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Algal- and soil-derived dissolved organic matter shapes bacteria-phytoplankton interactions in a high-altitude lake

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Abstract

1. Allochthonous and autochthonous dissolved organic matter (DOM) and nutrient availability (nitrogen [N], phosphorus [P]) are expected to increase in lakes as a consequence of climate change. These increases can alter the planktonic food web, especially in oligotrophic systems.
2. This study addresses the consequences of DOM and nutrient increase on heterotrophic bacterioplankton (Hbact) and phytoplankton in high-altitude lakes. A planktonic community from a high-altitude lake was incubated for 10 days and laboratory microcosms were subjected to a full factorial design $4 \times 2 \times 2$ of three factors: carbon (C-control, glucose, algal lysate, soil extract), inorganic nutrients (no added nutrients, addition of N and P) and light (light:dark cycle, dark).
3. Hbact was co-limited by organic C and inorganic nutrients, resulting in lower biomass and production with glucose or inorganic nutrients added alone than with the two sources added in combination. Algal-derived DOM was more labile, more consumed, and resulted in higher bacterial production, while soil-derived DOM was less labile and less consumed. Phytoplankton was strongly limited by inorganic nutrients. DOM additions, via the interactions with Hbact, increased phytoplankton biomass and modified the community composition. Mixotrophs increased with C under light or nutrient limitation, pointing them as major factors in controlling mixotrophy in high-altitude lakes. Soil-derived DOM led to higher algal biomass, higher proportion of mixotrophs, and more taxa adapted to nutrient-enriched conditions.
4. This study reveals a shift in planktonic interactions with changes in DOM quality and quantity. Under the projected climate change, we expect an increase in heterotrophy and a modification of trophic interactions with important repercussions on the C cycle in high-altitude lakes.

KEYWORDS

climate change, functional changes, nutrient limitation, oligotrophic lakes, planktonic food web

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1 | INTRODUCTION

Dissolved organic matter (DOM) plays a fundamental role in freshwater ecosystem structure and function through regulation at the base of the food web (Creed et al., 2018; Prairie, 2008; Williamson et al., 1999). DOM contains dissolved organic carbon (DOC), which acts as a source of energy that directly fuels heterotrophic bacterioplankton (Hbact) metabolism. Previous studies reported reduced Hbact reliance on phytoplankton-produced carbon (C) in response to increasing allochthonous sources of DOC (Figueroa et al., 2016; Jansson et al., 2000), and increased competition with phytoplankton for inorganic nutrients (Carney et al., 2016; Hitchcock & Mitrovic, 2013). These forms of competition, while they often result in decreased phytoplankton biomass (Drakare, 2002), also benefit eukaryotic heterotrophs and mixotrophs via grazing on bacteria (Deininger et al., 2017).

However, this pattern is not always observed and depends on organisms' nutrient limitation. For example, the degree of heterotrophy of mixotrophic taxa may differ according to whether they are limited by light, nutrient, or prey availability (Bergström et al., 2003; González-Olalla et al., 2021). Some mixotrophs require both light and nutrient limitation as well as sufficient prey to develop (Livanou et al., 2020; Modenutti, 2014). In oligotrophic systems, the inorganic nutrient limitation has been shown to limit Hbact consumption of allochthonous DOC (Nelson & Carlson, 2011), sucrose (Dorado-García et al., 2014) and glucose (Dory et al., 2021). By providing nutrient subsidies in addition to fixed carbon, DOM inputs have been shown to increase Hbact biomass (Pérez & Sommaruga, 2006), phytoplankton biomass (Kissman et al., 2017) or both (Hitchcock & Mitrovic, 2013). Assessing the limitation pattern of Hbact and phytoplankton is crucial to better understand the effect of DOM increase on planktonic communities (Lefébure et al., 2013).

The pool of DOM in freshwater ecosystems is particularly diverse owing to its multiple origins. The DOM pool includes autochthonous DOM, mainly derived from primary production, and allochthonous DOM transported from catchments to aquatic environments. Depending on its origin, DOM differs in its optical and chemical characteristics: autochthonous DOM is usually composed mainly of proteins and labile polysaccharides, whereas terrestrial DOM contains more humic compounds (Benner, 2003). Thus, autochthonous DOM is traditionally considered as more labile, and allochthonous DOM more recalcitrant for heterotrophic bacterial degradation (Del Giorgio & Davis, 2003).

However, this dichotomy may be more complex, as inputs of biolabile DOC may enhance (or reduce) the bioavailability of stable DOC components in aquatic systems, resulting in changing rates of microbial remineralisation, a mechanism referred to as priming effect (Bengtsson et al., 2018). For example, experimental studies have observed a higher DOC degradation, bacterial biomass and/or respiration when terrestrial DOC was mixed with algal-derived DOC, modifying food-web dynamics (Halvorson et al., 2020; Hotchkiss et al., 2014; Morling et al., 2017; Wyatt & Rober, 2020; Wyatt & Turetsky, 2015). The mixing of different organic matter

pools generally occurs in places and moments of transition in aquatic ecosystems, referred to as ecotones or ecohydrological interfaces (Krause et al., 2017). This may occur when phytoplankton blooms pulsing fresh autochthonous DOM interacts with older, resident DOM, or when water inlets bring terrestrially derived, allochthonous DOM in contact with lake autochthonous DOM (Bengtsson et al., 2018). However, the mixing of diverse DOM pools in aquatic ecosystems does not necessarily result in a change in DOM degradation rates (Bengtsson et al., 2018). In addition, the priming effect occurrence should be interpreted with caution, as it has been argued that increased DOC turnover as a result of inorganic nutrient additions is simply the product of nutrient limitation in the system of study and should not be interpreted as a priming effect (Textor et al., 2018).

The compositional differences of autochthonous versus allochthonous DOM are susceptible to influencing the structure of natural Hbact assemblages (Kritzberg et al., 2006), as well as the allocation of substrates by Hbact to biomass increase or respiration (Russell, 2007). Thus, it has recently been demonstrated that natural bacterial communities from lakes, once substrates are consumed, selectively allocate a greater fraction of autochthonous substrate to respiration, compared to terrestrial C which is preferentially allocated to biosynthesis (Guillemette et al., 2016). These DOM-source-dependent differences in bacterial metabolism response are likely to differentially influence trophic interactions with phytoplankton. For example, an increase in bacterial mineralisation of organic substrates may increase nutrient availability for phytoplankton, resulting in a positive effect on phytoplankton biomass (Li et al., 2014). However, the effect of different DOM sources on phytoplankton–Hbact relationships and phytoplankton community structure or functional traits is still poorly understood.

High-altitude lakes are generally oligotrophic (Catalan et al., 2006), located above the tree line, and characterised by small and poorly vegetated catchments. Consequently, both nutrient availability and DOC concentrations are usually low, and DOM exhibits a more autochthonous fluorescence signature (Bastidas Navarro et al., 2014; Sommaruga & Augustin, 2006). The exception is during snowmelt or after extreme rain events when DOM pools in lakes are dominated by allochthonous DOM (Miller & McKnight, 2010; Perga et al., 2018; Sadro et al., 2011). High-altitude lakes may thus respond strongly to a shift in the quantity and quality of DOM like that expected with the current global change in these ecosystems, especially in the alpine area (Ejarque et al., 2018). On the one hand, increased terrestrial DOC inputs in high-altitude lakes are expected as a consequence of higher air temperatures and more frequent extreme precipitation events (IPCC, 2021; Moser et al., 2019). On the other, higher temperatures and the associated reduction of the ice-cover period, as well as increased atmospheric deposition of nitrogen (N; Kirchner et al., 2014; Kopáček et al., 2005) and phosphorus (P; Kopáček et al., 2011), may significantly increase phytoplankton development (Bergström et al., 2005, 2008; Camarero & Catalan, 2012; Elser et al., 2009; Jacquemin et al., 2018) and thus the production of autochthonous DOM.

The aim of this study was to simulate two predictable DOM-increasing mechanisms in a climate-change scenario: autochthonous production and allochthonous inputs. To explore how a DOM shift could influence phytoplankton–Hbact relationships and phytoplankton community structure, we subjected a natural plankton assemblage from the high-altitude Lake Cordes to a full factorial design $4 \times 2 \times 2$ manipulating three factors: DOM (C-control, glucose, algal lysate, soil extract), nutrients (no added nutrient, addition of inorganic N and P) and light (light:dark cycle, dark). The following hypotheses were tested:

1. Hbact are co-limited by labile organic C and inorganic nutrients (N, P) in oligotrophic high-altitude lakes. This co-limitation will restrict Hbact development with glucose added alone and inorganic nutrients added alone, leading to low Hbact biomass and production.
2. Hbact biomass and production will increase with algal- and soil-derived DOM additions because of their potential content of bound nutrients (N, P). Further, higher DOC consumption, Hbact biomass and production are expected with algal lysate addition, given its theoretical higher lability.
3. Phytoplankton biomass will increase with increasing availability of inorganic nutrients in the microcosms, as a result of direct enrichments or interactions with Hbact.
4. Taxonomic and functional composition of phytoplankton will change according to the different treatments, with a higher proportion of mixotrophs when bacterial preys are abundant (high Hbact biomass), and light and/or nutrients limit autotrophy (i.e., in the dark and in unenriched nutrient treatments).

2 | METHODS

2.1 | Study area and sampling

Water for the experiment was sampled in Lake Cordes, an oligotrophic high mountain lake (2,446 m above sea level) located in the French Alps, adjacent to the Queyras Regional Natural Park. The lake is shallow (maximum depth=9 m) and small (1.8 ha), and the catchment is large (140 ha) with developed soil and meadows (c. 60% alpine grass cover). Water for the experiment (48 L) was sampled on 28 June 2021. It was collected using a Niskin Bottle every metre from 1 to 8 m to sample the entire water column and filtered through a 50- μm mesh to exclude any larger grazers on food-web components. Filtered water was stored in 8-L HDPE containers and transported in a cool box to the laboratory.

2.2 | Experimental design

After overnight acclimation at lake temperature (10°C), the water was divided among four sets of 12 glass bottles (1 L) previously soaked in hydrochloric acid (10% final concentration) and sterilised

(Figure S1). The first set of bottles (12 bottles) did not receive any DOM amendment. The second set of bottles (12 bottles) received glucose to reach a DOC concentration of 2 mg C L^{-1} , the maximum concentration measured in this lake during previous samplings (annual samplings realised since 2019 between mid-June and mid-October). The glucose treatment served as a simple C treatment to highlight the effect of nutrients (N, P) associated with DOM. The third set of bottles (12 bottles) received a filtrate from an algal lysate to reach a DOC concentration of 2 mg C L^{-1} . The algal lysate was obtained from a natural phytoplankton community of Lake Cordes cultured in the laboratory for several weeks and mainly dominated by the green algae *Chlorella minutissima* after several days. The culture was rinsed, disrupted (One Shot Cell Disruptor, CellD) at 2500 bars (40 mL min^{-1}), and filtered through a 0.22- μm polycarbonate membrane to eliminate algal remains and Hbact. The fourth set of bottles (12 bottles) received a filtrate from a soil extract to reach a DOC concentration of 2 mg C L^{-1} . The soil extract was obtained from soil samples collected randomly in the watershed the 28 June 2021. The soil was taken from alpine meadows on the first 20 cm. Stones, larger roots and larger vegetation were removed and the homogenised soil was transported to the laboratory. Soil samples were mixed, freeze-dried, ground and sifted through a 2-mm mesh sieve. The samples were extracted with Milli-Q water (solid to water ratio, $w/v=0.1$), and the suspensions were placed at 4°C and stirred for 24 h. The suspensions were then centrifuged (10,000 rpm, 20 min), filtered (0.2- μm) and subsequently stored at 4°C until the experiment.

Half of each of the four sets of bottles (six bottles) did not receive inorganic nutrients, and half (six bottles) were enriched with an excess of NH_4NO_3 (2 mg N L^{-1}) and KH_2PO_4 (0.6 mg P L^{-1}) (+N+P) (Figure S1). These concentrations were chosen to approximate a DIN:SRP ratio previously measured in summer on this lake even under the excess concentrations. All microcosms received an equivalent total volume of solution so as not to exceed 1.5% of the total volume of water in the microcosms, avoiding any dilution or shading effect. All 48 bottles were placed in growth chambers for 10 days, at the lake temperature measured on the sampling day (10°C). For each DOM and nutrient treatment, three bottles were placed under a circadian cycle at a 14 h:10 h, light:dark cycle (Light condition), at a fixed irradiance ($45 \pm 2 \mu\text{mol m}^{-2} \text{ s}^{-1}$). The irradiance of $45 \pm 2 \mu\text{mol m}^{-2} \text{ s}^{-1}$ was sufficiently high to avoid light limitation in the microcosms (Dubourg et al., 2015; Jacquemin et al., 2018). The three other bottles were placed in the dark (Dark condition) and surrounded by aluminium, to target heterotrophic processes while removing the influence of active and immediate photosynthesis.

2.3 | Water chemistry (C, N, P)

Subsamples for C and nutrient analysis were collected at Day (D)0 (beginning of the experiment) and D10 (end of the experiment). Subsamples for dissolved inorganic nitrogen ($\text{DIN}=\text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$), soluble reactive phosphorus ($\text{SRP}=\text{PO}_4^{2-}$) and DOC

concentrations were filtered through pre-combusted (4h, 450°C) 25-mm glass filters (Whatman GF/F, 0.7-µm). The dissolved fraction for DIN and SRP was stored in 125 mL HDPE bottles, and frozen (-18°C) until analysis. DIN and SRP were determined by ionic chromatography (Metrohm, 930 Compact IC Flex combined with 863 Compact Autosampler). The dissolved fraction for DOC was stored in two sets of 24-mL glass tubes (Wheaton equipped with Teflon/silicone septa) pre-acidified (HCl 10% final concentration) to remove dissolved inorganic carbon and pre-combusted (4h at 450°C). The samples were preserved in 25 µL of sodium azide solution (1 M N_aN₃) at +4°C. DOC concentrations were determined using a TOC-VCSH analyser (TOC-V; Shimadzu).

2.4 | Fluorescence measurements

Subsamples in one replicate for DOM absorbance measurements were filtered as described for DOC analysis. Samples were scanned in a spectrophotometer V-550 (Jasco) with 10-cm Suprasil quartz cells from 230 to 800 nm at a 1,000 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) equipped with a 450 W 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 Suprasil quartz cells. The scan speed was set at 2,400 nm min⁻¹, and the detector voltage was 700 V. Fluorescence intensity was normalised to Raman units (R.U.) using the daily-measured Raman peak of Milli-Q water ($\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 371\text{--}428 \text{ nm}$). The collected excitation–emission matrices (EEMs) were analysed at D0 and D10 using parallel factor analysis (PARAFAC), to identify the different components of the FDOM pool. PARAFAC was conducted on EEM dataset using PROGMEEF software (Redon & Mounier, 2018) in Matlab language.

2.5 | Heterotrophic bacterioplankton biomass

Subsamples of 1.5 mL were taken at D0 and thereafter every two experimental days in the microcosms for analyses of heterotrophic prokaryotes by flow cytometry. Given the low levels of archaea in oligotrophic waters (Wells & Deming, 2003), heterotrophic prokaryotes were termed heterotrophic bacterioplankton (Hbact). Subsamples were fixed with glutaraldehyde (0.25% final concentration) and stored at -80°C until flow cytometry analysis. Subsamples were stained with 1:10,000 (vol/vol) SYBR® Green II and incubated for 20 min in darkness. 50 µL of stained samples were run at medium speed (35 µL min⁻¹). Non-fluorescent polystyrene microspheres (Flow Cytometry Size Calibration Kit; Thermo Fisher Scientific) were used as a size standard. Hbact cells were detected by their small size (low FSC), low complexity (low SSC), high green fluorescence (530 ± 15 nm) and lack of red (>670 nm) fluorescence. Cell abundance (cell mL⁻¹) of bacterioplankton was converted to biomass (µg C L⁻¹)

using 20 fg C cell⁻¹ as constant conversion factor (Ducklow, 2000; Linacre et al., 2015).

2.6 | Heterotrophic bacterioplankton production

Hbact production (BP) was measured every day from D0 to D4, then on D6, D8 and D10. BP was estimated from rates of protein synthesis with ³H-leucine incorporation using the microcentrifugation technique (Smith & Azam, 1992) as detailed in Van Wambeke et al. (2021). Briefly, subsamples of 1.5 mL were incubated in the dark at 10°C. Leucine was added at 20 nM (final concentration), and the leucine-to-C conversion factor used was 1.5 kg C mol⁻¹. Cumulated BP (ng C L⁻¹) was calculated as time-integrated bacterioplankton production from t_0 to t_{10} with data and assuming exponential growth (or decay) between two time-points.

2.7 | Phytoplankton biomass and ciliate abundance

Subsamples for phytoplankton were fixed with a formaldehyde solution (5%) and stored in 250-mL HDPE bottles. Phytoplankton counts were performed at D0 and D10 according to the Utermöhl (1958) method, at ×40 magnification under an inverted microscope (IX 70; Olympus). Phytoplankton taxa were identified at the genus level or at the species level when it was possible using appropriate taxonomic guides (mainly from the Süßwasserflora von Mitteleuropa and specific manuals of Das Phytoplankton des Süßwassers). 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 C content defined for each class by Wetzel and Likens (2000). Phytoplankton taxa were classified in three groups (Table S1): (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) morpho-functional groups (MFG) based on their morphological and functional characteristics (Salmaso & Padisák, 2007). Phytoplankton diversity was characterised according to two indexes: specific richness (S) and Pielou evenness (J), calculated as the Shannon diversity index (H') divided by H'_{max} :

$$J = H' / H'_{\text{max}} = H' / \ln(S)$$

In order to determine whether any predator development occurred in our microcosms, ciliate abundances were estimated via the Utermöhl (1958) method.

2.8 | Carbon budget estimation

In order to estimate the net fluxes between C mineralisation and C production in the microcosms, a C budget was calculated based on

TABLE 1 Carbon and nutrient concentrations measured in the microcosms at the beginning of the experiment (t_0), just after DOM and nutrient (+N+P) amendments.

DOM	Nutrient	DOC (mg L ⁻¹)	DIN (mg L ⁻¹)	SRP (μg L ⁻¹)	C1 (%)	C2 (%)	C3 (%)	C4 (%)
Control	No nutrient	0.86 ± 0.08	0.42 ± 0.04	<10	45	25	19	11
Control	+N+P	0.86 ± 0.08	2.74 ± 0.53	710 ± 129	45	25	19	11
Glucose	No nutrient	2.03 ± 0.09	0.47 ± 0.06	<10	47	24	20	10
Glucose	+N+P	2.03 ± 0.09	2.30 ± 0.42	588 ± 123	47	24	20	10
Algae	No nutrient	2.28 ± 0.13	0.45 ± 0.4	<10	40	16	24	20
Algae	+N+P	2.28 ± 0.13	2.52 ± 0.57	698 ± 194	40	16	24	20
Soil	No nutrient	2.13 ± 0.04	0.45 ± 0.06	<10	43	22	24	10
Soil	+N+P	2.13 ± 0.04	2.39 ± 0.39	622 ± 124	43	22	24	10

Note: Means ± standard deviations are shown. Each treatment was performed in triplicate except for the fluorescent components. DIN, dissolved inorganic nitrogen; DOC, dissolved organic carbon; SRP, soluble reactive phosphorus. C1, C2, C3 C4 are the principal components of DOM obtained by PARAFAC. We indicated SRP < 10 μg L⁻¹ when SRP concentrations were below the detection limit.

the organic C stocks by comparing the sum of initial and final stocks according to the following formula: C-budget = (POC_i + DOC_i) - (POC_f + DOC_f). Particulate organic carbon (POC) stock was assumed to be represented by C_{phytoplankton} + C_{Hbact} · C_{phytoplankton} was obtained from phytoplankton biomasses calculated from biovolumes (see Section 2.7) and C_{Hbact} was obtained from Hbact biomasses, calculated from Hbact abundance converted using 20 fg C cell⁻¹ (see Section 2.5). The percentages of C change in the microcosms were calculated as follows: (C-budget / (POC_i + DOC_i)) * 100. In the light, a negative percentage of C change indicates net autotrophy, whereas a positive percentage of C change indicates net heterotrophy. In the dark, DOC changes represent the organic C which has been disappearing through mineralisation, and thus in that case the percentage of C changes represents the percentage of initial C stocks that was used for mineralisation.

2.9 | Statistical analyses

Two-way analysis of variance (ANOVA), with DOM source and inorganic nutrients as factors, was used to compare changes in C and nutrient concentrations at the beginning of the experiment, and to test significant differences among DOM source and nutrient treatments, in light for the net percentage of C change, in the dark for the percentage of C mineralised. Linear models were used to investigate how DOM sources combined with nutrient enrichment and light conditions affect abiotic and biological responses. Response variables were log-transformed if this resulted in a more uniform spread of data points. Pairwise differences were tested using *post hoc* Tukey's honestly significant difference (HSD) tests. The *emmeans* library was used for the HSD tests. We analysed changes in DOC concentrations between the beginning (t_0) and the end (t_{10}) of the experiment in the different treatments as the delta of change. Multivariate regression trees (MRT) were constructed to display changes in phytoplankton community composition according to experimental factors (De'ath, 2002). MRTs create dichotomies, where samples that share similar species patterns in relation to experimental factors are

clustered together. This hierarchical method aims to minimise the least sum of squares of the response data within a cluster by repeatedly splitting the data. A 1,000 cross-validation process based on the "lse" method was used. MRT analysis was carried out using R and the *mvpart* package (Therneau et al., 2013). A hierarchical cluster analysis with associated dendrogram and heatmap was performed on the phytoplankton community at the species level. All statistical analyses were performed with R 3.6.3 (R Core Team, 2018).

3 | RESULTS

3.1 | Initial conditions in the microcosms

Initial DOC concentrations in the control were low (<1 mg C L⁻¹), and DOM amendments (algal lysate, soil extract and glucose) significantly increased DOC concentrations, which reached between 2 and 2.3 mg C L⁻¹ at the beginning of the experiment (F value = 50.7, p < 0.001) (Tables 1 and S2). Initial DIN concentrations were low in the unenriched treatments (<0.5 mg N L⁻¹) and were not significantly increased by the amendments of algal lysate and soil extract (Table S2). The nutrient enrichment significantly increased DIN concentrations, which rose to between 2.3 and 2.8 mg N L⁻¹ (F = 426.1, p < 0.001). Initial SRP concentrations were below 10 μg P L⁻¹ in the unenriched treatments and were not significantly increased by the amendments of algal lysate and soil extract (Table S2). The nutrient enrichments significantly increased SRP concentrations, which rose to between 500 and 700 μg P L⁻¹ (F = 381.6, p < 0.001).

PARAFAC analysis distinguished between four components at the beginning of the experiment. Components C1 (ex/em = 250–310/440 nm), C2 (ex/em = 260–390/460 nm) and C4 (ex/em = 490/520 nm) have been described in the literature as humic compounds. Component C3 (ex/em = 270/340 nm) has been linked to protein-like compounds with low molecular weight (Chen et al., 2003). At the beginning of the experiment, the proportion of C1 was high in all the treatments (Table 1). The proportion of C2 was higher in control, glucose and soil treatments. The proportion

of C3 was higher in algal and soil treatments than in control and glucose treatments. Finally, the algal treatment was characterised by a higher proportion of C4.

Concerning biological parameters, initial phytoplankton biomass was very low in the microcosms ($4.67 \pm 0.68 \mu\text{g C L}^{-1}$) and the phytoplankton community was almost equally composed of mixotrophs ($52.79 \pm 4.9\%$) and autotrophs. Initial bacterioplankton biomass also was low ($17.33 \pm 5.3 \mu\text{g C L}^{-1}$).

3.2 | Changes in DOM quantity and quality

Change in DOC concentrations between the beginning and the end of the experiment varied significantly according to the DOM source ($F=6761.6$, $p<0.001$), the nutrient treatments ($F=1488.0$, $p<0.001$), and the light treatments ($F=803.8$, $p<0.001$). Additionally, the effect of the DOM source on DOC concentrations interacted with nutrients ($F=1633.4$, $p<0.001$) and light treatments ($F=126.9$, $p<0.001$) (Table S3). In controls, DOC concentrations moderately decreased between the beginning and the end of the experiment ($-38 \pm 18\%$) and DOC decreased more in the nutrient-enriched treatments ($-42 \pm 16\%$) than in unenriched treatments ($-35 \pm 20\%$), regardless of the light condition (Figures 1 and S2). In treatments receiving glucose, DOC decreased slightly in unenriched treatments ($-11 \pm 14\%$), whereas DOC decreased more significantly in nutrient-enriched treatments ($-71 \pm 7\%$) ($p<0.001$). In the treatments receiving algal lysate and soil extract, DOC concentrations

decreased drastically over time, whatever the nutrient enrichment. DOC concentrations decreased more in treatments receiving algal lysate ($-67 \pm 6\%$) than in those receiving soil extract ($-59 \pm 4\%$) ($p<0.001$).

There were few variations in DOM quality in controls between the beginning and the end of the experiment, and the percentages of variation in the contributions of the four components were close to 0% (between -4.6% and 4.9%) (Figure 2). Likewise, there was little variation in the contributions of the four components in glucose treatments without nutrients (Figure 2). However, the contribution of the humic compound C2 increased between the beginning and the end of the experiment in treatments receiving algal lysate, soil extract, and combined glucose and nutrients (Figures 2 and S3). Additionally, the contribution of the protein-like compound C3 decreased in all treatments receiving algal lysate and soil extract, except in the light for the unenriched algal lysate treatment (Figure 2).

3.3 | Heterotrophic responses

Trends of Hbact biomass stocks and production differed significantly between controls, glucose, algal, and soil treatments (D8, $F=10.3$ and $F=1,697$, respectively, $p<0.001$). The effect of the DOM source significantly interacted with nutrients for both Hbact biomass ($F=7.3$, $p<0.001$) and production ($F=575.4$, $p<0.001$) (Table S3). In controls, bacterioplankton biomass increased slightly over the experiment, depending on nutrient enrichment (Figure 3).

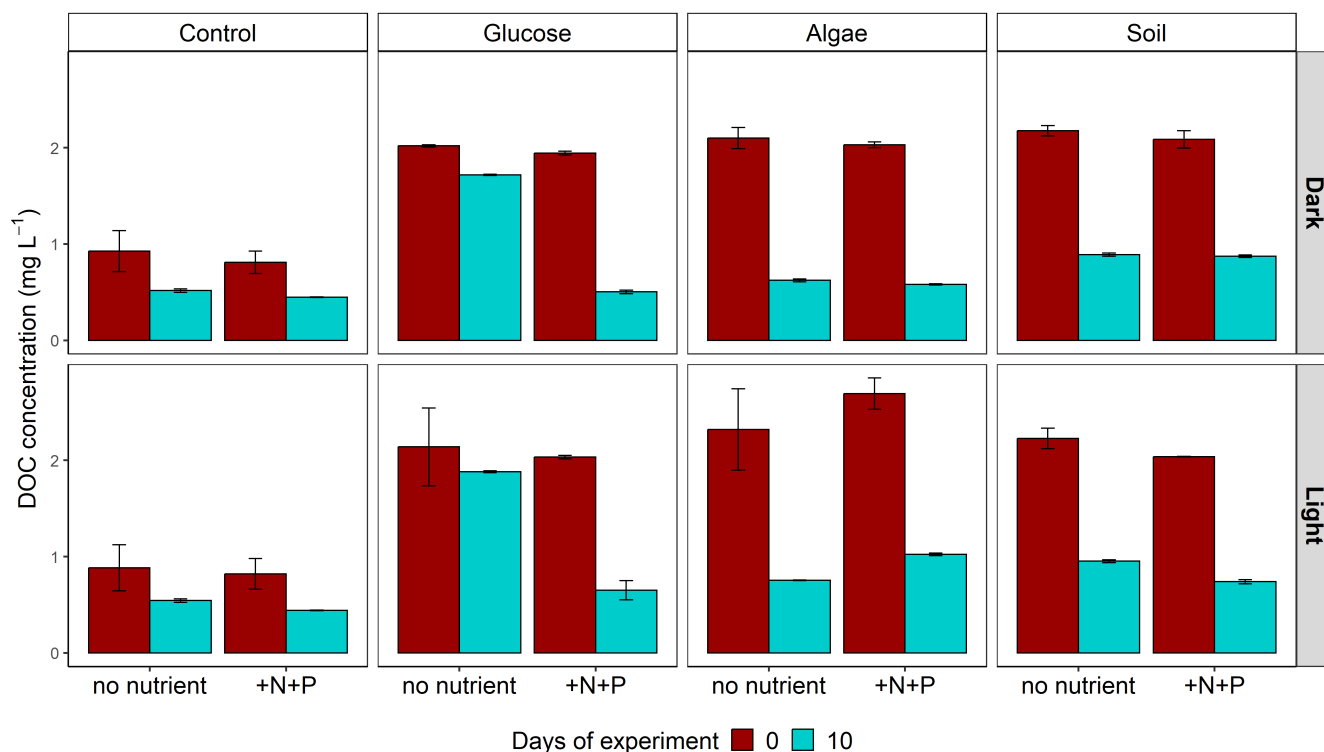


FIGURE 1 Average dissolved organic carbon (DOC) concentrations at the beginning (t_0) and the end (t_{10}) of the experiment in control, glucose, algal and soil treatments, without added nutrients (no nutrient) and with inorganic nutrients (+nitrogen [N]+phosphorus [P]), in the light and dark. Error bars represent standard error.

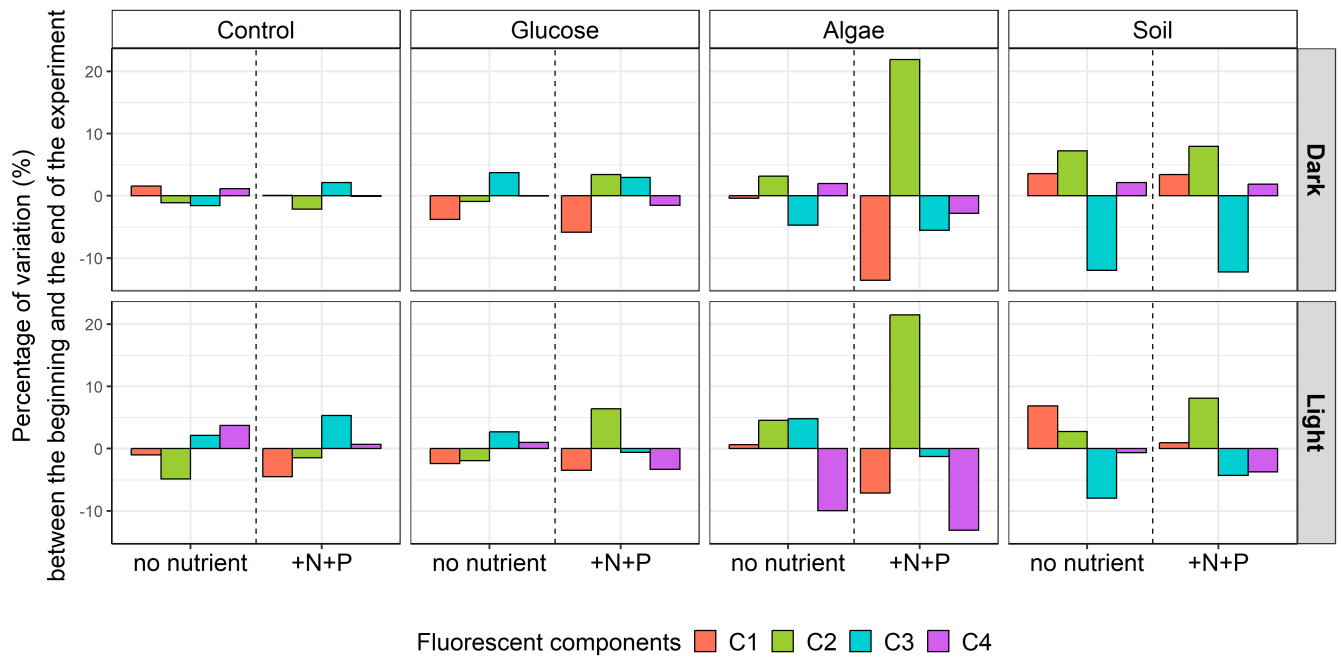


FIGURE 2 Percentage of variation in the contributions of the four fluorescent components (C1, C2, C3, and C4) between the beginning and the end of the experiment in control, glucose, algal and soil treatments, without added nutrients (no nutrient) and with inorganic nutrients (+nitrogen [N]+phosphorus [P]), in the light and dark.

In unenriched treatments, bacterioplankton biomass peaked on D8 in the dark ($84 \pm 8 \mu\text{g C L}^{-1}$). In nutrient-enriched treatments, bacterioplankton biomass peaked on D10 in the light ($141 \pm 37 \mu\text{g C L}^{-1}$). In treatments receiving glucose, bacterioplankton biomass increased rapidly and was significantly higher in nutrient-enriched than in unenriched treatments (D8, $p=0.001$). In unenriched treatments, bacterioplankton biomass peaked after 6 days in the dark ($92 \pm 23 \mu\text{g C L}^{-1}$), whereas in nutrient-enriched treatments, it peaked after 8 days in the light ($155 \pm 20 \mu\text{g C L}^{-1}$). In treatments receiving algal lysate, bacterioplankton biomass increased drastically over the experiment, reaching a peak on D6 in the dark. The magnitude of the peak depended on nutrient enrichment (D8, $p=0.01$): biomass reached $166 \pm 18 \mu\text{g C L}^{-1}$ in unenriched treatments and $218 \pm 21 \mu\text{g C L}^{-1}$ in nutrient-enriched treatments. In the soil treatments, bacterioplankton biomass increased drastically over the experiment, reaching its peak on D4 in the dark. Biomass reached $241 \pm 12 \mu\text{g C L}^{-1}$ in unenriched treatments and $246 \pm 21 \mu\text{g C L}^{-1}$ in nutrient-enriched treatments. In unenriched treatments, maximum bacterioplankton biomass was higher with soil extract than with algal lysate.

The effect of the DOM source on BP varied according to light conditions ($F=33.4$, $p<0.001$) and nutrient enrichment ($F=575.4$, $p<0.001$) (Table S3). BP did not increase in controls, except after 4 days in the light in nutrient-enriched treatments (from 325 to $500 \text{ ng C L}^{-1} \text{ h}^{-1}$ between D0 and D4 up to $1647 \pm 15 \text{ ng C L}^{-1} \text{ h}^{-1}$ at D10) (Figure 3). With glucose amendments, BP increased only in nutrient-enriched treatments, peaking on D4 in the light ($5553 \pm 99 \text{ ng C L}^{-1} \text{ h}^{-1}$). In algal treatments, BP increased drastically, depending on light conditions and nutrient enrichments. In unenriched treatments, BP reached its peak on D6 in the dark ($8334 \pm 80 \text{ ng C L}^{-1} \text{ h}^{-1}$),

whereas in nutrient-enriched treatments, it peaked on D4 in the dark ($17,307 \pm 690 \text{ ng C L}^{-1} \text{ h}^{-1}$). In soil treatments, BP increased over the experiment, reaching its peak after 4 days (without added nutrients) and 3 days (with inorganic nutrients) in the dark. The peak of production was $5245 \pm 176 \text{ ng C L}^{-1} \text{ h}^{-1}$ in unenriched treatments and $7755 \pm 555 \text{ ng C L}^{-1} \text{ h}^{-1}$ in nutrient-enriched treatments.

Time-integrated BP during the 10-day period of DOC consumption was systematically higher in treatments receiving algal lysate than soil extract, whatever the nutrient and light conditions (Figure S4). For example, in unenriched treatments in the light, time-integrated BP over 10 days was $53 \times 10^4 \text{ ng C L}^{-1}$ with algal lysate and $46 \times 10^4 \text{ ng C L}^{-1}$ with soil extract; In nutrient-enriched treatments in the light, time-integrated BP reached $11 \times 10^5 \text{ ng C L}^{-1}$ with algal lysate and $78 \times 10^4 \text{ ng C L}^{-1}$ with soil extract; In unenriched treatments in the dark, time-integrated BP was $67 \times 10^4 \text{ ng C L}^{-1}$ with algal lysate and $47 \times 10^4 \text{ ng C L}^{-1}$ with soil extract; Finally, in nutrient-enriched treatments in the dark, time-integrated BP reached $14 \times 10^5 \text{ ng C L}^{-1}$ with algal lysate and $63 \times 10^4 \text{ ng C L}^{-1}$ with soil extract.

The C budget, represented as the net percentage of C change in the microcosms, was negative (i.e., net autotrophy) only in controls in the light with nutrients (Figure S5 and Table S4). In all other treatments in the light, the C budget was positive (i.e., net heterotrophy). In the light, nutrient enrichment allowed a decrease in the C budget with algal lysate and soil extract, but the percentage of net C change remained positive. In the dark, the percentage of mineralised C was lower in the controls, and with glucose in unenriched treatments (values $<39\%$). With glucose, C mineralisation increased considerably and significantly in nutrient-enriched compared to unenriched treatments (from 39% to 80%) (Table S4).

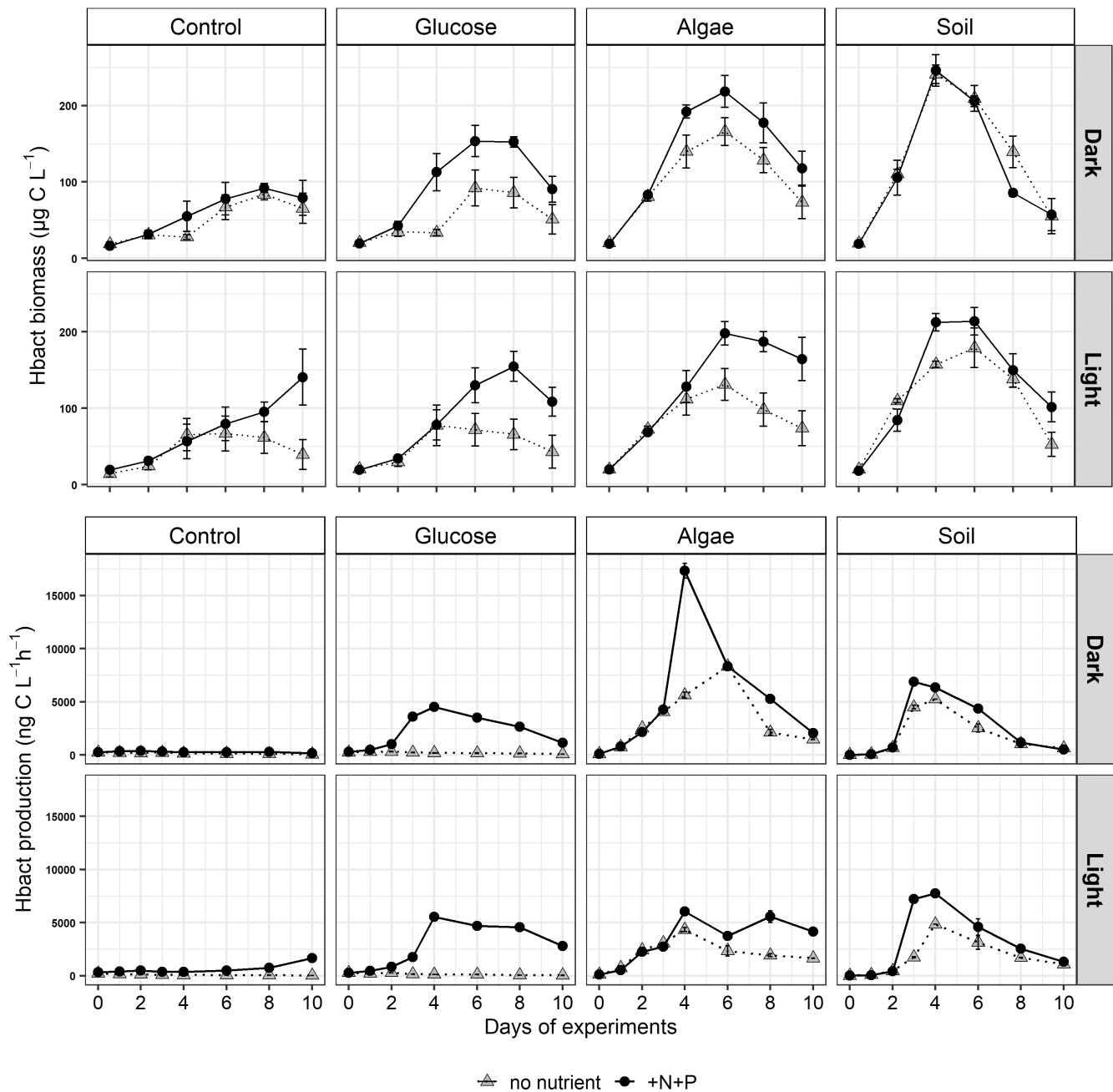


FIGURE 3 Temporal changes in heterotrophic bacterioplankton (Hbact) biomass and production in control, glucose, algal and soil treatments, without added nutrients (no nutrient) and with inorganic nutrients (+nitrogen [N]+phosphorus [P]), in the light and dark. Error bars represent the standard error of triplicate flasks.

The percentage of mineralised C did not change according to the nutrient enrichment in treatments receiving algal lysate and soil extract, and C mineralisation was higher with algal lysate than with soil extract.

3.4 | Phytoplankton responses

Concerning biomass, on D10 of the experiments, total phytoplankton biomass was lower in the dark ($<15.2 \mu\text{g C L}^{-1}$) than in the light ($>26.2 \mu\text{g C L}^{-1}$) ($F=1,672.4$, $p<0.001$) (Figure 4; Table S3). The

effect of DOM addition interacted with the light treatment ($F=4.0$; $p=0.01$) and in the dark, phytoplankton biomass was lower in controls ($2.1 \pm 0.4 \mu\text{g C L}^{-1}$) than in treatments receiving a C source (glucose, algal lysate and soil extract) where biomass ranged between 3.6 and $6.0 \mu\text{g C L}^{-1}$. In the light, nutrient enrichment greatly increased phytoplankton biomass and interacted with C additions: in unenriched treatments, phytoplankton biomass was low in the control ($27 \pm 2 \mu\text{g C L}^{-1}$) and increased significantly with amendments of algal lysate ($104 \pm 24 \mu\text{g C L}^{-1}$, $p=0.002$) and soil extract ($168 \pm 45 \mu\text{g C L}^{-1}$, $p<0.001$). In nutrient-enriched treatments, phytoplankton biomass reached on D10 in the control showed an increase compared to t_0

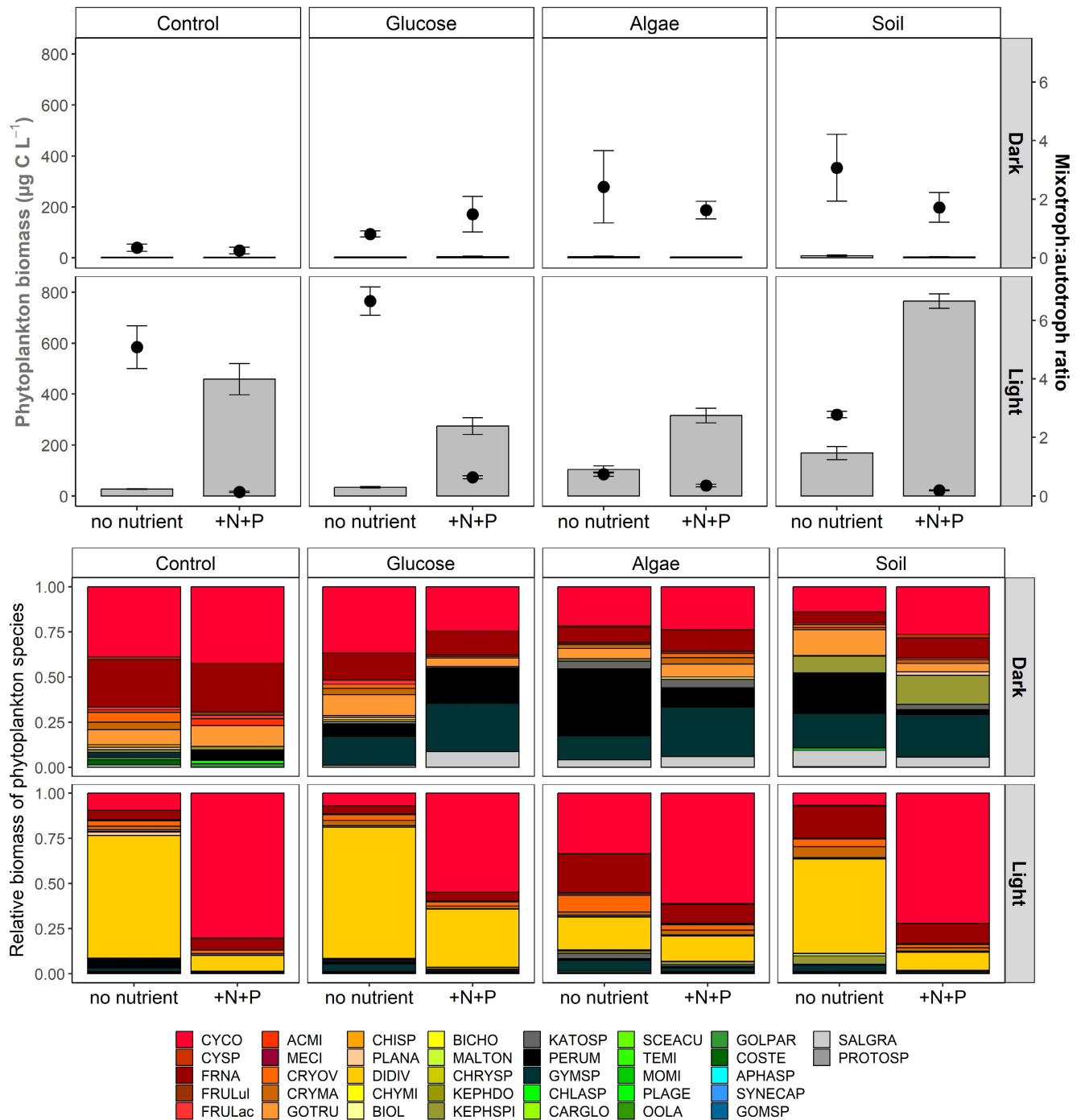


FIGURE 4 Total phytoplankton biomass (grey bars), mixotroph:autotroph ratio (black dots) and relative biomass of phytoplankton species in control, glucose, algal and soil treatments, without added nutrients (no nutrient) and with inorganic nutrients (+N+P), in the light and dark on D10 of the experiment. Species ID are detailed in [Table S1](#). Error bars represent standard error.

(5 ± 0.6 to $459 \pm 87 \mu\text{g C L}^{-1}$), and this increase was notably higher with soil extract amendment ($765 \pm 49 \mu\text{g C L}^{-1}$). It was the opposite with glucose and algal lysate, where phytoplankton biomass reached on D10 ($275 \pm 57 \mu\text{g C L}^{-1}$ and $316 \pm 43 \mu\text{g C L}^{-1}$, respectively) was significantly lower compared to the controls in the same conditions of light and nutrients ($459 \pm 87 \mu\text{g C L}^{-1}$).

On D10 of the experiment, mixotrophs:autotrophs biomass ratio (Mixo:Auto) varied according to DOM source, light and nutrient

enrichment ($F=3.0$, $p=0.04$) ([Table S3](#)). In the light in unenriched conditions, Mixo:Auto was lower with algal lysate (0.7 ± 0.07) and soil extract (2.8 ± 0.1) than in controls (5.0 ± 0.7) ([Figure 4](#)). Under glucose amendment, Mixo:Auto was higher than controls and reached 6.6 ± 0.5 . In the dark in unenriched treatments, Mixo:Auto was higher with algal lysate (2.5 ± 1.2 , $p=0.02$) and soil extract (3.0 ± 1.1 , $p=0.002$) than in controls (0.4 ± 0.1), whereas in nutrient-enriched treatments, Mixo:Auto was higher with glucose (1.5 ± 0.6 , $p=0.004$),

algal lysate (1.6 ± 0.3 , $p=0.001$), and soil extract (1.7 ± 0.5 , $p=0.001$) than in controls (0.2 ± 0.1).

Species richness on D10 was higher in light conditions ($F=306.3$, $p<0.001$) and with C amendments than in their corresponding controls ($F=4.1$, $p=0.01$). In the light, species richness was higher with algal lysate (26.3 ± 0.6), soil extract (25.7 ± 2.7) and in nutrient-enriched treatments (23.3 ± 0.6), compared to controls (21.3 ± 2.1) or glucose amendment alone (21.7 ± 0.6) (Figure S6). Conversely, evenness was higher in the dark than in the light ($p<0.001$). Species evenness differed among DOM sources ($F=10.7$, $p<0.001$) and was lower in the control and glucose treatments than in the algal lysate and soil extract treatments (Figure S6).

Concerning community composition, multivariate regression trees performed on the relative biomass of the MFG functional groups and phylogenetic affiliation (TAX) identified light as the major factor structuring community composition, regardless of the classification (Figures 5 and S7).

In the dark, the phytoplankton community was structured by the C treatments and the control was distinguished from the three other treatments (glucose, algal, soil) (Figures 5 and S8). In the control, the phytoplankton community was dominated by small centric diatoms (MFG, group 7a) and large pennate diatoms (MFG, group 6b). In the three treatments receiving C via glucose, algal lysate, or soil extract, the phytoplankton community was largely composed of mixotrophs (between 37% and 84%) and was dominated by large dinoflagellates (MFG, group 1b).

In the light, the phytoplankton community was mainly structured by nutrient enrichment (Figure 5). In nutrient-enriched treatments, it consisted mainly of small centric diatoms (MFG, group 7a). In unenriched treatments, the phytoplankton community differed according to the DOM source, with the algal treatment distinct from control, glucose and soil treatments. Under the algal treatment, the community consisted mainly of small centric diatoms, large pennate diatoms, and small Chrysophytes

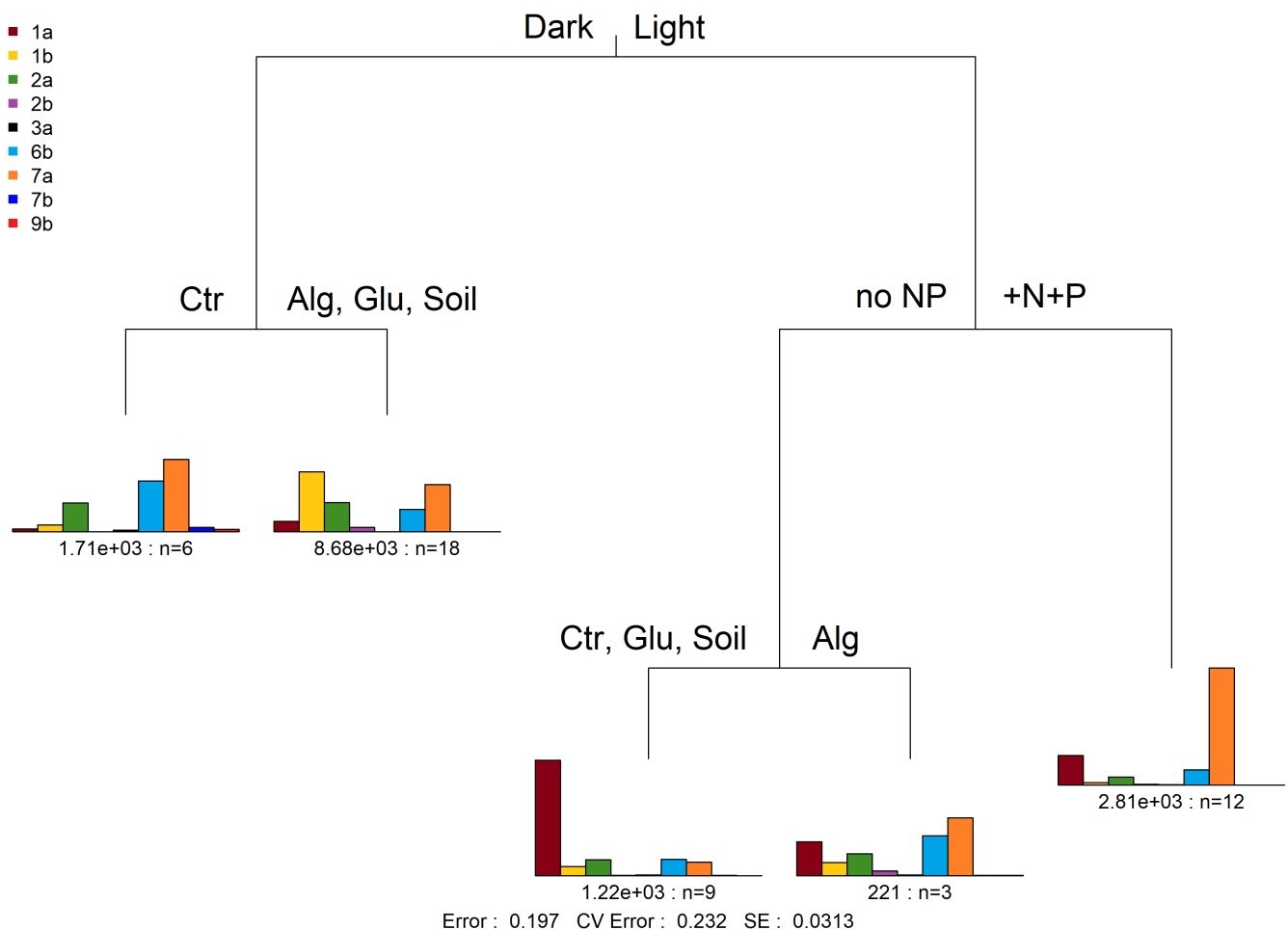


FIGURE 5 Multivariate regression tree analysis performed on relative biomass of phytoplankton MFG groups (>5% of the total biomass) on Day 10 of the experiment. Ctr, control; Glu, glucose; Alg, algal-derived DOM; Soil, soil-derived DOM; +N+P, inorganic nutrient (nitrogen and phosphorus)-enriched treatments; no NP, without added nutrients; Dark, dark conditions; Light, light:dark photoperiod conditions. 1a, Large Chrysophytes; 1b, Large Dinophytes; 2a, Small Chrysophytes; 2b, Small Dinophytes; 3a, Unicellular Phytomonada; 6b, Large Pennates; 7a, Small Centrics; 7b, Small Pennates; 9b, Small unicells Chlorococcales. Under each histogram, n is the number of observations; the value before n is the sum of squared errors for the group. Error is the total relative error of the tree with the chosen size ($k=4$ leaves); CV Error is the cross-validation error of the selected tree. SE is the standard error of the cross-validation statistic for the selected tree.

(MFG, groups 7a, 6b and 2a), as well as large Chrysophytes and small Dinoflagellates. In the control, glucose and soil treatments, the phytoplankton community was mainly composed of mixotrophic taxa, mostly Chrysophytes, large Cryptophytes and small Dinoflagellates.

Nevertheless, at the species level, the multivariate regression tree also distinguished between the soil treatment and the two others (glucose and control) in the light without nutrients (Figure S9). The soil treatment was shown to lead to a higher proportion of large pennate diatoms (*Fragilaria nanana*) than in glucose and control treatments, but a higher proportion of the large chrysophyte *Dinobryon divergens* than in algal treatments (Figures 4 and S8).

Finally, ciliate abundance varied according to DOM sources ($F=68.0$, $p<0.001$) and light conditions ($F=48.3$, $p<0.001$) (Figure 6 and Table S3). Ciliate abundance was low in the dark (1.8 ± 2.4 cell mL^{-1}) and did not significantly vary with the DOM source. In the light, under unenriched conditions, ciliate abundance increased with amendments of algal lysate (20.7 ± 8.2 cell mL^{-1}) and soil extract (25.1 ± 6.7 cell mL^{-1}) compared to control (undetectable levels set to 0 cell mL^{-1}) and glucose treatments (0.5 ± 0.8 cell mL^{-1} , $p=0.01$). In the light, under nutrient enrichment, ciliate abundance significantly increased with added soil extract (26.2 ± 14.3 cell mL^{-1}) compared to the control (undetectable levels set to 0 cell mL^{-1} , $p=0.006$).

4 | DISCUSSION

4.1 | Heterotrophic bacterioplankton co-limitation

Given the usual oligotrophic state and the low DOC concentrations in high-altitude lakes, heterotrophic bacterioplankton should be co-limited by organic C and inorganic nutrients (N, P) in these environments. Our results demonstrated a lower DOC decrease and a lower BP and lower peaks of Hbact biomass with glucose added alone and nutrient added alone compared to combined additions of glucose and inorganic nutrients. These results offer support for the first hypothesis that Hbact was co-limited by labile organic carbon and inorganic nutrients in the microcosms. Co-limitation of bacteria by both inorganic nutrients and organic C has previously been observed in high-altitude lakes (Dorado-García et al., 2014; Dory et al., 2021). Our results also confirm the co-limitation pattern already observed in other nutrient-depleted systems such as reservoirs, lakes (Grover, 2000; Ogebo & Ochs, 2008) or marine systems (Mills et al., 2008). This co-limitation may influence bacterioplankton utilisation of DOM, especially in the context of increasing atmospheric nutrient deposition that has already been documented in this region of the Alps (Rogora et al., 2006). Indeed, an increase in bacterial uptake of allochthonous DOM has already been reported with increasing P concentrations in high-altitude lakes (Nelson & Carlson, 2011).

4.2 | Effects of DOM, nutrients and light on bacterioplankton

As expected from our second hypothesis, we found a strong increase of Hbact biomass and production, and a strong DOC consumption, in response to both DOM sources, given the potential content of nutrients (N, P) bound to DOM. Further, because of the different properties of DOM sources depending on their origin (i.e., autochthonous vs. terrestrial), we expected different responses of Hbact to DOM source additions in the microcosms. Two major findings supported our second hypothesis of different Hbact metabolism depending on the DOM source added to the microcosms. Firstly, DOC declined more with algal lysate ($-67\pm 6\%$) than with soil extract ($-59\pm 4\%$), glucose ($-41\pm 10\%$) and controls ($-38\pm 18\%$). Secondly, the dynamics of fluorescent compounds differed between treatments. The contribution of humic-like compound C2 increased between t_0 and t_{10} in treatments receiving algal lysate, soil extract and glucose combined with nutrient enrichment, and this increase was more important with algal lysate (+13%) than in the other C treatments. It was previously demonstrated that biodegradation of organic matter can lead to the production of high-molecular-weight aromatic material, through the alteration of existing compounds and/or the production of new compounds by heterotrophic bacteria (Guillemette & del Giorgio, 2012; Jiao et al., 2010). Our results confirm the dual role of heterotrophic bacterioplankton in DOM dynamics as both consumers and producers of humic-like compounds. Furthermore, it demonstrates that patterns of bacterial DOM consumption and transformation vary according to the nature of the DOM.

We observed higher net peaks of bacterioplankton biomass and production with algal lysate and soil extract than in control, glucose alone or nutrients alone. Regarding the Hbact co-limitation, these results suggest that algal-derived and soil-derived DOM provided nutrients in organic form to Hbact. Indeed, our study checked DIN and SRP concentrations under initial conditions just after amendments, and neither of these DOM sources increased DIN and SRP concentrations in the microcosms. Additionally, the C budget in the dark showed that the addition of inorganic nutrients in algal lysate and soil extract treatments did not change the proportion of mineralised C, supporting that these two DOM pools contained enough N and P not to limit the use of organic C by Hbact. It has been shown that DOM can contain N and P in organic form, and that these two organic nutrients have been considered potentially very important sources of N and P for heterotrophic bacterioplankton (Fuhrman, 1992). Hence, it is reasonable to assume that organic nutrients (N, P) were provided by algal lysate and soil extract in our experiment.

Another striking feature was the discrepancy of DOC consumption, Hbact biomass stocks reached, and Hbact production in response to algal lysate versus soil extract amendments. Maximum Hbact biomass stocks reached were sooner (D4) and higher with soil-extract. Additionally, the Hbact production at the beginning of

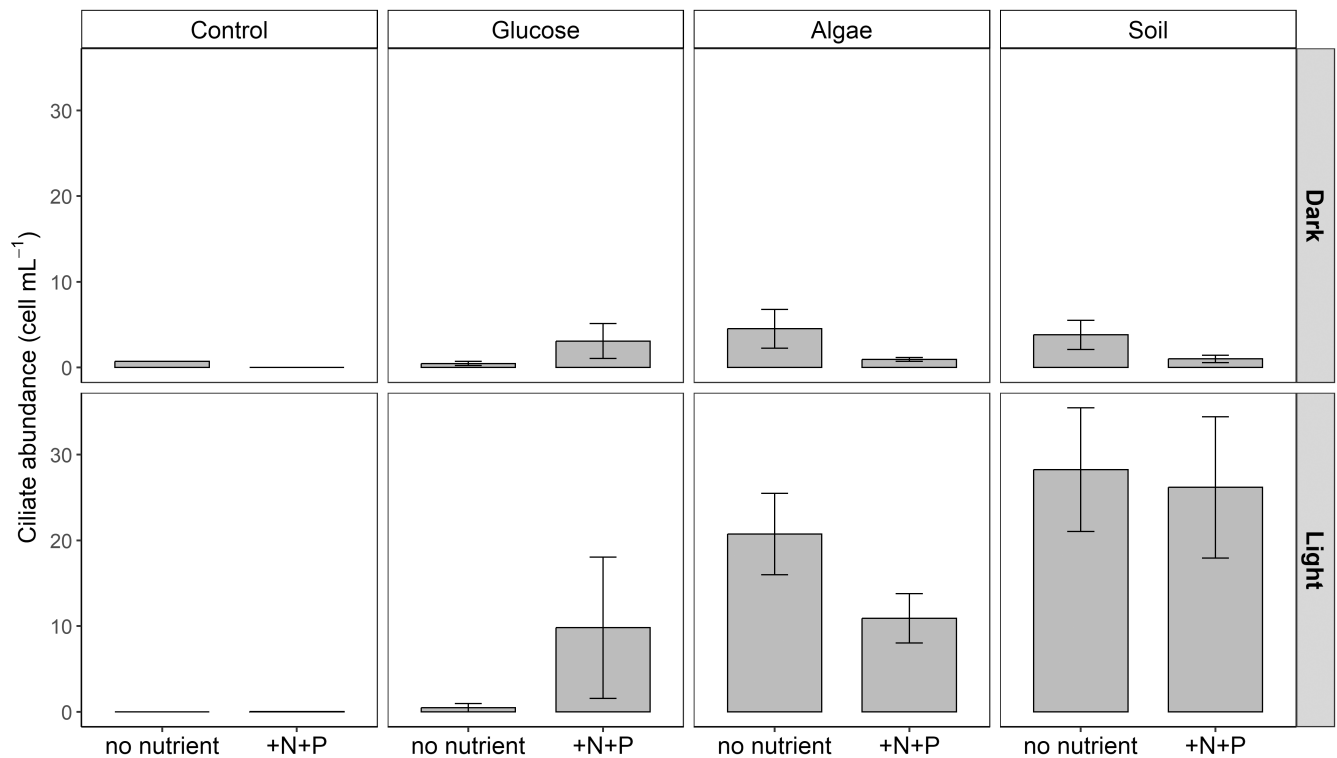


FIGURE 6 Ciliate abundance on Day 10 of the experiment in control, glucose, algal and soil treatments, without added nutrients (no nutrient) and with inorganic nutrients (+nitrogen [N]+phosphorus [P]), in the light and dark on Day 10 of the experiment. Error bars represent standard error.

the experiment increased faster with soil extract. However, in treatments receiving algal lysate, DOC was more consumed and peaks of Hbact production were higher. Time-integrated bacterioplankton production (assuming constant leucine-C conversion factors according to treatments and time) also was observed to be higher with algal lysate. Finally, the C budgets calculated on organic carbon stocks in the light showed a higher net heterotrophy at the end of the experiment with algal lysate than with soil extract. In the dark, the C budgets allowed us to demonstrate that the proportion of initial total C which was mineralised was slightly higher with algal lysate than with soil extract. However in both cases, the essential of the fate of C is through mineralisation (range 67%–76%).

These complex results suggest that (i) soil-derived DOM was less labile and less consumed by Hbact, (ii) the labile fraction of both soil-derived DOM and algal lysate were mainly mineralised, and (iii) the rapid response of Hbact production at the beginning of the experiment with soil extract also suggests that bacterial assemblage was effective in using this type of recalcitrant DOM pool. This last result is not surprising, as the microbial community was sampled in June, just after the snowmelt. During this period, allochthonous DOM has been showed to dominate the DOM pool in high-altitude lakes (Dory et al., 2022; Sadro et al., 2011). In addition, this rapid response of Hbact production led to a greater lag between the peaks of Hbact and predators, such as ciliates or mixotrophs, that have slower growth rates. However, algal-derived DOM was more labile, and more consumed, but, as for soil DOM, with a high energy cost. Our results are fully in line with previous studies showing

that lake bacteria preferentially consumed algal DOC rather than soil extract (Guillemette et al., 2013; Kritzberg et al., 2004; Pérez & Sommaruga, 2006), but also highlighted that the DOM preferences of the natural assemblage and the trophic interactions with predators increase considerably the complexity of the bacterial response in the microcosms.

It cannot be concluded that elevated phytoplankton biomass stimulated heterotrophic bacterial degradation of soil-derived DOM (i.e., a priming effect) as we did not observe higher Hbact metabolism in the light (with algae) than in the dark (without algae) in treatments receiving soil extract.

4.3 | Effects of DOM, nutrients, and light on phytoplankton biomass

In oligotrophic systems such as high-altitude lakes, phytoplankton growth is generally limited by nutrients during the ice-free season (Camarero & Catalan, 2012; Elser et al., 2009; Jacquemin et al., 2018). In our study, phytoplankton biomass increased after inorganic N and P enrichment, especially in the light. This result is consistent with our third hypothesis, indicating a strong limitation of phytoplankton by nutrients. This limitation was closely linked to the low concentrations of DIN and SRP in the water at the initial conditions of the experiment. Our results confirm those of Jacquemin et al. (2018), who demonstrated that phytoplankton growth was limited by inorganic nutrients (N, P) in Lake Cordes in early summer.

In freshwater environments, increasing allochthonous sources of DOC may decrease phytoplankton biomass, as a result of competition between phytoplankton and heterotrophic bacterioplankton for nutrients (Carney et al., 2016), or to a shading effect (Seekell et al., 2015; Thrane et al., 2014). However, in clear lakes such as arctic lakes in Sweden and Alaska, or high-altitude lakes, DOM increase has been shown to lead to increased phytoplankton biomass by raising the quantity of additional nutrients (Kissman et al., 2017; Seekell et al., 2015). In our study, in the dark, mixotrophic phytoplankton biomass increased with C-source amendments compared to controls. In the light, phytoplankton biomass increased with algal lysate and soil extract in unenriched treatments. This result also supports our third hypothesis of increasing phytoplankton biomass owing to interactions with Hbact. Indeed, based on the deduction discussed above, that algal lysate and soil extract contain N and P in organic form, we can assume that the observed increase in phytoplankton biomass in these treatments is linked to Hbact metabolism, via the bacterial mineralisation of organic N and P, and/or via direct ingestion of Hbact by mixotrophs, as mixotrophs represent a large part of the phytoplankton community in these conditions.

However, in the light and nutrient-enriched treatments, phytoplankton biomass reached at D10 was lower when ciliate abundance was low (in the microcosms with glucose and algal lysate), whereas phytoplankton biomass peaked coinciding with a high ciliate abundance (in the microcosms with soil extract). Ciliates are acknowledged to be major consumers of bacteria (Ameryk et al., 2005). DOC additions have been shown to extend the transfer of bacterial production to flagellates and ciliates, resulting in an increase of these predators in experimental conditions (Blomqvist et al., 2001; Dory et al., 2021). Our results thus suggest that the development of ciliates in our microcosms could supersede competition between bacterioplankton and phytoplankton, allowing phytoplankton to take advantage of nutrient recycling carried out by ciliates. Conversely, there could be strong competition between phytoplankton and bacterioplankton in the absence of any top-down regulation of bacterioplankton by ciliates, resulting in lower phytoplankton biomass.

4.4 | Effects of DOM, nutrients and light on phytoplankton community composition

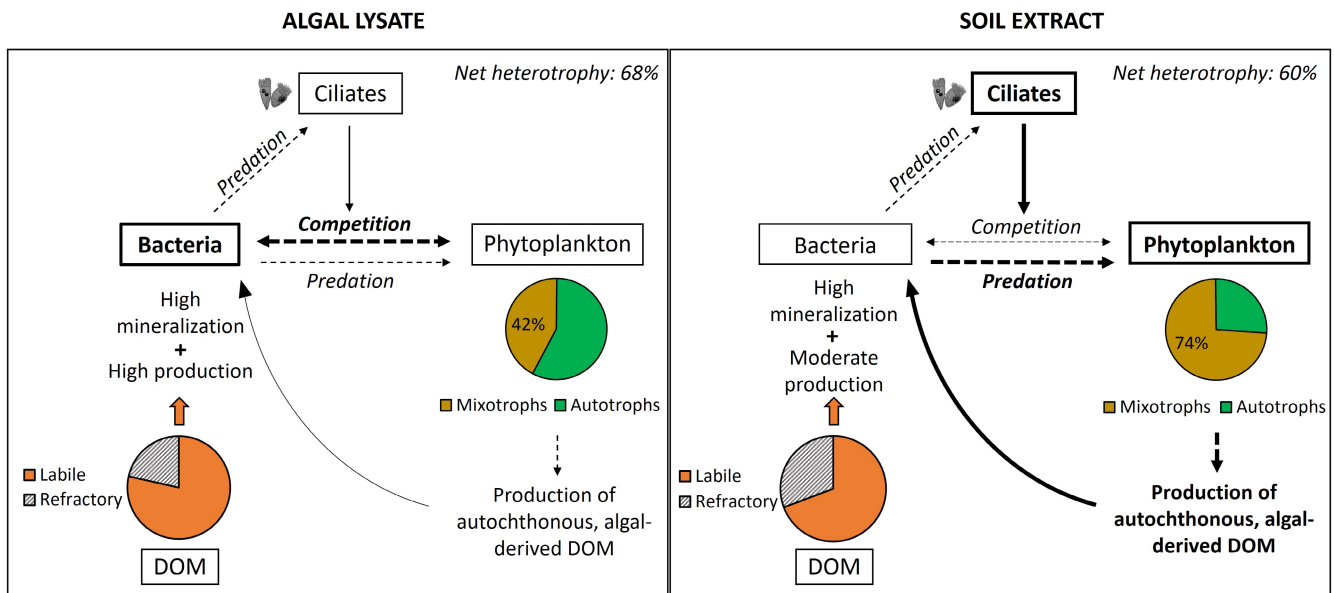
Increased mixotrophy is classically reported with increasing C subsidies supplied by terrestrial DOC, related to increased bacterial production (Bergström et al., 2003; Paczkowska et al., 2019). Ingestion of bacteria by mixotrophs prevents the negative effects of competition and bacterial dominance in the system (Thingstad et al., 1996). However, the nutritional behaviour patterns of mixotrophs vary widely according to both factors at the organism level and to environmental factors (Bergström et al., 2003; Flynn et al., 2019; Saad et al., 2016). Our results reveal two patterns of phytoplankton response to increased C, depending on light or nutrient regime. In the dark, the proportion of mixotrophic biomass increased with increasing glucose, algal lysate and soil extract, regardless of

whether or not there was nutrient enrichment. In the light, C enrichment was more favourable to the development of autotrophs, and the mixotrophs:autotrophs ratio decreased, except with glucose in unenriched treatments. Mixotrophy was previously reported to be an advantageous strategy to acquire organic C under light-limiting conditions, but also under nutrient-limiting conditions, as bacterial ingestion may provide nutrients poorly available for autotrophs (Flynn & Mitra, 2009; Modenutti, 2014). In our study, mixotrophs were favoured by C amendments under light-limiting conditions or nutrient-limiting conditions. These results confirm our last hypothesis and suggest that ingesting bacteria in our microcosms was an efficient strategy to acquire C and/or nutrients under light or nutrient limitations.

In the light under unenriched conditions, species richness was systematically higher with algal lysate and soil extract than in control or treatments receiving glucose alone and inorganic nutrients alone. Increased phytoplankton richness has previously been observed with nutrient enrichment from autumn rainfalls (Spatharis et al., 2007) or from snowmelt inputs (Parker et al., 2008). This result supports our previous assumption that DOM amendments constituted a source of nutrients (via Hbact metabolism) that had a positive effect on phytoplankton richness. Interestingly, in treatments receiving algal lysate, autotrophs dominated the community and were mainly represented by small centric diatoms. In treatments receiving soil extract, although mixotroph biomass was reduced, they remained dominant in the community, and autotrophs were mainly represented by large pennate diatoms. Small phytoplankton cells show better resource acquisition and a higher growth rate and photosynthetic rate than larger cells (Grover, 1989; Raven, 1998) and, because they grow faster, small diatoms are usually more efficient at nutrient assimilation (Litchman et al., 2009, 2010). Hence, small phytoplankton cells generally dominate under oligotrophic conditions while large cells are more associated with nutrient-rich environments (Irwin et al., 2006). One explanation for the observed difference in diatom composition between soil extract and algal lysate may be related to a difference in inorganic nutrient availability in these two treatments, and therefore indirectly to a difference in bacterial metabolism and ciliate regulation. We suggest that the lower top-down regulation of bacterioplankton by ciliates and thus the higher competition between phytoplankton and Hbact with algal lysate could lead to lower inorganic nutrient availability, promoting small centric diatoms. By contrast, with soil extract, the DOM mineralisation by Hbact coupled to the higher top-down regulation and the lower competition with phytoplankton could have led to higher inorganic nutrient availability, promoting large pennate diatoms.

5 | CONCLUSION AND IMPLICATIONS

Our study addressed the consequences of DOM and nutrient increase on a natural planktonic assemblage in a high-altitude lake. The experimental addition of DOM with contrasting properties enhanced heterotrophy and modified the interactions between



CLIMATE CHANGE SCENARIO

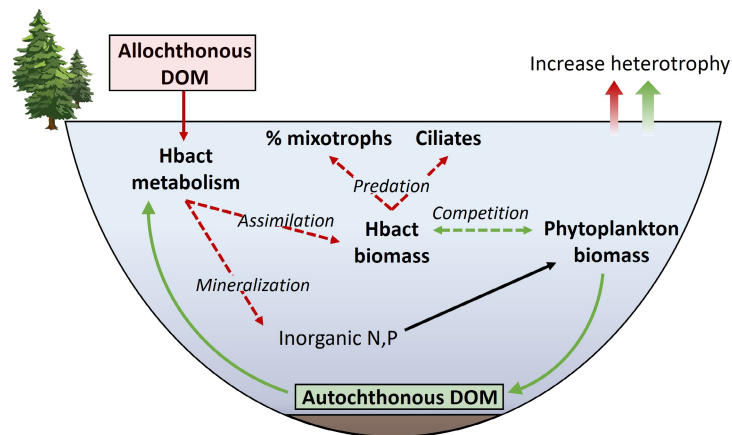


FIGURE 7 Above, graphical representation of changes in interactions between heterotrophic bacteria (Hbact) and phytoplankton based on the experimental results observed after 10 days with algal lysate versus soil extract amendments. These interactions are deduced from biological responses measured in the different DOM treatments, with or without added inorganic nutrients (N, P), in the dark or in the light. Below, expected changes in high-altitude lakes with large, vegetised catchments in a climate change scenario of increasing DOM just after snowmelt. Changes at a short time-scale are represented (~10 days after allochthonous DOM inputs). Inputs of allochthonous DOM are expected to occur first at this period of the year and will result in an increase of autochthonous DOM as a result of the pulse of fresh, phytoplankton-derived DOM. Dashed arrows represent ecological interactions and biological processes and the direction follows that of carbon transfers. Above, thick arrows and bold text represent exacerbated effects or processes. Below, red arrows represent the privileged pathway of allochthonous DOM, while green arrows represent the privileged pathways of autochthonous DOM, and black arrows represent the privileged pathways of inorganic nutrients.

heterotrophic bacteria and phytoplankton, with important implications for the trophic food web (Figure 7; top).

Under a climate change scenario, the expected variations in high-altitude lakes are depicted in Figure 7 (bottom). An increase in allochthonous DOM just after snowmelt will initially accentuate heterotrophy and maintain a high proportion of mixotrophs in the community. However, these allochthonous inputs also will pulse fresh phytoplankton DOM, which could create a "second-phase effect" similar to autochthonous DOM inputs. Ultimately, this will result in an increase in bacteria growth rate, a decrease in mixotrophs and

a decrease in cell size, with major repercussions on predation and therefore on the C transfer within the food web. In a climate change scenario of DOM increase, we expect that the increase in heterotrophy and the modification of trophic interactions will affect the C cycle and the role of the C sink or source of high-altitude lakes.

AUTHOR CONTRIBUTIONS

Conceptualisation: Flavia Dory, Laurent Cavalli, Evelyne Franquet, Céline Bertrand. Developing methods: Flavia Dory, Laurent Cavalli, Evelyne Franquet, Céline Bertrand, Benjamin Misson, France

Van Wambeke. Data analysis: Flavia Dory, France Van Wambeke, Christian Martino, Benjamin Oursel. Preparation figures & tables: Flavia Dory. Conducting the research, data interpretation, writing: Flavia Dory, Laurent Cavalli, Evelyne Franquet, Céline Bertrand, Benjamin Misson, France Van Wambeke, Thierry Tatoni, Stéphane Mounier, Christian Martino, Benjamin Oursel.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed in this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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