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ABSTRACT

1. Seasonal variations in mountain lakes regulate phytoplankton communities, which are thus considered as useful indicators mirroring environmental conditions. Climate change is altering both dissolved organic matter (DOM) properties and phytoplankton dynamics in sentinel lakes. Here we examined the relationship between dissolved organic matter (DOM) and planktonic communities to investigate how ecological functions in an oligotrophic lake change over time. Physical, chemical, and biological data were analyzed during three pre-defined periods running from complete ice-cover right to the end of the ice-free season to assess the relationship between DOM, bacteria, and phytoplankton functional phenology.

2. The results showed that phytoplankton functional change occurred with dissolved organic matter variation. Phytoplankton community was dominated by small size autotrophs and mixotrophic flagellates during the *ice-influenced* period when DOM from sediment and terrestrial origin dominated the DOM pool of the lake. Phytoplankton diversity and richness increased during the post-snowmelt *overturn* period when terrestrial DOM dominated the DOM pool of the lake. Finally, large siliceous autotrophs, competitive under small nitrogen concentrations and high temperature, dominated almost exclusively the community during the *late summer* period. Because increasing of phytoplankton biomass, phytoplankton-derived DOM was dominant during the *late summer* period.

3. These phenological changes in phytoplankton community resulted in functional shifts at the base of the food web: the nature of the relationship between phytoplankton and bacteria progressively shifted, from strong top-down control exerted by phytoplankton over bacteria toward predominantly bottom-up control at the end of the ice-free season.

4. *Synthesis*. Phenological functional shifts occur rapidly and are related to local processes, like change in dissolved organic matter properties, nutrient limitation, temperature, and light

availability. Phytoplankton functional traits are useful indicators of whole ecosystem ecological functioning.

KEYWORDS

Mountain lakes; Functional traits; Global change; Trophic interactions; Biogeochemical cycles

1. INTRODUCTION

In mountain lakes, phenological variations regulate phytoplankton communities, making them useful indicators mirroring environmental conditions (Kuefner et al., 2021; Oleksy et al., 2020). Phytoplankton communities during the ice-free season in high-altitude lakes have been widely described. A large peak of phytoplankton biomass is typically observed in late summer (Pulido-Villena et al., 2008; Tiberti et al., 2013), usually associated with shifts in community composition and functional traits. Small unicellular flagellates have been observed in early summer, followed by a shift toward less edible taxa in late summer, like large mucilaginous colonies, flagellates (McKnight et al., 1990; Tiberti et al., 2013) or chlorophytes (Pulido-Villena et al., 2008). However, this successional pattern is not always observed and depends on the characteristics of the lakes (Jacquemin et al., 2019). Increasing data recorded on phytoplankton community structure during winter in high-altitude lakes reveals patterns similar to other seasonally ice-covered lakes (Özkundakci et al., 2016). Phytoplankton taxa show functional traits that enable them to survive during winter under ice (Bertilsson et al., 2013), like small size, motility, or mixotrophy (Rue et al., 2020). However, there are gaps in our knowledge of how phytoplankton shifts occur between ice-covered and ice-free seasons, and how functional shifts may affect whole lake ecosystem functioning.

Dissolved organic matter (DOM) is a very good sensor for mountain lake structure and function (Aiken, 2014). High-altitude lakes traditionally exhibit an autochthonous DOM fluorescence signature (Bastidas Navarro et al., 2014; Sommaruga & Augustin, 2006). Nevertheless, DOM origin can vary at short-term, seasonal, and interannual time scales. For example, allochthonous DOM can be transferred to lakes during snowmelt and extreme rainfall events at high altitude (Perga et al., 2018; Sadro et al., 2011). An advancing treeline due to temperature increase, as well as more frequent extreme precipitation events, may enhance allochthonous inputs in mountain lakes and their connectivity with their surrounding catchments (Harsch et al., 2009).

DOM dynamics strongly affect internal lake processes through direct and indirect transformations at the base of the food web (Creed et al., 2018). Seasonal variations in DOM origin and nutrient limitation patterns in high-altitude lakes should lead to temporal variations in microbial interactions (Jacquemin et al., 2018). Depending on the nutrient limitation, increase of allochthonous C has been shown to reduce bacterial reliance on phytoplankton-produced carbon (Jansson et al., 2000), stimulate bacterial biomass, and increase predation over bacteria by mixotrophs (Bergström, 2009). Experimental DOC and nutrient additions have induced different interaction between bacterioplankton and phytoplankton in winter, early summer, or in late summer in a high-altitude lake (Dory et al., 2021; Dory et al., unpublished results). Thus, a clearer picture is needed of how the phytoplankton-bacterioplankton relationship varies from the ice-covered to the ice-free season according to DOM dynamics in high-altitude lakes.

High-altitude lakes have been identified as sentinels of global change (Moser et al., 2019). However, their value as sentinels is highly dependent on how well we understand their internal processes, determined to a large extent by climate seasonality (Catalan, Ventura, et al., 2002). The functioning of high-altitude lakes is traditionally divided into ice-covered and ice-free periods, and most studies focus on one period. Based on various lake indicators, however, some studies track temporal changes on a finer scale to further describe internal lake processes. By analyzing changes in microbial assemblages, Felip et al. (2002) discriminated between three periods of winter cover in an alpine high mountain lake. Ecosystem metabolism (Sadro et al., 2011) and DOM dynamics (Olson et al., 2021) allowed to highlight a snowmelt influence until late July or after the beginning of August. Tiberti et al. (2013) observed a late summer peak of phytoplankton biomass in October, slightly behind the physico-chemical variations.

We considered three periods ranging from complete ice-cover to the end of the ice-free season in an alpine lake: (i) the ice-influenced period; (ii) the overturn period; (iii) the late summer period. The temporal dynamics of dissolved organic matter (DOM) and planktonic

communities over these three periods were examined to better understand how lake ecological functions change over time. We hypothesized that the signature of DOM would be less influenced by allochthonous inputs and phytoplankton production during the ice-influenced period, mainly determined by allochthonous inputs during the overturn period, and mainly determined by primary production during the late summer period. We also anticipated phytoplankton functional changes according to environmental variations, with more winter-adapted traits and mixotrophic taxa during the ice-influenced period and more autotrophy during the ice-free season. Finally, we expected functional shifts at the base of the food web with a modified balance between heterotrophy and autotrophy, linked to the temporal dynamics of DOM, bacterioplankton, and phytoplankton communities.

2. MATERIALS AND METHODS

2.1. Lake area, sampling, and definition of time periods

Samplings were performed in the oligotrophic high-altitude Lake Cordes (Dory et al., 2021a). The lake was sampled 11 times between February 2020 and July 2021, divided into three periods. The lake was sampled four times during the ice-influenced period (IIP, from February to mid-June), four times during the overturn period (OP, from mid-June to late July), and three times during the late summer period (LSP, from August to October) (**Table A1**). On every date, samples were collected from the deepest area of the lake. Water was sampled using a Niskin Bottle 1 m above the bottom and 1 m below the surface. All the samples were realized in triplicate and then fixed and stored in the field in containers appropriate to the analyses to be performed in the laboratory. Abiotic parameters of temperature, dissolved oxygen, and turbidity were monitored using an Exo2 multiparameter probe (YSI, United States) placed 1 m above the lake bottom.

2.2. Biotic and abiotic parameters

2.2.1. Environmental and climate parameters

Water properties of the lake (temperature, dissolved oxygen, and turbidity) were averaged from continuous data measured by the multiparameter probe. Data were obtained every hour and a daily average was calculated for each sampling day. Daily precipitations were taken from a weather station located about 14 km from the lake, and data were collected from the alpine meteorological observation network (ROMMA, http://romma.org/). Average precipitations were calculated from the 10 days preceding the sampling date.

2.2.2. Nutrients (C, N, P, Si)

Water samples of 1L for nutrient analysis were filtered through precombusted (4h, 450°C) GF/F glass filters (Whatman GF/F, 25 mm, 0.7 µm). A first fraction of the filtered water was stored in 24 ml precombusted (4h, 450°C) glass tubes (Wheaton equipped with Teflon/silicon septa) and preserved with 25 µL of Sodium Azide solution (1 M NaN_3) at 4°C for dissolved organic carbon (DOC) analysis and dissolved organic matter (DOM) fluorescence measurements. DOC concentrations were measured using a TOC-VCSH analyzer (Shimadzsu, TOC-V). A second filtered fraction was stored in 150 ml HDPE bottles and frozen (-18°C) for analysis of dissolved inorganic nitrogen (DIN = NH_4^+ + NO_2^- + NO_3^-), soluble reactive phosphorus (SRP = PO_4^{3-}), and Silica (Si). DIN, SRP, and Si were measured by ion chromatography (Metrohm, 930 Compact IC Flex).

2.2.3. C:N ratio and C isotopic analyses

C:N ratios and analyses of carbon stable isotope ($\delta^{13}C$) in particulate organic matter (POM) were performed from June 2020 to September 2020 and in May 2021. Water samples were filtered (0.7 – 20 µm) to concentrate the finest fraction of the seston, consisting mainly of phytoplankton. Filters were then dried at 60°C and decarbonated by acid-fuming (HCl 37%) for analysis. Samples were ground (RETSCH Mixer Mill MM 400), then between 0.2 and 0.6 mg of homogeneous samples were weighed into tin capsules (three capsules per sample). C and

N abundances and $\delta^{13}C$ ratios were analyzed using an Elemental analyzer (EA, Flash HT plus - Thermo Scientific) equipped with an autosampler configured with a combustion reactor for carbon and nitrogen analyses. The elemental analyzer is connected to an isotope ratio mass spectrometer (IrMS "Delta V Advantage", Thermo Scientific). Organic carbon and total nitrogen contents were determined from the signal of the TCD detector in the Elemental Analyzer. The C and N values obtained were used to calculate the C:N ratio of the suspended particulate organic matter (POM) in the water sample. C:N values were measured from June 2020 to September 2020 and in March and May 2021. $\delta^{13}C$ (‰) values were expressed relative to the international reference (Vienna-Pee Dee Belemnite, V-PDB) using the equation 1 and 0.011237 for $R_{standard}$:

$$\delta^{13}C\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (1)$$

The $\delta^{13}C$ determination was calibrated by a two-point method using the international standard NBS-22 and a laboratory standard for urea.

2.2.4. DOM extractions

Leachates of four sources of organic matter were used to characterize the fluorescence signature of dissolved organic matter in Lake Cordes. The soil and alpine grass were collected randomly in the watershed on each sample date. When present, common macrophyte species were collected and cleaned by repeated rinsing in distilled water. Sediment samples were taken at each sample date with an Ekman grab. All samples were freeze-dried, ground, and sifted through a 2 mm mesh sieve. The samples were extracted with Milli-Q water (solid to water ratio, w/v = 0.1), the suspensions were placed at 4°C and stirred manually within 24 h. The suspensions were then centrifuged (10 000 rpm, 20 min), filtered (pre-combusted GF/F), and subsequently stored at 4 °C until analysis.

2.2.5. Absorbance and fluorescence measurements

Leachates and natural water samples were analyzed for UV-vis spectra using a Spectrophotometer V-550 (Jasco) with 10-cm Suprasil quartz cells. Water was scanned between 230 and 800 nm using a 1000 $nm.min^{-1}$ scan rate and 0.5 nm resolution. Milli-Q water was used as a blank and subtracted from each sample. Fluorescence measurements were performed using a spectrofluorometer (F4500, Hitachi, Santa Clara, California, USA) equipped with a 450W xenon lamp. The spectra were acquired in the scan ranges of 200–600 nm for emission and excitation, with both slits fixed at 5 nm using 1 cm quatrz Suprasil cell. The scan speed was set at 2400 $nm.min^{-1}$, and the detector voltage was 700 V. Fluorescence intensity was normalized to Raman units (R.U.) using the daily-measured Raman peak of Mili-Q water (λ_{ex} = 350 nm, λ_{em} = 371–428 nm). The collected EEMs were analyzed using parallel factor analysis (PARAFAC), to identify the different components of the FDOM pool. PARAFAC was conducted on the EEM dataset using Progmeef software (Redon, 2018) in Matlab language. PARAFAC was applied to the complete dataset containing all the EEMs (leachates and water samples of DOM). Three fluorescence indexes were used to characterize the FDOM: the humification index (HIX), the freshness index (BIX), and the fluorescence index (FI). The HIX is calculated as the sum of emissions ranging between 435 and 480 nm, divided by the sum of emissions ranging between 300 and 345 nm, at an excitation intensity of 255 nm, corrected according to the procedure of Ohno (2002). The BIX is the ratio of emission intensity at 380 nm divided by maximum emission intensity between 420 and 435 nm, at an excitation intensity of 310 nm. The BIX value reflects the contribution of recent (autochthonous) organic matter to the organic matter pool. Low ratios are interpreted as a low contribution of autochthonous organic matter, while high ratios correlate with a strong contribution of recent autochthonous organic matter (Gabor et al., 2014). Finally, the FI is the ratio of emission intensity between 470 and 520 nm, at an excitation intensity of 370 nm (Cory & McKnight, 2005). The

fluorescence index indicates whether the DOM precursor material is more microbial in nature (FI ~ 1.8) or is of terrestrial origin (FI ~ 1.2).

2.2.6. Phytoplankton analyses

Phytoplankton samples were fixed with a formaldehyde solution (5%) and stored in 250 ml HDPE bottles. Phytoplankton counts were performed according to the Utermöhl (1958) method, at 40-fold magnification under an inverted microscope (Olympus IX 70). Phytoplankton samples were identified at genus level and species level when possible, using appropriate taxonomic guides. Phytoplankton biovolume was estimated by shape assimilation to known geometric forms and direct measurement of the main cell dimensions. Then the biovolume was converted into biomass using the particular carbon content defined for each class by Wetzel & Likens (2000). Phytoplankton taxa were classified in four groups: (i) taxonomic groups (**TAX**) based on their main phylogenetic affiliations; (ii) protist functional groups (**PFG**) based on their nutrient acquisition, *i.e.* photoautotrophs lacking phagotrophy capacity (PA) and constitutive mixotrophs (CM) (Mitra et al., 2016); (iii) morphology-based functional groups (**MBFG**) based on their morphological traits (Kruk et al., 2010); and (iv) functional groups (**FG**) based on their morphology and physiology and on the similarity of their ecological characteristics (Padisák et al., 2009; Reynolds et al., 2002). Phytoplankton diversity was characterized according to two indexes: the Species richness index (S) measures the total number of phytoplankton species in each sample, and the Shannon index (H') takes into account both the richness and evenness of the species present in each sample. Ciliate abundances were also estimated via the Utermöhl method.

2.2.7. Picoplankton

Water samples (1.5 ml) were filtered (20 µm) and analyzed for picoplankton analyses by flow cytometry. Samples were fixed with glutaraldehyde (0.25% final concentration) and stored at

225 −80℃ until flow cytometry analysis. Picoplankton was characterized and enumerated using an
226 Accuri C6 flow cytometer equipped with a blue laser (488 nm) and using BD Accuri CFlow
227 Plus Analysis software (BD-Biosciences). Non-fluorescent polystyrene microspheres (Flow
228 Cytometry Size Calibration Kit, Thermo Fisher Scientific) were used as a size standard. For
229 picocyanobacteria, 500 µl of sample were run at fast speed (66 µl/min). Picocyanobacteria
230 (PCY) were identified by their small size (FSC < 2 µm), low complexity (SSC), and
231 fluorescence (emissions in the orange and red wavelength ranges, respectively 585 ± 20 and
232 >670 nm). Flow cytometer analysis distinguished between two groups of picocyanobacteria:
233 cells with high orange fluorescence were classified as phycoerythrin-rich picocyanobacteria
234 (PE-Pcy); cells with low orange and high dark red fluorescence were classified as
235 phycocyanine-rich picocyanobacteria (PC-Pcy). For heterotrophic prokaryotes (HP), samples
236 were stained with 1:10,000 (vol/vol) SYBR® Green II and incubated 20 min in darkness. 50 µl
237 of stained samples were run at medium speed (35 µl/min). HP were identified by their small
238 size (low FSC), low complexity (low SSC), high green fluorescence (530 ± 15 nm), and lack
239 of red (> 670 nm) fluorescence. Picoplankton data were acquired and analyzed using BD Accuri
240 CFlow Plus Analysis software (BD-Biosciences). Abundances were transformed into carbon
241 content based on the literature. Cell abundance (cells/ml) of HP was converted to biomass (µg
242 C/L) using 20 fg C/cell as constant conversion factor (Linacre et al., 2015). A conversion factor
243 of 112 fg C/cell was used for picocyanobacteria (Linacre et al., 2015).

244 **2.3. Statistical analyses**

245 One-way ANOVA tests followed by pairwise comparisons were used to test the effect of period
246 on the various abiotic and biological variables measured, and to test differences in fluorescence
247 characteristics between the four leachates of organic matter sources. Non-metric
248 multidimensional scaling (NMDS) analysis was performed on a Bray-Curtis distance matrix to
249 visualize the differences in taxonomic structure of the phytoplankton community between

periods. To test the hypothesis that the taxonomic distribution of the phytoplankton community was structured by period, a multivariate permutation analysis of variance (permanova) was used with 999 permutations ($p = 0.05$) with the adonis function in R. A multivariate homogeneity analysis of group dispersion (β diversity) was used to assess the homogeneity of phytoplankton assemblages within a group of samples (betadisper function). Canonical Correspondence Analysis (CCA) was performed to explore the distribution of the different phytoplankton taxonomical groups in relation to the measured explanatory variables. The significance of the phytoplankton composition variance explained by the selected abiotic variables was assessed by permutation tests. All analyses were performed with R 3.6.3 (R Core Team, 2018).

3. RESULTS

3.1. Abiotic and climate parameters

The ice-influenced period (IIP) was characterized by significantly lower concentrations of dissolved oxygen, temperature, and precipitations (**Figure 1**). Turbidity was significantly greater during this period, but highly variable. SRP concentrations were low during the ice-influenced period, whereas silica and DIN concentrations were high and highly variable. Ammonium was the dominant N source of the DIN pool during IIP (73 ± 13%, data not showed). Particulate organic matter (POM) showed the most negative $\delta^{13}C$ values and low C:N values.

During the overturn period (OP), many abiotic parameters significantly increased, such as dissolved oxygen, temperature, precipitations, and SRP concentrations. The $\delta^{13}C$ values and C:N of POM also significantly increased during OP, whereas turbidity and silica concentrations were significantly lower than during IIP. While there was no significant change in DIN concentrations, they were less variable. Nevertheless, within the DIN pool, nitrite became dominant over ammonium during OP (88 ± 7%, data not showed).

The late summer period (LSP) did not significantly differ from OP for any abiotic parameters except $\delta^{13}C_{POM}$ which showed significantly higher values, and C:N of POM. However, LSP was significantly different from IIP for all parameters except SRP concentrations and C:N of POM. This period was characterized by higher variability in temperature and precipitations.

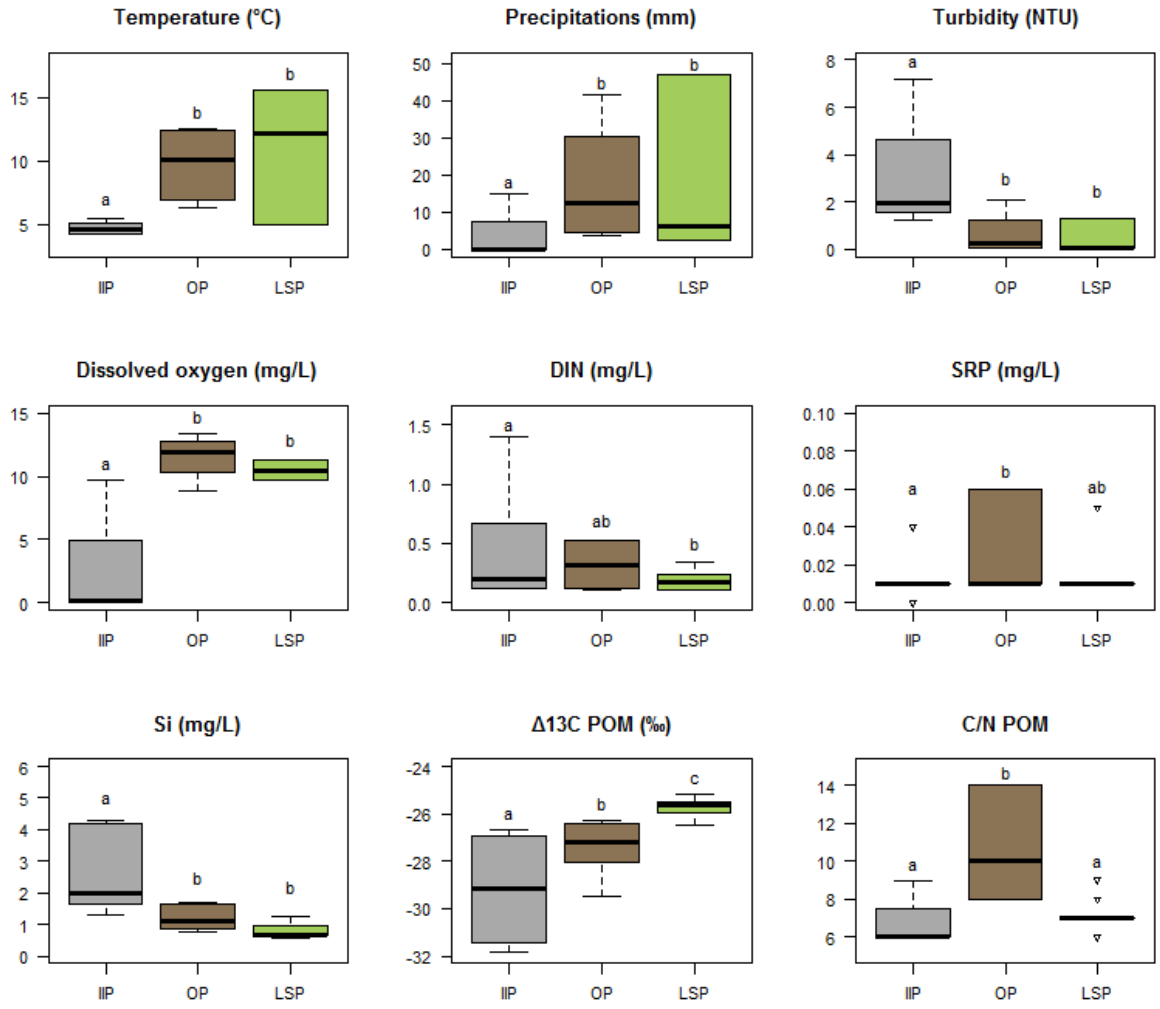

Figure 1: Abiotic and climate parameters measured in the three defined functional periods in Lake Cordes. DIN: dissolved inorganic nitrogen; SRP: soluble reactive phosphorus; Si: silica; POM: particulate organic matter.

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3.2. Characterization and dynamics of DOM

3.2.1. *Identification of the components by PARAFAC modeling*

PARAFAC analysis of the EEM spectra of leachates and water samples identified four fluorescent components (C1, C2, C3, and C4) (**Figure 2**). C1 and C4 are known to be non-humic compounds: C1 (Ex/Em = 300/350 nm) is linked to soluble microbial byproduct-like material (Chen et al., 2003), while C4 (Ex/Em = 230-270/350 nm) is often linked to simple aromatic proteins such as tyrosine. Conversely, C2 and C3 are considered humic-like compounds: C2 (Ex/Em = 250-310/450 nm) is referred to as a combined component constituted by humic acid-like and fulvic acid-like materials. C3 (Ex/Em = 270(360)/460 nm) has been associated with humic acid-like organics (Murphy et al., 2008).

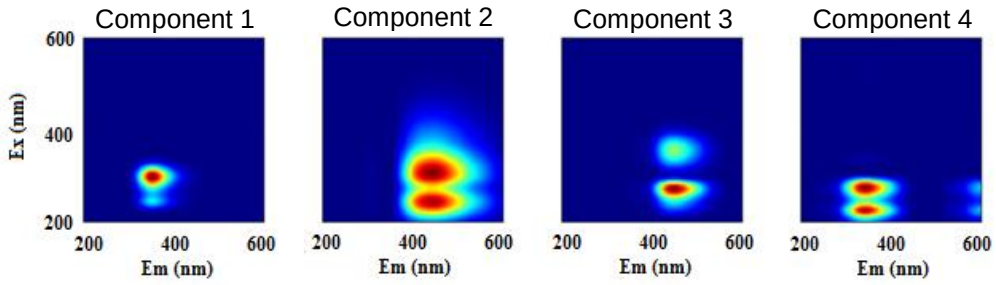

Figure 2: PARAFAC model output showing fluorescence signatures of the four fluorescent components identified in the 4 leachates and in water samples.

3.2.2. *Fluorescence signature of the sources of DOM*

Regarding the "internal" sources, the macrophytes leachate fluorescence response was mainly dominated by C4, while the sediment leachate fluorescence response was mainly dominated by C3 (**Figure 3A**). Regarding the "external" sources, the soil and the grass leachate fluorescence were mainly dominated by C2 and C4.

HIX values varied significantly between sources (Anova, F = 14.9, p-value<0.001), with a greater predominance of humic compounds in the sediment and soil leachates than in the macrophytes and grass leachates (**Figure 3B**). BIX values were significantly higher in the two internal sources than in the two external sources (Anova, F = 17.9, p-value<0.001). Finally, FI values were significantly higher in the sediment leachate (FI = 1.89 ± 0.2) than in the other sources, and were lowest in the soil leachate (FI = 1.16 ± 0.04) (Anova, F = 42.6, p-value<0.001) (**Figure 3C**).

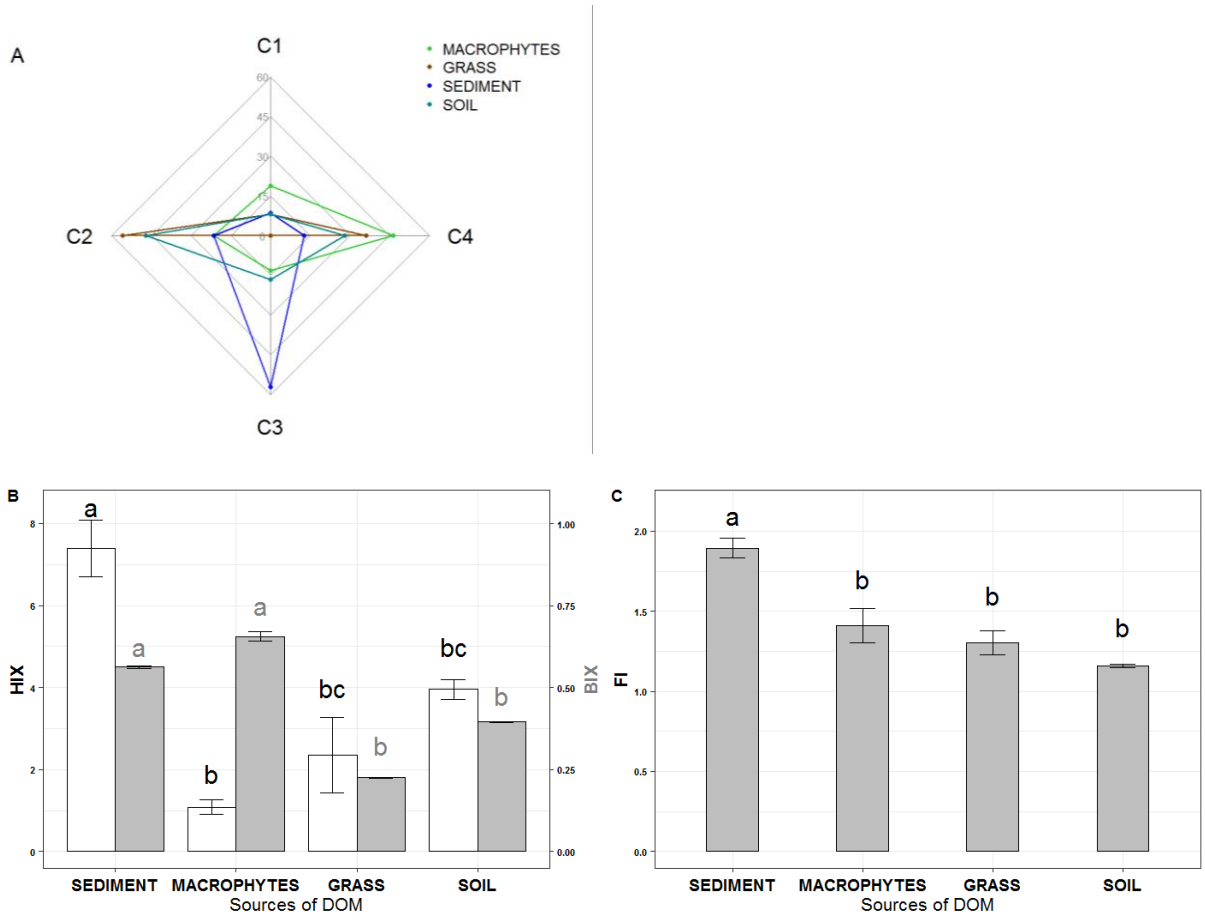

Figure 3: (A) Relative contribution of the four components for the four leachates of dissolved organic matter (DOM) sources; (B) Humification index (HIX) in white and freshness index (BIX) in grey of the four leachates of DOM sources; (C) Fluorescence index of the four leachates of DOM sources.

3.2.3. *Dynamic signature of lake DOM*

During the ice-influenced period (IIP), significantly higher values for FI, HIX, and the proportion of C3 were observed than during the two other periods (**Figure 4**). The proportion of C2 was also high. This period was also characterized by moderate but variable DOC concentrations and a moderate proportion of C4. The overturn period (OP) was characterized by significantly lower HIX and FI values than during IIP, while BIX values did not differ significantly, remaining low. Measured DOC concentrations were low. OP was characterized by a high proportion of C2 and C4 and a low proportion of C1. Finally, during the late summer period (LSP), BIX values were significantly higher than during the two other periods, associated with low but variable values of FI and HIX. DOC concentrations and C1 proportion were higher in LSP, while the proportions of C2, C3 and C4 were low. It is noteworthy that, the late summer period was characterized by higher variability in all parameters except DOC.

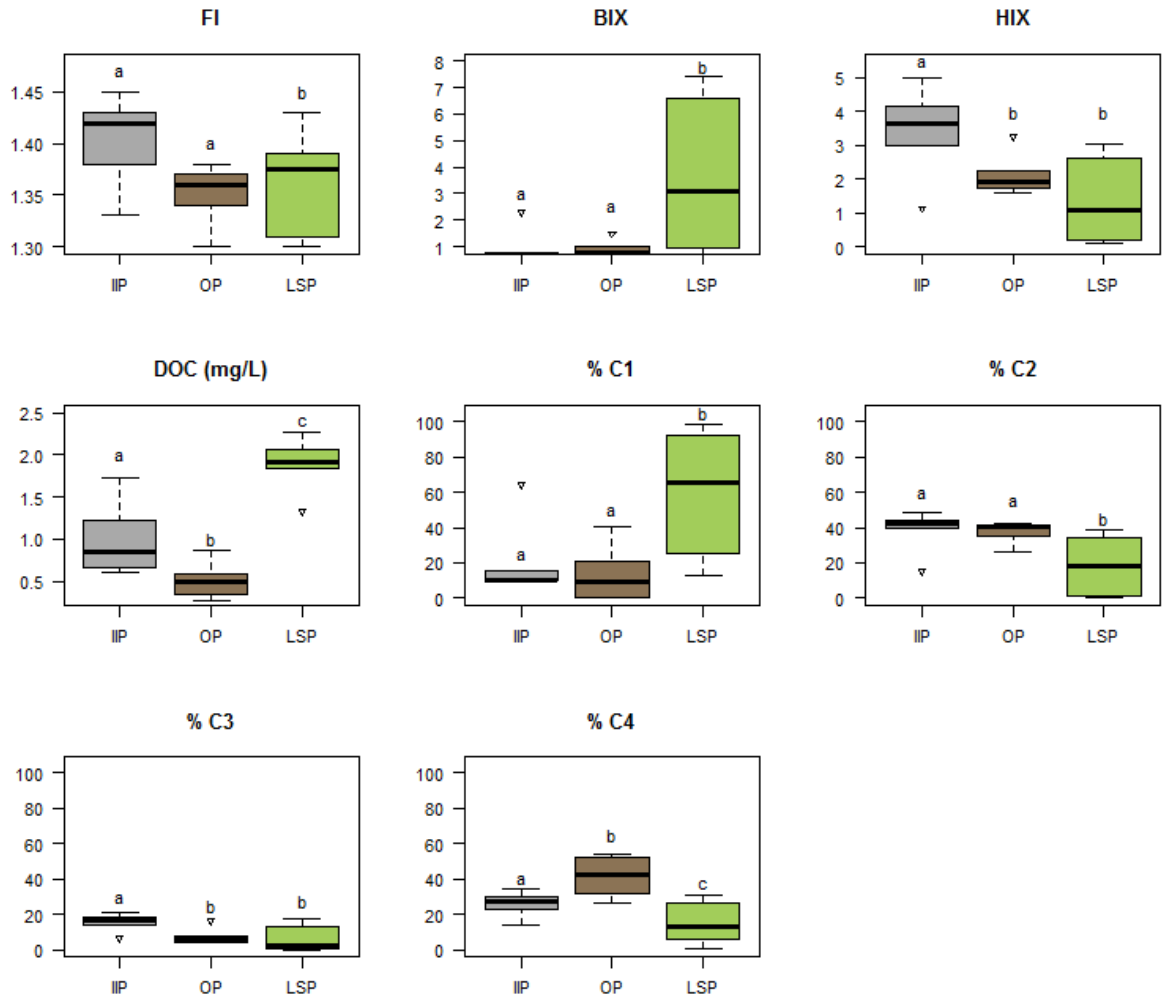

Figure 4: DOM characteristics measured in the three defined periods in Lake Cordes. DOC: dissolved organic carbon.

317

318 **3.3. Planktonic community dynamics**

319 Phytoplankton biomass was low during the ice-influenced period (IIP) and the overturn period
320 (OP), increasing significantly during the late summer period (LSP) (**Figure 5**). While specific
321 richness increased progressively from IIP to LSP, the Shannon diversity index was significantly
322 higher in OP than during the two other periods. HP biomass increased progressively over the
323 three periods. Ciliate abundance was low during IIP and OP, then significantly increased during
324 LSP.

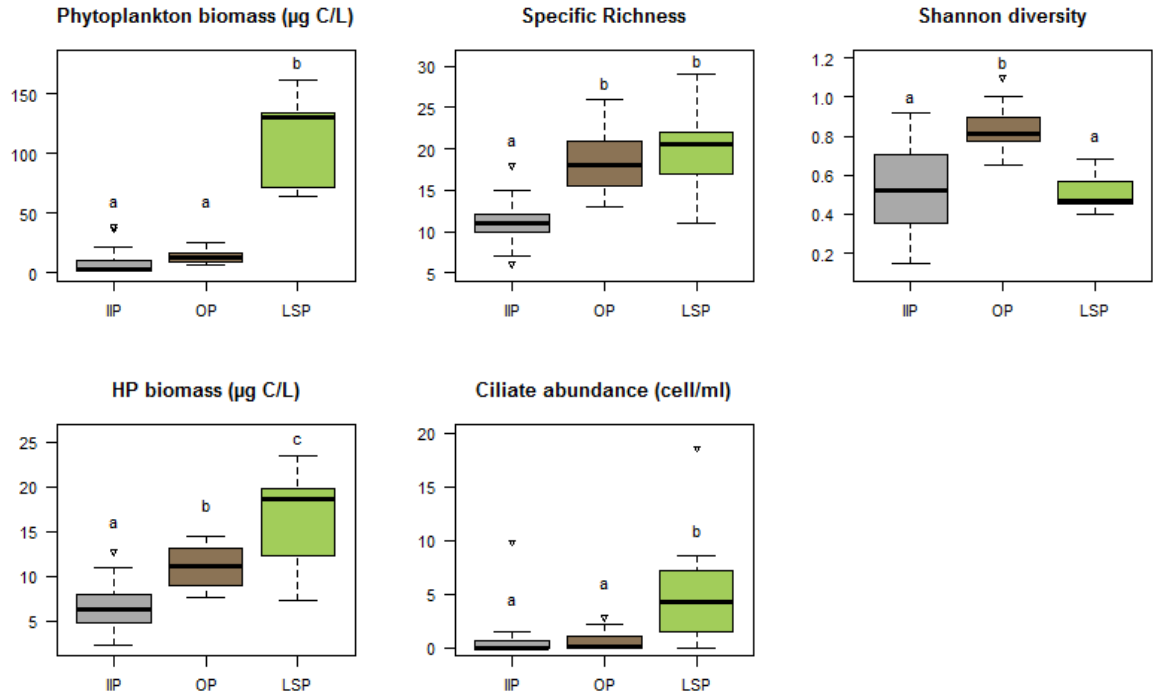

Figure 5: Biological parameters measured in the three defined periods in Lake Cordes. HP: heterotrophic prokaryotes.

325

326 Concerning taxonomical and functional changes in phytoplankton assemblages, a significant
327 shift in phytoplankton community structure was observed over the three periods (**Figure A1,**
328 Adonis, F = 29.362, r2 = 0.57, p-value < 0.001). Cyanobacteria dominated the other
329 phytoplankton groups during IIP (**Figure 6**), which also showed a high proportion of
330 cryptophytes, dinoflagellates, and phycoerythrin-rich picocyanobacteria (PE_Pcy). A relatively
331 high proportion of constitutive mixotrophs was found during IIP (45%), as well as MBFG
332 groups I (small cells with high surface/volume ratio) and V (flagellates of medium to large size)
333 and FG groups K (shallow, nutrient-rich water column) and Y (small, enriched lakes, lentic
334 ecosystems with low grazer pressure). OP was characterized by an increased proportion of
335 diatoms (51%), which became dominant over the other phytoplankton groups. Dominance by
336 the PE_rich PCy was replaced by that of the phycocyanin-rich picocyanobacteria (PC_Pcy).
337 The relative proportion of mixotrophs remained high (45%). Among the mixotrophic taxa,

while the proportion of dinoflagellates remained the same as during IIP, an increase in the proportion of chrysophytes was observed together with a decrease in the proportion of cryptophytes. The proportion of MBFG groups II (small siliceous flagellates) and VI (non-flagellated with siliceous exoskeletons) increased, as did FG groups A (clear, deep, often well-mixed lakes), Lo (all lentic ecosystems), and MP (all types of lakes, or frequently stirred up, inorganically turbid shallow lakes). During LSP, the phytoplankton assemblage was mainly composed of diatoms (89%), corresponding to the high proportion of MBFG group VI and FG group MP. PC_Pcy dominated the pool of picocyanobacteria in LSP.

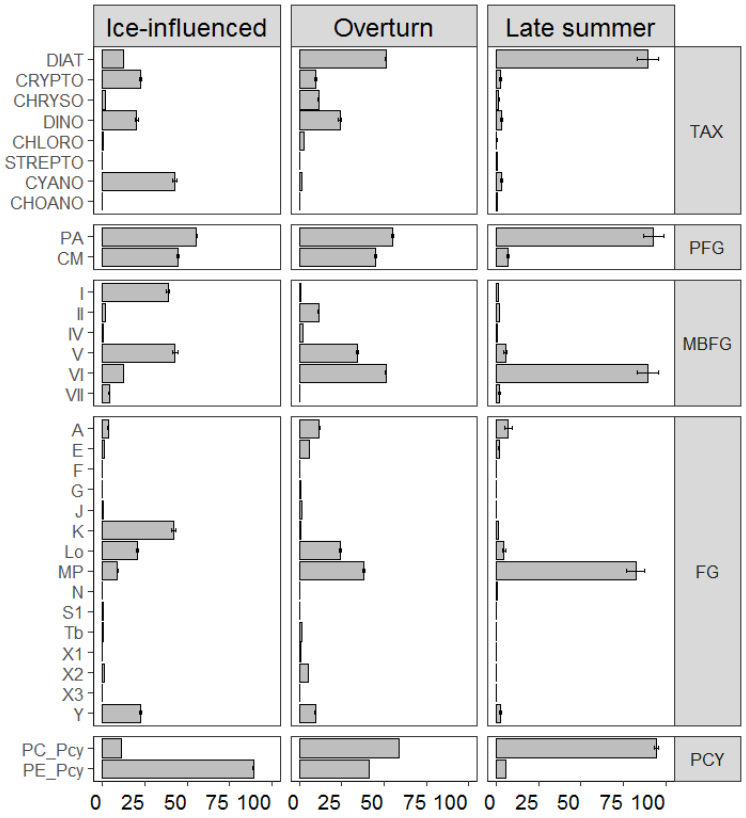

Figure 6: Phytoplankton composition in each period (error bars correspond to standard error). DIAT = diatoms; CRYPTO = cryptophytes; CHRYSO = chrysophytes; DINO = dinoflagellates; CHLORO = chlorophytes; STREPTO = Streptophytes; CYANO = cyanobacteria; CHOANO = choanoflagellates; PA = photoautotrophic phytoplankton; CM = constitutive mixotrophs; PE_Pcy = phycoerythrin-rich picocyanobacteria; PC_Pcy = phycocyanin-rich picocyanobacteria.

3.4. Links between microbial plankton and dissolved organic matter

CCA was conducted with phytoplankton taxonomical groups as response variables and abiotic parameters, and HP biomass and ciliate abundance as explanatory variables (**Figure 7**). The constrained variables explained 87% of inertia, and the eigenvalues for the first and second sorting axes were 0.178 and 0.075, respectively. Dissolved oxygen, HP biomass, FI, temperature, and turbidity showed far higher correlation coefficients with the first axis (-0.87, -0.63, 0.58, -0.56, and 0.55, respectively), suggesting that these explanatory variables played a predominant role in affecting the taxonomic composition of the phytoplankton community. PE_PCY, cyanobacteria and cryptophytes were associated with higher silica concentrations, higher proportions of C3, higher HIX values, higher turbidity, but lower dissolved oxygen concentrations during the ice-influenced period. The second axis discriminated between overturn and late summer periods, and DOC concentrations and C1 proportions were particularly high and positively correlated with the late summer period. Both OP and LSP were characterized by an increase in diatoms, while chrysophytes and chlorophytes were particularly prevalent during OP.

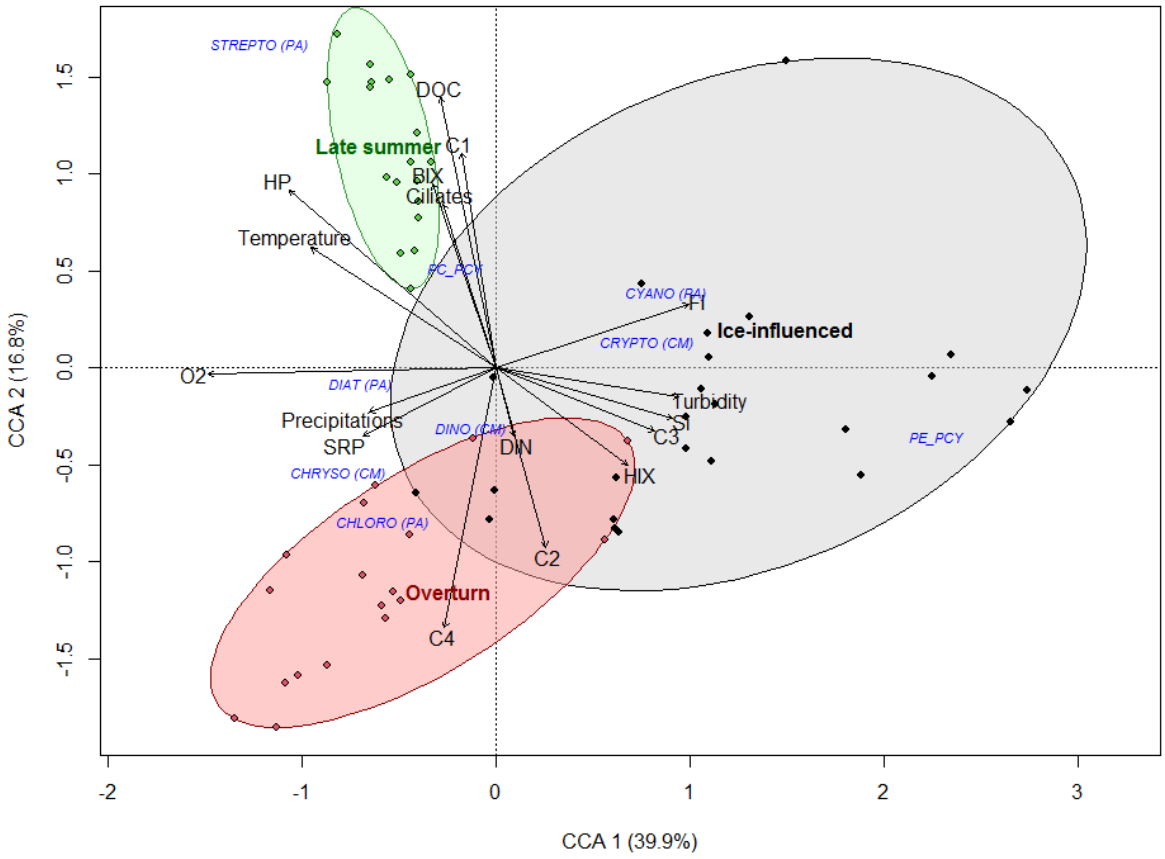

Figure 7: CCA performed on the taxonomic phytoplankton groups (and their corresponding PFG classification) and picophytoplankton groups (PCY) in relation to explanatory variables. DOC: dissolved organic carbon; HP: heterotrophic prokaryotes; O2: dissolved oxygen; SRP: soluble reactive phosphorus; DIN: dissolved inorganic nitrogen; Si: silica; CYANO: cyanobacteria; CRYPTO: cryptophytes; DINO: dinoflagellates; CHLORO: chlorophytes; CHRYSO: chrysophytes; DIAT: diatoms; STREPTO: Streptophytes; PC_PCY: phycocyanin-rich picocyanobacteria; PE_PCY: phycoerythrin-rich picocyanobacteria.

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4. DISCUSSION

4.1.Variation in DOM origin

We characterized the fluorescence signature of four sources of DOM to better understand DOM dynamics in Lake Cordes. All the DOM sources exhibited a specific fluorescent signature and, interestingly, none of the four sources of DOM exhibited a C1-dominated signature. Our findings demonstrate that the origin of DOM varied greatly over the three periods in Lake Cordes (**Figure 8**). The ice-influenced period exhibited the highest FI values (1.41). In previous studies in high-altitude lakes, FI values of 1.44 were considered to be low and to reflect a more allochthonous DOM pool (Miller et al., 2009; Rose et al., 2015). However, the associated high HIX values (3.43) and low BIX values (0.96) suggest a refractory and humic DOM linked to a microbial precursor material (Du et al., 2016; Ohno, 2002). The low C:N values observed for POM confirm a more autochthonous origin for DOM during the ice-influenced period. Moreover, the ice-influenced period showed the highest proportion of C3, indicating a higher contribution of the sediment to the DOM pool than during the two other periods. However, allochthonous inputs of DOM also occurred during the ice-influenced period, as suggested by the highly variable C:N values of POM and the relatively high proportion of C2 and C4. Similar results were previously observed during winter in a subarctic clear-water lake (Karlsson et al., 2008), where sediment organic carbon was the dominant carbon source for respiration under the ice, but both allochthonous and autochthonous carbon contributed to DIC accumulation in the lake. In our study, such allochthonous inputs likely started at the end of the ice-influenced period, when the snowmelt occurred.

During the overturn period, DOM showed the lowest FI (1.35) and BIX (0.90) values and moderate HIX (2.12) values, suggesting a more refractory, terrestrial origin for DOM than during the ice-influenced period. The C:N values of POM and the contribution of C2 and C4 to FDOM were highest during the overturn period, confirming the hypothesis of a higher

389 contribution of the DOM pool originating from the catchment area. Despite the predominant
390 soil signature of the DOM during the overturn period, however, low DOC concentrations were
391 measured in the water column. It is therefore possible that DOC inflows occurred earlier, at the
392 end of the ice-influenced period when the snow and ice started to melt. Indeed, DOC can rapidly
393 be degraded after allochthonous DOM inputs (Catalán et al., 2013). These low DOC
394 concentrations may also be explained by low DOC content in the allochthonous DOM
395 transferred to the lakes (Queimaliños et al., 2019) and a strong dilution effect during snowmelt
396 or rain events involving shorter water residence (Anderson & Stedmon, 2007).

397 During the late summer period of our study, C1 was predominant in DOM. This component
398 was not associated with a specific fluorescence signature of the four leachate sources of DOM
399 but was associated with higher DOC concentrations and higher phytoplankton biomass.
400 Moreover, DOM showed low but highly variable HIX values (1.35) and high BIX values (3.65),
401 suggesting a high contribution of fresh, autochthonous DOM. However, FI values were also
402 low (1.36) thus not indicative of organic material derived from algae and bacteria. Like the
403 other fluorescence indexes, FI was highly variable during the late summer period, and
404 particularly low at the October sampling. Heavy precipitations occurred during October,
405 possibly driving allochthonous inputs into the lake and modifying the autochthonous signature
406 of the DOM. Thus, despite a variable signature, our results suggest that DOM mainly originated
407 from phytoplankton development in the water column during the late summer period. These
408 findings are consistent with other studies in mountain lakes showing a higher contribution of
409 phytoplankton to the DOM pool at the end of the ice-free period (Sadro & Melack, 2012;
410 Sommaruga & Augustin, 2006).

411 **4.2. Phytoplankton functional changes**

412 Our results highlight the high sensitivity to environmental variations of phytoplankton
413 assemblages, whose structure and composition changed according to shifts in lake functioning

(**Figure 8**). During the ice-influenced period, phytoplankton biomass was low and the assemblage was dominated by small autotrophs with a high surface/volume ratio, and by large mixotrophic flagellates. Mixotrophs represented 45% of the phytoplankton assemblage, mainly cryptophytes (*Cryptomonas sp.*) and dinoflagellates (*Katodinium sp., Peridinium umbonatum, Gymnodinium sp.*). These results confirm that mixotrophic strategy and motility are critical for phytoplankton survival under ice (Özkundakci et al., 2016). The autotrophic fraction was mainly composed of small cyanobacteria (*Synechococcus sp.* and *Gomphosphaeria sp.*). This group is known to be cold-tolerant (Jungblut & Vincent, 2017) and has already been observed under ice in oligotrophic lakes (Bullerjahn et al., 2020). In addition, the pool of picocyanobacteria was dominated by phycoerythrin-rich picocyanobacteria (PE_Pcy) during the ice-influenced period. Picocyanobacteria seems to be well adapted to low levels of light and low nutrient concentrations (Callieri et al., 2007; Stomp et al., 2007). Because PE_Pcy use phycoerythrin for harvesting the prevailing green wavelengths, they are well-adapted to poor under-ice light conditions (Jungblut & Vincent, 2017).

The overturn period was characterized by a similar phytoplankton biomass but changes in species composition, with more taxa associated with well-mixed systems. Species richness substantially increased, and diversity was at a peak. Changes in phytoplankton community structure have previously been observed after snowmelt as a consequence of meltwater inputs in oligotrophic lakes (Williams et al., 2016), and episodic nutrient enrichments can increase species richness (Zufiaurre et al., 2021). The increased richness and diversity observed during the overturn period was therefore likely the result of nutrient inputs occurring during snowmelt. The proportion of mixotrophs remained the same as in the ice-influenced period and dinoflagellates remained dominant, but small chrysophytes (*Dinobryon divergens, Kephyrion spirale*) prevailed over cryptophytes during the overturn period. This replacement is not surprising: chrysophytes, especially *Dinobryon*, are known to be obligatory phototrophs

(Princiotta et al., 2016) and to form resting stages to withstand adverse environmental conditions (Özkundakci et al., 2016). Changes in light spectral composition have been shown to change dominance patterns between cryptophytes and chrysophytes in shallow North Patagonian lakes (Gerea et al., 2017). Here, in the autotrophic fraction of the phytoplankton assemblage, diatoms (*Cyclotella comensis* and *Fragilaria nanana*) replaced the small cyanobacteria. These two species are widely used as an indicator of physico-chemical changes (Cantonati et al., 2021), especially increased water temperatures in alpine lakes (Catalan, Pla, et al., 2002; Sochuliaková et al., 2018). In addition, the PE:PC picocyanobacteria changed during the overturn, and PC_Pcy became dominant over PE_Pcy. This result clearly indicates a modification of the underwater light spectrum, and consequently the pigment composition of picocyanobacteria (Bastidas Navarro et al., 2009). Our results suggest that the drastic changes in temperature, nutrients, and light availability after snowmelt lie behind the changes in phytoplankton community that we observed.

Phytoplankton biomass greatly increased during the late summer period, when the phytoplankton assemblage was almost exclusively composed of less edible, large diatoms (mainly *Fragilaria nanana*). While previous studies report that *Fragilaria nanana* prefers nutrient-enriched waters (Cantonati et al., 2019), this species has also been shown to tolerate very small concentrations of nitrogen (Van Dam et al., 1994). Strong diatom development has already been observed in high-altitude lakes in summer, when thermal stability is higher (Tolotti et al., 2007). It appears that the diatoms in our study were more competitive than the other phytoplankton genera during the late summer period, despite the theoretically successful mixotrophic strategy under limiting nutrients and warmer temperatures (Bhutiani et al., 2009).

Overall, we observed a major functional change in phytoplankton assemblages over the three periods. Heterotrophy stood out during the ice-influenced period, with the mixotrophy strategy playing a significant role in the community. Then, mixotrophy was progressively overtaken by

autotrophy, becoming negligible during the late summer period next to both the quantity of autotrophic biomass and the proportion of autotrophs in the phytoplankton assemblage.

4.3. Functional shifts at the base of the food web

Bacterial biomass showed the lower values during the ice-influenced period, then progressively increased until the last summer period. Despite the low biomass during the ice-influenced period, the observed hypoxia as well as the high proportion of ammonium in the DIN pool indicate that microbial respiration did occur during this period. Anaerobic metabolism of active psychrophilic bacteria certainly occurred, as it was previously demonstrated in during winter in ice-covered lakes (Bullerjahn et al., 2020; Margesin & Miteva, 2011; Bertilsson et al., 2013), and during the ice-free season in deep high-altitude lakes (Llorens-Marès et al., 2020).

Regardless of bacterial respiration, dissolved organic matter properties varied over time and may explain the observed variation in bacterial biomass. The refractory DOM may have insufficiently fueled bacteria during the ice-influenced period, as DOM's overall bioavailability typically declines over time when isolated from fresh inputs (Del Giorgio & Davis, 2003). The increase in bacterial biomass during the overturn period was associated to inputs of terrestrial DOM, which could constitute a valuable source of energy and/or inorganic nutrients for bacteria, stimulating more biomass production than respiration (Guillemette et al., 2016). The bacterial biomass continued to increase until the end of the late summer period, during which the high phytoplankton biomass constituted a highly available C source for bacterioplankton. However, temporal trends of bacterial biomass may also result from bacterial interaction with phytoplankton. The high abundance of large phytoflagellates during the ice-influenced period suggests high predation pressure by mixotrophs during this period. Then predation pressure progressively decreased with the observed change in phytoplankton community composition:

some large cryptophytes were replaced by small siliceous chrysophytes during the overturn period. In taxa such as *Dinobryon*, phagotrophy appears to be more a complementary nutritive strategy when light is limiting (Saad et al., 2016), and therefore enhanced light availability during the overturn period may have promoted photosynthesis rather than phagotrophy. Finally, autotrophs largely dominated the phytoplankton community during the late summer period, removing the top-down control over bacteria. Our results confirmed that, together with temporal variation in temperature, oxygen, and nutrient concentrations (Zhou et al., 2020), shifts in DOM properties and phytoplankton community exerted a key role in bacterioplankton regulation.

Thus, our result showed that phytoplankton control over bacteria changed over time, from a predominantly top-down control exerted by mixotrophs toward a bottom-up control as source of DOM. By occupying the niche of microheterotrophs and preventing the development of heterotrophic nanoflagellates and ciliates, mixotrophs are known to reduce the number of trophic levels, acting as a bypass of C flux toward the grazing chain (Medina-Sánchez et al., 2004; Ward & Follows, 2016). Indeed, we found low ciliate abundance during the ice-influenced period but higher ciliate abundance during the late summer period. These results also suggest a possibly top-down control of ciliates on heterotrophic nanoflagellates, which in turn allowed an increase in bacterial biomass (Haraguchi et al., 2018).

The progressively increase of the $\delta^{13}C_{POM}$ confirmed our previous assumptions on shifts occurring at the base of the food web. During the ice-influenced period, $\delta^{13}C_{POM}$ ranged between -31.80 ‰ at the bottom and -26.70 ‰ at the surface. These low values may confirm (i) the use of isotopically light DIC from anaerobic respiration for phytoplankton photosynthesis, which might contribute to $\delta^{13}C_{POM}$ depletion at the bottom (Cole et al., 2002); (ii) the ingestion of anaerobic bacterial prey by mixotrophs, as anaerobic bacteria usually exhibit further depletion in $\delta^{13}C$ (Grey, 2016). The increase in $\delta^{13}C_{POM}$ during the overturn period could result from multiple processes, like increase inputs of allochthonous DOM (Hou

et al., 2013), higher CO_2 uptake rates resulting from greater phytoplankton growth (Gu et al., 2006), or change in phytoplankton taxonomic composition (Golubkov et al., 2020; Trochine et al., 2015). In our study, phytoplankton biomass did not increase during the overturn, but both inputs of allochthonous DOM and change in phytoplankton composition with more efficient photosynthesis occurred. Finally, in late summer, the higher $\delta^{13}C_{POM}$ values corroborated the higher CO_2 uptake rates associated with greater autotrophic phytoplankton growth (Gu et al., 2006), although precipitation events at the end of this period may also contribute to higher values of $\delta^{13}C_{POM}$. Thus, if $\delta^{13}C$ values in POM did not allow to determine the part of each process during the different periods, the result confirmed that the origin of the carbon transferred in the planktonic food web varied over time.

To conclude, in accordance with our last hypothesis, we observed a temporal shift in phytoplankton – bacterioplankton relationships according to lake functioning patterns (**Figure 8**). During the ice-influenced period, mixotrophic taxa exerted a strong top-down control over bacteria. Then, phytoplankton control progressively shifted to a predominantly bottom-up control by autotrophs that governed the DOM pool of the lake. Considering our three pre-defined periods thus brings to light differences in the functioning of this high-altitude lake and provides new insights into the mechanisms controlling the phytoplankton community. Our study highlights the need to better assess the trophic processes at work between the planktonic constituents of the food web. Further studies should be conducted to determine the vulnerability of high-altitude lakes to the shortened ice-cover and increased connectivity with their catchments that are predicted.

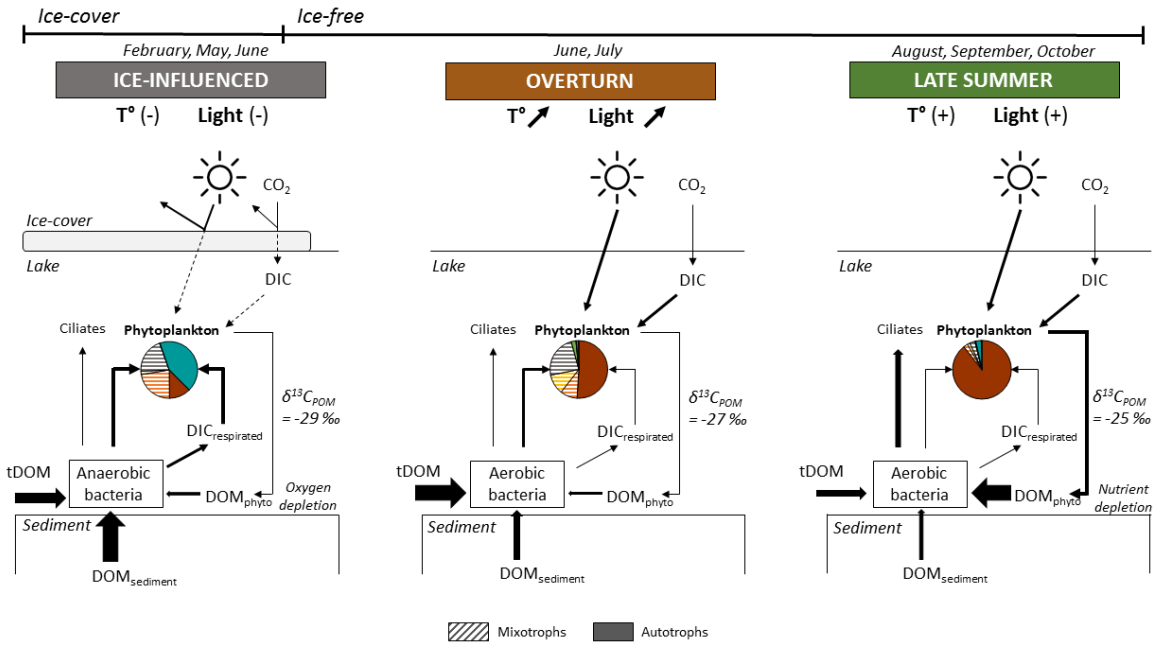

Figure 8: General outline reflecting shifts in phytoplankton control over bacteria in relation to dissolved organic matter properties in the Lake Cordes. tDOM: terrestrial allochthonous DOM; DOM_{phyto}: autochthonous DOM derived from phytoplankton; DIC: dissolved inorganic carbon; T°: water temperature.

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Conflict of interests

The authors declare no conflicts of interest.

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