



HAL
open science

Summer dynamics drive the microbial response to carbon and nutrient additions in a high-altitude lake

Flavia Dory, Laurent Cavalli, Evelyne Franquet, Magalie Claeys-Bruno,
Benjamin Misson, Thierry Tatoni, Céline Bertrand

► To cite this version:

Flavia Dory, Laurent Cavalli, Evelyne Franquet, Magalie Claeys-Bruno, Benjamin Misson, et al.. Summer dynamics drive the microbial response to carbon and nutrient additions in a high-altitude lake. *Limnology and Oceanography*, 2022, 67 (5), pp.1142-1156. 10.1002/lno.12062 . hal-03818173

HAL Id: hal-03818173

<https://hal.science/hal-03818173v1>

Submitted on 16 Feb 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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^{a*}, Laurent Cavalli^a, Evelyne Franquet^a, Magalie Claeys-Bruno^a, Benjamin
5 Misson^b, Thierry Tatoni^a, Céline Bertrand ^a

6 ^a Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France

7 ^b Université de Toulon, Aix Marseille Univ, CNRS, IRD, MIO, Toulon, France

8

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 ; Phone: 04 13 94 49 85

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

14

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

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

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

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

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

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

21

22 *Keywords:* DOC additions, phytoplankton-bacterioplankton relationship, high-altitude lakes,
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 7th July 2019 (early summer) and on 28th 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 NH₄NO₃ sufficient to increase N concentrations to
166 335 µg/L and P was added as KH₂PO₄ 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°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°C) glass tubes (Wheaton equipped
188 with Teflon/silicone septa) and preserved in 30 μl of sodium azide solution (1 M 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 ± 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 ± 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/ μm^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 ± 0.02 mg/L)

278

than in late summer (1.04 ± 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 ± 0.06 mg/L) than in late summer (0.15 ± 0.04 mg/L) ($t = 16.22$,

282

$df = 10.42$, $p < 0.001$). Water temperature was significantly lower in early summer (8.9°C)

283

than in late summer (14.6°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

288

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%),

290

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

291

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

292

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

293

higher in early summer (1.13 ± 0.44) than in late summer (0.45 ± 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 (μ g/L)	5 \pm 0,14	3 \pm 0,03
DIN (mg/L)	0,60 \pm 0,06	0,15 \pm 0,04 ^{***}
Temperature ($^{\circ}$ C)	8,98 \pm 0,08	14,60 \pm 0,18 ^{***}
Phytoplankton biomass (μ g C/L)	16,25 \pm 1,41	111,94 \pm 14,55 ^{**}
HPP biomass (μ 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 ^{**}
Ciliate abundance (cell/ml)	0,12 \pm 0,41	3,98 \pm 4,43 ^{***}

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.

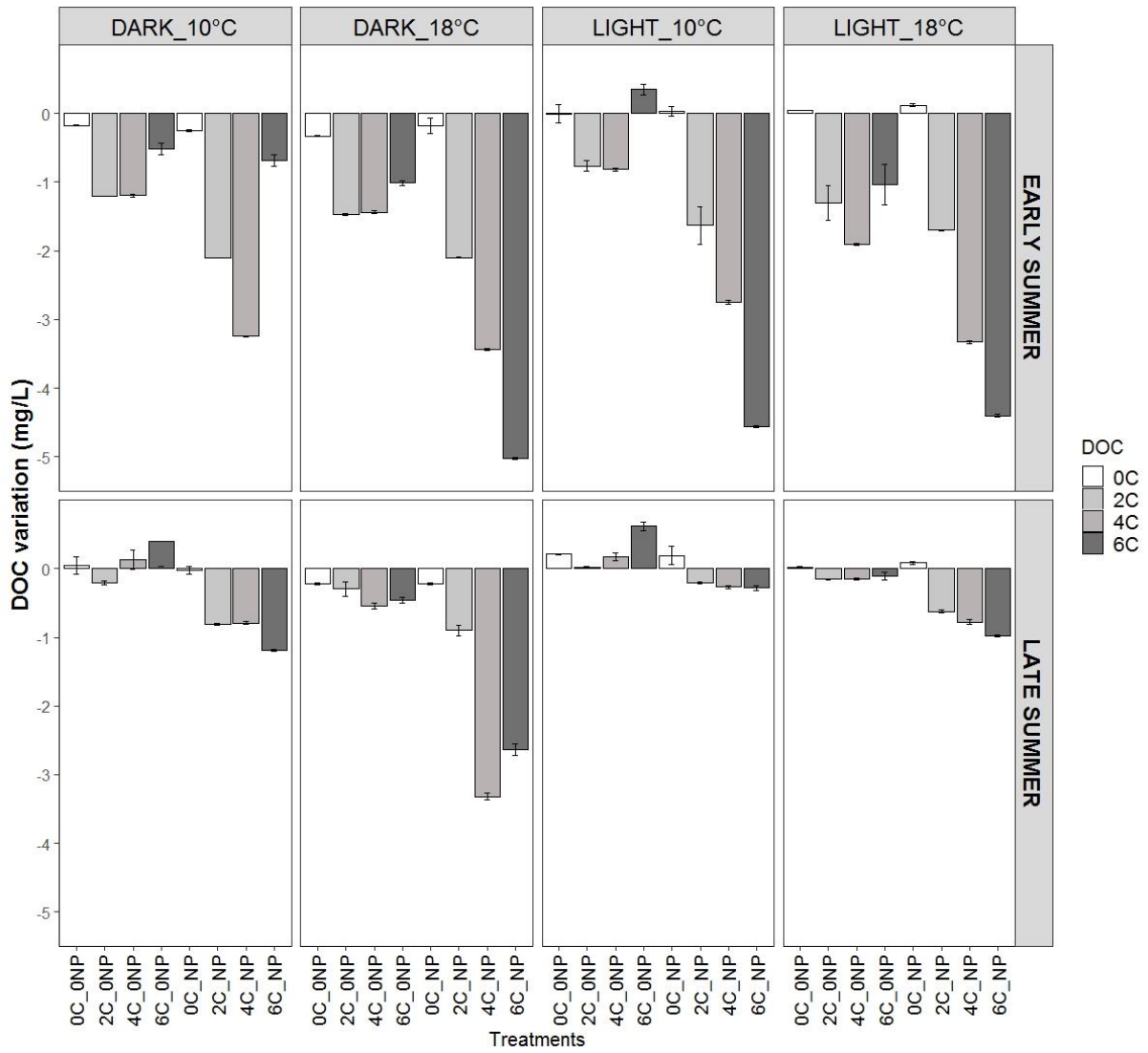
308 **3.2.1. Consumed DOC**

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**).



327

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).

328

329

330

331

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 8th day for all glucose concentrations.

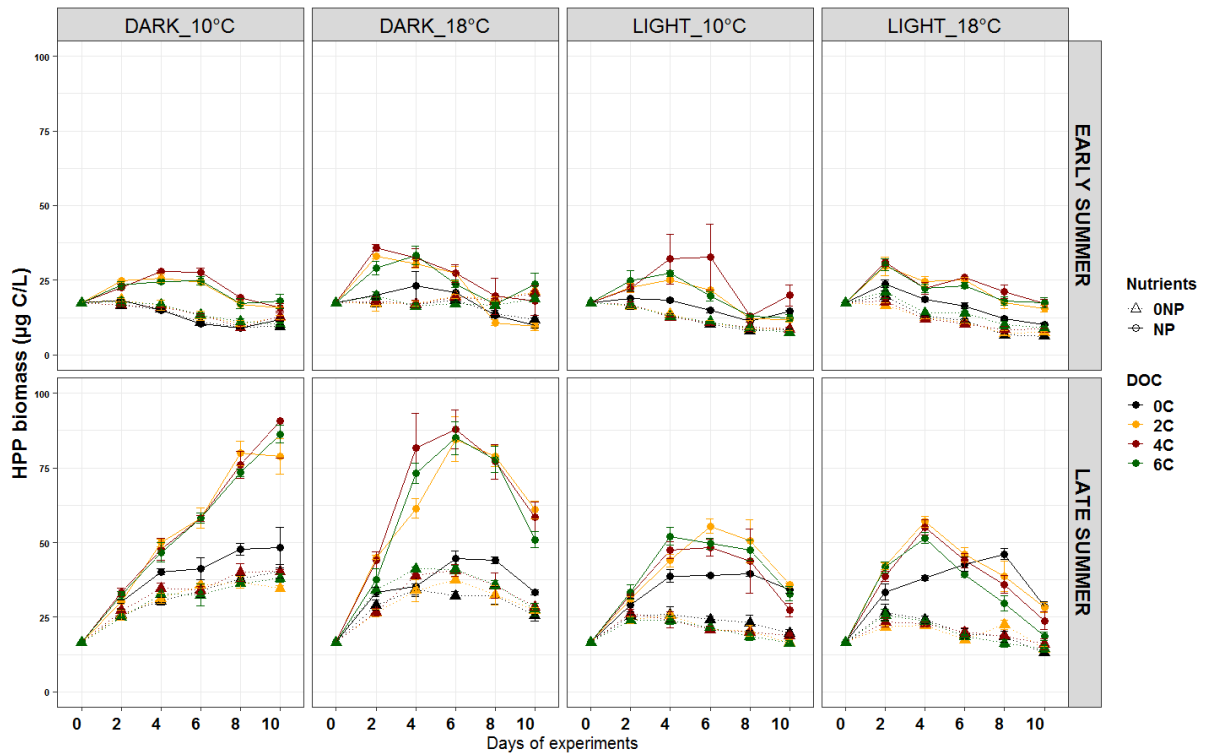
335 HPP biomass dynamic differed between early and late summer: in early summer, HPP
336 biomass rapidly decreased in almost all treatments except the CNP treatments (Figure 2). In
337 late summer however, HPP biomass increased in almost all treatments, including controls
338 (0CNP and 0C0NP) especially 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 4th 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 4th or 6th 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 2nd 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 4th or 6th day, then declined until the end of the experiments. In 0NP treatments,
352 glucose additions stimulated HPP biomass only at 18°C_DARK.

353



354

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).

355

356

3.2.3. Shifts in phytoplankton community biomass, structure and composition

357

358

359

Total phytoplankton biomass differed strongly between campaigns (CAMP effect,

360

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

361

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

362

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

363

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

364

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.

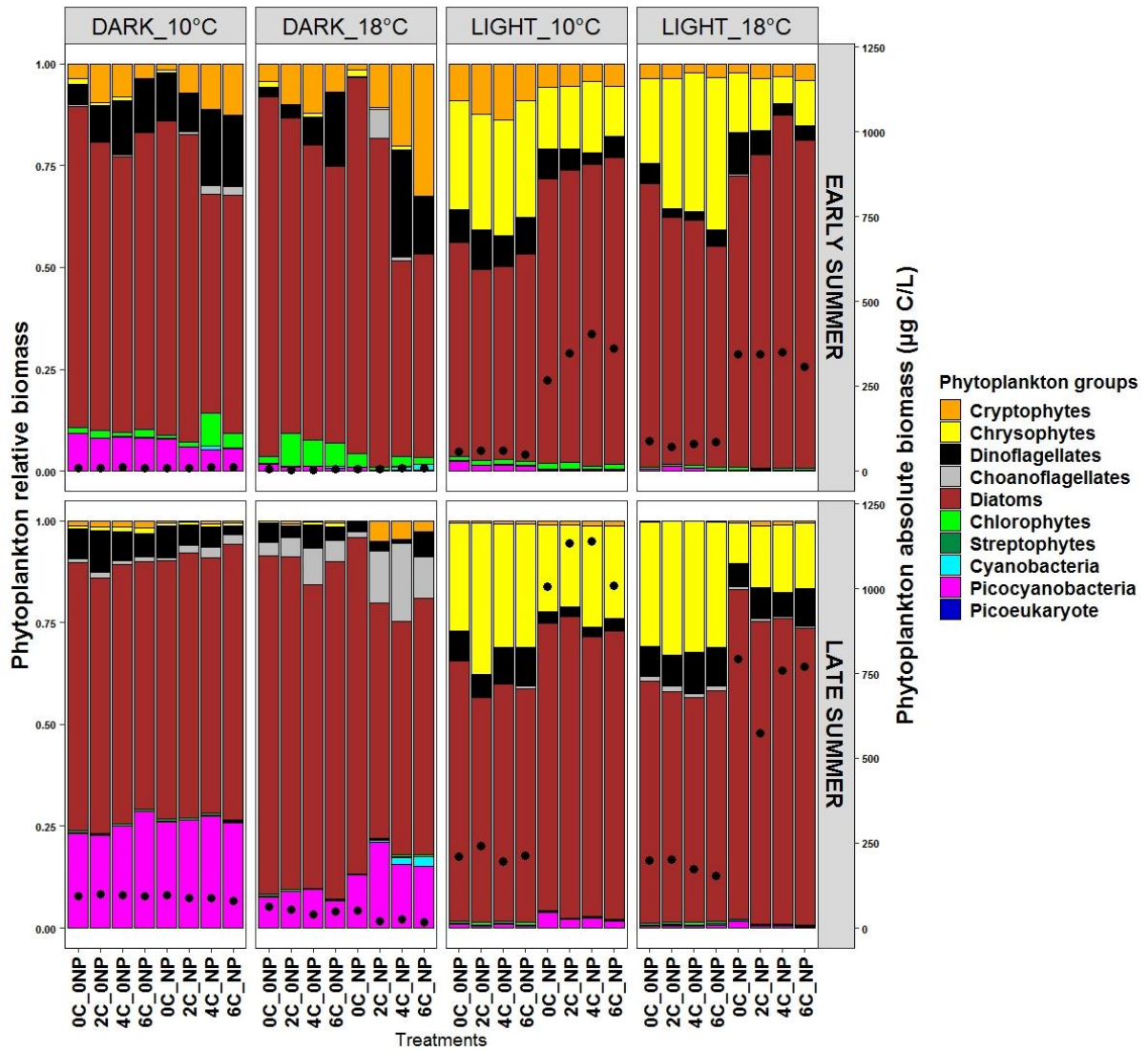
385 Based on the linear model, a quadruple interaction was detected on the
386 mixotrophs/autotrophs (M/A) ratio at the end of the experiments (DOC*NUT*LU*CAMP,
387 **Table S1**), when glucose was added at 4 mg/L and 6 mg/L.

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.

419

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 ($p = 0.02$).

428

429

430

431 **Discussion**

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

438 **1. Differential prokaryotic assimilation of DOC over the summer**

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

447 We observed that glucose was consumed more in early summer than in late summer
448 (Figure 4), whatever the conditions of temperature and light and the nutrient treatments. Initial
449 DOC concentrations were lower in early summer (0.61 mg/l) than in late summer (1.04 mg/l).
450 As the magnitude of plankton response to nutrient additions depends on the nutrient limitation
451 (O'Donnell et al., 2017), it could be that the greater glucose consumption observed in early
452 summer resulted in greater C limitation of bacteria. Initial DOC concentrations measured in
453 both campaigns varied with phytoplankton biomass, DOC concentrations being higher when
454 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 4th 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.

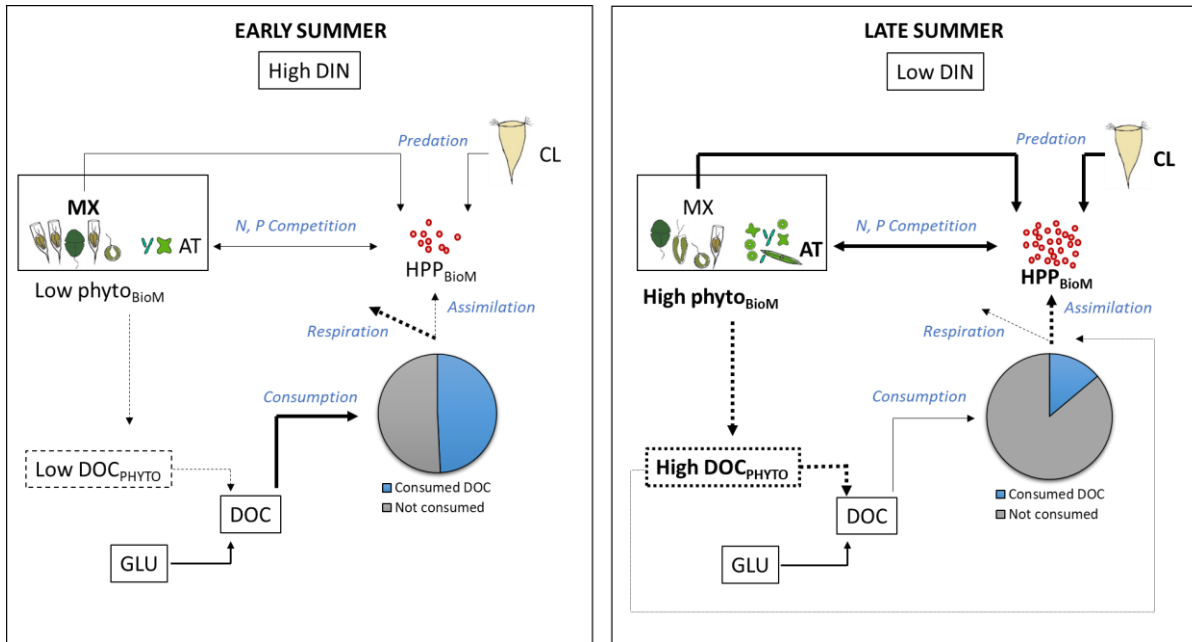
550 **3. Conclusion**

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.

576



577

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_{BioM} = Heterotrophic
 582 prokaryotic plankton biomass; Phyto_{BioM} = phytoplankton biomass; DOC_{PHYTO} = phytoplankton-
 583 derived DOC.

584

585

586 **Acknowledgments**

587 We are grateful to the members of the French Biodiversity Office, especially René
588 Conraud, for their help in the field. DOC analyses were carried out by the Mediterranean
589 Institute of Oceanography. Data used in this study were partly produced through the technical
590 facilities of the Biological and Chemical Analysis (IMBE, Marseille). We thank Marjorie
591 Sweetko for improving the English of this manuscript. This work was supported by the
592 French National program EC2CO (Ecosphère Continentale et Côtière). Flavia Dory received
593 a PhD fellowship from the Ministère de l'Enseignement Supérieur et de la Recherche and this
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.

600

601

602

603

604

605 **References**

- 606 Bergström, A. K., Jansson, M., Drakare, S., & Blomqvist, P. (2003). Occurrence of
607 mixotrophic flagellates in relation to bacterioplankton production, light regime and
608 availability of inorganic nutrients in unproductive lakes with differing humic contents.
609 *Freshwater Biology*, 48(5), 868-877. <https://doi.org/10.1046/j.1365-2427.2003.01061.x>
- 610 Bergström, A. K., Jonsson, A., Isles, P. D. F., Creed, I. F., & Lau, D. C. P. (2020). Changes in
611 nutritional quality and nutrient limitation regimes of phytoplankton in response to declining N
612 deposition in mountain lakes. *Aquatic Sciences*, 82(2), 31. [https://doi.org/10.1007/s00027-](https://doi.org/10.1007/s00027-020-0697-1)
613 [020-0697-1](https://doi.org/10.1007/s00027-020-0697-1)
- 614 Box, G. E., Hunter, W. H., & Hunter, S. (1978). *Statistics for experimenters* (Vol. 664). John
615 Wiley and sons New York.
- 616 Carney, R. L., Seymour, J. R., Westhorpe, D., & Mitrovic, S. M. (2016a). Lotic
617 bacterioplankton and phytoplankton community changes under dissolved organic-carbon
618 amendment : Evidence for competition for nutrients. *Marine and Freshwater Research*, 67(9),
619 1362-1373. <https://doi.org/10.1071/MF15372>
- 620 Carney, R. L., Seymour, J. R., Westhorpe, D., & Mitrovic, S. M. (2016b). Lotic
621 bacterioplankton and phytoplankton community changes under dissolved organic-carbon
622 amendment : Evidence for competition for nutrients. *Marine and Freshwater Research*, 67(9),
623 1362-1373. <https://doi.org/10.1071/MF15372>
- 624 Currie, D. J., & Kalff, J. (1984). A comparison of the abilities of freshwater algae and bacteria
625 to acquire and retain phosphorus1. *Limnology and Oceanography*, 29(2), 298-310.
626 <https://doi.org/10.4319/lo.1984.29.2.0298>

627 Dorado-García, I., Medina-Sánchez, J. M., Herrera, G., Cabrerizo, M. J., & Carrillo, P.
628 (2014). Quantification of Carbon and Phosphorus Co-Limitation in Bacterioplankton : New
629 Insights on an Old Topic. *PLOS ONE*, 9(6), e99288.
630 <https://doi.org/10.1371/journal.pone.0099288>

631 Dory, F., Cavalli, L., Franquet, E., Claeys-Bruno, M., Misson, B., Taton, T., & Bertrand, C.
632 (2021). Microbial consortia in an ice-covered high-altitude lake impacted by additions of
633 dissolved organic carbon and nutrients. *Freshwater Biology*, 00, 1-15.
634 <https://doi.org/10.1111/fwb.13781>

635 Ducklow, H. (2000). Bacterial production and biomass in the oceans. *Microbial ecology of the*
636 *oceans*, 1, 85-120.

637 Ejarque, E., Khan, S., Steniczka, G., Schelker, J., Kainz, M. J., & Battin, T. J. (2018).
638 Climate-induced hydrological variation controls the transformation of dissolved organic
639 matter in a subalpine lake. *Limnology and Oceanography*, 63(3), 1355-1371.
640 <https://doi.org/10.1002/lno.10777>

641 Elliott, G. P., & Kipfmüller, K. F. (2011). Multiscale Influences of Climate on Upper
642 Treeline Dynamics in the Southern Rocky Mountains, USA : Evidence of Intraregional
643 Variability and Bioclimatic Thresholds in Response to Twentieth-Century Warming. *Annals*
644 *of the Association of American Geographers*, 101(6), 1181-1203.
645 <https://doi.org/10.1080/00045608.2011.584288>

646 Evans, C. D., Monteith, D. T., & Cooper, D. M. (2005). Long-term increases in surface water
647 dissolved organic carbon : Observations, possible causes and environmental impacts.
648 *Environmental Pollution*, 137(1), 55-71. <https://doi.org/10.1016/j.envpol.2004.12.031>

649 Gasol, J. M., & Duarte, C. M. (2000). Comparative analyses in aquatic microbial ecology :
650 How far do they go? *FEMS Microbiology Ecology*, *31*(2), 99-106.
651 <https://doi.org/10.1111/j.1574-6941.2000.tb00675.x>

652 Gereá, M., Queimalinos, C., & Unrein, F. (2019). Grazing impact and prey selectivity of
653 picoplanktonic cells by mixotrophic flagellates in oligotrophic lakes. *Hydrobiologia*, *831*(1), 5
654 -21. <https://doi.org/10.1007/s10750-018-3610-3>

655 Guillemette, F., Leigh McCallister, S., & del Giorgio, P. A. (2016). Selective consumption
656 and metabolic allocation of terrestrial and algal carbon determine allochthony in lake bacteria.
657 *The ISME Journal*, *10*(6), 1373-1382. <https://doi.org/10.1038/ismej.2015.215>

658 Guillemette, F., McCallister, S. L., & Giorgio, P. A. del. (2013). Differentiating the
659 degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic
660 carbon in temperate lakes. *Journal of Geophysical Research: Biogeosciences*, *118*(3), 963-
661 973. <https://doi.org/10.1002/jgrg.20077>

662 Harsch, M. A., Hulme, P. E., McGlone, M. S., & Duncan, R. P. (2009). Are treelines
663 advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters*,
664 *12*(10), 1040-1049. <https://doi.org/10.1111/j.1461-0248.2009.01355.x>

665 Hazukova, V., Burpee, B. T., McFarlane-Wilson, I., & Saros, J. E. (2021). Under Ice and
666 Early Summer Phytoplankton Dynamics in Two Arctic Lakes with Differing DOC. *Journal of*
667 *Geophysical Research-Biogeosciences*, *126*(4), e2020JG005972.
668 <https://doi.org/10.1029/2020JG005972>

669 Hitchcock, J. N., & Mitrovic, S. M. (2013). Different resource limitation by carbon, nitrogen
670 and phosphorus between base flow and high flow conditions for estuarine bacteria and

671 phytoplankton. *Estuarine, Coastal and Shelf Science*, 135, 106-115.

672 <https://doi.org/10.1016/j.ecss.2013.05.001>

673 IPCC. (2013). Climate change 2013 : The Physical Science Basis. Contribution of Working
674 Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.
675 *GIEC*.

676 Isles, P. D. F., Creed, I. F., Jonsson, A., & Bergström, A.-K. (2021). Trade-offs Between
677 Light and Nutrient Availability Across Gradients of Dissolved Organic Carbon Lead to
678 Spatially and Temporally Variable Responses of Lake Phytoplankton Biomass to Browning.
679 *Ecosystems*. <https://doi.org/10.1007/s10021-021-00619-7>

680 Jacquemin, C., Bertrand, C., Oursel, B., Thorel, M., Franquet, E., & Cavalli, L. (2018a).
681 Growth rate of alpine phytoplankton assemblages from contrasting watersheds and N-
682 deposition regimes exposed to nitrogen and phosphorus enrichments. *Freshwater Biology*,
683 63(10), 1326-1339. <https://doi.org/10.1111/fwb.13160>

684 Jacquemin, C., Bertrand, C., Oursel, B., Thorel, M., Franquet, E., & Cavalli, L. (2018b).
685 Growth rate of alpine phytoplankton assemblages from contrasting watersheds and N-
686 deposition regimes exposed to nitrogen and phosphorus enrichments. *Freshwater Biology*,
687 63(10), 1326-1339. <https://doi.org/10.1111/fwb.13160>

688 Jansson, M., Bergström, A.-K., Blomqvist, P., & Drakare, S. (2000). Allochthonous Organic
689 Carbon and Phytoplankton/Bacterioplankton Production Relationships in Lakes. *Ecology*,
690 81(11), 3250-3255. [https://doi.org/10.1890/0012-9658\(2000\)081\[3250:AOCAPB\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[3250:AOCAPB]2.0.CO;2)

691 Jansson, M., Bergström, A.-K., Lymer, D., Vrede, K., & Karlsson, J. (2006).
692 Bacterioplankton growth and nutrient use efficiencies under variable organic carbon and
693 inorganic phosphorus ratios. *Microbial ecology*, 52(2), 358-364.

694 Jansson, M., Hickler, T., Jonsson, A., & Karlsson, J. (2008). Links between Terrestrial
695 Primary Production and Bacterial Production and Respiration in Lakes in a Climate Gradient
696 in Subarctic Sweden. *Ecosystems*, *11*(3), 367-376. [https://doi.org/10.1007/s10021-008-9127-](https://doi.org/10.1007/s10021-008-9127-2)
697 2

698 Kritzberg, E. S., Cole, J. J., Pace, M. M., & Granéli, W. (2005). Does autochthonous primary
699 production drive variability in bacterial metabolism and growth efficiency in lakes dominated
700 by terrestrial C inputs? *Aquatic Microbial Ecology*, *38*(2), 103-111.
701 <https://doi.org/10.3354/ame038103>

702 Kritzberg, E. S., Langenheder, S., & Lindström, E. S. (2006). Influence of dissolved organic
703 matter source on lake bacterioplankton structure and function – implications for seasonal
704 dynamics of community composition. *FEMS Microbiology Ecology*, *56*(3), 406-417.
705 <https://doi.org/10.1111/j.1574-6941.2006.00084.x>

706 Lagaria, A., Mandalakis, M., Mara, P., Papageorgiou, N., Pitta, P., Tsiola, A., Kagiorgi, M., &
707 Psarra, S. (2017). Phytoplankton Response to Saharan Dust Depositions in the Eastern
708 Mediterranean Sea : A Mesocosm Study. *Frontiers in Marine Science*, *3*.
709 <https://doi.org/10.3389/fmars.2016.00287>

710 Laurion, I., Ventura, M., Catalan, J., Psenner, R., & Sommaruga, R. (2000). Attenuation of
711 ultraviolet radiation in mountain lakes : Factors controlling the among- and within-lake
712 variability. *Limnology and Oceanography*, *45*(6), 1274-1288.
713 <https://doi.org/10.4319/lo.2000.45.6.1274>

714 Leppäranta, M. (2015). Ice-Covered Lakes Environment. In *Freezing of Lakes and the*
715 *Evolution of their Ice Cover* (p. 245-270). Springer.

716 Linacre, L., Lara-Lara, R., Camacho-Ibar, V., Herguera, J. C., Bazán-Guzmán, C., & Ferreira-
717 Bartrina, V. (2015). Distribution pattern of picoplankton carbon biomass linked to mesoscale
718 dynamics in the southern gulf of Mexico during winter conditions. *Deep Sea Research Part I:
719 Oceanographic Research Papers*, 106, 55-67. <https://doi.org/10.1016/j.dsr.2015.09.009>

720 Mitrovic, S. M., & Baldwin, D. S. (2016). Allochthonous dissolved organic carbon in river,
721 lake and coastal systems : Transport, function and ecological role. *Marine and Freshwater
722 Research*, 67(9), i. https://doi.org/10.1071/MFv67n9_ED

723 Montgomery, D. C. (2017). *Design and Analysis of Experiments*. John Wiley & Sons.

724 Morán, X. A. G., Calvo-Díaz, A., & Ducklow, H. W. (2010). Total and phytoplankton
725 mediated bottom-up control of bacterioplankton change with temperature in NE Atlantic shelf
726 waters. *Aquatic Microbial Ecology*, 58(3), 229-239. <https://doi.org/10.3354/ame01374>

727 Moser, K. A., Baron, J. S., Brahney, J., Oleksy, I. A., Saros, J. E., Hundey, E. J., Sadro, S. A.,
728 Kopáček, J., Sommaruga, R., Kainz, M. J., Strecker, A. L., Chandra, S., Walters, D. M.,
729 Preston, D. L., Michelutti, N., Lepori, F., Spaulding, S. A., Christianson, K. R., Melack, J. M.,
730 & Smol, J. P. (2019a). Mountain lakes : Eyes on global environmental change. *Global and
731 Planetary Change*, 178, 77-95. <https://doi.org/10.1016/j.gloplacha.2019.04.001>

732 Moser, K. A., Baron, J. S., Brahney, J., Oleksy, I. A., Saros, J. E., Hundey, E. J., Sadro, S.,
733 Kopáček, J., Sommaruga, R., Kainz, M. J., Strecker, A. L., Chandra, S., Walters, D. M.,
734 Preston, D. L., Michelutti, N., Lepori, F., Spaulding, S. A., Christianson, K. R., Melack, J. M.,
735 & Smol, J. P. (2019b). Mountain lakes : Eyes on global environmental change. *Global and
736 Planetary Change*, 178, 77-95. <https://doi.org/10.1016/j.gloplacha.2019.04.001>

737 O'Donnell, D. R., Wilburn, P., Silow, E. A., Yampolsky, L. Y., & Litchman, E. (2017).
738 Nitrogen and phosphorus colimitation of phytoplankton in Lake Baikal : Insights from a

739 spatial survey and nutrient enrichment experiments. *Limnology and Oceanography*, 62(4),
740 1383-1392. <https://doi.org/10.1002/lno.10505>

741 Oleksy, I. A., Beck, W. S., Lammers, R. W., Steger, C. E., Wilson, C., Christianson, K.,
742 Vincent, K., Johnson, G., Johnson, P. T. J., & Baron, J. S. (2020). The role of warm, dry
743 summers and variation in snowpack on phytoplankton dynamics in mountain lakes. *Ecology*,
744 101(10), e03132. <https://doi.org/10.1002/ecy.3132>

745 Reche, I., Pulido-Villena, E., Conde-Porcuna, J. M., & Carrillo, P. (2001). Photoreactivity of
746 Dissolved Organic Matter from High-Mountain Lakes of Sierra Nevada, Spain. *Arctic*,
747 *Antarctic, and Alpine Research*, 33(4), 426-434.
748 <https://doi.org/10.1080/15230430.2001.12003451>

749 Rottberger, J., Gruber, A., Boenigk, J., & Kroth, P. G. (2013). Influence of nutrients and light
750 on autotrophic, mixotrophic and heterotrophic freshwater chrysophytes. *Aquatic Microbial*
751 *Ecology*, 71(2), 179-191. <https://doi.org/10.3354/ame01662>

752 Saad, J. F., Unrein, F., Tribelli, P. M., López, N., & Izaguirre, I. (2016). Influence of lake
753 trophic conditions on the dominant mixotrophic algal assemblages. *Journal of Plankton*
754 *Research*, 38(4), 818-829. <https://doi.org/10.1093/plankt/fbw029>

755 Sadro, S., & Melack, J. M. (2012). The effect of an extreme rain event on the biogeochemistry
756 and ecosystem metabolism of an oligotrophic high-elevation lake. *Arctic, Antarctic, and*
757 *Alpine Research*, 44(2), 222-231. <https://doi.org/10.1657/1938-4246-44.2.222>

758 Sadro, S., Melack, J. M., Sickman, J. O., & Skeen, K. (2019). Climate warming response of
759 mountain lakes affected by variations in snow. *Limnology and Oceanography Letters*, 4(1), 9-
760 17. <https://doi.org/10.1002/lol2.10099>

761 Schulhof, M. A., Allen, A. E., Allen, E. E., Mladenov, N., McCrow, J. P., Jones, N. T.,
762 Blanton, J., Cavalheri, H. B., Kaul, D., Symons, C. C., & Shurin, J. B. (2020). Sierra Nevada
763 mountain lake microbial communities are structured by temperature, resources and
764 geographic location. *Molecular Ecology*, 29(11), 2080-2093.
765 <https://doi.org/10.1111/mec.15469>

766 Shapiro, S. S., & Wilk, M. B. (1965). An Analysis of Variance Test for Normality (Complete
767 Samples). *Biometrika*, 52(3/4), 591-611. <https://doi.org/10.2307/2333709>

768 Smits, A. P., MacIntyre, S., & Sadro, S. (2020). Snowpack determines relative importance of
769 climate factors driving summer lake warming. *Limnology and Oceanography Letters*, 5(3),
770 271-279. <https://doi.org/10.1002/lol2.10147>

771 Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., Larsen, S.,
772 Lennon, J. T., Read, J. S., Sadro, S., & Saros, J. E. (2015). Ecosystem consequences of
773 changing inputs of terrestrial dissolved organic matter to lakes : Current knowledge and future
774 challenges. *Ecosystems*, 18(3), 376-389. <https://doi.org/10.1007/s10021-015-9848-y>

775 Thingstad, T. F., Havskum, H., Garde, K., & Riemann, B. (1996). On the Strategy of 'Eating
776 Your Competitor' : A Mathematical Analysis of Algal Mixotrophy. *Ecology*, 77(7), 2108-
777 2118. <https://doi.org/10.2307/2265705>

778 Tiberti, R., Metta, S., Austoni, M., Callieri, C., Giuseppe, M., Aldo, M., Rogora, M., Tartari,
779 G. A., Hardenberg, J. V., & Provenzale, A. (2013). Ecological dynamics of two remote alpine
780 lakes during ice-free season. *Journal of Limnology*, 72(3), e33-e33.
781 <https://doi.org/10.4081/jlimnol.2013.e33>

782 Utermöhl, H. (1958). Methods of collecting plankton for various purposes are discussed. *SIL*
783 *Communications, 1953-1996*, 9(1), 1-38. <https://doi.org/10.1080/05384680.1958.11904091>

784 Ventura, M., CAMARERO, L., BUCHACA, T., BARTUMEUS, F., LIVINGSTONE, D. M.,
785 & CATALAN, J. (2000). The main features of seasonal variability in the external forcing and
786 dynamics of a deep mountain lake (Redó, Pyrenees). *Journal of Limnology*, 59(1s), 97-108.

787 Waibel, A., Peter, H., & Sommaruga, R. (2019). Importance of mixotrophic flagellates during
788 the ice-free season in lakes located along an elevational gradient. *Aquatic Sciences*, 81(3), 45.
789 <https://doi.org/10.1007/s00027-019-0643-2>

790 Warner, K. A., Fowler, R. A., & Saros, J. E. (2020). Differences in the Effects of Storms on
791 Dissolved Organic Carbon (DOC) in Boreal Lakes during an Early Summer Storm and an
792 Autumn Storm. *Water*, 12(5), 1452. <https://doi.org/10.3390/w12051452>

793 Wetzel, R. G., & Likens, G. E. (2000). Composition and Biomass of Phytoplankton. In R. G.
794 Wetzel & G. E. Likens (Éds.), *Limnological Analyses* (p. 147-174). Springer.
795 https://doi.org/10.1007/978-1-4757-3250-4_10

796 Williamson, C. E., Morris, D. P., Pace, M. L., & Olson, O. G. (1999). Dissolved organic
797 carbon and nutrients as regulators of lake ecosystems : Resurrection of a more integrated
798 paradigm. *Limnology and Oceanography*, 44(3part2), 795-803.
799 https://doi.org/10.4319/lo.1999.44.3_part_2.0795

800

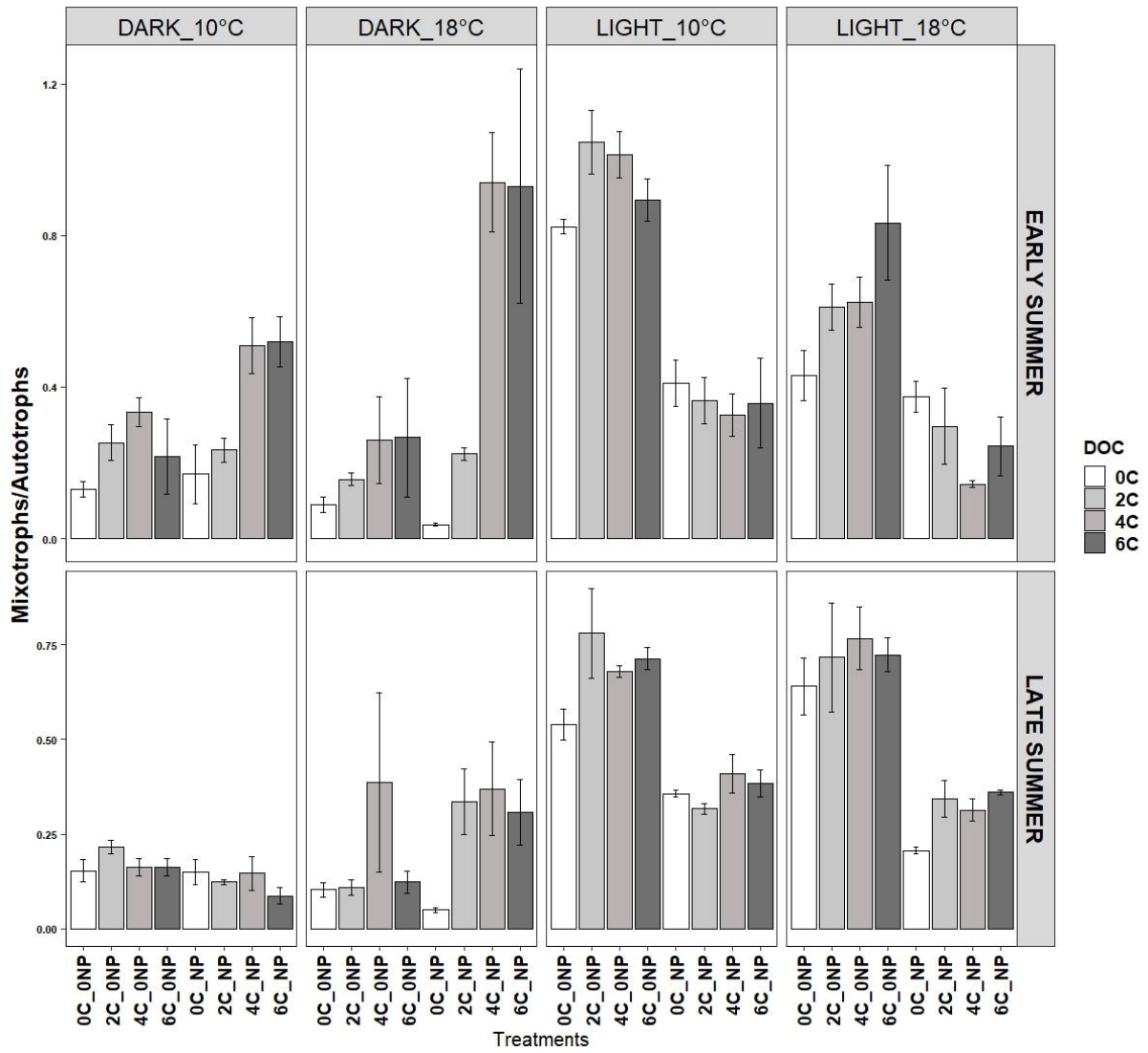
801 **Supplementary information**

802

803

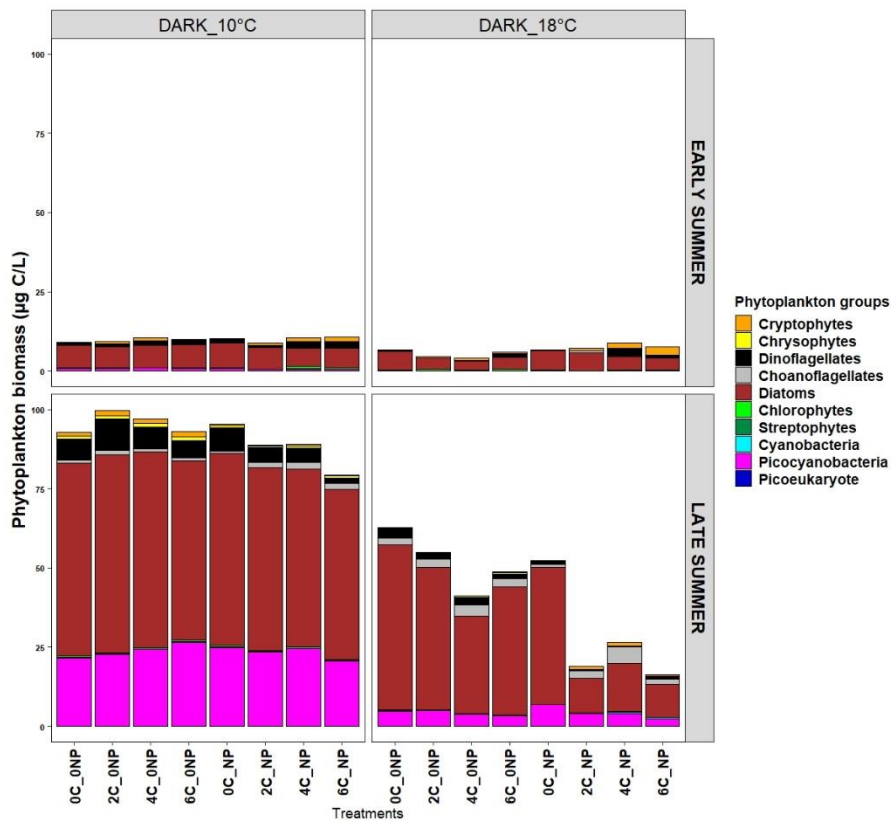
804 Table S1: Simple effects and interaction effects detected with the linear model from Design of Experiments on abiotic and biotic variables. Phyto_Total: total phytoplankton
 805 biomass on the 10th day; HPP_{2...10}: Biomass of heterotrophic prokaryotic plankton on the 2nd, 4th, 6th, 8th and 10th 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 ₂			HPP ₄			HPP ₆			HPP ₈			HPP ₁₀			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
[GLUCOSE]																								
DOC	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
NUT	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
LU	***	***	***	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
T	***	***	***	***	***	***	**	*	***	***	*	***	ns	ns	ns	***	***	***	***	***	***	***	ns	ns
CAMP	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	ns	**	***
DOC*NUT	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***	***	***	ns	ns	ns
DOC*LU	ns	ns	ns	*	*	ns	*	**	***	***	***	***	***	***	***	***	***	***	ns	***	*	ns	***	*
DOC*T	**	*	ns	ns	ns	*	*	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	***	ns	*	*
DOC*CAMP	ns	ns	ns	ns	ns	ns	*	*	***	**	ns	**	***	ns	ns	*	ns	ns	***	***	***	ns	ns	*
NUT*LU	***	***	***	ns	ns	*	**	ns	**	*	ns	ns	ns	ns	ns	ns	ns	*	ns	***	*	***	***	***
NUT*T	***	***	**	***	***	ns	**	ns	ns	**	ns	*	ns	ns	ns	*	ns	***	ns	**	***	**	ns	ns
NUT*CAMP	***	***	***	*	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns	ns
LU*T	**	*	ns	ns	ns	ns	*	**	***	***	***	***	ns	ns	*	*	*	**	ns	***	***	*	***	*
LU*CAMP	***	***	***	ns	ns	ns	ns	*	**	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns	ns
T*CAMP	***	***	***	ns	ns	ns	ns	*	ns	ns	ns	ns	**	*	***	***	***	***	ns	***	*	**	*	ns
DOC*NUT*CAMP	ns	ns	ns	ns	ns	ns	**	ns	**	***	ns	***	***	*	***	***	*	***	*	***	***	ns	ns	ns
DOC*LU*CAMP	ns	ns	ns	ns	ns	ns	ns	ns	*	**	**	***	***	***	***	***	***	***	ns	***	***	ns	**	**
DOC*T*CAMP	ns	ns	ns	ns	ns	ns	**	***	***	ns	**	**	ns	ns	ns	ns	ns	***	ns	***	***	ns	ns	ns
NUT*LU*CAMP	***	***	***	ns	ns	ns	ns	ns	*	ns	ns	ns	***	ns	**	***	***	***	ns	***	***	ns	**	*
NUT*T*CAMP	***	***	*	ns	ns	ns	ns	ns	ns	*	**	*	ns	ns	ns	ns	ns	**	*	***	**	ns	***	ns
LU*T*CAMP	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	***	***	***	ns	***	***	ns	*	ns
DOC*NUT*LU*CAMP	ns	ns	*	ns	ns	ns	ns	ns		*	*	**	***	**	***	***	***	***	ns	***	***	ns	***	**
DOC*NUT*T*CAMP	ns	ns	ns	ns	ns	ns	**	**	**	ns	ns	ns	ns	ns	*	ns	ns	***	ns	***	**	ns	ns	ns
DOC*LU*T*CAMP	ns	ns	ns	ns	ns	ns	ns	ns	**	***	ns	***	*	ns	***	ns	ns	ns	ns	***	***	ns	ns	ns
NUT*LU*T*CAMP	***	***	*	ns	ns	ns	*	ns	ns	*	ns	*	*	ns	*	ns	ns	ns	ns	***	***	***	ns	ns
DOC*NUT*LU*T*CAMP	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	***	*	***	ns	ns	ns	ns	**	***	ns	ns	ns



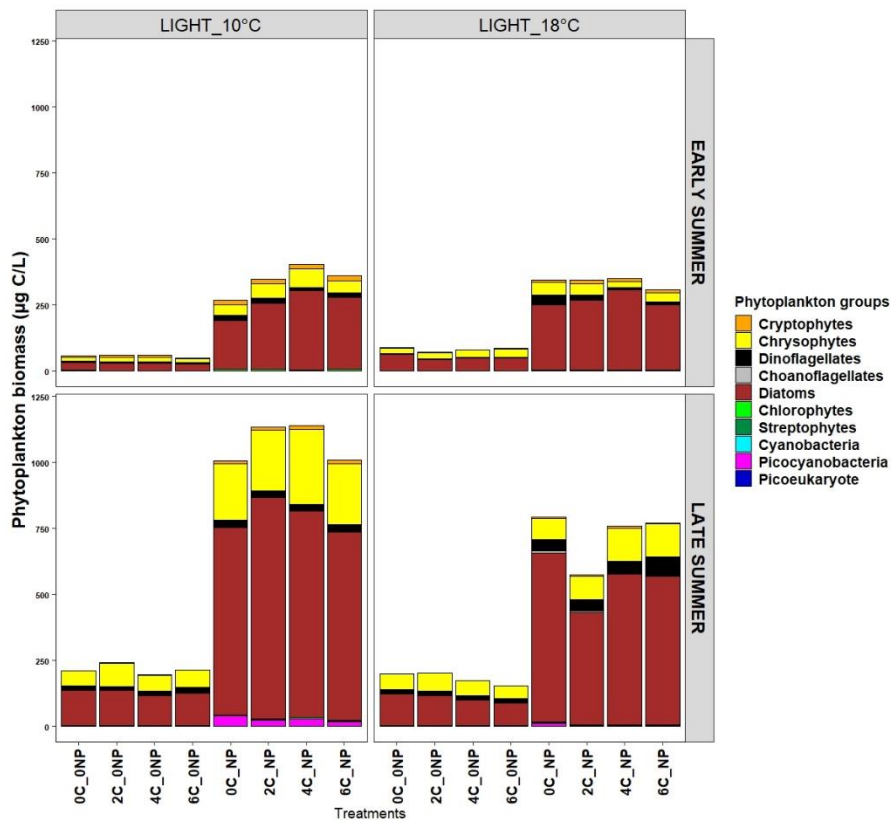
809

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)



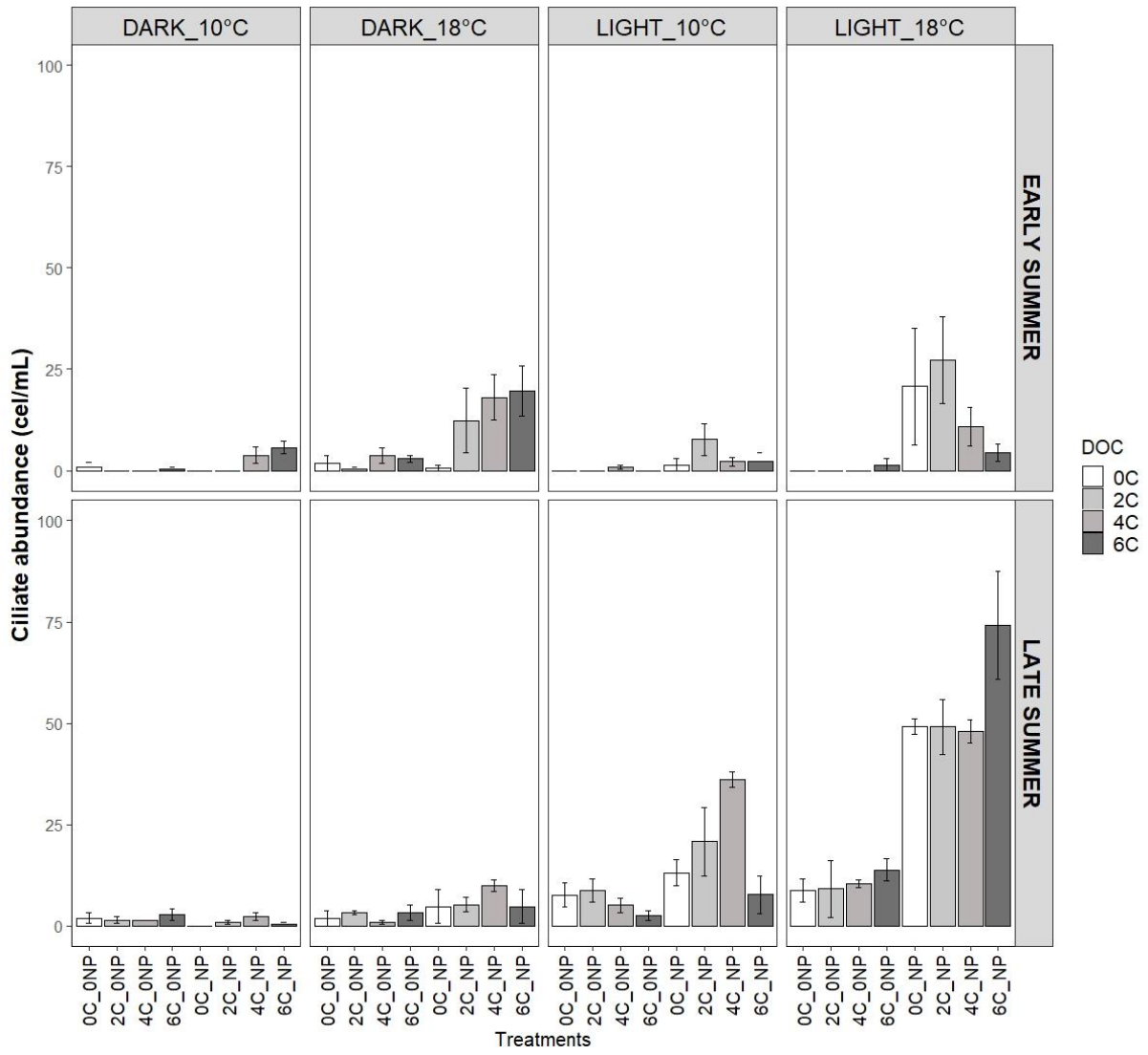
810

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).