\*light conditions. Error-bars represent standard error (n = 3)



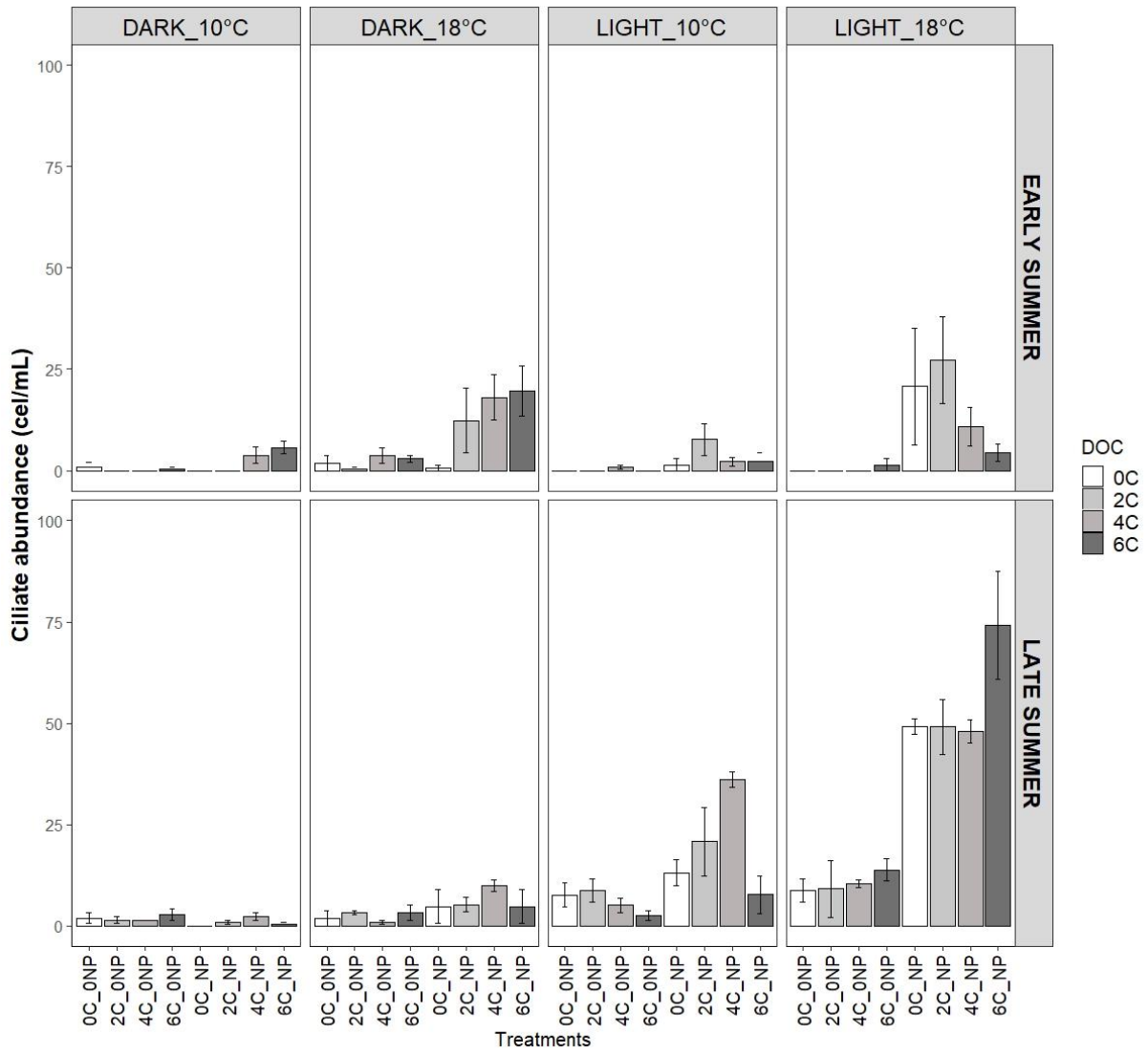
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811 Figure S2 : Phytoplankton biomass in DARK conditions in early summer and late summer



812

813 Figure S3 : Phytoplankton biomass in LIGHT conditions in early summer and late summer



814

Figure S4: Ciliate abundances at the end of experiments, in early summer and late summer. Abundances are shown for each DOC\*nutrient treatment under the four temperature\*light conditions. Error-bars represent standard error (n = 3).