NNT/NL : 2022AIXM0146/013ED251

THÈSE DE DOCTORAT

Soutenue à Aix-Marseille Université
le 04 mai 2022 par

Flavia Dory

Sensibilité des communautés planctoniques à la matière organique dissoute : vers une compréhension de la vulnérabilité des lacs de haute altitude aux changements globaux

Discipline
Sciences de l'Environnement

Spécialité
Ecologie

École doctorale
Sciences de l'Environnement (ED 251)

Laboratoire
Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE)

Composition du jury

Nico SALMASO, Pr. Fondazione Edmund Mach	Rapporteur
François GUILLEMETTE, Pr. Université du Québec, Trois-Rivières	Rapporteur
Vanina PASQUALINI, Pr. Université de Corse	Examinatrice
Stéphan JACQUET, DR Université de Savoie	Président du jury
Thierry TATONI, Pr. Aix-Marseille Université	Directeur de thèse
Céline BERTRAND, MCF Aix-Marseille Université	Co-directrice de thèse
Evelyne Franquet, Pr. Aix-Marseille Université	Co-encadrante
Laurent Cavalli, MCF Aix-Marseille Université	Co-encadrant

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Vanina PASQUALINI, Pr. Université of Corsica	Examinator
Stéphan JACQUET, DR INRAE, Thonon-les-Bains	Président of jury
Thierry TATONI Pr. Aix-Marseille University	Thesis director
Céline BERTRAND, MCF Aix-Marseille University	Thesis co-director
Evelyne FRANQUET, Pr. Aix-Marseille Université	Co-supervisor
Laurent CAVALLI, MCF Aix-Marseille Université	Co-supervisor

"Accommode-toi aux événements que le sort te destine ; et les hommes avec lesquels ton partage est de vivre, aime-les, et d'un amour véritable."

Pensées de Marc-Aurèle, Livre VI

Affidavit

Je soussignée, Flavia Dory, déclare par la présente que le travail présenté dans ce manuscrit est mon propre travail, réalisé sous la direction scientifique de Thierry Tatoni et Céline Bertrand, dans le respect des principes d'honnêteté, d'intégrité et de responsabilité inhérents à la mission de recherche. Les travaux de recherche et la rédaction de ce manuscrit ont été réalisés dans le respect à la fois de la charte nationale de déontologie des métiers de la recherche et de la charte d'Aix-Marseille Université relative à la lutte contre le plagiat.

Ce travail n'a pas été précédemment soumis en France ou à l'étranger dans une version identique ou similaire à un organisme examinateur.

Fait à Marseille, le 24 février 2022

Affidavit

I, undersigned, Flavia Dory, hereby declare that the work presented in this manuscript is my own work, carried out under the scientific direction of Thierry Tatoni and Céline Bertrand, in accordance with the principles of honesty, integrity and responsibility inherent to the research mission. The research work and the writing of this manuscript have been carried out in compliance with both the French national charter for Research Integrity and the Aix-Marseille University charter on the fight against plagiarism.

This work has not been submitted previously either in this country or in another country in the same or in a similar version to any other examination body.

Place Marseille, date 24 février 2022

Dory

Liste de publications et participation aux conférences

1) Liste des publications[1] réalisées dans le cadre du projet de thèse :

   1. **Dory, F**., Cavalli, L., Franquet, E., Claeys‐ Bruno, M., Misson, B., Tatoni, T., & Bertrand, C. (2021). Microbial consortia in an ice‐ covered high‐ altitude lake impacted by additions of dissolved organic carbon and nutrients. Freshwater Biology, 66(8), 1648-1662. https://doi.org/10.1111/fwb.13781

   2. **Dory, F**., Cavalli, L., Franquet, E., Claeys‐ Bruno, M., Misson, B., Tatoni, T., & Bertrand, C. (2022). Summer dynamics drive the microbial response to carbon and nutrient additions in a high-altitude lake. Limnology and Oceanography. https://doi.org/10.1002/lno.12062

   3. **Dory, F.,** Cavalli, L., Franquet, E., Mounier, S., Höhener, P., Misson, B., Martin, M., Arnault, Q., Tatoni, T., Bertrand, C. Planktonic shift with dissolved organic matter properties: a functional perspective for sentinel lakes. *Under review*, Journal of Ecology

   4. **Dory, F.,** Cavalli, L., Franquet, F., Van Wambeke, F., Misson, B., Mounier, S., Martino, C., Oursel, B., Tatoni, T., Bertrand, C. Rising algal- and soil-derived dissolved organic matter modifies interactions between bacteria and phytoplankton in a high-altitude lake. In preparation.

   5. **Dory, F**., **& Claeys-Bruno**, **M**., Tatoni, T., Cavalli, L., Franquet, E., Bertrand, C. Using Design of Experiments in experimental ecology. In preparation.

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[1] Cette liste comprend les articles publiés, les articles soumis à publication et les articles en préparation ainsi que les livres, chapitres de livre et/ou toutes formes de valorisation des résultats des travaux propres à la discipline du projet de thèse. La référence aux publications doit suivre les règles standards de bibliographie et doit être conforme à la charte des publications d'AMU.

2) Participation aux conférences[2] et écoles d'été au cours de la période de thèse :

1. **AFL2018 (Strasbourg)**
   Dory, F., Jacquemin, C., Dutilleul, M., Cavalli, L., Franquet, E., Bertrand, C. Phytoplankton response to increasing temperature under different nutrient limitation pattern. 2018.

2. **SEFS12 Symposium for European Freshwater Sciences**
   Dory, F., Cavalli, L., Franquet, E., Claeys- Bruno, M., Misson, B., Tatoni, T., & Bertrand, C. Planktonic response to dissolved organic carbon and nutrients in a high-altitude lake. 2021.

3. **ASLO 2021 Aquatic Sciences Meeting**
   Dory, F., Cavalli, L., Franquet, E., Claeys- Bruno, M., Misson, B., Tatoni, T., & Bertrand, C. Microbial consortia in an ice-covered high-altitude lake impacted by additions of dissolved organic carbon and nutrients. 2021.

4. **GIS Rencontres lacs sentinelles 2021 (Chamrousse)**
   Sensibilité des communautés phytoplanctoniques des lacs de haute altitude aux apports de COD. 2021.

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[2] Le terme « conférence » est générique. Il désigne à la fois « conférence », « congrès », « workshop », « colloques », « rencontres nationales et/ou internationales » … etc.

Indiquer si vous avez fait une présentation orale ou sous forme de poster.

Résumé

Les lacs de haute altitude sont des écosystèmes sentinelles, vulnérables aux variations des facteurs environnementaux. Dans le cadre des changements globaux, l'augmentation de la matière organique dissoute a été pointée comme un élément clé, capable de modifier les réseaux trophiques planctoniques à la base du fonctionnement des lacs. Ce travail de thèse a pour but de mieux comprendre les effets de la variation quantitative et qualitative de la matière organique dissoute sur les interactions entre bactéries hétérotrophes et phytoplancton.

La sensibilité des communautés planctoniques a été abordée à travers deux approches, expérimentale et *in situ*. Le suivi *in situ* a été réalisé sur le lac des Cordes, un lac de haute altitude oligotrophe peu profond à grand bassin versant. A travers une série d'expérimentations en laboratoire, les communautés de ce lac ont été exposées à différents enrichissements en carbone organique dissous (glucose), nutriments (azote, phosphore), et en matière organique dissoute.

Les résultats mettent en évidence une composition taxonomique et fonctionnelle du phytoplancton et une biomasse bactérienne fortement régulées par la dynamique saisonnière de la matière organique dissoute et par le contexte de limitation en carbone et en nutriments. En conditions expérimentales, l'augmentation de la concentration en carbone organique et en nutriments stimule le métabolisme bactérien et favorise les taxa mixotrophes dans les communautés hivernales et de fin d'été. En revanche, dans ces conditions d'enrichissement, la communauté phytoplanctonique de début d'été est dominée par les autotrophes. En lien avec l'utilisation de la matière organique dissoute par les bactéries, un apport autochtone entraîne un déclin plus important des mixotrophes qu'un apport allochtone.

Ce travail fournit des éléments de discussion sur l'évolution des relations entre bactéries et phytoplancton et démontre le rôle clé de la matière organique dissoute dans le fonctionnement des communautés planctoniques en lac de haute altitude. Cette thèse ouvre des perspectives sur les trajectoires évolutives potentielles des lacs de haute altitude au regard des changements globaux.

Mots clés : Phytoplancton, Changements fonctionnels, Matière organique dissoute, Dynamique saisonnière, Interactions trophiques

Abstract

High-altitude lakes are sentinel ecosystems, vulnerable to environmental variations. The increase of dissolved organic matter forecasted with global changes has been recognized as a key factor regulating the planktonic food web at the base of the functioning of the lakes. This thesis aims to better understand how qualitative and quantitative variations of dissolved organic matter could affect interactions between bacterioplankton and phytoplankton.

The sensitivity of planktonic communities has been addressed through an *in-situ* survey and an experimental approach. The *in-situ* survey was carried out on the Lake Cordes, a shallow oligotrophic high-altitude lake with a large catchment. Through laboratory experiments, the communities of this lake were exposed to different enrichments in dissolved organic carbon (glucose), nutrients (nitrogen, phosphorus), and dissolved organic matter.

Our results showed that the taxonomic and functional composition of phytoplankton and bacterial biomass are highly regulated by the seasonal dynamics of dissolved organic matter and by the context of carbon and nutrient limitation. In experimental conditions, increase concentrations of organic carbon and nutrients stimulates the bacterial metabolism and benefit mixotrophic taxa in winter and late summer communities. By contrast, the early summer phytoplankton community is dominated by autotrophs under these enrichment conditions. In connection with the use of dissolved organic matter by bacterioplankton, an autochthonous input induces a greater decline of mixotrophs than an allochthonous input.

Our work provides insights on the relationships between bacterioplankton and phytoplankton and demonstrates the key role of dissolved organic matter in the functioning of planktonic communities of high-altitude lakes. This thesis opens perspectives on the potential trajectories of high-altitude lakes regarding global change.

Keywords: Phytoplankton, Functional changes, Dissolved organic matter, Seasonal dynamics, Trophic interactions

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GENERAL DISCUSSION

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Abbreviations

DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DIN	Dissolved inorganic nitrogen
SRP	Soluble reactive phosphorus
DIC	Dissolved inorganic carbon
TP	Total phosphorus
IIP	Ice-influenced period
OP	Overturn period
LSP	Late summer period
TAX	Taxonomical groups
PFG	Protist functional groups
MBFG	Morphology-based functional groups
FG	Functional groups
MFG	Morpho-functional groups
POM	Particulate organic matter
EEM	Excitation-emission matrix
Si	Silica

Abbreviations and names used in the different papers to designate bacterioplankton

HP	Heterotrophic prokaryotes
HPP	Heterotrophic prokaryotic plankton

French synthesis

Introduction générale

Les changements globaux affectent les propriétés physiques, chimiques et biologiques des écosystèmes lacustres (Adrian et al., 2009). Les variations attendues de la matière organique dissoute en lien avec les changements globaux devraient modifier le métabolisme des lacs et leur rôle dans cycle global du carbone (Perga et al., 2018; Rofner et al., 2017).

La matière organique dissoute (MOD) joue un rôle fondamental dans le fonctionnement des écosystèmes lacustres (Creed et al., 2018; Prairie, 2008; Williamson et al., 1999). A travers son action sur les organismes à la base du réseau trophique, la matière organique dissoute régule les voies de transfert du carbone vers les niveaux trophiques supérieurs et donc le bilan carbone des lacs (Jansson et al., 2000). Une augmentation des concentrations en matière organique dissoute a été reportée dans de nombreux écosystèmes aquatiques de l'hémisphère Nord en lien avec les changements globaux d'origine anthropique (Monteith et al., 2007; Solomon et al., 2015). Par exemple, l'augmentation de la température influence la productivité terrestre des bassins versants et augmente la quantité de matière organique d'origine terrestre potentiellement transférée dans les lacs (Larsen et al., 2011). L'augmentation des événements pluvieux extrêmes affecte le transport hydrologique, la connectivité entre lacs et bassins versants et accentue le transfert de la matière organique vers la colonne d'eau (Perga et al., 2018; Wu et al., 2011). Les concentrations en matière organique dissoute d'origine phytoplanctonique sont également susceptibles d'augmenter en lien avec l'eutrophisation des masses d'eau (Zhou et al., 2018). Mieux comprendre l'influence qu'exerce la matière organique sur les écosystèmes aquatiques est indispensable au regard des changements globaux à venir.

La matière organique dissoute est un ensemble de composés rassemblant des lipides, des carbohydrates et des protéines, de taille inférieure à 0,7 µm (Aitkenhead-Peterson et al., 2003). Le pool de matière organique des lacs peut-être d'origine allochtone, provenant des apports terrestres des bassins versants et autochtone, issue de la productivité primaire benthique, des macrophytes et du phytoplancton (Bertilsson & Jones, 2003; Canham et al., 2004). Ces différences d'origine entraînent des différences de composition chimique et de propriétés optiques de la matière organique, avec pour conséquence des effets variables sur les communautés. Par exemple, la matière organique dissoute autochtone, de plus faible poids moléculaire, est souvent reportée comme plus labile et donc plus facilement assimilable par les bactéries hétérotrophes que la MOD allochtone (McKnight & Aiken, 1998). Une utilisation différente de la MOD par les bactéries en fonction de son origine a également été observée. Ainsi, il a été montré que la MOD autochtone était davantage utilisée par les bactéries pour la respiration, tandis que la MOD allochtone était davantage utilisée pour la croissance (Guillemette et al., 2016). Les conséquences de ces différences d'utilisation sur le compartiment phytoplanctonique restent toutefois peu étudiées.

Parce qu'elle contient du carbone organique dissous (COD) et des nutriments (azote, phosphore), la matière organique dissoute peut agir comme source d'énergie et/ou comme source de nutriments pour les bactéries et le phytoplancton (Creed et al., 2018).

La mixotrophie est une stratégie nutritionnelle qui fait combiner l'autotrophie et l'hétérotrophie chez un même organisme (Flynn et al., 2019). Chez les taxa phytoplanctoniques, le mode d'autotrophie correspond à l'acquisition du CO_2 atmosphérique par la photosynthèse. L'hétérotrophie correspond quant à elle à l'acquisition du carbone organique dans la colonne d'eau, par osmotrophie et/ou phagotrophie (ingestion des proies). L'osmotrophie, la capacité à utiliser des matières organiques dissoutes telles que les acides aminés et les vitamines, est courante chez le phytoplancton (Droop, 2007; Flynn et al., 2019; Våge et al., 2013). En raison du caractère ubiquiste de l'osmotrophie, la mixotrophie fait souvent référence aux organismes qui « expriment, ou ont le potentiel d'exprimer, la phototrophie et la phagotrophie » (Flynn et al., 2019). La notion de potentiel d'expression est importante, car si des organismes ont la capacité d'être mixotrophes, l'expression réelle et le degré d'hétérotrophie des taxa en conditions naturelles est dépendante des limitations environnementales comme la lumière, les nutriments, ou la disponibilité des proies (Bergström et al., 2003; Mitra et al., 2016; Saad et al., 2016).

La matière organique dissoute peut induire des réponses variables des bactéries et du phytoplancton selon sa composition et selon les besoins nutritionnels des organismes. En fonction de son action en tant que source de carbone et/ou de nutriments, l'augmentation de la matière organique pourrait renforcer ou atténuer les interactions commensalistes, de compétition, ou de prédation, dans les communautés planctoniques (Castillo et al., 2010; Flynn et al., 2008; Livanou et al., 2020).

Le commensalisme fait référence à l'utilisation par les bactéries du carbone organique excrété par le phytoplancton et est souvent associé à l'augmentation de la production phytoplanctonique (Carrillo et al., 2015). La compétition fait référence à la limitation par les mêmes nutriments de la croissance bactérienne et phytoplanctonique (Carney et al., 2016). La prédation fait référence à l'ingestion de proies, généralement des bactéries, par le phytoplancton mixotrophe pour contrecarrer la limitation par la lumière ou les nutriments inorganiques (Thingstad et al., 1996).

Lorsque les concentrations en COD augmentent, la croissance bactérienne n'est plus limitée par le carbone et devient principalement limitée par les nutriments (N, P), ce qui a pour effet d'augmenter la compétition avec le phytoplancton pour les nutriments inorganiques (Almeida et al., 2005; Pinhassi et al., 2006). Les bactéries sont généralement plus efficaces que le phytoplancton pour assimiler ces nutriments (Currie & Kalff, 1984; Jansson et al., 2006). L'augmentation des concentrations en COD se traduit donc généralement par une stimulation du métabolisme bactérien et par une diminution de la production primaire phytoplanctonique dans les écosystèmes aquatiques (Hitchcock et al., 2010; Joint et al., 2002; Westhorpe et al., 2010). Des modifications de la composition taxonomique et fonctionnelle des communautés phytoplanctoniques sont également souvent observées, avec une favorisation des groupes possédant des capacités mixotrophes, comme les cyanobactéries (Maranger & Pullin, 2003) ou les phytoflagellés (Bergström et al., 2003; Paczkowska et al., 2017). Des études rapportent donc une augmentation de l'abondance des mixotrophes dans les communautés phytoplanctoniques après l'augmentation d'une source de carbone organique, associée à une plus forte compétition entre le phytoplancton et les bactéries pour les nutriments (Carney et al., 2016; Creed et al., 2018; Jansson et al., 2006).

Parce que la matière organique dissoute contient aussi des nutriments, l'augmentation des concentrations en MOD peut également entraîner une augmentation de la disponibilité en azote et en phosphore dans la colonne d'eau (Engel et al., 2018). Si ces concentrations excèdent les demandes biologiques des organismes, une augmentation de la production bactérienne mais aussi phytoplanctonique peut être observée en réponse à l'augmentation de la matière organique (Hitchcock & Mitrovic, 2013; Tanentzap et al., 2017). Les effets de l'augmentation de la MOD sont donc complexes et peuvent fortement modifier les interactions biotiques entre bactéries et phytoplancton.

L'action de la matière organique en tant que source de carbone et/ou de nutriments est fonction des besoins nutritionnels des organismes et de la disponibilité des ressources dans le milieu naturel (Pete et al., 2010). Par exemple, dans des écosystèmes oligotrophes caractérisés par de faibles concentrations en nutriments inorganiques, il a été montré que la limitation des bactéries par les nutriments limitait leur consommation de COD allochtone (Nelson & Carlson,

2011) et de sucrose (Dorado-García et al., 2014) en conditions expérimentales. Toutefois, l’influence du contexte de limitation sur la réponse planctonique à l’augmentation de la MOD est traditionnellement étudiée sur les bactéries et le transfert de ces résultats au compartiment phytoplanctonique manque. L’approfondissement des connaissances sur la limitation des organismes et sur l’effet de la matière organique en tant que source de carbone et/ou de nutriments sur les interactions entre bactéries et phytoplancton est crucial pour mieux comprendre les mécanismes de transfert du carbone au sein des lacs.

Les lacs de haute altitude sont des écosystèmes oligotrophes et ont été identifiés comme des sentinelles des changements globaux (Moser et al., 2019; Williamson et al., 2009). En effet, éloignés et difficiles d’accès, ils sont essentiellement impactés par les effets du changement climatique et par les retombées atmosphériques (Psenner, 2003). Leurs caractéristiques les rendent extrêmement vulnérables à l’augmentation de la matière organique dissoute (Moser et al., 2019; Schindler, 2009). Cette vulnérabilité est liée à leur forte exposition et leur forte sensibilité aux variations environnementales. Par exemple, ces lacs sont exposés à une augmentation des apports de matière organique en provenance des bassins versants en lien avec l’élévation de la limite supérieure des forêts et l’augmentation de la fréquence et de l’intensité des événements pluvieux extrêmes prévues dans le cadre du changement global (Ejarque et al., 2018; IPCC, 2021).

Leurs conditions environnementales extrêmes et fluctuantes (**Illustration S1**) (lumière extrêmement faible et extrêmement forte, faible température, faibles concentrations en nutriments) sélectionnent un pool restreint d’espèces capables de se maintenir dans ces milieux. De fait, leurs réseaux trophiques sont plus simples qu’en lacs de plaine et les organismes sont particulièrement sensibles aux variations, même faibles, des facteurs environnementaux (Catalan et al., 2006). De plus, ces contraintes sélectionnent un ensemble de traits fonctionnels au sein des communautés phytoplanctoniques (Caroni et al., 2012; Jacquemin et al., 2019). Ces traits fonctionnels déterminent les performances écologiques des taxa au sein des lacs, à savoir : leur capacité à croître, à exploiter les ressources lumineuses et nutritives, à se maintenir dans la masse d’eau et à résister aux différents processus de perte tels que la prédation ou la sédimentation (Hulot et al., 2014; Reynolds, 1984; Salmaso & Padisák, 2007). Par exemple, la petite taille, la motilité, les formes de résistance, ou encore la mixotrophie, sont reconnues pour être des stratégies avantageuses pour les organismes phytoplanctoniques en lacs de haute altitude (Medina-Sánchez et al., 2004; Trevisan et al., 2014). Les changements fonctionnels des communautés phytoplanctoniques des lacs d'altitude constituent donc un indicateur particulièrement pertinent pour comprendre la réponse des organismes aux variations environnementales.

Illustration S1 : Conditions environnementales contrastées en lacs de haute altitude entre la période hivernale sous glace (gauche) et la période estivale (droite).

En altitude, la faible superficie et la faible végétalisation des bassins versants entraînent de faibles apports allochtones. Les concentrations en nutriments inorganiques et en COD sont donc généralement faibles dans ces milieux et la matière organique dissoute possède une signature principalement autochtone (Bastidas Navarro et al., 2014; Rose et al., 2015). La saisonnalité très contrastée induit une dynamique de la matière organique (Olson et al., 2021) et une dynamique de la limitation des organismes par les nutriments et par le carbone organique dans ces milieux. Par exemple, une importante quantité de matière organique dissoute allochtone peut être transférée aux lacs au moment de la fonte (Catalan, 1992; Olson et al., 2021; Sadro et al., 2018) ou lors des événements pluvieux extrêmes (**Illustration S2**) (Perga et al., 2018; Sadro & Melack, 2012).

En lien avec la dynamique saisonnière des ressources lumineuses et nutritives, des variations de biomasse et de composition des communautés phytoplanctoniques ont pu être observées en lacs de haute altitude. Par exemple, la limitation par la lumière en période hivernale induit classiquement une dominance des taxa mixotrophes dans les communautés sous glace (Rue et al., 2020). Au cours de la saison estivale, une évolution de la structure et de la composition des communautés phytoplanctoniques est observée en lien avec les contextes de limitation en nutriments inorganiques. Les communautés de fin d'été sont généralement dominées par des taxa plus résistants à la prédation, à plus faibles taux de croissance et efficaces pour assimiler les ressources lorsqu'elles sont rares (Jacquemin et al., 2018; McKnight et al., 1990). En ce qui concerne la biomasse, un schéma bimodal est souvent observé, avec un premier pic de biomasse phytoplanctonique immédiatement après la fonte et un second pic à la fin de la période estivale (Tiberti et al., 2013).

Illustration S2: Différences de coloration d'un même lac de haute altitude en fonction des saisons : en juin après la fonte (gauche) ; en juillet après un épisode de forte pluie (centre) ; en août (droite).

Des travaux précédents ont souligné l'importance du double contrôle exercé par le phytoplancton sur les bactéries en lacs de haute altitude : (i) top-down, à travers la prédation par les mixotrophes et (ii) bottom-up, à travers la production de matière organique dissoute autochtone (Medina-Sánchez et al., 2004). La biomasse et la composition taxonomique et fonctionnelle des communautés phytoplanctoniques, notamment à travers la proportion des mixotrophes, constituent donc des indicateurs particulièrement pertinents pour comprendre la réponse des organismes aux variations environnementales, en particulier à l'augmentation de la matière organique dissoute. Toutefois, le lien entre dynamique de la matière organique et communautés planctoniques en lac de haute altitude est surtout étudié sur des périodes temporelles courtes et principalement en période estivale. Mieux comprendre comment évoluent les communautés planctoniques (bactéries et phytoplancton) en lien avec la qualité et la quantité de matière organique dissoute à l'échelle saisonnière est nécessaire pour évaluer à la fois la sensibilité des communautés et la vulnérabilité des lacs de haute altitude aux changement globaux.

Cette thèse se propose d'apporter une meilleure compréhension de la sensibilité des communautés planctoniques (bactéries et phytoplancton) à l'augmentation de la matière organique dissoute, source de carbone et de nutriments, en lac de haute altitude. Elle permet de fournir de nouvelles connaissances fondamentales sur le rôle de la matière organique dissoute dans la régulation des réseaux trophiques en lac d'altitude, mais également sur le fonctionnement général de ces lacs à l'échelle saisonnière. *In fine*, elle permet d'ouvrir des perspectives sur la compréhension de la vulnérabilité des lacs de haute altitude aux effets des changements globaux.

Cette synthèse comprend une description des méthodes et des objectifs de la thèse. Ces objectifs seront traités à travers quatre articles, correspondant aux chapitres 2 à 5 de ce manuscrit. Une discussion générale termine cette synthèse, mettant en relation les résultats des différents chapitres et fournissant des pistes de réflexion pour de nouvelles perspectives d'études.

Méthodes et objectifs de la thèse

Méthodes

1.1. Choix du lac étudié

Les Alpes du Sud constituent l'ensemble des massifs situés dans la partie méridionale de la chaîne alpine française, rassemblant entre autres les massifs du Mercantour, du Queyras et des Ecrins (**Figure S1**). La configuration géographique des Alpes du Sud induit des caractéristiques environnementales contrastées le long d'un gradient nord-sud, notamment en termes d'influence climatique et de dépôts atmosphériques. Par exemple, les massifs du sud (Mercantour) sont soumis à un climat plus méditerranéen et sont plus exposés aux retombées atmosphériques d'azote (N) (Fagerli et al., 2015) et de phosphore (P), étant situés dans le périmètre d'influence d'aérosols enrichis en phosphore émis au niveau des régions désertiques sahariennes (Moulin & Chiapello, 2004). A l'opposé, les massifs situés plus au nord des Alpes du Sud (Queyras, Ecrins) sont sous l'influence d'un climat plus continental et sont moins exposés aux retombées atmosphériques d'azote et de phosphore (Jacquemin, 2019).

Le lac des Cordes se situe dans la partie nord des Alpes du sud, dans la vallée des Fonds de Cervières, entre les massifs des Ecrins et du Queyras. Par sa localisation, le lac des Cordes est faiblement exposé aux dépôts atmosphériques : par exemple, les taux annuels de dépôt d'azote mesurés dans cette région entre 2000 et 2013 étaient de 435 ± 14 kg N km^{-2} an^{-1}, contre 1123 ± 49 kg N km^{-2} an^{-1} dans les régions du Sud (Mercantour) (Fagerli et al., 2015). Ce lac est caractérisé par une petite taille (1,8 ha), une faible profondeur (profondeur maximale = 9 m) et un grand bassin versant (170 ha) recouvert de pelouse alpine sur environ 60%. Les travaux précédents qui ont été menés sur ce lac ont mis en évidence une contribution forte des taxa mixotrophes à la communauté phytoplanctonique en 2015 et 2016 (Jacquemin, 2019). Les travaux de Jacquemin et al. (2019) ont notamment permis de montrer que les communautés phytoplanctoniques observées sur ce lac étaient le résultat non seulement du contexte d'exposition aux retombées atmosphériques, qui détermine la limitation par les nutriments, mais également des caractéristiques du bassin versant, qui déterminent les apports nutritifs transférés au lac.

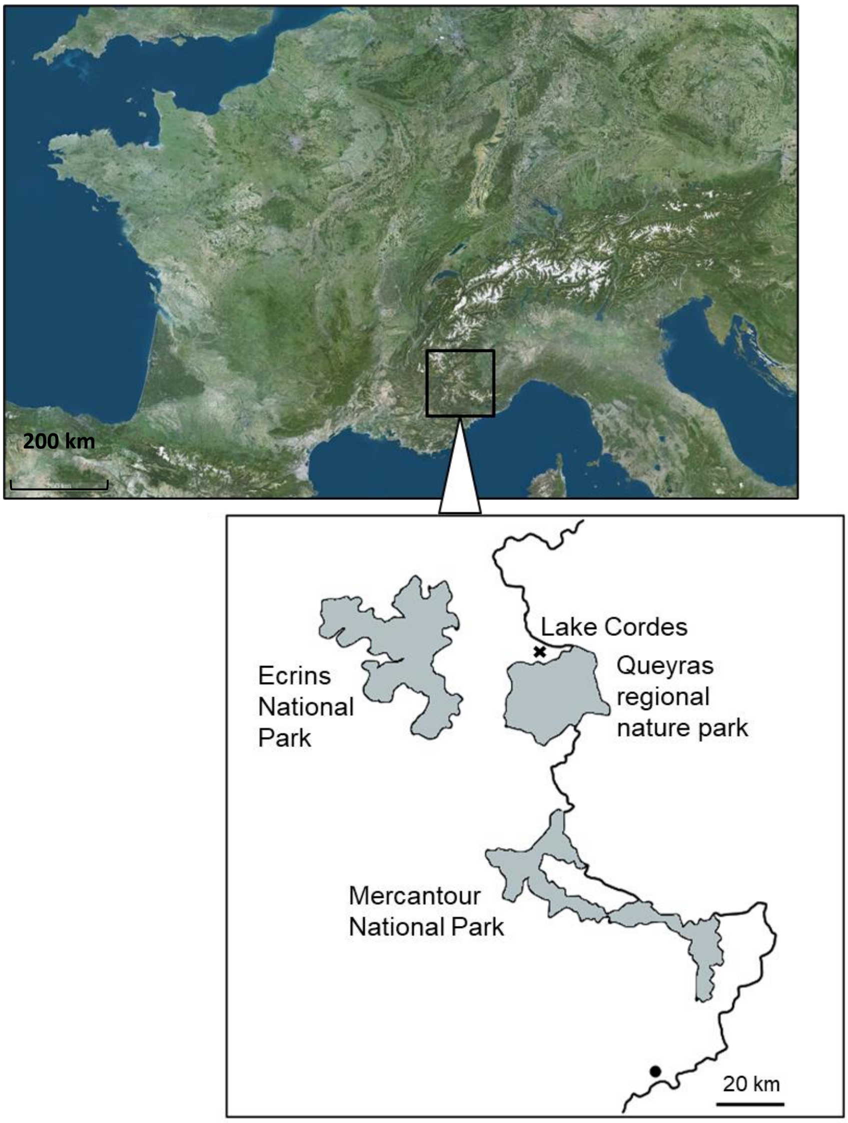

Figure S1 : Localisation des principaux massifs protégés des Alpes du Sud et du lac des Cordes.

Les concentrations en nutriments mesurées en 2016 au cours de la saison estivale indiquent des valeurs relativement faibles, que ce soit pour l'azote inorganique dissous (0,140 ± 0.051 mg L^{-1}), le phosphore réactif soluble (5 ± 2 µg L^{-1}), ou le carbone organique dissous (2,631 ± 1,13 mg L^{-1}) et définissent ce lac comme un lac clair oligotrophe. Il a été montré que la limitation du phytoplancton par les nutriments évolue au cours de l'été, passant d'une limitation par P en début d'été vers une co-limitation par NP en fin d'été (Jacquemin et al., 2018). En lien notamment avec ces modifications de limitation, des modifications des assemblages phytoplanctoniques ont pu être observées au cours de la saison végétative (**Figure S2**).

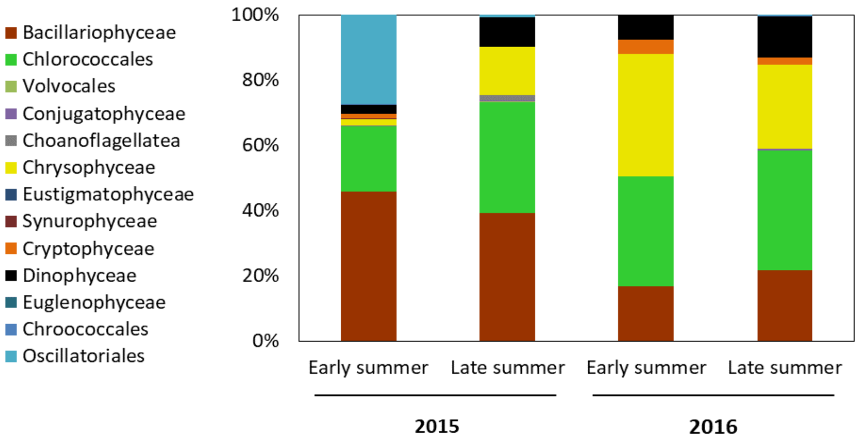

Figure S2 : Composition taxonomique des communautés phytoplanctoniques du lac des Cordes en début d'été et fin d'été, en 2015 et 2016. Données issues de la thèse de Jacquemin (2019).

L'exposition du lac des Cordes aux dépôt atmosphériques, les caractéristiques de sa masse d'eau et de son bassin versant, font de ce lac un site d'étude privilégié pour analyser les impacts de l'augmentation de la matière organique dissoute sur les communautés planctoniques. Le présent travail de thèse s'intègre dans l'ensemble des travaux précédemment menés sur les lacs de haute altitude des Pyrénées et des Alpes, portant sur des sujets variés comme l'écologie des populations de poissons et leurs impacts sur l'environnement (Cavalli et al., 2001, 2002; Tiberti et al., 2014), sur des approches paléo-limnologiques (Cantonati et al., 2021; Cartier et al., 2019; Rivera-Rondón & Catalan, 2020), ou encore sur l'écologie des diatomées benthiques (Birck et al., 2013; Feret et al., 2017).

1.2. Combiner les approches expérimentales et *in situ*

L'étude de la dynamique de la matière organique dissoute ainsi que des successions phytoplanctoniques en lien avec les facteurs environnementaux nécessite un échantillonnage régulier et fournit des éléments de compréhension sur le fonctionnement global de ces écosystèmes. Le suivi *in situ* de la dynamique de la matière organique est particulièrement important pour comprendre les flux de matière entre les bassins versants, les lacs et les compartiments trophiques (Cole et al., 2011; Miller et al., 2009; Olson et al., 2021). La dynamique phytoplanctonique est quant à elle le reflet des variations environnementales et donne des informations précieuses sur les effets potentiels des changements globaux sur le fonctionnement des lacs (Jacquemin et al., 2019; Miller & McKnight, 2015; Rue et al., 2020).

Toutefois, les suivis *in situ* sont limités pour identifier l'effet direct d'un ou de plusieurs facteurs environnementaux sur les populations biologiques (Thackeray et al., 2008). La distinction des effets spécifiques de certains facteurs parmi les nombreux effets d'interaction est souvent complexe. En ce sens, les méthodes expérimentales peuvent aider à distinguer l'effet direct d'un facteur parmi des variations simultanées des conditions environnementales. Par exemple, l'ajout expérimental d'une source simple de carbone organique dissous comme le glucose, permet de distinguer les effets de la source de carbone de ceux des nutriments associés lors des apports de matière organique (Hitchcock et al., 2010; Liao et al., 2019). De même, l'exclusion de certains maillons trophiques, comme le zooplancton prédateur, permet de s'assurer que la réponse phytoplanctonique observée est bien celle générée par le facteur expérimental et qu'elle n'est pas associée à un effet conjoint ou indirect de la prédation par le zooplancton (Hitchcock & Mitrovic, 2013; Hrycik & Stockwell, 2021). Bien que les conditions expérimentales ne permettent pas une entière représentativité des effets des facteurs environnementaux sur les communautés biologiques, la simplification expérimentale est une première étape indispensable pour évaluer les effets de certains facteurs et leurs interactions (Srivastava et al., 2004).

Dans cette thèse, des approches *in situ* et expérimentales ont été combinées pour étudier la sensibilité des communautés planctoniques à l'augmentation de la matière organique dissoute (**Figure S3**). L'approche *in situ*, qui constitue le premier volet présenté dans la thèse (chapitre 2), fournit une caractérisation de la dynamique de la matière organique dissoute et des communautés phytoplanctoniques en lien avec les variations de la biomasse bactérienne et des facteurs environnementaux. Cette première approche a pour objectif d'identifier les changements saisonniers de concentrations et de qualité de la matière organique dissoute sur le lac des Cordes et de suivre la dynamique des communautés phytoplanctoniques en lien avec la matière organique dissoute.

Le volet expérimental se décompose en deux parties. Une première série d'expérimentations avait pour objectif d'identifier les limitations potentielles des bactéries hétérotrophes et du phytoplancton et d'évaluer la réponse de ces deux compartiments à des enrichissements en COD (glucose), en nutriments (azote et phosphore) et en une combinaison des deux (chapitres 3 et 4). Ces expérimentations ont été effectuée à différentes périodes clef du fonctionnement du lac (période hivernale, début d'été, fin d'été) afin d'évaluer comment la saisonnalité peut moduler la réponse des organismes aux différents enrichissements. Une seconde expérimentation avait pour objectif d'évaluer la réponse des organismes planctoniques à la variation de qualité de la matière organique dissoute (chapitre 5) dans des communautés de début d'été. Des enrichissements en extrait de sol issu du bassin versant et en lixiviat d'algues issues du lac des Cordes ont permis de caractériser la réponse à des sources allochtones et autochtone de matière organique dissoute et de comparer cette réponse à celle obtenue avec du glucose et des nutriments.

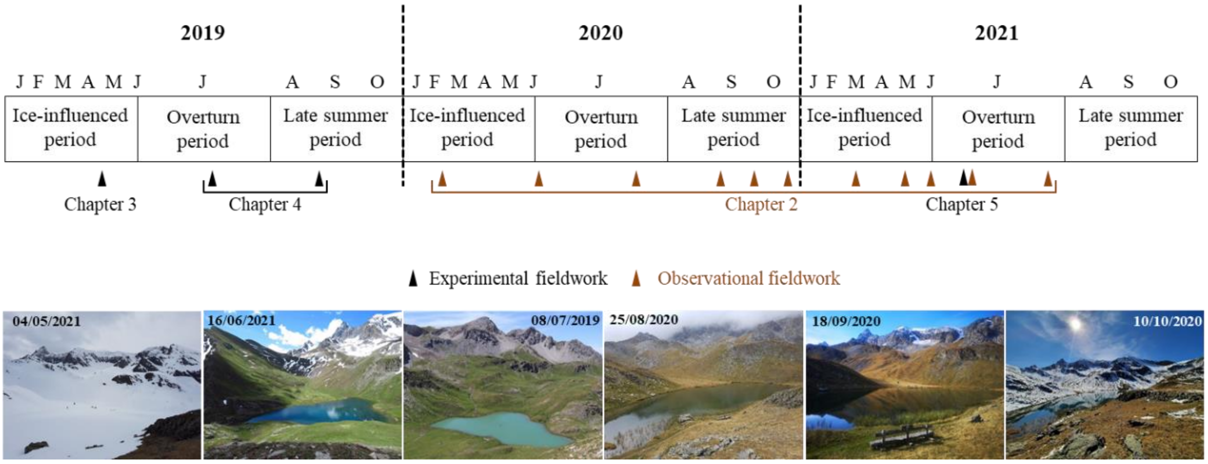

Figure S3 : Chronologie des différents échantillonnages réalisés sur le lac des Cordes pour les expérimentations en laboratoire (chapitres 3, 4 et 5) et pour le suivi in situ (chapitre 2) entre 2019 et 2021. Les différents échantillonnages ont été répartis dans les trois périodes clef de fonctionnement du lac, la période hivernale (« Ice-influenced period »), la période de début d'été (« overturn period »), et la période de fin d'été (« Late summer period »). La variation saisonnière du lac des Cordes entre la période hivernale et la période de fin d'été a été capturée au cours des différentes années.

1.3. L'approche par plans d'expériences

L'échantillonnage sous la glace en période hivernale est un grand défi en lacs de haute altitude et cela est particulièrement vrai pour les expérimentations en laboratoire qui nécessitent le prélèvement d'une grande quantité d'eau (plusieurs dizaines de litres) (**Illustration S3**). De plus, la quantification des effets d'interaction requiert la multiplication (i) de la taille des échantillons, (ii) du nombre de combinaisons expérimentales, (iii) des mesures et des analyses et par conséquent de la quantité d'eau à prélever. Les plans d'expériences sont un outil statistique intéressant pouvant permettre, dans certains cas, une diminution du nombre d'essais et par conséquent un allègement du protocole expérimental pour les trois aspects évoqués plus haut (Droesbeke et al., 1997; Tinsson, 2010).

Illustration S3: Conditions d’accès et échantillonnage difficile en période hivernale en lac de haute altitude.

En effet, on montre que la qualité de l'information extraite d’un ensemble d’essais expérimentaux dépend du choix des expériences, c’est-à-dire de la position des points expérimentaux dans l'espace expérimental à explorer. Ces expériences doivent permettre de répondre à des questionnements et selon l'objectif de l'étude, le choix du plan d'expériences le mieux adapté s'impose. La construction de nos stratégies expérimentales nécessite donc un choix *a priori*, en amont de l’expérimentation, des effets qui seront supposés négligeables et donc des effets à quantifier et leurs interactions (Carlson & Carlson, 2009). Si le but recherché est une réduction de la taille de l’échantillonnage, de l’expérimentation et des analyses, tout en gardant une qualité d’information correcte, une sélection *a priori* des effets à quantifier et de leurs interactions est nécessaire. Il est donc assumé que les interactions supplémentaires qui ne répondent pas spécifiquement à la question posée ne seront pas analysées et que leurs effets sur la réponse biologique seront supposés négligeables.

En outre, la présence d’interactions multiples rend l’interprétation de la réponse biologique complexe et cette réponse peut être difficilement interprétable au-delà de trois facteurs (Scheiner & Gurevitch, 2001). L’expérimentation du chapitre 3 sera utilisée à titre d’exemple : les objectifs principaux de cette expérimentation étaient (i) de tester l’effet de l’augmentation du glucose sur l’abondance phytoplanctonique et (ii) d’analyser comment l’augmentation du glucose interagissait avec d'autres facteurs, tels que les nutriments, la lumière, ou la température, sur cette réponse. Dans le cas de cette étude, le prélèvement de l'eau sous la glace avec des moyens humains et matériels limités ne permettait pas la réalisation d'un design expérimental complet (matrice factorielle complète). Un design expérimental fractionné dans le cadre d'un plan d'expériences a donc été réalisé.

Dans le cadre d'un design expérimental fractionné (dans le cas d'étude, d'une matrice de Rechtschaffner), le choix *a priori* est fait d'analyser uniquement les interactions d'ordre 1 avec le glucose, soit dans l'exemple (i) l'interaction du glucose avec les nutriments, (ii), l'interaction du glucose avec la température, (iii) l'interaction du glucose avec la lumière. Les interactions supplémentaires (par exemple, l'interaction des nutriments avec la température) et les interactions d'ordre 2 et 3 sont supposées négligeables. Les expériences sont ensuite choisies et organisées de manière à obtenir la meilleure qualité d'information possible avec un nombre minimum de combinaisons expérimentales (Manzon et al., 2020). Dans l'exemple, cela implique la réalisation d'une expérience à 24 combinaisons en triplicats au lieu de 32 combinaisons dans le cas d'un design expérimental complet (**Figure S4**). La réponse mesurée peut ensuite être statistiquement analysée de façon classique, par exemple à l'aide de modèles linéaires en ne testant que les effets d'interactions souhaités. Dans cette thèse, l'approche par plans d'expériences a été utilisée pour l'expérimentation hivernale (chapitre 3).

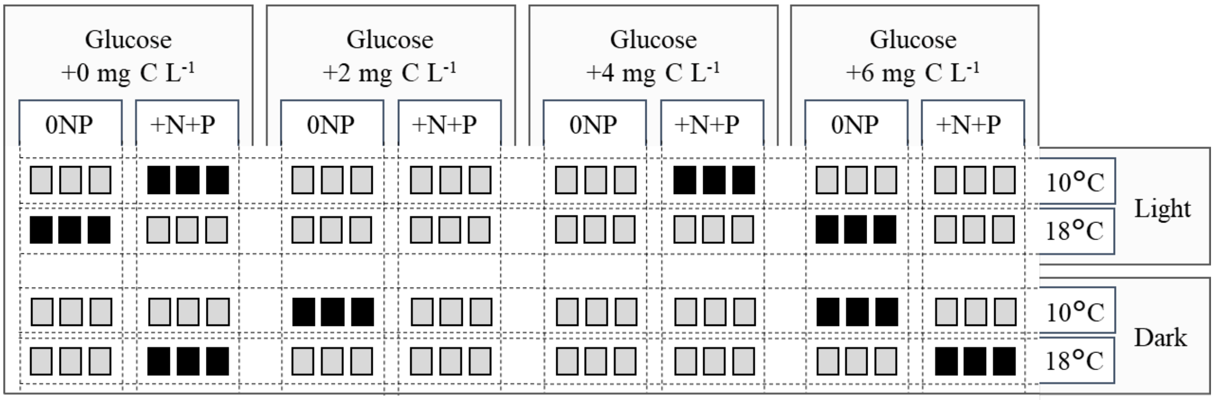

Figure S4 : Exemple d'un design expérimental permettant de tester l'effet de l'ajout de glucose en interaction avec les nutriments (0NP ou +N+P), la température (10°C ou 18°C), et la lumière (Light ou Dark) sur l'abondance phytoplanctonique. Le design complet nécessite 32 combinaisons réalisées en triplicat, soit l'échantillonnage de 33,6 L d'eau pour des microcosmes de 350 ml. Le design expérimental sélectionné par le plan d'expérience implique la réalisation de 24 combinaisons expérimentales (les combinaisons non réalisées sont colorées en noir), soit l'échantillonnage de 25,2 L d'eau pour des microcosmes de 350 ml.

Objectifs de la thèse

Les lacs de haute altitude sont des sentinelles des changements globaux (Moser et al., 2019). Leurs caractéristiques particulières les rendent vulnérables aux variations environnementales, notamment à l'augmentation de la matière organique dissoute (Ejarque et al., 2018). L'évaluation de cette vulnérabilité implique une meilleure compréhension de la dynamique de la matière organique et de son influence sur les communautés planctoniques. Le lien entre dynamiques phytoplanctoniques et matière organique est loin d'être totalement compris en altitude, en particulier à une échelle saisonnière entre la période hivernale et la phase de dégel. A l'échelle des communautés, l'effet de l'augmentation de la manière organique en tant que source de carbone organique et/ou de nutriments sur la structure des communautés phytoplanctoniques et sur les interactions entre bactéries et phytoplancton est encore difficilement prévisible.

L'objectif de cette thèse est d'apporter une meilleure compréhension de la sensibilité des communautés planctoniques (bactéries et phytoplancton) à l'augmentation de la matière organique dissoute, source de carbone et de nutriments, en lac de haute altitude. En particulier, les travaux de thèse visent à répondre à deux grandes questions :

(i) Comment les communautés planctoniques (bactéries et phytoplancton) répondent-elles aux variations saisonnières de la qualité et de la quantité de matière organique dissoute dans un lac de haute altitude ?

(ii) Comment l'origine de la matière organique, le contexte de limitation et la nature du peuplement phytoplanctonique initial peuvent-ils moduler la sensibilité des communautés planctoniques à l'augmentation d'une source de carbone organique et de nutriments en lac de haute altitude ?

Ces deux grands aspects ont été traités en quatre chapitres :

Le chapitre 2 a pour objectif de caractériser la dynamique saisonnière de la matière organique dissoute et des communautés phytoplanctoniques en lien avec les variations environnementales. Ce chapitre présente un suivi *in situ* réalisé sur le lac des Cordes entre février 2020 et juillet 2021 et met en relation les modifications fonctionnelles du phytoplancton avec la dynamique de la matière organique dissoute ainsi qu’avec un ensemble de variables biologiques et environnementales indicatrices du fonctionnement du lac.

Le chapitre 3 présente une expérimentation en conditions contrôlées dont l’objectif est de caractériser la réponse d’assemblages planctoniques (bactéries et phytoplancton) prélevés sous la glace à l’augmentation du carbone organique dissous (glucose) et des nutriments (N et P). Pour cette expérimentation, un design expérimental fractionné dans le cadre d’un plan d’expériences a été réalisé. (Expérimentation 1).

Le chapitre 4 présente une série de deux expérimentations en conditions contrôlées, dont l’objectif est d’appréhender comment la composition et la biomasse phytoplanctonique et le contexte de limitation des organismes conditionnent la réponse bactérienne et phytoplanctonique à une augmentation du carbone organique dissous (glucose) et des nutriments (N et P). (Expérimentation 2).

Le chapitre 5 présente une expérimentation en conditions contrôlées ayant pour objectif de mieux comprendre comment la qualité de la matière organique dissoute peut influencer l’activité bactérienne et la composition fonctionnelle des communautés phytoplanctoniques issues du lac des Cordes. (Expérimentation 3).

Enfin, ce manuscrit présente une discussion générale, présentant une synthèse des principaux résultats, une mise en relation des conclusions des différents chapitres et l’implication et les perspectives de recherches soulevées par le travail de thèse.

Discussion générale

L'effet de l'augmentation de la matière organique dissoute dans le cadre du changement global est un enjeu majeur pour le devenir des écosystèmes lacustres (Ejarque et al., 2018; Solomon et al., 2015). Les lacs de haute altitude constituent des sentinelles des changements globaux (Moser et al., 2019) et devraient être particulièrement impactés par l'augmentation des concentrations en matière organique dissoute. Leurs communautés planctoniques, en particulier les bactéries et le phytoplancton, se situent à la base du réseau trophique et jouent un rôle déterminant dans les voies de transfert du carbone et le fonctionnement métabolique de ces écosystèmes (Medina- Sánchez et al., 2004). La matière organique dissoute, par ses propriétés optiques et chimiques et parce qu'elle contient des sources de carbone et de nutriments, peut influencer différemment les taxa phytoplanctoniques et les bactéries hétérotrophes (Creed et al., 2018). L'objectif de cette thèse est d'apporter une meilleure compréhension de la sensibilité des communautés planctoniques à l'augmentation de la matière organique dissoute en lac de haute altitude. Pour répondre à cet objectif, deux grandes questions ont été posées et ont été traitées à travers quatre articles :

(iii) Comment les communautés planctoniques (bactéries et phytoplancton) répondent-elles aux variations saisonnières de la qualité et de la quantité de matière organique dissoute dans un lac de haute altitude ?

(iv) Comment l'origine de la matière organique, le contexte de limitation et la nature du peuplement phytoplanctonique initial peuvent-ils moduler la sensibilité des communautés planctoniques à l'augmentation d'une source de carbone organique et de nutriments en lac de haute altitude ?

La présente discussion met en relation les résultats des différents articles et vise à apporter des éléments nouveaux dans la compréhension de la réponse des communautés planctoniques aux variations de la matière organique dissoute. De ces différents éléments émergent également des questionnements et des pistes de réflexion pour de nouvelles perspectives d'études.

Dans un premier temps, nous discuterons du contexte de limitation par le carbone et les nutriments des organismes, puis nous aborderons la *sensibilité* des communautés planctoniques à la variation qualitative et quantitative de la matière organique dissoute à une échelle saisonnière. Enfin, nous discuterons de la *vulnérabilité* des lacs de haute altitude à l'augmentation de la matière organique dissoute.

1. Contexte de limitation

La notion de sensibilité peut être définie comme une réponse rapide et de grande ampleur des organismes aux variations des conditions environnementales (Adrian et al., 2009). Dans les milieux oligotrophes comme les lacs de haute altitude, la réponse des communautés aux variations des ressources nutritives, en particulier du carbone organique et des nutriments inorganiques, est fonction de leur contexte de limitation (Dorado-García et al., 2014).

Les lacs de haute altitude sont des environnements contraints d'un point de vue des ressources nutritives (Bergström et al., 2013; Lewis, 2011). Le suivi *in situ* du **chapitre 2** a permis une meilleure compréhension du fonctionnement saisonnier du lac des Cordes et de la dynamique des ressources. En période hivernale, le lac est caractérisé par une forte limitation par la lumière sous la glace, une température froide et une hypoxie au fond de la colonne d'eau. Ces conditions sont classiques sous glace en lac de haute altitude (Catalan et al., 2006). En fin de période hivernale, les concentrations en nutriments (N et P) sont relativement faibles, de même que les concentrations en COD (0,9 ± 0,3 $mg.L^{-1}$) et le pool de matière organique du lac est essentiellement dominé par de la matière organique dissoute d'origine sédimentaire et allochtone. La labilité de la matière organique diminue lorsqu'elle est isolée des apports extérieurs (Del Giorgio & Davis, 2003) ce qui suggère une nature réfractaire de la matière organique dans le lac des Cordes avant les apports allochtones liés à la fonte.

En début d'été, le lac est caractérisé par une augmentation de la disponibilité en lumière, de la température et des concentrations en oxygène sur toute la colonne d'eau. Ces modifications sont classiques pendant la période de mélange printanier en lac de haute altitude (Catalan et al., 2006). Le pool de matière organique du lac est essentiellement d'origine allochtone, lié au transfert depuis le bassin versant lors de la fonte du manteau neigeux. Les concentrations en COD restent faibles (0,5 ± 0,2 $mg.L^{-1}$), mais les concentrations en nutriments, en particulier en phosphore, sont plus élevées.

En fin d'été, le lac est caractérisé par une augmentation forte de la température de l'eau et une diminution des concentrations en nutriments, en particulier en azote inorganique dissous.

En revanche, les concentrations en carbone organique dissous augmentent fortement (1,9 ± 0,2 mg.L^{-1}) et la matière organique est principalement d'origine phytoplanctonique.

Dans les systèmes oligotrophes, les bactéries hétérotrophes peuvent être limitées par les nutriments, principalement le phosphore (Bertoni et al., 2008), par le carbone organique (Kritzberg et al., 2005), ou co-limitées par les nutriments et le carbone (Vidal et al., 2011). Les résultats expérimentaux des **chapitres 3 et 4** ont permis de mettre en évidence une co-limitation par le carbone et les nutriments des bactéries hétérotrophes quelle que soit la saison. Toutefois, en fin d'été, les bactéries sont davantage limitées par les nutriments inorganiques en lien avec une plus grande disponibilité du COD d'origine phytoplanctonique en conditions initiales.

Dans le suivi *in situ* du **chapitre 2**, cette dynamique de limitation s'est manifestée par de très faibles biomasses bactériennes sous la glace et par une augmentation progressive de cette biomasse pour atteindre des valeurs maximales en fin d'été. Des patterns de limitation des bactéries par les nutriments inorganiques (en particulier P) ont déjà pu être observés tout au long de l'été dans des lacs de haute altitude des Pyrénées (Zufiaurre, 2019). Notre étude confirme la co-limitation par le carbone et par les nutriments des bactéries hétérotrophes en lac de haute altitude (Dorado-García et al., 2014) et l'évolution saisonnière de cette limitation en lien avec l'augmentation de la matière organique issue des excrétions phytoplanctoniques en fin d'été (Villar-Argaiz et al., 2002).

Les résultats expérimentaux des **chapitres 3 et 4** ont mis en évidence une forte limitation par les nutriments inorganiques de la biomasse phytoplanctonique, variable selon les saisons. La biomasse phytoplanctonique était modérément limitée par les nutriments dans les communautés sous glace, faiblement limitée dans les communautés de début d'été, et très fortement limitée par les nutriments dans les communautés de fin d'été. Ces résultats confirment la limitation par les nutriments inorganiques de la croissance phytoplanctonique en lac de haute altitude (Bergström et al., 2013; Lewis, 2011) et l'évolution saisonnière de cette limitation, vers une limitation très prononcée en fin d'été (Jacquemin et al., 2018). Dans le suivi *in situ* du **chapitre 2**, cette dynamique de limitation s'est traduite par de faibles biomasses phytoplanctoniques mesurées sous la glace et en début d'été et une biomasse maximale en fin d'été. Comme cela a été montré dans de nombreuses études, le type de limitation de la croissance phytoplanctonique par les nutriments est étroitement lié aux concentrations en N et P dans la colonne d'eau (Leruste et al., 2019; Morris & Lewis, 1988).

Grâce aux résultats expérimentaux des **chapitres 3 et 4,** nous avons pu identifier une forte limitation par les nutriments inorganiques des bactéries hétérotrophes et du phytoplancton dans le lac des Cordes, tout au long des saisons étudiées. La limitation par les mêmes nutriments inorganiques des bactéries et du phytoplancton induit des mécanismes de compétition pour l'acquisition de ces nutriments (Carney et al., 2016). Nos résultats confirment une concurrence forte entre bactéries et phytoplancton pour les nutriments, comme cela a déjà été observé dans un lac de la Sierra Nevada espagnole (González-Olalla et al., 2018). En outre, nous montrons que cette concurrence a lieu tout au long des périodes étudiées sur le lac des Cordes. Dans le suivi *in situ* du **chapitre 2**, nous avons observé des concentrations en COD d'origine phytoplanctonique plus élevées en fin d'été dans le lac des Cordes. Dans l'expérimentation du **chapitre 4**, cela s'est traduit par une limitation par le carbone des bactéries hétérotrophes moins forte en fin d'été. Le commensalisme se produisant lorsque les bactéries utilisent le carbone organique excrété par le phytoplancton (Carrillo et al., 2015), nos résultats suggèrent un renforcement des interactions commensalistes entre bactéries et phytoplancton au cours de l'été sur le lac des Cordes.

2. Sensibilité des communautés planctoniques à la matière organique dissoute

2.1. Sensibilité des bactéries hétérotrophes

Dans les expérimentations des **chapitres 3 et 4**, des différences saisonnières de réponse des bactéries hétérotrophes à l'augmentation du COD et des nutriments en conditions contrôlées ont pu être observées. Dans les communautés sous glace, le COD est fortement consommé par les bactéries et l'augmentation du COD et des nutriments stimule la production de biomasse, bien qu'une régulation top-down par les ciliés et les mixotrophes régule rapidement la croissance bactérienne. Dans les communautés de début d'été, le COD est fortement consommé mais l'augmentation du COD et des nutriments stimule peu la production de biomasse. Dans les communautés de fin d'été, le COD est faiblement consommé mais l'augmentation du COD et des nutriments stimule fortement la production de biomasse des bactéries. Il a précédemment été montré que la matière organique dissoute autochtone est facilement et rapidement consommée par les bactéries hétérotrophes (Guillemette et al., 2013). Nos résultats confirment qu'une augmentation de la biomasse phytoplanctonique en fin d'été peut être un facteur diminuant la limitation des bactéries par le carbone et a conditionné une plus faible consommation du COD en conditions expérimentales dans des communautés de fin d'été.

La réponse rapide des bactéries à une source de carbone dans les lacs d'altitude est parfois accompagnée d'un remplacement d'espèces comme des taxa copiotrophes, c'est à dires des taxa adaptés à des environnements riches en nutriments (Rofner et al., 2017). En lac de haute altitude, une modification taxonomique des communautés bactériennes a précédemment été observée après des ajouts expérimentaux en matière organique dissoute (Pérez & Sommaruga, 2006b). L'augmentation de substrats organiques en conditions expérimentales a également entraîné des différences de structure, de production, de respiration et de rendement de croissance dans une communauté bactérienne lacustre (Kritzberg et al., 2006). Dans le lac des Cordes, les apports allochtones liés à la fonte en début d'été ont pu sélectionner des taxa bactériens possédant une stratégie nutritionnelle d'avantage portée sur la respiration que sur la croissance. Nous suggérons qu'une modification taxonomique ou fonctionnelle des bactéries entre les périodes a pu stimuler plus fortement la respiration avec l'augmentation en conditions contrôlées du COD et des nutriments dans des communautés de début d'été.

- Il serait intéressant de savoir si l'hétérogénéité saisonnière de la réponse bactérienne à l'augmentation du COD et des nutriments en conditions expérimentales est due à des modifications taxonomiques, fonctionnelles, ou physiologiques des communautés bactériennes ?

2.2. Sensibilité du phytoplancton

En lac de haute altitude, l'évolution des conditions environnementales induit une dynamique saisonnière de la biomasse et de la composition des communautés phytoplanctoniques (McKnight et al., 1990; Tiberti et al., 2013). Cette dynamique entraîne à son tour une variabilité de réponse des communautés à l'augmentation des nutriments (Jacquemin et al., 2018). Les résultats expérimentaux des **chapitres 3 et 4** ont mis en évidence une hétérogénéité saisonnière dans la réponse phytoplanctonique à l'augmentation en conditions contrôlées du COD et des nutriments. A la lumière, nous avons observé une tendance à la diminution de la biomasse phytoplanctonique avec l'augmentation combinée du COD et des nutriments dans des communautés sous glace par rapport à des ajouts de nutriments seuls. Au contraire, dans des communautés de début et fin d'été, l'augmentation combinée du COD ne modifie pas l'effet positif des nutriments (à 18°C) ou a tendance à accentuer l'effet positif des nutriments (à 10°C) sur la biomasse phytoplanctonique. Nos résultats confirment que l'augmentation des concentrations en COD pourrait modifier l'action positive des nutriments sur la biomasse phytoplanctonique, comme cela a déjà été montré dans des lacs boréaux (Deininger et al., 2017) et soulignent la variabilité de cet effet selon la température et les saisons.

Certains groupes phytoplanctoniques, comme les cryptophytes, ont présenté une réponse saisonnière marquée à l'augmentation expérimentale du COD et des nutriments en interaction avec la lumière. Pourtant, les assemblages spécifiques au sein de ce groupe étaient relativement identiques au cours des saisons. Dans les communautés sous glace et de début d'été, les cryptophytes étaient fortement favorisées par l'augmentation expérimentale du carbone organique et des nutriments à l'obscurité. Au contraire, dans les communautés de fin d'été, ces mêmes espèces n'étaient que très peu favorisées à l'obscurité avec du COD et des nutriments, et uniquement à 18°C. Des études précédentes ont mis en évidence que certaines espèces de cryptophytes étaient capables de se développer à l'obscurité uniquement grâce à l'ingestion de proies (McKie-Krisberg et al., 2015) et ce groupe est souvent rencontré dans des milieux sous glace où la lumière est limitante (Laybourn-Parry & Marshall, 2003; Marshall & Laybourn-Parry, 2002; Rue et al., 2020). L'histoire de vie des organismes et notamment l'intensité lumineuse, peut induire une variabilité saisonnière de la réponse planctonique aux changements environnementaux (Ji et al., 2010). Nos études confirment le potentiel de croissance des cryptophytes uniquement par phagotrophie en conditions limitantes par la lumière, mais démontrent également un changement de la physiologie et de la stratégie nutritive des taxa en fonction des conditions environnementales et de l'histoire de vie des organismes en lac d'altitude. Nous supposons que la faible disponibilité en lumière dans le milieu naturel sélectionne un comportement majoritairement mixotrophe en hiver et en début d'été et que la stratégie alimentaire des cryptophytes change lorsque les conditions de lumière redeviennent favorables à l'autotrophie.

Le suivi *in situ* du **chapitre 2** a mis en évidence que les cryptophytes sont particulièrement bien représentées dans les communautés naturelles en période hivernale et leur proportion diminue progressivement au cours de l'été. Ces résultats confortent l'idée que la mixotrophie est un avantage compétitif majeur pour les cryptophytes en condition de limitation par la lumière en lac de haute altitude, comme cela a précédemment été suggéré (Rue et al., 2020). Les cryptophytes semblent perdre leur avantage compétitif face aux grandes diatomées pennées en lien avec l'augmentation de la lumière en fin d'été dans la communauté naturelle. La présence de vacuoles chez les grandes diatomées a été montrée comme un trait fonctionnel compétitif face aux mixotrophes (Cadier et al., 2020) et a pu expliquer la dominance des grandes diatomées pennées par rapport aux cryptophytes en fin d'été dans le lac des Cordes. Les diatomées de grande taille développent des stratégies efficaces d'acquisition des nutriments lorsqu'ils sont rares (Endo et al., 2018; Litchman et al., 2009, 2010) et cette stratégie (stratégie K) leur permet de dominer en fin de succession estivale en lac oligotrophe (Bürgi et al., 2003). En outre, les cellules de grande taille sont plus résistantes à la prédation (Litchman et al., 2010; Salmaso et al., 2015) et la forte abondance des ciliés observée en fin d'été dans le lac des Cordes a pu être un facteur de régulation du phytoplancton mixotrophe (Haraguchi et al., 2018).

Les expérimentations des **chapitres 3 et 4** ont montré une favorisation des grandes diatomées pennées dans des communautés de début d'été avec l'augmentation en conditions contrôlées du COD et des nutriments. La favorisation de diatomées pennées a déjà été observée après des ajouts expérimentaux de matière organique dissoute en lac de haute altitude (Kissman et al., 2013) et cette réponse est expliquée par l'effet positif des nutriments associés à l'augmentation de matière organique. Nos résultats confirment le caractère autotrophe et le fort potentiel compétitif des diatomées en réponse à l'augmentation des nutriments (Garrido et al., 2016). En outre, des études précédentes ont démontré que certaines diatomées étaient capables d'assimiler des composés organiques (Tuchman et al., 2006) et de concurrencer les bactéries hétérotrophes pour l'assimilation des nutriments lorsqu'elles n'étaient pas limitées par d'autres ressources, comme la silice (Havskum et al., 2003). En lac de haute altitude, la période de début d'été est une période peu contrainte du point de vue de la lumière et des nutriments par rapport à la période sous glace ou la fin de la saison estivale (Jacquemin et al., 2018; Rue et al., 2020). Ces résultats soulignent que le potentiel compétitif des diatomées varie de façon saisonnière avec l'augmentation du COD. Nous suggérons que la diminution des contraintes naturelles par la lumière ou les nutriments en début d'été a pu favoriser les diatomées autotrophes au détriment des autres groupes phytoplanctoniques après l'augmentation en conditions contrôlées du COD et des nutriments.

Les expérimentations des **chapitres 3 et 4** ont mis en évidence que les chrysophytes sont essentiellement favorisées par l'augmentation expérimentale du COD et des nutriments lorsque la lumière est non limitante. Ces chrysophytes sont majoritairement représentées par le genre *Dinobryon* sp. qui a été reporté dans la littérature comme étant phototrophe obligatoire, c'est-à-dire ayant besoin d'une source de lumière suffisamment disponible pour réaliser la mixotrophie (Rottberger et al., 2013). Une exception a toutefois pu être observée dans les communautés sous glace à 18°C, où des chrysophytes appartenant au genre *Ochromonas* sp. se sont développées à l'obscurité après des ajouts de COD. Ce taxa, dont les capacités phagotrophes ont été observées à l'obscurité dans des études antérieures (Lie et al., 2017), était peu présent dans les communautés initiales des expérimentations de début d'été et de fin d'été. Les travaux de thèse confortent l'idée que les chrysophytes du genre *Dinobryon* sp. sont phototrophes obligatoires quelle que soit la saison et qu'une variabilité de la réponse phytoplanctonique à l'augmentation du COD peut être observée en fonction de la composition initiale de la communauté et des stratégies nutritives des organismes.

Le suivi *in situ* du **chapitre 2** a permis de mettre en évidence une forte représentativité des chrysophytes dans les communautés de début d'été, ce qui souligne l'importance de la disponibilité en lumière dans la composition taxonomique et fonctionnelle des communautés phytoplanctoniques en lac de haute altitude. Comme pour les cryptophytes, les chrysophytes ont perdu leur avantage compétitif face aux diatomées autotrophes dans la communauté naturelle de fin d'été.

Nos résultats mettent en évidence une variabilité des stratégies nutritionnelles et des traits fonctionnels en fonction des groupes phytoplanctoniques mais aussi des conditions environnementales. En lien avec les différences de réponse observées dans communautés planctoniques, des modifications des relations entre bactéries et phytoplancton sont attendues avec les variations des concentrations en COD et en nutriments.

2.3. Interactions entre bactéries et phytoplancton

Les résultats expérimentaux des **chapitres 3 et 4** ont permis de mieux comprendre dans quelles mesures l'augmentation de la concentration en COD et en nutriments pouvait modifier les interactions existantes entre bactéries et phytoplancton.

Nous avons constaté une augmentation du ratio entre organismes mixotrophes et autotrophes dans les communautés issues de la période sous glace et de fin d'été avec l'augmentation du COD et des nutriments, mais une diminution du ratio dans la communauté de début d'été. Selon Modenutti (2014), la mixotrophie est une stratégie avantageuse en conditions de limitation par la lumière ou en conditions de limitation par les nutriments. En lac de haute altitude, la limitation du phytoplancton par la lumière est traditionnellement observée en période hivernale (Catalan et al., 2006), tandis que la limitation par les nutriments se renforce au cours de la saison de croissance pour devenir maximale en fin d'été (Jacquemin et al., 2018). Nous suggérons que l'augmentation du carbone organique et des nutriments en conditions expérimentales favorise l'expression de la mixotrophie dans des assemblages phytoplanctoniques issues des périodes sous glace et de fin d'été, en lien avec la limitation initiale de ces assemblages par la lumière ou les nutriments (**Figure S5**). Les résultats expérimentaux du **chapitre 4** corroborent cette hypothèse. En effet, l'apport de carbone organique augmente la proportion d'organismes mixotrophes dans des assemblages phytoplanctoniques de début d'été mais uniquement dans des conditions expérimentales limitées par la lumière ou par les nutriments. Ainsi, la composition taxonomique et la stratégie nutritionnelle des assemblages mixotrophes, elles-mêmes déterminées par le contexte environnemental, ont été des éléments déterminants dans l'expression saisonnière de la mixotrophie après des apports en COD et en nutriments.

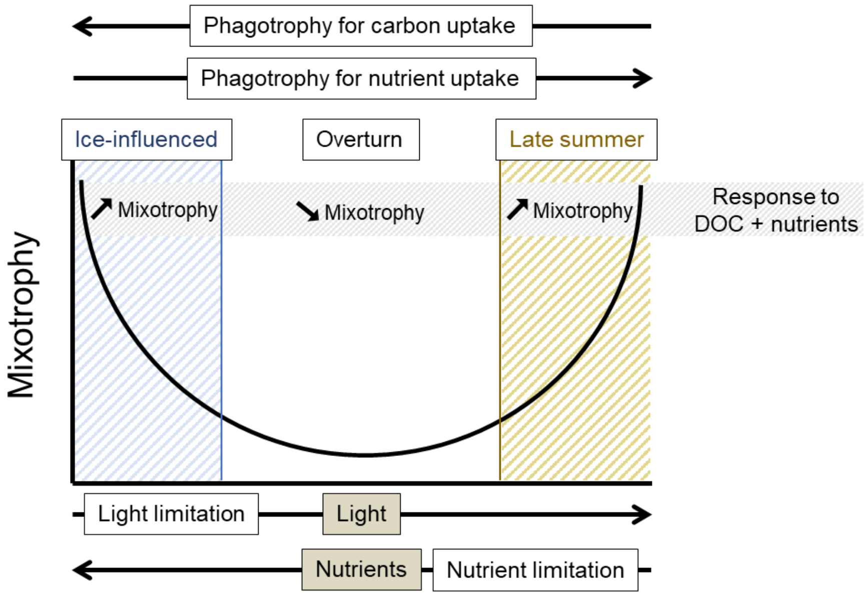

Figure S5 : Replacement des différentes périodes de fonctionnement du lac des Cordes et des résultats expérimentaux observés à chaque période dans le modèle général des facteurs d'influence de la mixotrophie développé par Modenutti (2014).

Le suivi *in situ* du **chapitre 2** n'a pas mis en évidence d'augmentation de la mixotrophie en fin d'été en lien avec l'augmentation des concentrations en COD dans la colonne d'eau. En fin d'été, lorsque les concentrations en COD sont maximales dans la colonne d'eau, l'autotrophie est favorisée avec un avantage compétitif très fort des grandes diatomées pennées. Toutefois, la nature de la matière organique pourrait expliquer la différence de réponse observée entre les résultats expérimentaux et *in situ*.

En effet, dans les expérimentations du **chapitre 5**, nous avons mis en évidence une diminution très forte de la proportion du phytoplancton mixotrophe dans des communautés de début d'été après des enrichissements expérimentaux en matière organique dissoute autochtone (lysat d'algues) par rapport à des enrichissements en matière organique allochtone (extraits de sol). Ces modifications phytoplanctoniques sont étroitement liées à l'utilisation par les bactéries de la matière organique, plus fortement consommée lorsqu'il s'agit du lysat d'algues mais plus fortement utilisée pour la production de biomasse lorsqu'il s'agit de l'extrait de sol. Ces résultats confirment une consommation plus efficace de la matière organique dissoute autochtone par les bactéries en lien avec la qualité et la labilité de la matière organique (Guillemette et al., 2013), mais une plus forte production de biomasse bactérienne avec de la matière organique dissoute allochtone (Guillemette et al., 2016). Nous suggérons que la nature autochtone de la matière organique dissoute en fin d'été dans le lac des Cordes a pu favoriser le développement des autotrophes grâce à (i) leur fort potentiel compétitif face aux mixotrophes pour l'acquisition des nutriments et (ii) leur stratégie d'évitement des prédateurs (grande taille) lorsque les ciliés sont plus abondants en fin d'été.

Les résultats de cette thèse ont mis en évidence une forte sensibilité des bactéries et du phytoplancton à l'augmentation du carbone organique dissous et des nutriments, ainsi qu'à la variation qualitative et quantitative de la matière organique dissoute. Ces résultats ont permis d'identifier des modalités de réponse différentes des bactéries et du phytoplancton en fonction de la saison, de la nature du peuplement phytoplanctonique et de la qualité de la matière organique dissoute. Un certain nombre de questions mériteraient d'être approfondies à l'avenir :

- Les communautés phytoplanctoniques sont le reflet des conditions environnementales contrastées et fluctuantes en lacs de haute altitude (Kuefner et al., 2021). La théorie métabolique de Wilken et al. (2013) prédit une augmentation de l'hétérotrophie chez les taxa mixotrophes avec l'augmentation de la température. Chez certains mixotrophes, la mixotrophie effective ne s'exprime que lorsque les proies et les conditions lumineuses atteignent des quantités suffisantes dans l'environnement (Livanou et al., 2020). Comment le mode de nutrition d'un même organisme pourrait' il évoluer en fonction de son contexte environnemental ? La réponse des mixotrophes aux apports inorganiques et organiques pourra t'elle varier sous différentes contraintes environnementales liées aux changements globaux ?

- L’augmentation des taxa mixotrophes ne se traduit pas toujours par une augmentation proportionnelle de l’ingestion des proies (Gerea et al., 2016). Comment varient les taux d’ingestions des proies (mixotrophie effective) en lien avec l’augmentation de la matière organique dissoute ?

- La quantification du carbone organique excrété par le phytoplancton et de la proportion de ce carbone utilisé par les bactéries permettrait d’augmenter notre compréhension des processus impliqués dans le transfert du carbone en réponse à l’augmentation de la matière organique dissoute en lac d’altitude (Durán et al., 2016).

Les résultats expérimentaux et *in situ* des différents chapitres ont souligné l’importance du contrôle de prédation exercé par les ciliés dans la réponse bactérienne et phytoplanctonique. La complexité de la dynamique et de la structure du réseau trophique peut créer des voies alternatives de flux d'énergie jusqu'au niveau trophique le plus élevé (Hulot et al., 2014; Vadeboncoeur et al., 2005). L’augmentation du carbone organique dissous peut augmenter ou diminuer l’efficacité du transfert d’énergie dans le réseau trophique selon la présence de zooplancton ou de poissons (Degerman et al., 2018). La plupart des lacs de haute altitude des Alpes du Sud sont soumis à l’introduction de poissons, ce qui affecte directement la structure des assemblages zooplanctoniques (Cavalli et al., 2001; Tiberti et al., 2014). Dans des lacs périalpins, le zooplancton a été pointé comme un bon indicateur du fonctionnement des lacs (Jacquet et al., 2014).

- Il serait intéressant d’intégrer l’ensemble du réseau trophique dans les études futures pour savoir comment le contrôle top-down par les ciliés, le zooplancton et les poissons régule la réponse des bactéries et du phytoplancton à l’augmentation de la MOD.

3. Vulnérabilité du lac des Cordes

La vulnérabilité d’un écosystème aux changements globaux fait référence au niveau de menace de cet écosystème et est le résultat du degré d’exposition aux variations climatiques et anthropiques, de la sensibilité des organismes et de la capacité adaptative de l’écosystème (Kling et al., 2020). Les lacs de haute altitude sons soumis à des filtres du paysage (position géographique, caractéristiques des bassins versants, morphologie des lacs) et à des filtres internes (interactions abiotiques et biotiques) qui conditionnent les modalités de réponse de l'écosystème aux variations environnementales (Blenckner, 2005).

Le lac des Cordes est localisé dans les Alpes du Sud, dans une zone géographique faiblement exposée aux dépôts atmosphériques d'azote et de phosphore (Fagerli et al., 2015). En ce sens, il est peu exposé à une augmentation des concentrations en nutriments inorganiques en lien avec les retombées atmosphériques. Sur ce lac, une forte limitation de la croissance phytoplanctonique par les nutriments inorganiques a été montré en période estivale, qui s'accentue au cours de la saison de croissance avec l'épuisement des ressources (Jacquemin et al., 2018). En lien avec la forte limitation des organismes, l'augmentation des concentrations en nutriments devrait entraîner une réponse plus importante des communautés planctoniques sur ce lac (O'Donnell et al., 2017). Le lac des Cordes est caractérisé par un bassin versant de grande superficie (170 ha), majoritairement recouvert de pelouse alpine. La superficie des bassins versants et leur végétalisation conditionnent la quantité de matière transférée vers les lacs (Rose et al., 2015). La quantité de matière organique allochtone transférée vers les lacs devrait être plus importante dans les lacs à grands bassins versants végétalisés. Les principales contributions des résultats de la thèse à l'évaluation de la sensibilité des communautés planctoniques et la vulnérabilité du lac des Cordes à l'augmentation de la matière organique sont synthétisées dans la **Figure S6**.

Dans le cadre du changement global, les lacs de haute altitude des Alpes du Sud devraient connaître des changements environnementaux majeurs (Gobiet et al., 2014; Moser et al., 2019). Des évènements pluvieux extrêmes plus intenses et plus fréquents devraient induire une augmentation des apports ponctuels de la matière organique dissoute au sein des lacs (Perga et al., 2018), bien que des effets de dilution pourraient également entraîner une réduction de la quantité de nutriments organiques et inorganiques (Anderson & Stedmon, 2007). L'augmentation de la température entraînera une augmentation de la végétalisation des bassins versants en altitude et de leur productivité (Elliott & Kipfmueller, 2011) donc de la quantité de matière organique allochtone transférée des bassins versants vers les lacs (Ejarque et al., 2018). Un allongement de la phase de dégel estivale entraînera une augmentation de la fenêtre temporelle de lumière non limitante et d'augmentation de la température (De Senerpont Domis et al., 2013). Des périodes hivernales plus courtes devraient augmenter la productivité primaire des lacs donc la quantité de matière organique autochtone (Oleksy et al., 2020b; Roberts et al., 2017) et augmenter la proportion de diatomées dans les communautés phytoplanctoniques (Rühland et al., 2008; Rühland et al., 2015). Toutefois, une plus faible couverture neigeuse en hiver en lien avec l'augmentation de la température pourrait également diminuer la quantité des apports allochtones transférés au lac au moment de la fonte.

Dans le cadre des changements globaux, l'augmentation de la concentration en matière organique dans le lac des Cordes devrait augmenter la croissance et/ou la respiration des bactéries hétérotrophes (**Figure S6**). L'augmentation de la biomasse bactérienne pourrait augmenter la probabilité de développement du phytoplancton mixotrophe et des ciliés hétérotrophes dans les communautés de fin de période hivernale et de fin d'été. Une meilleure disponibilité en nutriments inorganiques associée aux apports de matière organique dissoute devrait induire une augmentation de la biomasse phytoplanctonique. En début d'été, une augmentation de la matière organique dissoute autochtone devrait entrainer une diminution importante du phytoplancton mixotrophe dans les communautés phytoplanctoniques. L'augmentation de la matière organique dissoute allochtone devrait induire un déclin plus léger des taxa mixotrophes dans les communautés phytoplanctoniques de début d'été. Les effets de l'augmentation de la matière organique devraient interagir avec l'augmentation des températures de l'eau et de la disponibilité en lumière et induire une complexification de la réponse des communautés planctoniques et des interactions entre phytoplancton et bactérioplancton sur le lac des Cordes. Toutefois, si elle est provoquée par des évènements de tempêtes, l'augmentation des concentrations en matière organique devrait être associée à une diminution de la température de l'eau (Doubek et al., 2021), ce qui pourrait atténuer l'effet d'interaction de l'augmentation la température avec l'augmentation des concentrations en MOD.

Les modifications attendues des communautés bactériennes et phytoplanctoniques devraient altérer le fonctionnement global du lac des Cordes et ses services écosystémiques. Si l'augmentation des concentrations en nutriments inorganiques associés à la matière organique dissoute dépasse les besoins nutritionnels à la fois des bactéries et du phytoplancton, l'augmentation de la biomasse phytoplanctonique pourrait conduire à des phénomènes d'eutrophisation du lac, comme cela a déjà été observé dans certains lacs des Alpes du Sud en lien avec le pastoralisme ou l'activité touristique (Jacquemin, 2019). Une diminution du ratio entre phytoplancton mixotrophe et autotrophe en début d'été devrait augmenter le nombre de niveaux trophiques entre la boucle microbienne et les niveaux trophiques supérieurs (zooplancton, poissons). En conséquence, une diminution de l'efficacité de transfert du carbone au sein de la chaine trophique est attendue (González-Olalla et al., 2018; Medina-Sánchez et al., 2004). Ces conséquences ne devraient pas être observées en fin de période hivernale ou en fin d’été. La dynamique saisonnière de la matière organique dissoute devrait déséquilibrer la balance entre autotrophie et hétérotrophie et donc le métabolisme global du lac à une échelle saisonnière.

4. Vulnérabilité des lacs de haute altitude

L’augmentation de la concentration en matière organique dissoute en lacs de haute altitude devrait entraîner (i) une modification taxonomique et fonctionnelle des assemblages phytoplanctoniques et (ii) une modification des interactions avec les bactéries hétérotrophes avec des effets variables à l’échelle saisonnière et avec la nature de la matière organique. Toutefois, on s’attend à des différences de vulnérabilité des lacs aux variations de la matière organique dissoute dans des contextes différents d’exposition aux dépôts atmosphériques et de caractéristiques des bassins versants.

Dans des lacs soumis à des dépôts atmosphériques élevés de N et P, les bactéries hétérotrophes devraient être principalement limitées par le carbone organique. On s’attend donc à des phénomènes de compétition moins importants entre bactéries et phytoplancton pour les nutriments inorganiques après des apports de COD, ce qui pourrait induire un découplage entre la réponse bactérienne et phytoplanctonique (Nelson & Carlson, 2012) et une diminution des interactions entre phytoplancton et bactéries.

Dans la mesure où les caractéristiques des bassins versants déterminent la proportion de mixotrophes et la composition des communautés phytoplanctoniques (Jacquemin et al., 2019; Tolotti et al., 2003, 2006), une superficie et une végétalisation plus faible des bassins versants pourrait être un facteur de diminution du contrôle top-down de prédation par les mixotrophes sur les bactéries hétérotrophes après des apports de matière organique. Il se pourrait que le contrôle top-down sur les bactéries soit principalement effectué par les ciliés et les hétérotrophes nanoflagellés dans ce type de lacs. En fonction des caractéristiques des lacs et des bassins versants, les communautés phytoplanctoniques de fin de d’été peuvent être dominées par des assemblages phytoplanctoniques contrastés taxonomiquement et fonctionnellement en lacs de haute altitude (McKnight et al., 1990; Tiberti et al., 2013), comme des mixotrophes ou des chlorophytes. Les bactéries hétérotrophes pourraient être plus impactées par l’augmentation de la compétition pour les nutriments inorganiques si les assemblages phytoplanctoniques sont dominés par des chlorophytes qui pourraient avoir des potentiels compétitifs plus forts que les diatomées (Oleksy et al., 2020a).

Il serait intéressant de comprendre plus précisément l'influence des caractéristiques des bassins versants et des contextes d'expositions aux dépôts atmosphériques d'azote et de phosphore sur l'évolution des interactions entre bactéries et phytoplancton en lien avec l'augmentation de la matière organique dissoute.

L'ampleur des modifications des réseaux trophiques planctoniques induite par les variations de la matière organique dissoute en lacs de haute altitude sera amenée à varier en fonction (i) du contexte d'exposition des lacs aux dépôts atmosphériques d'azote et de phosphore (Camarero & Catalan, 2012; Jacquemin et al., 2019); (ii) de la morphologie des lacs et des caractéristiques de leurs bassins versants (Blenckner, 2005; Jacquemin et al., 2019); (iii) des interactions biotiques des bactéries et du phytoplancton avec d'autres compartiments trophiques, comme le compartiment benthique, les macrophytes, le zooplancton prédateur, ou les poissons (Degerman et al., 2018; Kissman et al., 2017); (vi) des interactions de la matière organique avec d'autres facteurs environnementaux, comme la température, la lumière, la stratification, ou la durée de la période estivale (Cabrerizo et al., 2017; Carrillo et al., 2015; Durán et al., 2016).

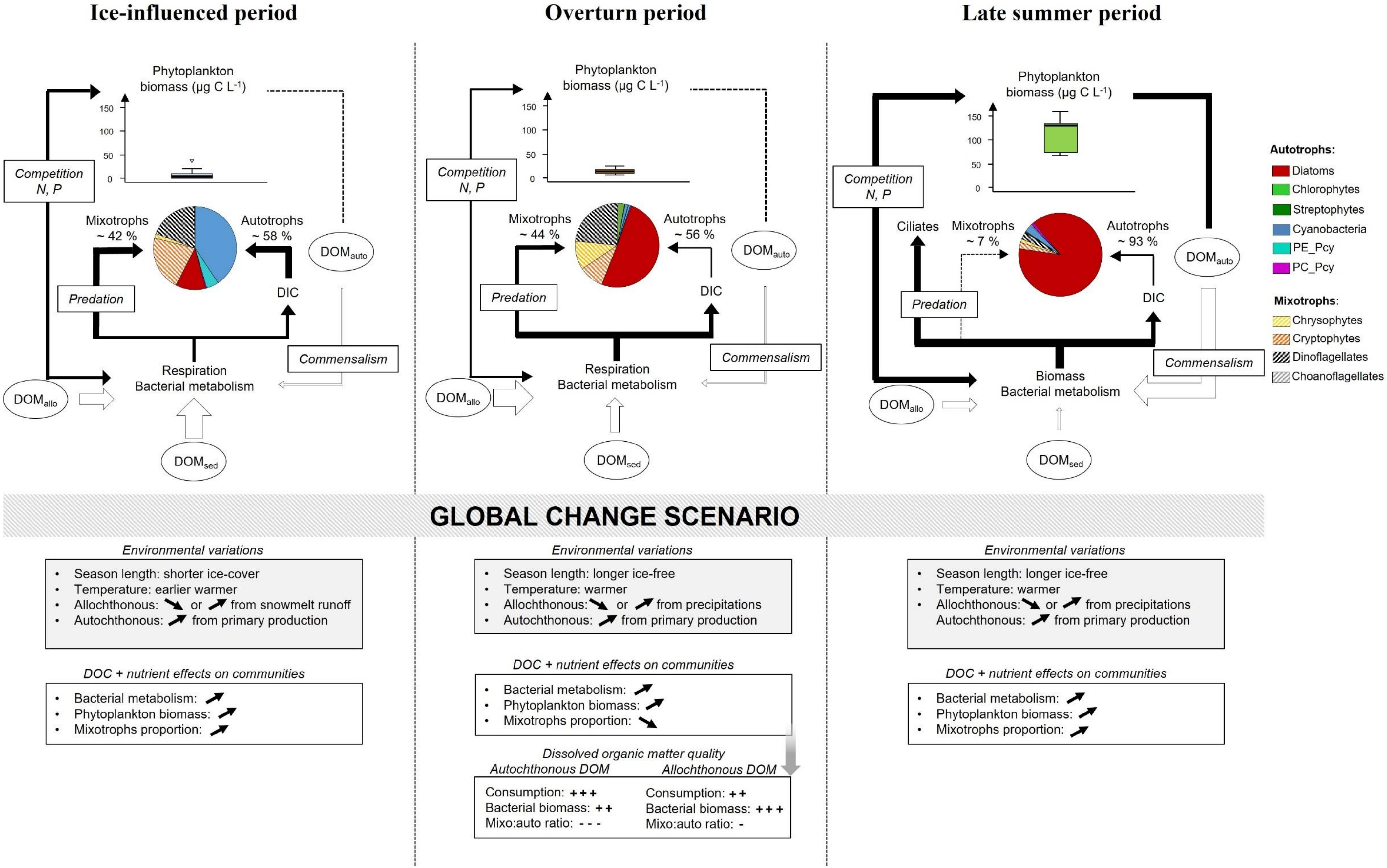

Figure S6 : Schéma synthétique des relations entre matière organique dissoute et communautés planctoniques observées sur le lac des Cordes au cours de la dynamique saisonnière, et de la sensibilité de ces communautés et de leurs interactions à l’augmentation de la matière organique dissoute dans un scénario de changement global.

Conclusions et perspectives

Les principales conclusions de cette thèse sont les suivantes :

- L'origine et la concentration en matière organique dissoute est très dynamique à l'échelle annuelle, et cette variabilité peut être reliée à la structure de la communauté phytoplanctonique. La matière organique dissoute agit en interaction avec d'autres facteurs environnementaux comme la lumière, la température et les nutriments et détermine la variation saisonnière des interactions entre bactéries et phytoplancton.

- La qualité de la matière organique influence la réponse bactérienne et phytoplanctonique à l'augmentation des concentrations en MOD. La matière organique dissoute autochtone est plus utilisée par les bactéries et la proportion de mixotrophes diminue drastiquement en présence de lumière. La matière organique dissoute allochtone est moins utilisée par les bactéries mais stimule plus la production de biomasse, et la proportion de mixotrophes ne diminue que légèrement dans la communauté phytoplanctonique en présence de lumière.

- Sur le lac des Cordes, les conditions environnementales influencent la nature du peuplement phytoplanctonique qui elle-même détermine la réponse des bactéries et du phytoplancton aux variations qualitatives et quantitative de la matière organique dissoute. La réponse observée est à la fois le résultat de la réponse intrinsèque des organismes et des conditions environnementales fluctuantes. Nos travaux de thèse soulignent l'importance d'intégrer la matière organique dissoute dans l'évaluation de la vulnérabilité des lacs de haute altitude. Cette évaluation de la matière organique doit être associée au contexte environnemental qui agit comme un modulateur de la réponse des organismes.

Les résultats de cette thèse font émerger de nouvelles perspectives d'études :

- Au regard des résultats de thèse, la quantification des interactions entre bactéries et phytoplancton est intéressante pour approfondir l'hypothèse que l'augmentation de la matière organique dissoute en lac de haute altitude devrait altérer les voies de transfert du carbone vers les niveaux trophiques supérieurs. Cette quantification des interactions implique des mesures du taux d'ingestion des bactéries par les taxa mixotrophes (bactérivorie), de la quantité de carbone organique excrété par le phytoplancton, et de la proportion de ce carbone utilisée par les bactéries hétérotrophes.

- Pour améliorer notre compréhension des processus d'influence des interactions entre bactéries et phytoplancton, il serait intéressant de savoir dans quelle mesure les modifications taxonomiques et fonctionnelles des bactéries peuvent à leur tour influencer la réponse phytoplanctonique à l'augmentation de la matière organique.

- L'élargissement des résultats obtenus sur le lac des Cordes est indispensable pour une évaluation précise de la vulnérabilité des lacs de haute altitude à l'augmentation de la matière organique dissoute. Il pourrait être intéressant de caractériser la dynamique saisonnière de la matière organique en lien avec les communautés planctoniques dans des lacs soumis à différents contextes d'exposition aux dépôts atmosphériques et caractéristiques de bassins versant, donc des contextes de limitation différents. L'évaluation de la réponse de communautés phytoplanctoniques taxonomiquement et fonctionnellement très contrastées permettrait de mieux comprendre le rôle de la communauté phytoplanctonique dans la réponse des organismes à la matière organique dissoute.

Chapter 1

General introduction

Global changes affect the physical, chemical, and biological properties of lake ecosystems (Adrian et al., 2009). Variations in dissolved organic matter are expected in relation to global changes and may alter the lake metabolism and their role in the global carbon cycle (Perga et al., 2018; Rofner et al., 2017).

Dissolved organic matter (DOM) plays a fundamental role in freshwater ecosystems functioning (Creed et al., 2018; Prairie, 2008; Williamson et al., 1999). Through its action at the base of the food web, dissolved organic matter regulates the pathways of carbon transfer to higher trophic levels and therefore the carbon balance of lakes (Jansson et al., 2000). An increase of dissolved organic matter concentrations has been observed in many aquatic ecosystems in the northern hemisphere and anthropogenic factors have been identified as the main cause of this increase (Monteith et al., 2007; Solomon et al., 2015). For example, warming influences the terrestrial productivity of watersheds, thus increases the quantity of terrestrial dissolved organic matter potentially transferred to the lakes (Larsen et al., 2011). The increase of extreme rainfall events affects hydrological transport, connectivity between catchments and lakes, and accentuates the transfer of dissolved organic matter to the water column (Wu et al., 2011). Autochthonous dissolved organic matter concentrations will also increase due to the expected eutrophication of water (Zhou et al., 2018). There is a crucial need to better understand the influence of dissolved organic matter on aquatic ecosystems regarding the forecasted global changes.

Dissolved organic matter refers to a set of compounds gathering lipids, carbohydrates, proteins inferior to 0,7 µm (Aitkenhead-Peterson et al., 2003). The dissolved organic matter pool of the lakes may derived from catchments terrestrial inputs, named allochthonous DOM, and from benthic, macrophytes and phytoplankton primary production, named autochthonous DOM (Bertilsson & Jones, 2003; Canham et al., 2004). Dissolved organic matter origin induces differences in optical properties and chemical composition that in turn induce various effects on communities. For example, it has traditionally been assumed that bacteria preferentially remove autochthonous dissolved organic matter than allochthonous DOM, owing to the presence of more simple and lower molecular weight carbon compounds (McKnight & Aiken, 1998). Depending on the DOM pool origin, bacterial utilization of organic matter should differ.

Thus, it has been demonstrated that bacterial communities selectively allocate autochthonous substrate to respiration, whereas terrestrial carbon is preferentially allocated to biosynthesis (Guillemette et al., 2016). However, we do not know how these strategies of bacterial resource utilization influence the phytoplankton community structure and composition.

Dissolved organic matter contains dissolved organic carbon (DOC) and nutrients, thus may act as a source of energy and/or nutrients for bacterioplankton and phytoplankton (Creed et al., 2018).

Mixotrophy refers to a nutritional strategy combining autotrophy and heterotrophy in a single organism (Flynn et al., 2019). In phytoplankton taxa, autotrophic nutritional mode corresponds to the acquisition of atmospheric CO_2 by photosynthesis. Heterotrophy corresponds to the acquisition of organic carbon in the water column, by osmotrophy and/or phagotrophy (prey ingestion). Osmotrophy, the ability to use dissolved organic compounds such as amino acids and vitamins, is common in phytoplankton taxa (Droop, 2007; Flynn et al., 2019; Våge et al., 2013). Because of the ubiquity of osmotrophy, mixotrophy often refers to organisms that “express, or have potential to express, phototrophy and phagotrophy” (Flynn et al., 2019). The word “potential” is important, because while some species can combine both autotrophic and heterotrophic metabolism, the real expression and the degree of heterotrophy of organism in natural conditions vary according to specific environmental limitations like light, nutrients, prey availability, or a combination of these (Bergström et al., 2003; Saad et al., 2016).

The response of bacteria and phytoplankton to dissolved organic matter variations should depend on dissolved organic matter chemical composition, elemental stoichiometry and on nutritional requirements of organisms. Depending on its action as a source of carbon and/or nutrient, increased DOM could enhance or attenuate commensalistic, competitive, or predation interactions in planktonic communities (Castillo et al., 2010; Flynn et al., 2008; Livanou et al., 2020).

Commensalism refers to the use by bacteria of organic carbon excreted by phytoplankton and is often associated to phytoplankton production increase (Carrillo et al., 2015). Competition refers to the limitation by the same nutrients of bacterial and phytoplankton growth (Carney et al., 2016). Predation refers to the ingestion of bacteria by mixotrophs to counteract limitation by light or nutrient (Thingstad et al., 1996).

When DOC increases in excess, nutrients (mainly nitrogen and phosphorus) become the major limiting factors for bacterial growth (Almeida et al., 2005; Pinhassi et al., 2006), leading to direct competition with phytoplankton for these nutrients. Bacteria have an inherent advantage over phytoplankton to acquire inorganic nutrients (Currie & Kalff, 1984; Jansson et al., 2006). Increase of dissolved organic carbon concentrations therefore generally results in a stimulation of bacterial metabolism and a decrease of primary phytoplankton production in aquatic ecosystems (Hitchcock et al., 2010; Joint et al., 2002; Westhorpe et al., 2010). Changes in the taxonomic and functional composition of phytoplankton communities are also often observed, with an increase of groups owing mixotrophic capacities, such as cyanobacteria (Maranger & Pullin, 2003) or phytoflagellates (Bergström et al., 2003). For example, studies report an increase of the abundance of mixotrophs in phytoplankton community after increase of a dissolved organic carbon source, related to an increase of bacterial biomass and of competition with bacteria for nutrients (Carney et al., 2016; Creed et al., 2018; Jansson et al., 2006).

Because dissolved organic matter also contains nutrients, increased DOM concentrations can also lead to increased nitrogen and phosphorus availability in the water column (Engel et al., 2018). If these concentrations exceed the biological demands of the organisms, an increase in phytoplankton production can also be observed in response to increasing organic matter (Hitchcock & Mitrovic, 2013; Tanentzap et al., 2017). The effects of dissolved organic matter increase are therefore complex and can strongly modify the biotic interactions between bacteria and phytoplankton.

The effects of dissolved organic matter depend on the nutritional requirements of organisms, and therefore the resource availability in the natural water column (Pete et al., 2010). For example, in oligotrophic ecosystems characterized by low nutrient concentrations, nutrient limitation of bacteria has been shown to limit their consumption of allochthonous DOC (Nelson & Carlson, 2011) and sucrose (Dorado-García et al., 2014) in experimental conditions. However, the influence of resource limitation pattern of organisms on their response to DOM is traditionally studied on bacteria and the transfer of these results to the phytoplankton is lacking. There is a crucial need to better understand the limitation patterns of organisms and the effect of dissolved organic matter as a source of carbon and/or nutrients on the interactions between bacteria and phytoplankton in lake ecosystems.

High-altitude lakes are oligotrophic systems and have been identified as sentinels of global changes (Moser et al., 2019; Williamson et al., 2009). Indeed, they are remote and difficult to reach, thus mainly affected by global impacts such as climate change or atmospheric deposition (Psenner, 2003). The characteristics of high-altitude lakes make them vulnerable to dissolved organic matter variation (Moser et al., 2019; Schindler, 2009). This vulnerability is related to their high exposition and their high sensitivity to environmental changes. For example, these lakes are exposed to increase of allochthonous dissolved organic matter from catchments in relation to the upward shift of treeline and the higher frequency and intensity of extreme rainfall events forecasted with global change (Ejarque et al., 2018; IPCC, 2021).

The extreme and fluctuating environmental conditions (extremely low and extremely high light, low temperature, low nutrient concentrations, **Illustration 1.1**) select a restricted pool of species capable to live in these harsh environments. Thus, food webs are more simple than in lower-elevation lakes and organisms are highly sensitive to environmental variations, even small (Catalan et al., 2006). In addition, environmental constraints in high-altitude lakes select a set of functional traits within phytoplankton communities (Caroni et al., 2012; Jacquemin et al., 2019). These functional traits determine the ecological performance of taxa: their ability to grow, to exploit light and nutrient resources, to maintain in the water and to resist to loss processes such as predation or sedimentation (Hulot et al., 2014; Reynolds, 1984; Salmaso & Padisák, 2007). For example, small size, motility, resting stages, or mixotrophy, are known to be advantageous strategies for phytoplankton taxa in high-altitude lakes (Medina-Sánchez et al., 2004; Trevisan et al., 2014). Functional modification of phytoplankton communities thus constitutes a relevant indicator of environmental variations in high-altitude lakes.

Illustration 1.1. Contrasted environmental conditions between ice-covered (left) and ice-free season (right) in high-altitude lakes.

At higher elevation, catchment size, vegetation cover, and thus allochthonous inputs can be very low. Dissolved organic carbon concentrations and nutrients are traditionally low, and dissolved organic matter exhibits a dominant autochthonous signature (Bastidas Navarro et al., 2014; Rose et al., 2015). However, highly contrasted seasonality induces a dynamic of dissolved organic matter (Olson et al., 2021) and a dynamic of limitation pattern by nutrient and carbon of organisms. For example, a large quantity of allochthonous dissolved organic matter can be transferred to lakes during snowmelt (Catalan, 1992; Olson et al., 2021; Sadro et al., 2018) or during extreme precipitation events (**Illustration 1.2)** (Perga et al., 2018; Sadro & Melack, 2012).

Seasonal variations in light and nutrients induce a seasonal dynamic of phytoplankton biomass and community composition in high-altitude lakes. For example, phytoplankton communities dominated by mixotrophs are classically observed during ice-covered winter in relation to light limitation (Rue et al., 2020). During the summer season, inorganic nutrient limitation drives the structure and composition of phytoplankton communities (Jacquemin et al., 2018; McKnight et al., 1990). Regarding phytoplankton biomass, a bimodal pattern is often observed, with a first peak in phytoplankton biomass immediately after snowmelt and a second peak at the end of the summer period (Tiberti et al., 2013).

Illustration 1.2. Contrasted water color in a same lake between season: in June after snowmelt (left); in July after an extreme rainfall event (center); in August (right).

A previous work highlighted the importance of the dual control exerted by phytoplankton over bacteria in high altitude lakes: (i) top-down, through predation by mixotrophs and (ii) bottom-up, through the production of autochthonous dissolved organic matter (Medina-Sánchez et al., 2004). The phytoplankton biomass and the community composition, especially through the proportion of mixotrophs, are important factors regulating the bacterial response to carbon and nutrient increase and therefore the interactions between bacteria and phytoplankton. However, the link between dissolved organic matter dynamic and planktonic communities is mainly studied over short periods and mainly during summer period. We need to better understand how plankton communities evolve in relation to the quality and quantity of dissolved organic matter at a seasonal scale to assess both the sensitivity of communities and vulnerability of high-altitude lakes to global changes.

This thesis aims to provide a better understanding of the sensitivity of planktonic organisms (bacteria and phytoplankton) to increase of dissolved organic matter, source of carbon and nutrients, in high-altitude lakes. The thesis provides new fundamental knowledge on the role of dissolved organic matter in the regulation of food webs in high-altitude lakes, but also on the general functioning of these lakes at a seasonal scale. Ultimately, this work should give perspectives to better understand the vulnerability of high-altitude lakes to the potential effects of global change.

After a background of existing literature, we will describe the implementation and objectives of the thesis. The objectives of the thesis will be addressed through four articles presented in the chapters 2, 3, 4 and 5. A general discussion linking the results of the articles and opening new perspectives will end this manuscript.

Background

1. Dissolved organic matter as regulator of lake ecosystems

The primary component of dissolved organic matter (DOM) is dissolved organic carbon (DOC) (McCaul et al., 2011) that acts as a major regulator of lake ecosystems (Williamson et al., 1999). The importance of DOC in lakes has long been recognized. The first studies date back to 1921 when the term "dystrophic" was used to refer to high DOC lakes (Rodhe, 1969). However, the potential role of DOC in lakes was rapidly eclipsed by the focus on the recently discovered chlorophyll-phosphorus paradigm. The interest of DOC importance has re-emerged from studies of northern humic lakes with high DOC concentrations (> 10 mg L^{-1}) (Salonen et al., 1992), and from the Wetzel's limnology study (Wetzel, 1983). These pioneer studies already recognized the central role of DOC in many ecosystem processes, particularly in regulating microbial growth (Cole et al., 1982).

At the end of the 20th century, the review of Williamson et al. (1999) acted as a turning point by proposing a model that includes DOC as well as nutrients to better understand the structure, function, and response to disturbance of lake ecosystems. This review not only highlighted the DOC importance in many ecosystem processes, but also underlined the need to incorporate DOC into studies to better understand how lakes will respond to stressors. More recently, Prairie (2008) extended this paradigm and defined DOC as "a great modulator, the variable that modify the influence of other variables". In this work, DOC has been discussed through its role (i) at the landscape level, especially with DOC inputs into lakes from watersheds, and (ii) at the microbial level, acting in the whole lake metabolism and global carbon budget.

From that, studying DOC at global and microbial scales became a major issue in the understanding of lake functioning. Because DOC contained in dissolved organic matter is associated to various molecules including micro- and macronutrients, more recent studies seek to understand the differential influence of DOC and nutrients (Degerman et al., 2018; McGuirk

Flynn, 2008) to better assess the role of DOM in freshwater systems (Creed et al., 2018). The effect of DOM in the lake structure and function received increased attention in recent years, because strong modifications of DOM are expected as a consequence of the current global change (Solomon et al., 2015).

1.1. Origin and transformation of dissolved organic matter

Dissolved organic carbon originates from a complex mixture of lipids, carbohydrates, proteins, and other biochemical components produced by various organisms that have live in and around the lake (Moser et al., 2019). DOC concentrations can range from values < 0.5 mg L^{-1} in seas and oligotrophic clear-water lakes, to 30 mg L^{-1} in colored lakes and up to 60 mg L^{-1} in swamps, marshes and bogs (Tulonen, 2004).

Dissolved organic carbon is a component of dissolved organic matter which includes carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, and other heteroatoms within its molecular structures. Dissolved compounds are defined as the fraction that passes through a pore size filter of less than 0.7 µm, whereas larger compounds are particulates compounds. Thus, dissolved organic matter comprises a continuum of small organic molecules (molecular weight about 100 Da) to highly polymeric humic substances (molecular weight about 100 000 Da) (Aitkenhead-Peterson et al., 2003). DOM pool in freshwater ecosystems derives from a wide variety of sources. These sources may include internally produced (autochthonous) and externally produced (allochthonous) DOM (**Figure 1.1**).

Autochthonous DOM essentially derives from algae (phytoplankton and periphyton) and macrophytes. Autochthonous DOM release from algae can occur upon cell death, directly released to the surrounding waters as dissolved compounds or as particulate detritus. Autochthonous DOM can also result from herbivores grazing, viral lysis, or from actively growing cells (Bertilsson & Jones, 2003). Phytoplankton cells are composed of biopolymers, mostly proteins, polymeric sugar, and lipids, whereas a major fraction of the total cellular biomass (10-35%) consists of monomeric compounds of low molecular weight (< 600 Da).

As lakes drain their surrounding landscape, they also receive allochthonous DOM inputs from their catchments. Allochthonous DOM typically derives from atmospheric compounds, throughfall, leaf and root litter, and metabolites of soil microorganisms (Aitkenhead-Peterson et al., 2003). Allochthonous DOM is exported to aquatic systems with hydrological processes through surface water or groundwater, and export may vary according to terrestrial net primary production, catchment size, or slopes (Canham et al., 2004). It is traditionally assumed that allochthonous DOM is more recalcitrant than autochthonous DOM, because labile compounds are degraded before the DOM reaches the pelagic zone (Tranvik, 1992). Thus, allochthonous DOM is largely composed of humic substances, defined as relatively high molecular weight, yellow- to black-colored substances formed by secondary synthesis reactions. Most of these humic compounds are fulvic acids that result from microbial degradation of plant and animal remains (Aitkenhead-Peterson et al., 2003).

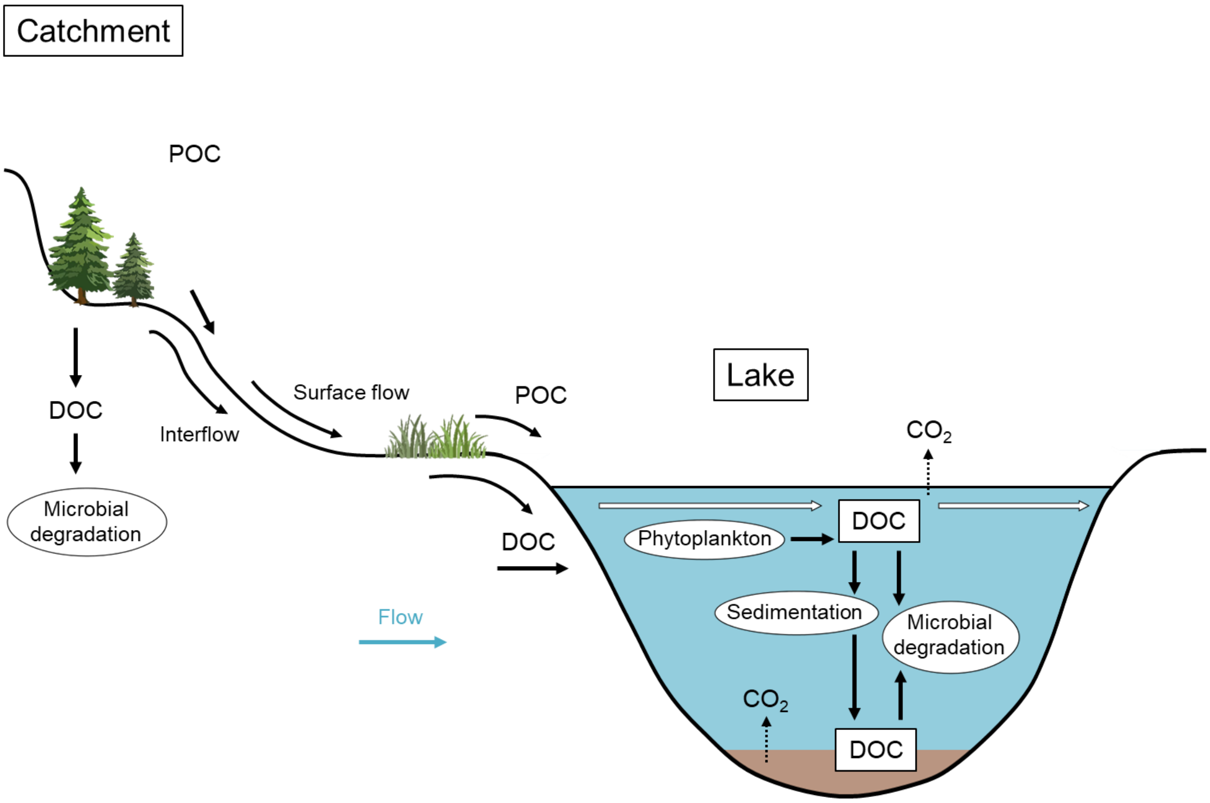

Figure 1.1. Schematic presentation of organic carbon (dissolved and particulate) sources according to their origin. Autochthonous dissolved organic carbon mainly results from phytoplankton, whilst allochthonous dissolved organic carbon mainly derives from the surrounding catchment. Modified from Morling et al. (2017) and Tulonen (2004). DOC: dissolved organic carbon; POC: particulate organic carbon.

Differences in the DOM origin therefore significantly determines the DOM composition in freshwater environments. Moreover, both DOM amount and composition also vary spatially and temporally due to its exposure to environmental and biological processing (Hansen et al., 2016). The two major processes affecting DOM content and composition in aquatic systems are **photodegradation** and **biodegradation**.

- **Photodegradation** is related to chromophoric dissolved organic matter, the fraction of DOM that absorbs ultraviolet and visible light (Helms et al., 2008; Kirk, 1994). Photodegradation results in the action of solar radiations inducing a modification of the average molecular weight of DOM, a modification of the light-absorbing capabilities through "bleaching", and a formation of a range of photoproducts. These photoproducts can be inorganic compounds, that represent direct photochemical mineralization of carbon, or organic compounds (Moran & Covert, 2003).

- **Biodegradation** mainly results in the action of heterotrophic bacteria to convert organic matter either into biomass through bacterial production or into CO_2 as bacterial respiration (Del Giorgio & Davis, 2003). Both in the water column and in the sediments, bacteria are the main biological organisms involved in the degradation and mineralization of DOM, that supports their energy and carbon demands. Assimilated organic substrates are needed to generate energy in the form of ATP (adenosine triphosphate), involved in essential cell processes like enzymatic biosynthesis or growth. DOM can be used by heterotrophic bacteria to produce either essential organic molecules through biosynthesis of new bacterial biomass, or inorganic molecules through respiration (Fuhrman, 1992). The degradation of organic matter is largely dependent on the metabolic capability of bacteria to synthesize the required enzyme and transport systems (Münster et al., 1992). Production of extracellular enzymes is essential to cleave the DOM into smaller molecules, especially those that are too large to be readily assimilable, like polymeric compounds (Tulonen, 2004).

Both biodegradation and photodegradation alter DOM from larger molecules to labile, lower molecular weights compounds (Moran et al., 2000; Moran & Zepp, 1997; Wetzel et al., 1995). However, biodegradation can also lead to the production of high molecular weight aromatic material (through alteration of existing compounds and/or production of new compounds by heterotrophs) (Guillemette & del Giorgio, 2012; Jiao et al., 2010). Photodegradation can also transform labile compounds into higher refractory material, when the ratio bacterial activity:DOC concentration is high (Obernosterer et al., 1999). The molecular weight, the nutrient contents, and the relative contribution of compounds of the DOM pool determine its utilization in the trophic food web and influence its role in the aquatic ecosystem.

1.2. The roles of dissolved organic matter in lake functioning

Dissolved organic matter affects the structure and function of aquatic ecosystems *via* complex, interacting, direct and indirect effects. DOM have two direct major effects that arise from its two major properties, resulting in various indirect effects:

(i) First, the colored or chromophoric fraction of the DOM pool absorbs solar radiations in the water column, changing the vertical distribution of light and heat (Fee et al., 1996; Kirk, 1994).

(ii) Second, DOM constitutes a source of carbon and energy to microbial food webs and supports catabolic and anabolic metabolism (Del Giorgio & Davis, 2003).

1.2.1. Light attenuation

The absorption of solar radiation by chromophoric DOM is primarily related to the allochthonous fraction of the DOM pool, as terrestrial DOM contains a large proportion of colored humic substances. However, phytoplankton may also contribute significantly to the UV attenuation either as particles or as a source of chromophoric DOM (Laurion et al., 2000).

Light absorption by chromophoric DOM directly alters water transparency and decreases the depth of UV light penetration in the water column (Williamson et al., 1996) (**Figure 1.2**). Biologically, light attenuation directly limits light availability to primary producers and alters interactions between visual predators and their prey (Solomon et al., 2015). Physically, the extinction of light nearest to the surface structures the vertical distribution of water temperature and leads to shallower surface mixed layers. Consequently, lakes with higher DOM concentrations are generally colder overall, thermal stratification occurs closer to the surface and tends to be more stable (Read & Rose, 2013). Stratification reduces the amount of vertical mixing, decreasing oxygen concentration in the hypolimnion and altering nutrient loading from sediment and nutrient vertical gradient in the water column (Wilhelm & Adrian, 2008). By modifying temperature, oxygen, and nutrient concentrations, DOM indirectly impacts metabolic rates of organisms, the growth, and the composition of planktonic communities.

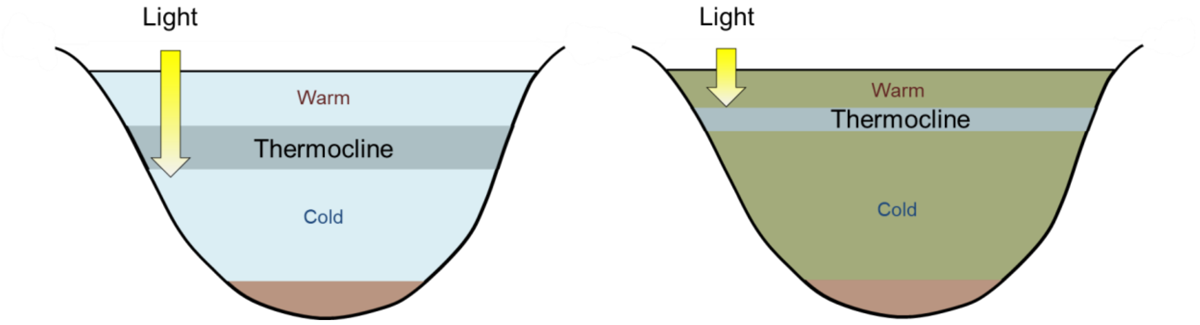

Figure 1.2. Light attenuation occurs deepest in water column in lakes with low concentrations of chromophoric dissolved organic matter (left) than in lakes with high concentrations of chromophoric dissolved organic matter (right). From Solomon et al. (2015).

1.2.2. Supporting planktonic food web

Heterotrophic bacteria are primarily responsible for the bulk degradation of organic matter. The low-molecular weight fraction of the DOM pool can be rapidly consumed, whereas the higher-molecular weight fraction can be slowly consumed if residence times are sufficient (Aitkenhead-Peterson et al., 2003). Because organic matter also contains nutrients, short-term degradation of DOM by bacteria is highly implicated in the cycling and bioavailability of nitrogen and phosphorus (Del Giorgio & Davis, 2003).

The organic carbon of the DOM pool can follow two major pathways: firstly, bacteria may allocate substrates for growth, incorporate them into cellular structure, making them available for higher consumers like zooplankton and fishes (anabolism) (Grey et al., 2001; Guillemette et al., 2016). Secondly, organic carbon may be respirated into CO_2 and thus contributes to the dissolved inorganic carbon (DIC) pool of the ecosystem (catabolism). This DIC produced by mineralization can then be loss *via* direct emission to the atmosphere, incorporated into the food web *via* consumption by benthic and pelagic primary producers, or transformed *via* carbonate precipitation (Engel et al., 2018) (**Figure 1.3**).

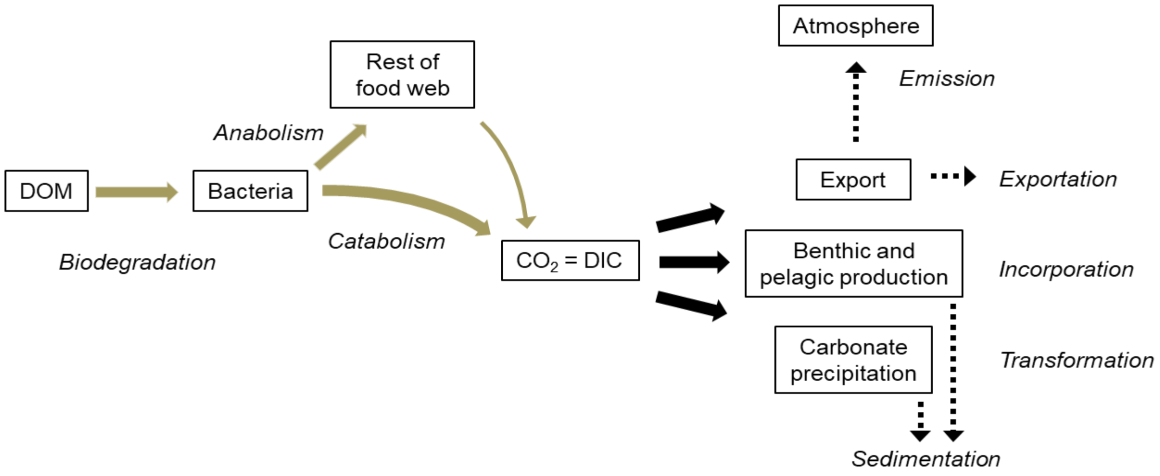

Figure 1.3. Schematic representation of carbon pathways via the biodegradation of dissolved organic matter by bacteria. Modified from Solomon et al. (2015) and Engel et al. (2018).

The quantity and the origin of the DOM in lakes is a key factor determining the microbial interactions between bacterioplankton and phytoplankton. In freshwater ecosystems receiving little allochthonous DOM inputs, most of the DOM is phytoplankton-derived DOM (**Figure 1.4**). Bacterioplankton is usually considered to be dependent on organic carbon supplied by phytoplankton in such ecosystems. The energy mobilization at the base of the food web is mostly based on phytoplankton photosynthesis, the CO_2 fixation exceeds the respiration, and ecosystems act as a net C sink (Jansson et al., 2000).

In ecosystems receiving high quantities of allochthonous DOM (about > 10 mg L^{-1}), the stimulation of bacterioplankton production by allochthonous dissolved organic carbon results in bacteria being relieved of their dependence on autochthonous carbon (Jansson, 1998). Consequently, bacteria become limited by inorganic nutrients rather than by organic carbon, resulting in an increase competition for nutrients between bacterioplankton and phytoplankton (Jansson et al., 2001). As bacteria are efficient competitors for inorganic nutrients (Currie & Kalff, 1984), high-allochthonous inputs ecosystems have high bacteria:phytoplankton biomass ratios compared with oligotrophic clearwater lakes (Tranvik, 1992). The energy mobilized by bacteria is transferred to higher trophic levels mainly via heterotrophic and mixotrophic flagellates (Jansson et al., 2000). In such systems, whole-lake respiration exceeds CO_2 fixation, and lakes act as net source of CO_2 (Cole et al., 1994).

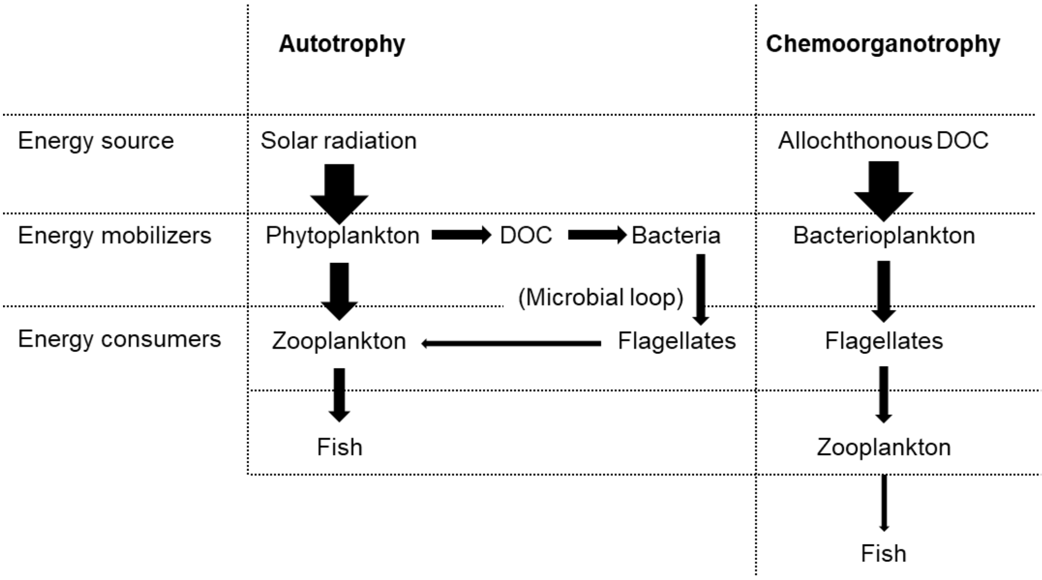

Figure 1.4. Schematic illustration of the pelagic energy flux in autotrophic and chemoorganotrophic aquatic systems. Natural lakes are mixtures of these different pathways. Autotrophy (system production depends on photosynthesis) dominates in clearwater lakes and eutrophic lakes. Chemoorganotrophy (system production depends on energy mobilization from organic compounds) may dominate in lakes with high concentrations of colored organic substances and low primary productivity. From Jansson et al. (2000).

Increase of DOM, however, not always results in a decrease of phytoplankton production and in higher bacterial biomass/phytoplankton biomass ratios. Firstly, because DOM contains nutrients, nitrogen and phosphorus can be provided in such surplus supply, exceeding the demand for both communities and allowing high growth for both (Hitchcock & Mitrovic, 2013; Kissman et al., 2017). Secondly, the phytoplankton community may not be outcompeted for nutrients. Whilst many studies have shown bacteria can outcompete phytoplankton for available nutrients, some species, in particular diatoms that have a high surface area to volume ratio, may in fact be able to outcompete bacteria under certain conditions (Havskum et al., 2003; Hitchcock & Mitrovic, 2013). Thirdly, mutualism between phytoplankton and bacterioplankton can occur. In this case, the DOM-induced stimulation of bacterial activity may lead to mineralization of organic nitrogen into inorganic forms that supports increase of primary production (Kirchman, 2000; Wheeler & Kirchman, 1986). This produced autochthonous DOC may in turn support increased bacterial production.

1.3. Dissolved organic matter and global changes

The influence of human activities is detected by global warming of the atmosphere and the ocean, modification of global water cycle, changes in concentration of atmospheric anthropogenic compounds, and modification of extreme climate events (IPCC, 2021). Global surface temperature has increased of +0.2°C per decade in the past 30 years (Hansen et al., 2006), and mean temperatures will continue to rise over the 21st century if greenhouse gas emissions continue unabated. In the long term, global precipitation will increase with increased global mean surface temperature, however changes in average precipitation will exhibit substantial spatial variation (IPCC, 2021).

The dissolved organic matter in freshwater ecosystems have changed substantially in recent decades and will likely continue to change (Williams et al., 2016a). Both autochthonous and allochthonous DOM are expected to increase with the current global change (Creed et al., 2018). Various mechanisms related to climate change, atmospheric deposition, and hydrology have been pointed as contributors to observed changes in DOM (Solomon et al., 2015).

1.3.1. Changes in allochthonous DOM

Allochthonous DOM concentration is directly related to the lake's connectivity with its surrounding catchment. Increased temperature may increase the terrestrial net primary productivity and vegetation, leading to more productive catchment (Solomon et al., 2015). Moreover, warming directly affects the solubility of soil organic matter by increasing extracellular enzyme activity (Freeman et al., 2001). Hydrological processes influence the hydrologic transport of DOM to surface waters. For example, increased mean precipitations may induce higher gross primary productivity of terrestrial vegetation, and increased the organic matter soil pool (Wu et al., 2011). Increased runoff associated to extreme precipitation events directly enhances the transport of terrestrial DOM through catchment (Hongve et al., 2004; Schindler et al., 1997). Therefore, a global increase of dissolved organic matter in surface waters is expected with the forecast climate change, even if drought events have been shown to decrease the allochthonous DOM transport through catchment (Schindler et al., 1997).

1.3.2. Changes in autochthonous DOM

Autochthonous DOM concentration is directly related to variation in primary production. Increase of nutrient inputs from watershed, from atmospheric deposition of nitrogen and phosphorus (Tranvik et al., 2009), and from sediments through warmer waters (Wilhelm & Adrian, 2008), may increase the autochthonous production in lakes, decreasing the CO_2 emission to the atmosphere (Jansson et al., 2008). In addition, water temperature directly affects phytoplankton physiology and metabolic rates. The basic metabolic processes of a phytoplankton cell, such as photosynthesis, respiration, growth, and nutrient uptakes, increase with increasing temperatures (Zohary et al., 2021). Thus, by direct effect on phytoplankton metabolic activity, and by lengthening the duration of optimal growth period for phytoplankton, warmer climate may increase the primary production and thus the autochthonous DOM concentration in lakes.

As climate and atmospheric modification vary at a regional scale, the impacts of change in dissolved organic matter quantity and quality are diverse, difficult to predict, and will depend on the magnitude of those change. Identifying vulnerable ecosystems and better understand the role of organic matter in their functioning is an important challenge for their protection.

High-altitude lakes: sentinels and vulnerable

2.1. High-altitude lakes are sensitive

Lakes are sentinels of climate change (Williamson, Saros, Vincent, et al., 2009), because they provide signals that reflect the influence of climate change in their much broader landscape (Williamson, Saros, & Schindler, 2009). The responses of lakes as sentinels include changes in physical, chemical, and biological characteristics (Adrian et al., 2009; Schindler, 2009; Williamson, Saros, Vincent, et al., 2009) (**Figure 1.5**).

High-altitude lake are generally small (area <5 ha), shallow (Z_{max} < 15 m), and oligotrophic systems (Catalan et al., 2013) situated above the tree line (between 2000 and 3000 m a.s.l. in the Alps) (Sommaruga, 2001). Most of the high-altitude lakes in the Alpine region are of glacial origin, resulting of the last glaciers retreated (~10,000 years ago) (Jacobsen & Dangles, 2017). High-altitude lakes are among the ecosystems with larger similarities throughout the planet (Catalan et al., 2006). Because they are often remote, they are relatively distant from direct anthropic disturbance and nutrient sources.

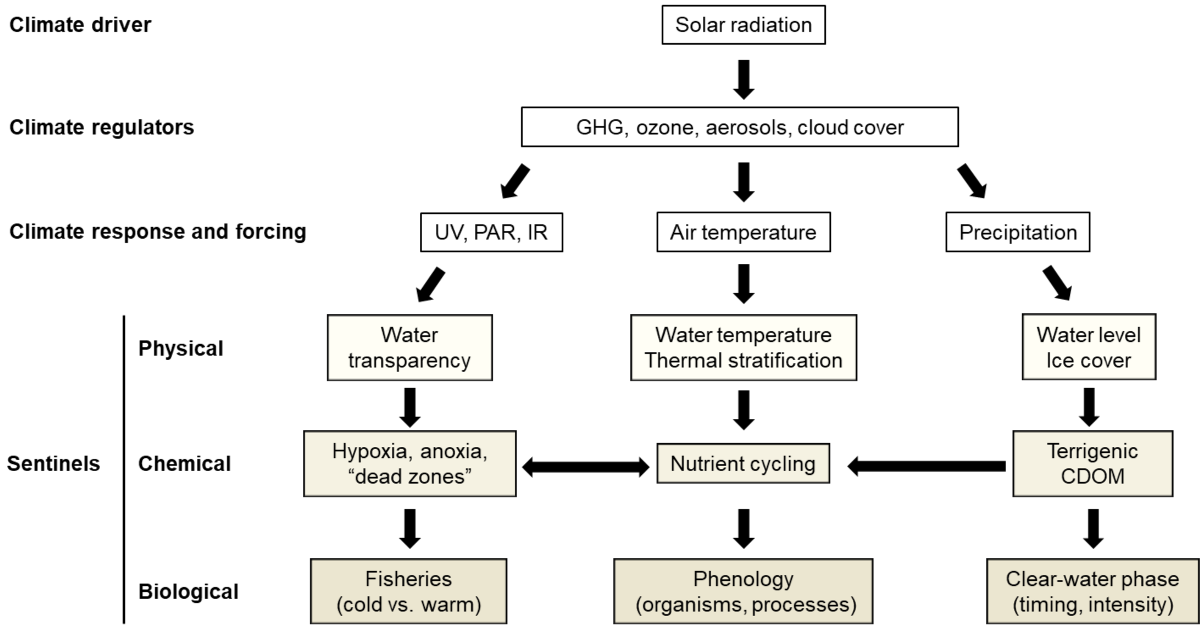

Figure 1.5. Flow diagram of lakes as sentinels of climate change showing major climate regulators, climate response and forcing, and a few of the many physical, chemical, and biological sentinels that can be quantified in lakes as they respond to climate change. UV: ultraviolet radiations; PAR: photosynthetically active radiations; IR: infrared; CDOM: chromophoric dissolved organic matter. From Williamson et al. (2009).

However, regardless of their location and remoteness, high-altitude lakes are situated in extreme environments, characterized by cold temperatures, high and low solar radiations, and prolonged ice and snow cover. These characteristics, in turn, affect mountain lake ecosystem structure, diversity, and productivity (Catalan et al., 2006; Hobbs et al., 2010; Wolfe et al., 2003) (**Figure 1.6**). Extreme characteristics induce the presence of highly adapted organisms to low food availability, low temperature, short growing season (3 – 5 months), low pH, and extreme change in light conditions. High-altitude lakes are thus traditionally seen as species-poor ecosystems with low functional redundancy (Vinebrooke & Leavitt, 2005). Both extreme conditions and simple food web induce a rapid and immediate response of organisms to environmental variation, even small. In this sense, high-altitude lakes are particularly *sensitive*[3] to environmental changes and are good sentinels of global changes.

In high-altitude lakes, irradiance fluctuates between extremely low and extremely high values. When lakes freeze, 1% of the subsurface irradiance may reach a depth of 10 m (Catalan et al., 2006). During the ice-cover, the accumulation of snow reduces underwater irradiance below compensatory levels for phytoplankton. During the ice-free season, light penetration into the water column is very high, and organisms may be exposed to high ultraviolet radiations because of its natural increase with elevation (Sommaruga, 2001).

High-altitude lakes are nutrient poor and are also characterized by a large elemental imbalance stoichiometric in regard to the ideal proportion for life. According to the total phosphorus concentrations, high-altitude lakes are mostly ultra-oligotrophic ($TP < 4.7\ \mu g.L^{-1}$), or oligotrophic ($4.7 < TP < 9.3\ \mu g.L^{-1}$), but certain can also be mesotrophic ($TP < 31\ \mu g.L^{-1}$) (Catalan et al., 2006). Because of usually low nutrient concentrations, loading from catchments are fundamental in determining the lake chemistry, and water chemistry in high-altitude lakes is thus highly related to catchment lithological composition and soil characteristics (Camarero et al., 2009; Mosello et al., 2002). For example, it has been found a positive correlation between the percentage of the catchment covered by soil and dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and total phosphorus (TP) (Catalan et al., 2006).

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[3] The *sensitivity* of lakes to climate change can be defined as the potentiality of physical, chemical, and biological lake properties to respond rapidly to climate-related changes (Adrian et al., 2009).

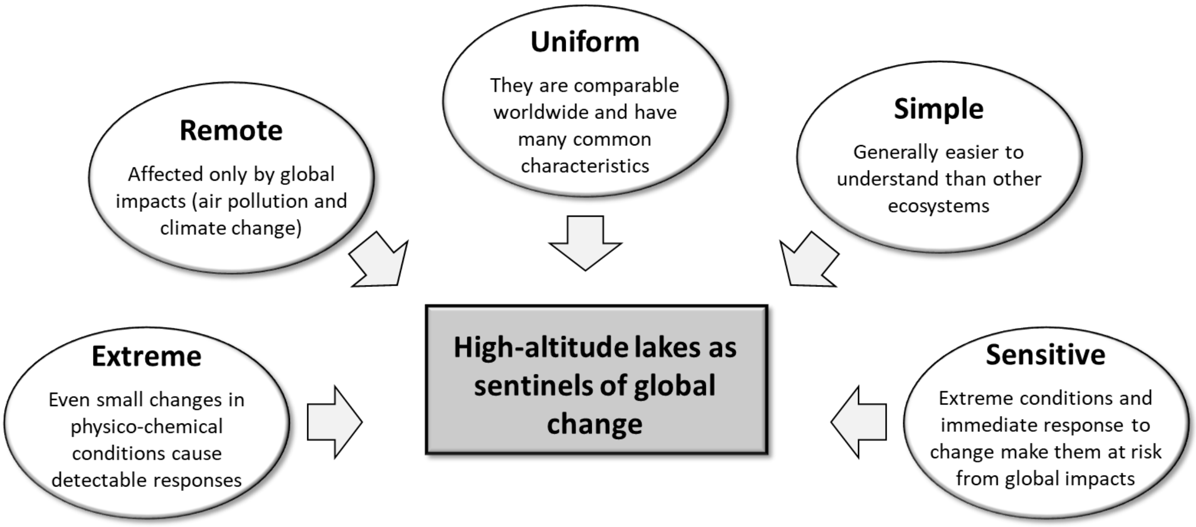

Figure 1.6. The five properties making high mountain lakes ideal indicators of global change (Psenner, 2003).

2.2. High-altitude lakes are vulnerable

According to climate change projection, climate change will be greater at high latitude and elevations (Auer et al., 2007; Beniston, 2003). In particular, the Alps are expected to be considerably affected by rising temperatures, changes in the seasonal cycle of precipitation, global radiation, and closely related impacts like floods, droughts, snow cover, and natural hazards (Gobiet et al., 2014). For example, in the Alps, mean snow depth, snow cover duration and number of snowfall days have decreased since the early 1980s (Laternser & Schneebeli, 2003), and a widespread greening has been observed for the 2000–2020 period, consistent with the overall increase in summer temperature (Choler et al., 2021). The high-altitude lakes in the alpine region are exposed to a number of threats that make them particularly *vulnerable*[4] to climate and global changes.

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[4] Climate change *vulnerability* is defined as the degree of threat to an ecosystem in response to changing climate, and differences in vulnerability across systems are often conceived of as resulting from their differing levels of exposure, *sensitivity*, and adaptive capacity (Kling et al., 2020).

Atmospheric deposition

The high elevation makes the high-altitude lakes particularly vulnerable to atmospheric deposition of pollutant and nutrients because mountain act as natural barriers to atmospheric transported particulates derived from lowland areas. Atmospheric compounds come from a number of natural and anthropogenic sources, including soil erosion, industrial emissions, transportation, civil engineering, and biomass burning (Field et al., 2010; Mahowald et al., 2008). The composition of atmospheric compounds varies according to the origin, the source region and factors that alter composition during transport. For example, dusts from Sahara are minerogenic and contain a few fraction of organic matter, while dusts generated from agricultural regions tend to contain a more organic and nutrient-rich fraction (Eglinton et al., 2002; Malm et al., 2004).

Atmospheric depositions interact with catchments characteristics and have been shown to increase the concentrations and the availability of nitrogen (Kirchner et al., 2014; Kopáček et al., 2005; Weintraub et al., 2017), phosphorus (Kopàček et al., 2011), and DOC (Monteith et al., 2007; Rodríguez-Murillo & Filella, 2015) in high-altitude lakes. Because high-altitude lakes are usually nutrient poor, they respond rapidly to nutrient concentration and availability increase from atmospheric deposition. Such nutrients have the potential to alter algal community structure, and to further support growth of heterotrophic bacteria and phytoplankton in alpine lakes (de Vicente et al., 2012; Pulido-Villena et al., 2008; Reche et al., 2009).

Temperature

Temperatures are increasing faster in mountain regions than in adjacent low elevation sites (Mountain Research Initiative Edw Working Group et al., 2015). In the European Alps, temperature increased by ca. +2°C since 1880, i.e., twice as much as the global average, and the trend has accelerated since 1970 (Rottler et al., 2019). Warmer surface temperatures generally increase the duration and intensity of thermal stratification, which can in turn decrease oxygen concentration at the bottom and affect nutrients and carbon cycling (Lepori et al., 2018; Missaghi et al., 2017).

A warming climate is expected to shorten the ice-cover duration in altitude (Preston et al., 2016). Already, the seasonal snowline therein increased by ca. +150 m for +1°C in temperature, and less snow accumulates at the lowest altitudes (Fuso et al., 2021). More frequent and severe drought and warmer air temperatures have caused snowpack in mid-latitude mountains to decrease in extent and duration, particularly in spring, although with large spatial variability (Beniston, 2003; Brown, 2018). Earlier melting are expected in spring, and are yet observed two to three weeks earlier in the Colorado Rockies (Clow, 2010), which can directly cause earlier seasonal runoff and influence lake hydrology (Huss et al., 2017). Snowmelt plays an important role in numerous physical and chemical processes in high-altitude lakes, including summer temperature, timing of ice-out, onset of thermal stratification, and transfer of allochthonous materials through the air-to-sediment continuum, which can affect algal production (Miller & McKnight, 2015; Parker et al., 2008; Sadro et al., 2018, 2019).

Finally, warming indirectly influences mountain lake structure and function trough changes in their surrounding landscapes, strengthening connectivity of the lake to the watershed. The upward shift of tree line and ranges of mountain plant species will cause shift in vegetation type and increase vegetation cover in the catchments (Gottfried et al., 2012; Harsch et al., 2009).

Precipitation

All climate models for 21st century project an intensification of heavy precipitation events during autumn and spring in the Alpine region (Rajczak et al., 2013). These changes are variable according to the season and the region of the Alps (Smiatek et al., 2009). For example, a reduction in mean precipitation and precipitation frequency is expected in summer across the entire Alpine arch. In terms of annual mean precipitation, Brunetti et al. (2006) show that the north-western parts experienced slight precipitation increases during the 20th century, while the south-eastern Alps have been subject to a significant drying. Despite the general drying of future summers, the occurrence of heavy or extended precipitation events will likely increase in a future greenhouse climate and possibly cause more frequent severe flooding events in Europe (Gobiet et al., 2014). In addition, higher frequency and severity of drought events is projected for the near and the long-term future in the Alpine region, although these changes are subject to uncertainty related to the different climate scenarios (Gobiet et al., 2014).

2.3. Vulnerability to dissolved organic matter variations

Catchment characteristics (watershed area, vegetation and soil type, slope) explain a large proportion of allochthonous inputs that lakes receive (Jacquemin et al., 2019; Xenopoulos et al., 2003). At higher elevations, catchments are usually small with poorly developed soils, and vegetation may be scarce. Consequently, allochthonous organic inputs to high-altitude lakes may be very low compared to other ecosystems, similar to what has been observed in Antarctic lakes (McKnight et al., 2001). High-altitude lakes thus exhibit a more autochthonous signature of DOM than the lower-elevation lakes (Bastidas Navarro et al., 2014; Mladenov et al., 2008). Alternatively, because nutrient concentrations that stimulate in-lake primary productivity are low, oligotrophy typically keeps autotrophy low in high-altitude lakes. Both low inputs and low primary productivity make DOC concentrations low in high-altitude lakes. For example, 26 lakes in the Alps and the Pyrenees situated at elevations ranging from 422 to 2 799 m a.s.l had DOC concentrations ranging from 0.2 to 3.5 mg.L^{-1} (Laurion et al., 2000). More recently, a study including 11 subalpine and seven alpine lakes in USA from 2 340 to 3 205 m a.s.l showed DOC concentrations ranging from 0.3 to 10.1 mg.L^{-1} (Rose et al., 2015). Comparatively, humic lakes in Sweden, Norway and Finland traditionally exhibit DOC concentrations from 2-3 to 25 mg.L^{-1} (Bergström & Karlsson, 2019; Jansson et al., 2000) while typically highly humic systems like coastal lagoon can reach DOC concentrations averaged 160 mg.L^{-1} (Farjalla et al., 2009).

Despite this, DOM concentration and origin can be very dynamic at an annual scale in high-altitude lakes, as DOM characteristics is determined by the seasonal functioning of lakes. In similarly ice-covered lakes, allochthonous DOM and organic matter originating from sediments have been showed to supports microbial respiration during winter (Karlsson et al., 2008). In high-altitude lakes, large quantities of allochthonous DOM can be transferred to lakes during snowmelt and extreme rainfall events (Miller & McKnight, 2010; Perga et al., 2018; Sadro et al., 2011a). During the summer and fall, autochthonous DOM derived from algal and microbial biomass become a more important source of organic matter (Hood et al., 2003; Miller & McKnight, 2010, 2015).

Upward elevational migration of mountain treelines is occurring across the globe (Daniels & Veblen, 2004; Elliott & Kipfmueller, 2011). For example, the reconstruction of the altitudinal dynamics of treeline in the western Italian Alps revealed that the treeline shifted upwards of 115 m over the period 1901–2000, reaching the altitude of 2505 m in 2000 and 2515 m in 2008 (Leonelli et al., 2011). Afforestation and the predicted increase of unusually high precipitation events (IPCC, 2013) are likely to increase particulate and dissolved C fluxes to mountain lakes (Moser et al., 2019). In addition, combined effects of increasing nutrient concentrations and rising water temperature may enhance autochthonous DOM concentrations, through increasing phytoplankton development and trophic level of less productive lakes (Tolotti et al., 2012).

Identically to lower elevation sites (see section 1.4.), carbon cycling is susceptible to change in high-elevation lakes as a consequence of global change. Because the Alpine region is particularly exposed to these changes, and because lake's characteristics make them particularly sensitive, the expected variations in DOM source and supply may have profound effects in high elevation lakes (Moser et al., 2019). However, current knowledge on DOM dynamics in mountain lakes is still scarce (Ejarque et al., 2018).

Planktonic communities

3.1. Phytoplankton is indicator of environmental changes

Planktonic organisms refer to the organisms that are adapted to spend part or all of their lives in apparent suspension in the open water of the sea, of lakes, ponds and rivers (Reynolds, 2006). Phytoplankton refers to the collective of photosynthetic plankton, adapted to live partly or continuously in open water (Reynolds, 2006). Phytoplankton is responsible for over half of the net primary production on Earth (Behrenfeld et al., 2001). Phytoplankton organisms belong to a wide range of extremely diverse polyphyletic groups of photosynthetic protists and cyanobacteria, which fuel food webs and drive biogeochemical cycling in marine and freshwater systems (Rousseaux & Gregg, 2014; Salmaso et al., 2015) (**Figure 1.7**). This apparent taxonomical diversity is supported by a commensurate diversity of form, function, and adaptive strategies.

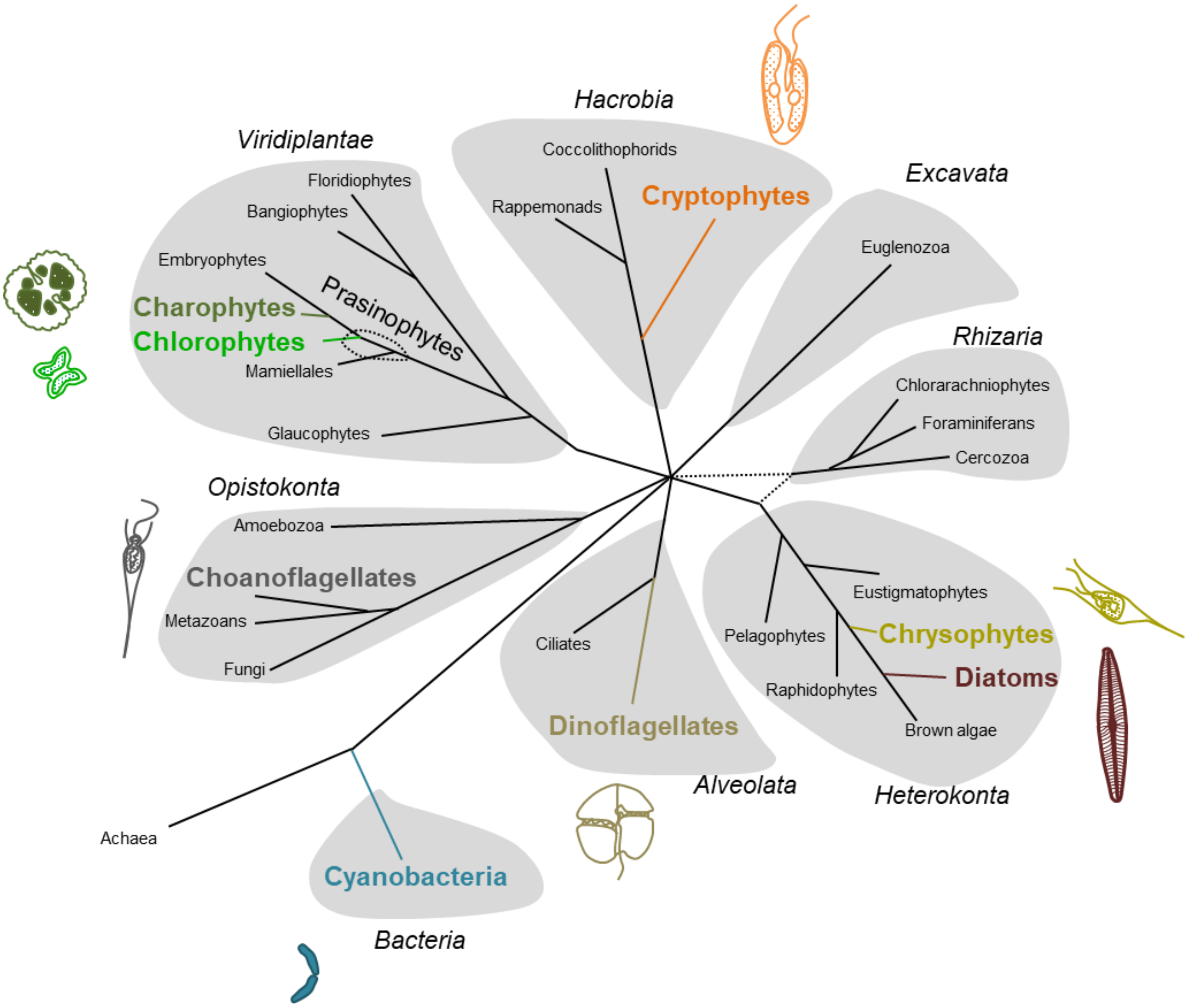


Figure 1.7. Schematic phylogenetic tree representing the distribution of phytoplanktonic taxa and choanoflagellates across eukaryote and prokaryote (cyanobacteria) lineages. Branches in color represent the main taxa discussed in this manuscript. Adapted from Collins et al. (2014) and Not et al. (2012).

Because of their rapid responses to water quality and anthropogenic pressures, phytoplankton communities are commonly used as trophic and ecological indicators (Laplace-Treyture et al., 2021). Predicting how phytoplankton communities will change in the response to global changes is a major challenging.

In order to better investigate links between phytoplankton communities and the main environmental factors, various attempts have been made during the last decades to categorize traits and functions in the phytoplankton (Salmaso et al., 2015). As outlined by Litchman et al. (2010), trait-based approach is widely used to explain and predict species distributions along environmental gradients. Some traits are particularly relevant in shaping the phytoplankton distribution, like resource utilization, morphological, grazer resistance, and temperature response traits. For example, phytoplankton cell size is widely recognized to be an important feature related to physiological and ecological performances of organisms (Naselli-Flores & Barone, 2011) (**Figure 1.8**).

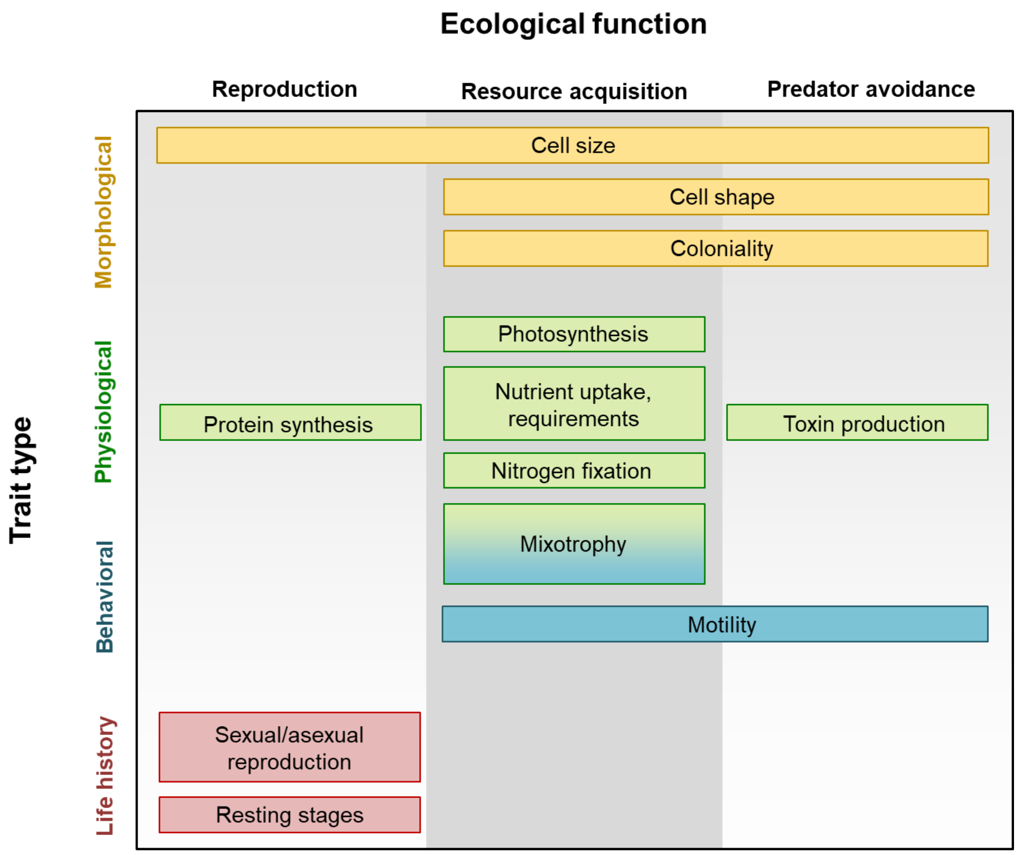


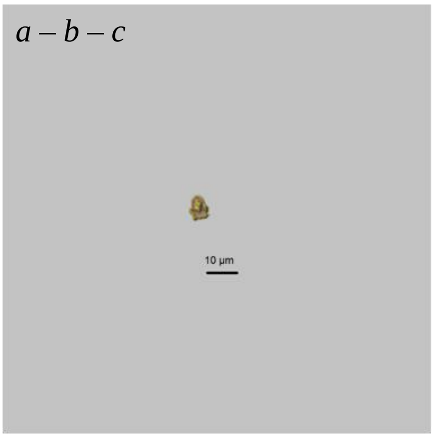
Figure 1.8. A typology of phytoplankton functional traits. From Litchman & Klausmeier (2008).

3.2. Adapted traits to altitude

The study of phytoplankton ecology is particularly relevant in high mountain lakes. First studies on phytoplankton assemblages led to the assumption that high mountain lakes were extremely low productive environments, not able to sustain a persistent phytoplankton community. These finding were mainly caused by the utilization of large mesh plankton nets (over 80 µm) for sampling, which restricted phytoplankton studies to larger species (Tolotti et al., 2006). It was generally assumed that phytoplankton in high altitude lakes is not dominated by endemic species, but results from a rarefaction of taxa commonly occurring at lower elevation lakes (Pechlaner, 1971; Rott, 1988). High-altitude lakes were recognized as autotrophic ecosystems after the late 1950s (Rodhe, 1962), which stimulated a series of intensive investigation that significantly improved knowledge of phytoplankton distribution an ecology in high-altitude lakes (Tolotti et al., 2006).

The harsh physical conditions, characterized by low temperatures and seasonally ice-cover, are reflected by a seasonality in water chemistry composition and physical processes that in turn affects lake biota (Trevisan et al., 2014). The selected phytoplankton taxa are thus extremely adapted to the short vegetation period, extreme and variable light conditions and the scarce nutrient resource (Pechlaner, 1971). Among the strategies involved, small cell size, motility, pigment adaptation, and mixotrophy are reported (Trevisan et al., 2014) (**Figure 1.9**).

Mixotrophy is among the most important traits reported in altitude lakes (Medina-Sánchez et al., 2004). Mixotrophy refers to a nutritional strategy combining autotrophy and heterotrophy in a single organism (Flynn et al., 2019). In phytoplankton taxa, autotrophic nutritional mode is photosynthesis, whereas heterotrophy may be realized by both osmotrophy and phagotrophy (prey ingestion). Osmotrophy, the ability to use dissolved organic compounds such as amino acids and vitamins, is common in phytoplankton taxa (Droop, 2007; Flynn et al., 2019; Våge et al., 2013). Because of the ubiquity of osmotrophy, mixotrophy often refers to organisms that "express, or have potential to express, phototrophy and phagotrophy" (Flynn et al., 2019). The word "potential" is important, because while some species can combine both autotrophic and heterotrophic metabolism, the real expression and the degree of heterotrophy of organism in natural conditions vary according to specific environmental limitations like light, nutrients, prey availability, or a combination of these (Bergström et al., 2003; Saad et al., 2016). For example, studies report an increase of the abundance of mixotrophs in phytoplankton community after increase of a dissolved organic carbon source, related to an increase of bacterial biomass and of competition with bacteria for nutrients (Carney et al., 2016; Creed et al., 2018; Jansson et al., 2006).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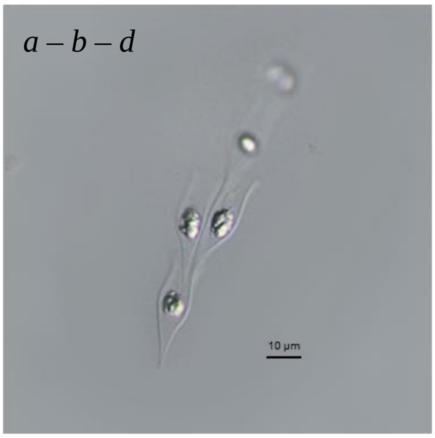
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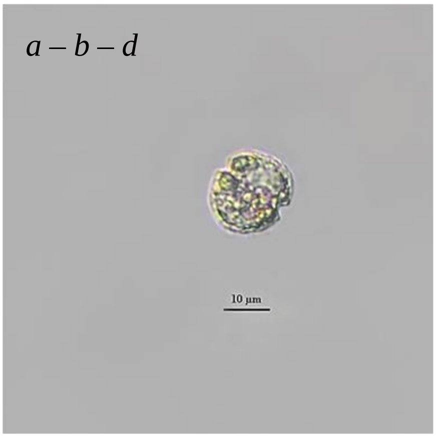
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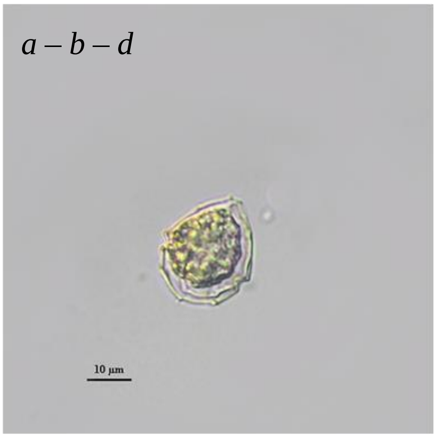
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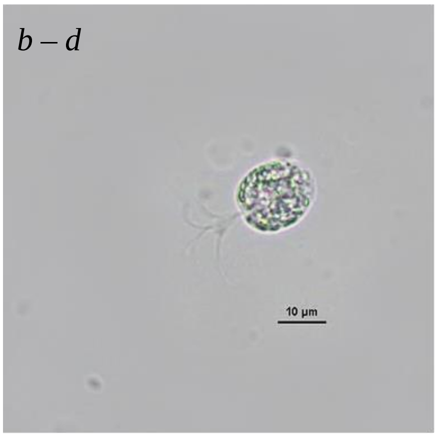
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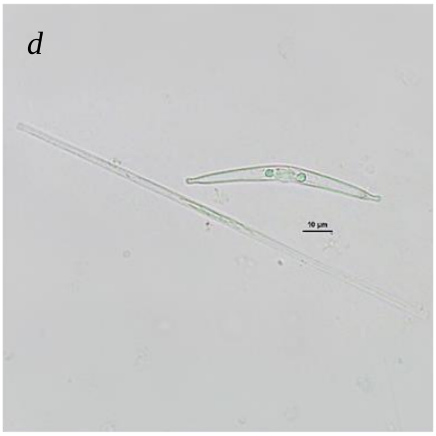
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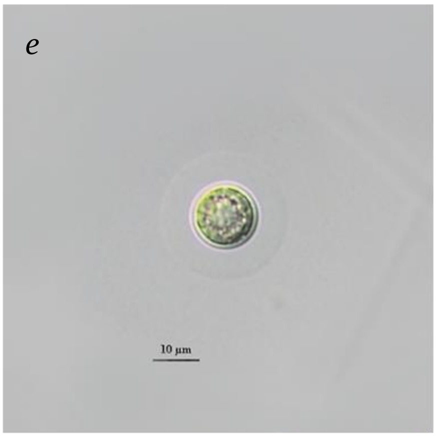
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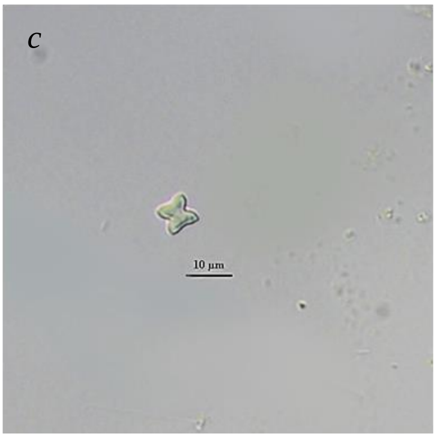
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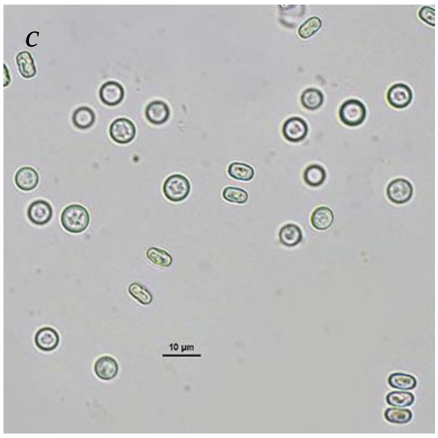
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◀ **Figure 1.9**. Example of phytoplankton traits commonly observed in high-altitude lakes: (a) mixotrophy; (b) motility; (c) small size; (d) large size; (e) mucilage. From right to left: *Kephyrion spirale* (Chrysophyceae), *Mallomonas* sp. (Chrysophyceae), *Cryptomonas* sp. (Cryptophyceae), *Dinobryon divergens* (Chrysophyceae), *Gymnodinium* sp. (Dinoflagellate), *Perdinium* sp. (Dinoflagellate), *Carteria* sp. (Chlorophyceae), *Hannaea arcu*s (Bacillariophyceae), *Planktosphaeria gelatinosa* (Chlorophyceae); *Synechococcus* sp. (Cyanobacteria), *Tetraedron minimum* (Chlorophyceae), *Cyclotella comensis* (Bacillariophyceae).

Mixotrophy is an advantageous strategy for phytoplankton in high-altitude lakes (Medina-Sánchez et al., 2004; Waibel et al., 2019). Mixotrophs have been showed to account for 68 % of total phytoplanktonic cells in Austrian and Italian lakes (Tolotti et al., 2003) and up to 80-90 % in North Patagonian Andean lakes form Argentina (Callieri et al., 2007; Queimaliños, 2002). The dual nutrition mode, despite its higher energetic cost compared with that of obligate autotrophic or heterotrophic metabolism (Rothhaupt, 1996), is a suitable strategy in environments with contrasting light and low nutrient regime (Modenutti, 2014) (**Figure 1.10**). Thus, during ice-covered winter, phytoplankton communities are often dominated by mixotrophic taxa, because prey ingestion allows them to maintain their growth when light is limiting (Rue et al., 2020).

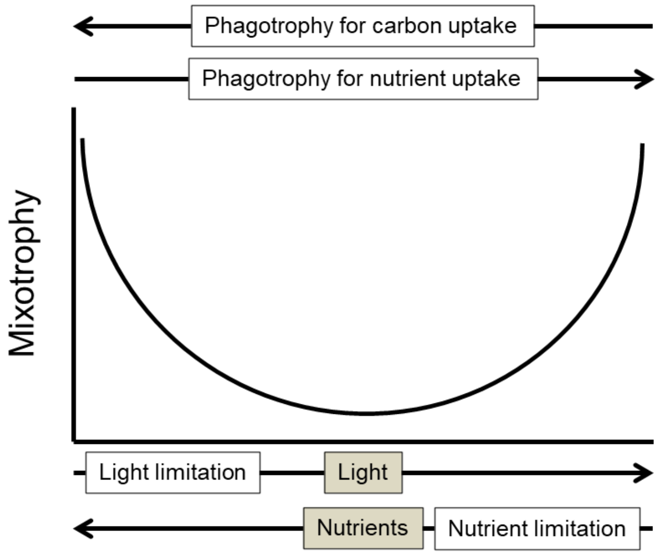

Figure 1.10. Simplified model relating phagotrophy and light and nutrient limitation influencing mixotrophy. From Modenutti (2014).

Other functional traits may be advantageous during winter under ice-cover. Motility refers to the ability of species to move in the water column, thanks to gas vacuoles or flagella. Motility allows to phytoplankton species to acquire nutrients at the bottom during lake's stratification (Özkundakci et al., 2016). Small size also allows organisms to be more efficient to acquire nutrients when they are scarce (Litchman et al., 2010; Queimaliños, 2002), and small species are often observed under nutrient limitation in high-altitude lakes (Magnea et al., 2013; Rue et al., 2020). However, large, mucilaginous, or colonial species are also reported in mountain lakes (McKnight et al., 1990; Tiberti et al., 2013) because large cell sizes or colonial morphology increase grazer resistance and thus reduce overall mortality (Herrera-Martínez et al., 2017; Litchman et al., 2010). Finally, resting stages offer a temporal escape to endure adverse environmental conditions while providing an inoculum population for rapid population growth when conditions have improved (Cirés et al., 2013; Özkundakci et al., 2016). For example, siliceous resting stages of chrysophytes are common in sediment of mountain lakes or ice-covered lakes (Özkundakci et al., 2016; Pla et al., 2003). As the composition of the assemblages reflects their surrounding physico-chemical environment, they are often used in paleolimnological studies to assist in environmental reconstructions (Catalan et al., 2006; Kamenik & Schmidt, 2005).

The study of functional traits is thus a relevant approach for monitoring environmental variations in high-altitude lakes. However, most studies focus on a short period and knowledges about evolution of functional traits in relation to seasonal environmental variations are missing. Because mixotrophy is often associated to increased competition and predation, mixotrophs proportion therefore appears to be a good indicator of interactions between phytoplankton and bacterioplankton, especially in a DOM-increase context.

3.3. Interactions between phytoplankton and bacteria

The functional response of phytoplankton to environmental changes in high-altitude lakes can directly alter the phytoplankton connection to the microbial loop and to higher trophic levels. Phytoplankton and bacterioplankton are positioned at the base of the food web, and their relationships exerts a major influence in regulating the fundamental processes in mountain lakes (Medina-Sánchez et al., 2004). Phytoplankton – bacterioplankton relationships encompass commensalism, competition, predation, and mutualism (González-Olalla et al., 2018; Reche et al., 1997).

- **Commensalism** occurs when bacteria acquire carbon for growth from the excreted organic carbon provide by phytoplankton.

- **Predation** occurs when bacteria constitute a prey for mixotrophs, supplying carbon and nutrients to mixotrophic phytoplankton.

- **Mutualism** is established when bacteria, during organic matter decomposition, supply mineral nutrients or vitamins for algal growth, while phytoplankton supply carbon for bacterial growth.

- **Competition** occurs when bacterioplankton and phytoplankton growth are limited by the same inorganic nutrients (usually N and P).

Global change is expected to alter the responses and interactions between primary producers and decomposers in the future. Recent studies, mainly in the Sierra Nevada Mountains lakes (Spain) studied how bacterioplankton – phytoplankton interactions respond to multiple global change stressors: for example, P enrichment (> 30 µg $P.L^{-1}$) have been showed to reinforce commensalistic interaction by favoring the development of strict autotrophs (Cabrerizo et al., 2017). However, moderated nutrients amendments may shift an algal–bacterial interaction from commensalism to competition depending on bacterial N:P ratio (Gurung et al., 1999; Villar-Argaiz et al., 2002). Additionally, increased temperature and Saharan dust depositions led to a shift from a bacterivory (predation) control exerted by algae to commensalism between 2005 *versus* 2015 (González-Olalla et al., 2018). It has also recently been shown that the phytoplankton – bacterioplankton relationship's response to ultraviolet radiation changed under nutrients acclimatation and abrupt temperature shifts (Durán-Romero et al., 2020).

The impact of DOM variations on the phytoplankton – bacterioplankton relationship in high-altitude lakes is far to be completely understood. For now, studies of DOM effect on high-altitude lakes biota focused on bacterioplankton assemblages (Pérez & Sommaruga, 2006), phytoplankton (Kissman et al., 2013), or interactions between zooplankton and phytoplankton (Kissman et al., 2017). These experimental studies showed that DOM increase modified bacterial community structure and activity (Pérez & Sommaruga, 2006), phytoplankton biomass and community composition (Kissman et al., 2013, 2017), and zooplankton biomass (Kissman et al., 2017).

These changes occurring at the base of the food web with DOM increase could have critical impact on the whole lake functioning. For example, mixotrophs have been showed to exert an important net predatory control bacterioplankton in high-altitude lakes. Mixotrophic bacterivory would act as a bypass of carbon flux toward the grazing chain, preventing the development of the heterotrophic microbial food web (Medina-Sánchez et al., 2004). Thus, mixotrophs would occupy the niche of microheterotrophs, implying a simplification of planktonic structure and improving the energetic transfer efficiency through the food web by the reduction in the number of trophic levels and the consequent loss of energy of each trophic link (Medina-Sánchez et al., 2004) (**Figure 1.11**).

A scenario in which autotrophic phytoplankton may dominate over mixotrophs after increase DOM concentrations would certainly have critical implications for carbon transfer in oligotrophic high-altitude lakes. Understand how dissolved organic matter variations, both in quantity and quality, may affect phytoplankton communities and their interactions with the microbial loop will contribute to a better predictability of ecological trajectories of high-altitude lakes in a context of global change.

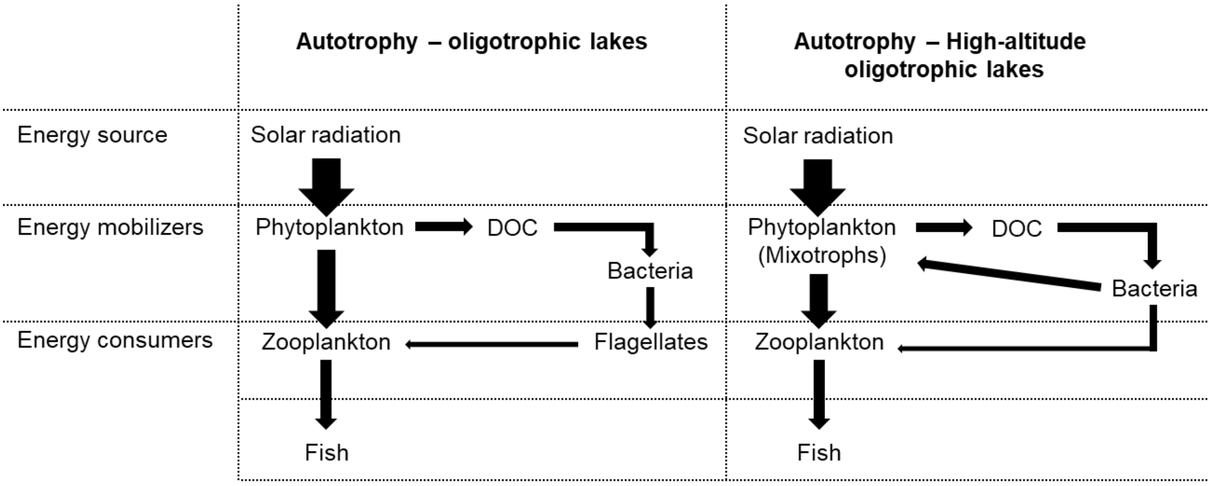

Figure 1.11. Schematic illustration of the pelagic energy flux in oligotrophic lakes versus oligotrophic clear-water high mountain lakes. Adapted from Medina-Sánchez et al. (2004).

Thesis methods and objectives

1. Methods

1.1. Study lake

The Southern Alps constitute all the massifs located in the southern part of the French Alpine chain, among others, the Mercantour, Queyras and Ecrins massifs (**Figure 1.12**). The geographical configuration of the Southern Alps induces contrasting environmental characteristics along a north-south gradient, particularly in terms of climatic influence and atmospheric deposition. For example, the southern massifs (Mercantour) are subject to a more Mediterranean climate and are more exposed to atmospheric deposition of nitrogen (N) (Fagerli et al., 2015) and phosphorus (P), being located within the perimeter influence of aerosols enriched in phosphorus emitted at the level of the Saharan desert regions (Moulin & Chiapello, 2004). In contrast, the massifs located to the north of the Southern Alps (Queyras, Ecrins) are exposed to a more continental climate and are less exposed to atmospheric deposition of nitrogen and phosphorus (Jacquemin, 2019).

Lake Cordes is located in the north part of southern Alps, in the Fonds de Cervières valley. Due to its location, Lake Cordes is poorly exposed to atmospheric deposition: for example, the annual rates of nitrogen deposition measured in this region between 2000 and 2013 were 435 ± 14 kg N km^{-2} $year^{-1}$, compared to 1123 ± 49 kg N km^{-2} yr^{-1} in the southern regions (Mercantour) (Fagerli et al., 2015). This lake is characterized by a small size (1.8 ha), a shallow depth (maximum depth = 9 m) and a large catchment area (170 ha) covered with alpine meadow for about 60%. Previous works on this lake highlighted a strong contribution of mixotrophic taxa to the phytoplankton community in 2015 and 2016 (Jacquemin, 2019). Jacquemin et al. (2019) demonstrated that the composition of phytoplankton communities of the Lake Cordes was determined both by the lake's exposition to atmospheric deposition but also by catchment characteristics.

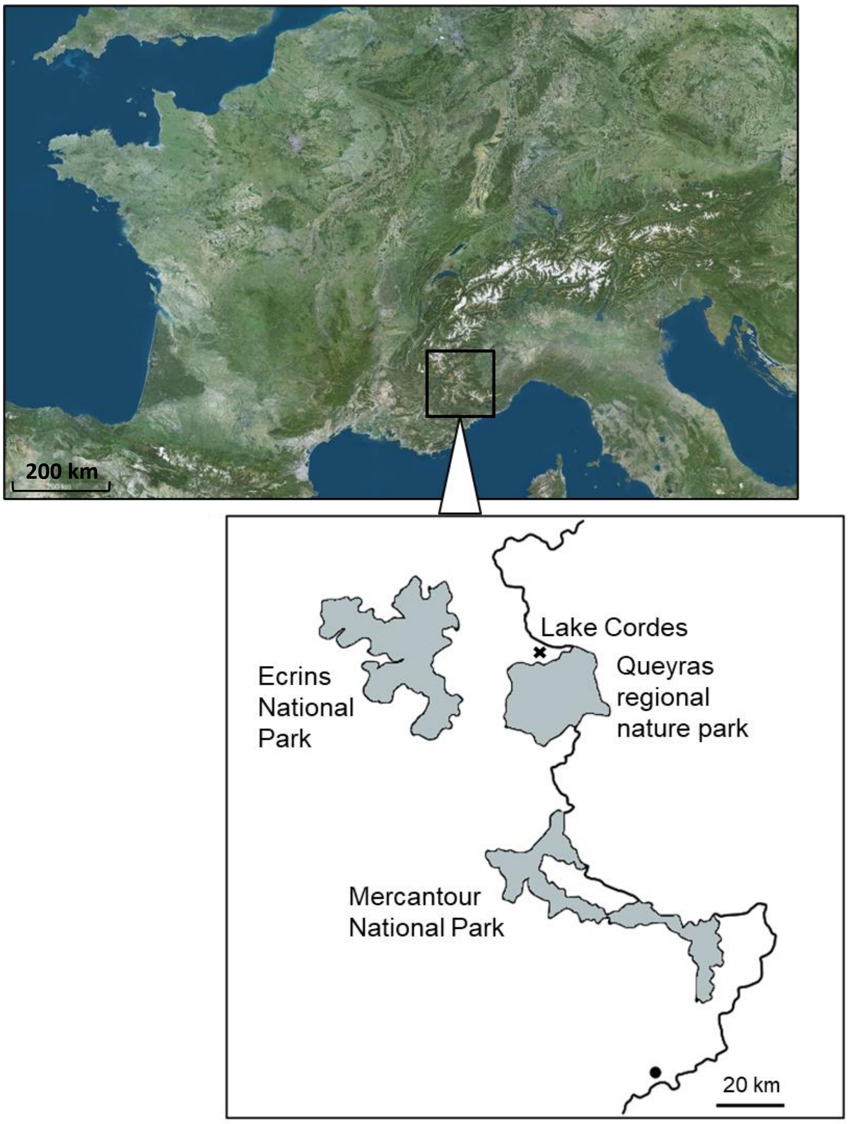

Figure 1.12. Localization of the main protected massifs of the southern Alps and of the Lake Cordes.

Nutrient concentrations measured in 2016 during the summer season indicate relatively low values, whether for dissolved inorganic nitrogen (0.140 ± 0.051 mg L^{-1}), soluble reactive phosphorus (5 ± 2 µg L^{-1}) or dissolved organic carbon (2.631 ± 1.13 mg L^{-1}) and define this lake as a clear oligotrophic lake. It has been shown that phytoplankton limitation by nutrients evolves during the summer, from a limitation by P at the beginning of summer towards a co-limitation by NP at the end of summer (Jacquemin et al., 2018). In relation to these changes in limitation patterns, phytoplankton successions could be observed during the growing season (**Figure 1.13**).

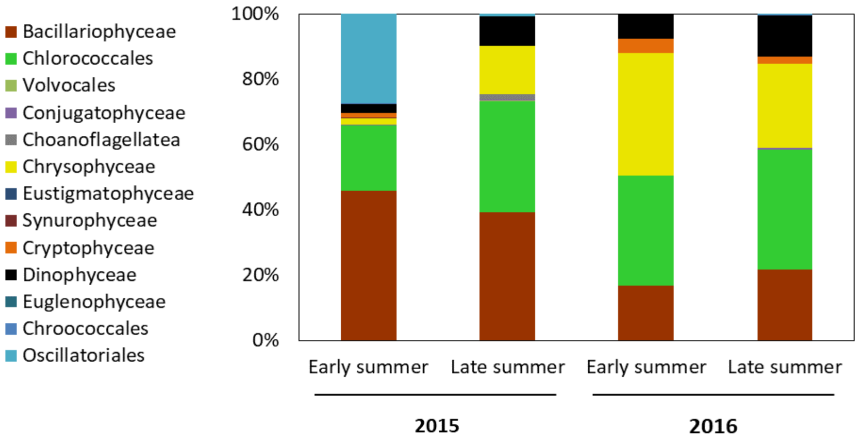

Figure 1.13. Taxonomical composition of phytoplankton communities from the Lake Cordes in early summer and late summer, in 2015 and 2016. Non-published data extracted from the thesis of Jacquemin (2019).

The exposition of Lake Cordes to atmospheric deposition, the characteristics of the lake and of its watershed, make this lake a privileged study site for analyzing the impacts of dissolved organic matter increase on planktonic communities. This thesis work is an extension of research previously carried out on the high-altitude lakes of the Pyrenees and the Alps, on, among others, the ecology of fish populations and their impacts on the environment (Cavalli et al., 2001, 2002; Tiberti et al., 2014), on paleo-limnological approaches (Cantonati et al., 2021; Cartier et al., 2019; Rivera-Rondón & Catalan, 2020), or on ecology of benthic diatoms (Feret et al., 2017).

1.2. Combine field survey and experimental approaches

Analyzing the dynamic of dissolved organic matter and phytoplankton successions in relation to environmental factors requires regular sampling and provides a better understanding of ecosystems functioning. Field survey on dissolved organic matter dynamics is crucial to understand organic matter transfers from the watershed to the lake and trough the trophic levels of the food web in the lake (Cole et al., 2011; Miller et al., 2009; Olson et al., 2021). Phytoplankton successions reflect environmental variations and provide valuable information on the potential effects of global change on lake functioning (Jacquemin et al., 2019; Miller & McKnight, 2015; Rue et al., 2020).

However, field observational approaches are limited to identify the direct effect of one or multiple environmental factors on biological populations (Thackeray et al., 2008) and the distinction of specific effects is complex. In this sense, experimental approaches can help to distinguish the direct effect of a factor from simultaneous variations in environmental conditions. For example, the experimental addition of a single source of dissolved organic carbon such as glucose is essential to separate the effects of the carbon source from those of the associated nutrients during increase of organic matter (Hitchcock et al., 2010; Liao et al., 2019). Similarly, the exclusion of some trophic links, such as predatory zooplankton, allows to be sure that the phytoplankton response observed is that generated by the experimental factor and not associated with a joint or indirect effect of zooplankton predation (Hitchcock & Mitrovic, 2013; Hrycik & Stockwell, 2021). Although the experimental conditions do not allow full representativeness of the effects of environmental factors on biological communities, experimental simplification is an essential first step in evaluating the direct effects of specific factors and their interactions (Srivastava et al., 2004).

In this thesis, field survey and experimental approaches were combined to analyze the sensitivity of planktonic communities to dissolved organic matter increase (**Figure 1.14**). The observational approach constitutes the first part of the thesis (chapter 2) and provides a characterization of the dynamics of dissolved organic matter and phytoplankton communities in relation to biological and environmental factors. This first approach aimed to identify seasonal changes in concentrations and quality of dissolved organic matter in Lake Cordes and to follow the successional patterns of phytoplankton in relation to dissolved organic matter.

The experimental approach is divided into two parts. Firstly, the objective was to identify potential limitations of heterotrophic bacteria and phytoplankton and to evaluate the response of these two compartments to enrichments in DOC (glucose), nutrients (nitrogen and phosphorus) and a combination of them (Chapters 3 and 4). These experiments were carried out at three key periods of the lake functioning (ice-influenced period, overturn, late summer period) to assess how seasonality can modulate the response of organisms to different enrichments. Secondly, the objective was to evaluate the response of planktonic organisms to the variation in the quality of dissolved organic matter (chapter 5). Enrichments in soil extract from the watershed and in algal lysate were used to characterize the response to allochthonous and autochthonous sources of dissolved organic matter and to compare this response to that obtained with glucose and nutrients.

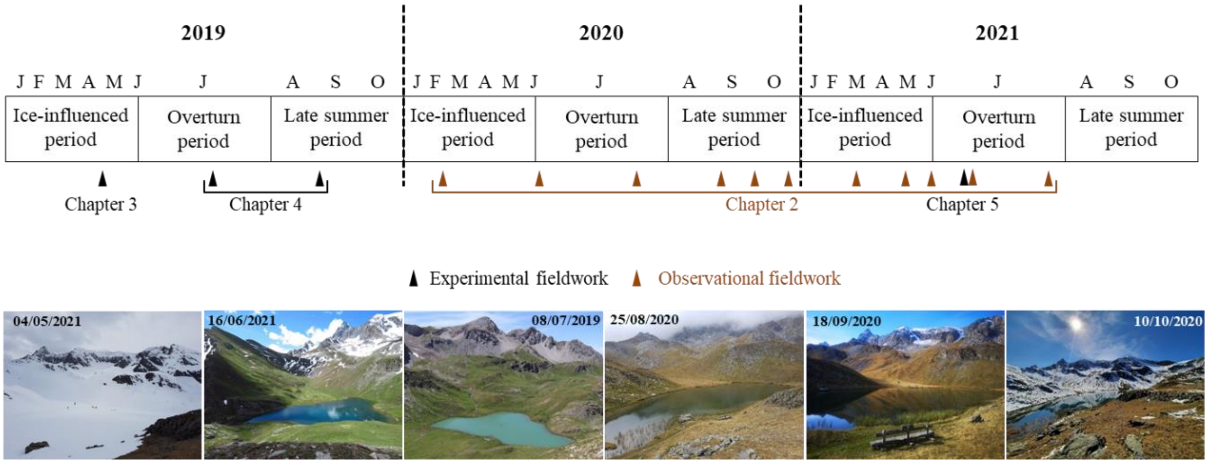

Figure 1.14. Chronological sampling performed on the Lake Cordes between 2019 and 2021 for field survey (chapter 2) or experiments (chapters 3, 4, and 5). Samplings were performed during the three key periods of the lake functioning: ice-influenced, overturn, and late summer period. Seasonal variation of the lake was captured throughout the years.

1.3. Design of experiments

Water sampling under ice during winter is a major challenge in high-altitude lakes and this is particularly true for laboratory experiments which require a large quantity of water (several tens of liters) (**Illustration 1.3**). Moreover, the quantification of the interaction effects requires the multiplication of (i) the size of the samples, (ii) the number of experimental combinations, (iii) the measurements and analyzes and consequently the quantity of water to be sampled. Designs of experiments are interesting statistical tools, which could, in some cases, reduce the number of essays and consequently a simplification of the design for the three aspects mentioned above (Droesbeke et al., 1997; Tinsson, 2010).

Illustration 1.3. Difficult access conditions and sampling during the ice-covered season in high-altitude lakes.

Indeed, we show that the quality of the information extracted from a set of experimental essays depends on the choice of experiments, i.e. on the position of the experimental points in the experimental space to be explored. These experiments must make it possible to answer questions and, according to the objective of the study, the choice of the most suitable experimental plan is required. Performing a design of experiments implies to choose *a priori*, before the experiment realization, the most important effects to be quantified and their interactions. If the final goal is to reduce the size of sampling, experimental combinations, and analyzes, while maintaining a high quality of statistical result, a selection *a priori* of the effects to be quantified and their interactions is necessary. Thus, it is assumed that additional interactions that do not specifically address the question of the study are not analyzed, and their effects on the biological response are assumed to be negligible.

In addition, the presence of multiple interactions makes the interpretation of the biological response complex and this response can be difficult to interpret beyond three factors (Scheiner & Gurevitch, 2001). The experiment presented in the chapter 3 will serve as an example: the main objectives of this experiment were (i) to test the effect of increased glucose on phytoplankton abundance and (ii) to analyze how the increased glucose interacts with other factors, such as nutrients, light, or temperature, on this response. In the case of this study, the sampling of water under the ice with limited human and material resources did not allow the realization of a complete experimental design (complete factorial matrix). A fractionated experimental design within the framework of a Design of Experiments was thus performed.

The realization of a fractionated experimental design implies to prioritize the most important factors, in this case we only choose the order 1 interactions with glucose, i.e. (i) glucose with nutrients, (ii) glucose with temperature, (iii) glucose with light. Additional interactions (e.g. interaction of nutrients with temperature) and 2nd and 3rd order interactions are assumed to be negligible. The essays are then chosen and organized to obtain the best possible quality of information with a minimum number of experimental combinations (Manzon et al., 2020). In the example, this involves an experiment with 24 combinations in triplicates instead of 32 combinations in the case of a complete experimental design (**Figure 1.15**). The measured response can then be statistically analyzed in a conventional way, for example using linear models by testing only the desired interaction effects. In this thesis, the design of experiments approach was used for the winter experiment (chapter 3).

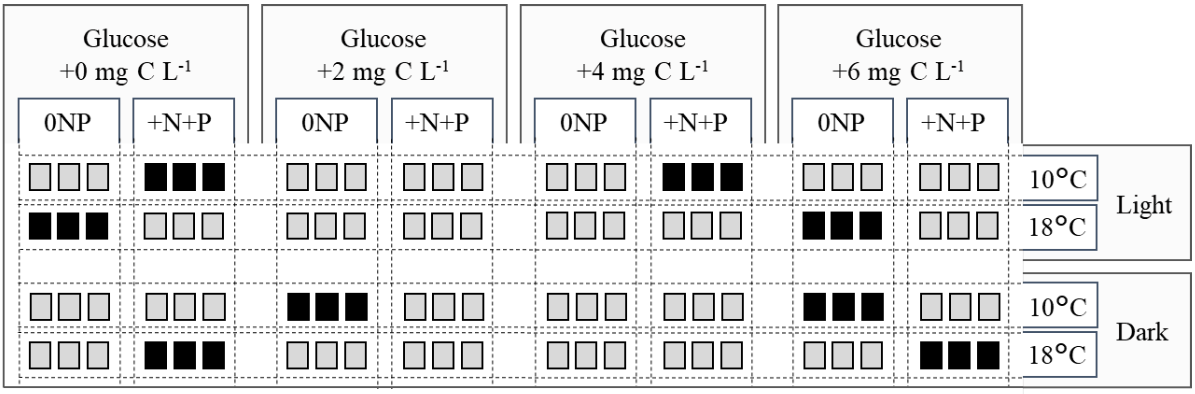

Figure 1.15. Example of an experimental design to test the effect of glucose addition in interaction with nutrients (0NP or +N+P), light (Light or Dark), and temperature (10°C or 18°C). Complete experiment requires 32 combinations in triplicates, thus a sampling of 33, 6 L of water for 350 ml microcosms. The fractioned experimental design selected by the Design of Experiments requires 24 combinations in triplicates (non-experimentally performed combinations are colored in black), thus a sampling of 25,2 L of water.

2. Thesis objectives

High-altitude lakes are sentinels of global change (Moser et al., 2019). Because they are remote and exposed to extreme conditions, they are particularly vulnerable to environmental variations, especially to dissolved organic matter increase (Ejarque et al., 2018). Assessing this vulnerability involves a better understanding of the dynamics of organic matter and its influence on planktonic communities. The link between phytoplankton successions and organic matter dynamics of is far from being completely understood in altitude, especially at a seasonal scale between the ice-covered period and the ice-free period. At the community level, the effect of increasing organic matter, acting as a source of organic carbon and/or nutrients, on phytoplankton communities and on the interactions between bacteria and phytoplankton is still difficult to predict.

This thesis aims to provide a better understanding of the sensitivity of planktonic organisms (bacteria and phytoplankton) to increase of dissolved organic matter, source of carbon and nutrients, in high-altitude lakes. Two main questions were asked:

(i) How do plankton communities (bacteria and phytoplankton) respond to seasonal variations in dissolved organic matter quality and quantity in a high-altitude lake?

(ii) How could the origin of organic matter, the limitation patterns and the nature of the phytoplankton community modulate the planktonic sensitivity to organic carbon and nutrient increase in high altitude-lakes?

These two major aspects have been addressed in four chapters:

Chapter 2 aims to characterize the seasonal dynamics of dissolved organic matter and phytoplankton communities in relation to environmental variations. This chapter presents a field survey of the Lake Cordes performed between February 2020 and July 2021 and relates the functional modifications of phytoplankton to the dynamics of dissolved organic matter as well as to a set of biological and environmental variables indicative of the lake functioning.

Chapter 3 presents a laboratory experiment aiming to characterize the response of planktonic assemblages sampled under the ice to an increase in dissolved organic carbon (glucose) and nutrients (N and P). For this experiment, a fractioned experimental design within the framework of a Design of Experiments was performed. (Experiments 1).

Chapter 4 presents a series of two laboratory experiments, aiming to understand how the initial phytoplankton assemblage and the context of limitation of the organisms modulate the bacterial and phytoplankton response to an increase in dissolved organic carbon (glucose) and nutrients (N and P). (Experiments 2).

Chapter 5 presents a laboratory experiment aiming to better understand how the quality of dissolved organic matter can influence bacterial activity and the functional composition of phytoplankton communities from Lake Cordes. (Experiments 3).

Finally, this manuscript presents a general discussion, presenting a synthesis of the main results, a linking of the conclusions of the different chapters and the implications and research perspectives raised by the thesis work.

Chapter 2

Chapter 2

Planktonic shift with dissolved organic matter properties: a functional perspective for sentinel lakes

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Keywords

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

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.

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.

1. Introduction

In mountain lakes, phenological variations regulate phytoplankton communities, making them useful indicators mirroring environmental conditions (Kuefner et al., 2021; Oleksy et al., 2020b). 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, Melack, 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., 2022). 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, Melack, et al., 2011a) 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. Methods

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

Samplings were performed in the oligotrophic high-altitude Lake Cordes (Dory et al., 2021). 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 A1.1**). 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 $R_{standard}$:

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

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

2.3. Statistical analyses

One-way ANOVA tests followed by pairwise comparisons were used to test the effect of period on the various abiotic and biological variables measured, and to test differences in fluorescence characteristics between the four leachates of organic matter sources. Non-metric multidimensional scaling (NMDS) analysis was performed on a Bray-Curtis distance matrix to 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 2.1**). 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.

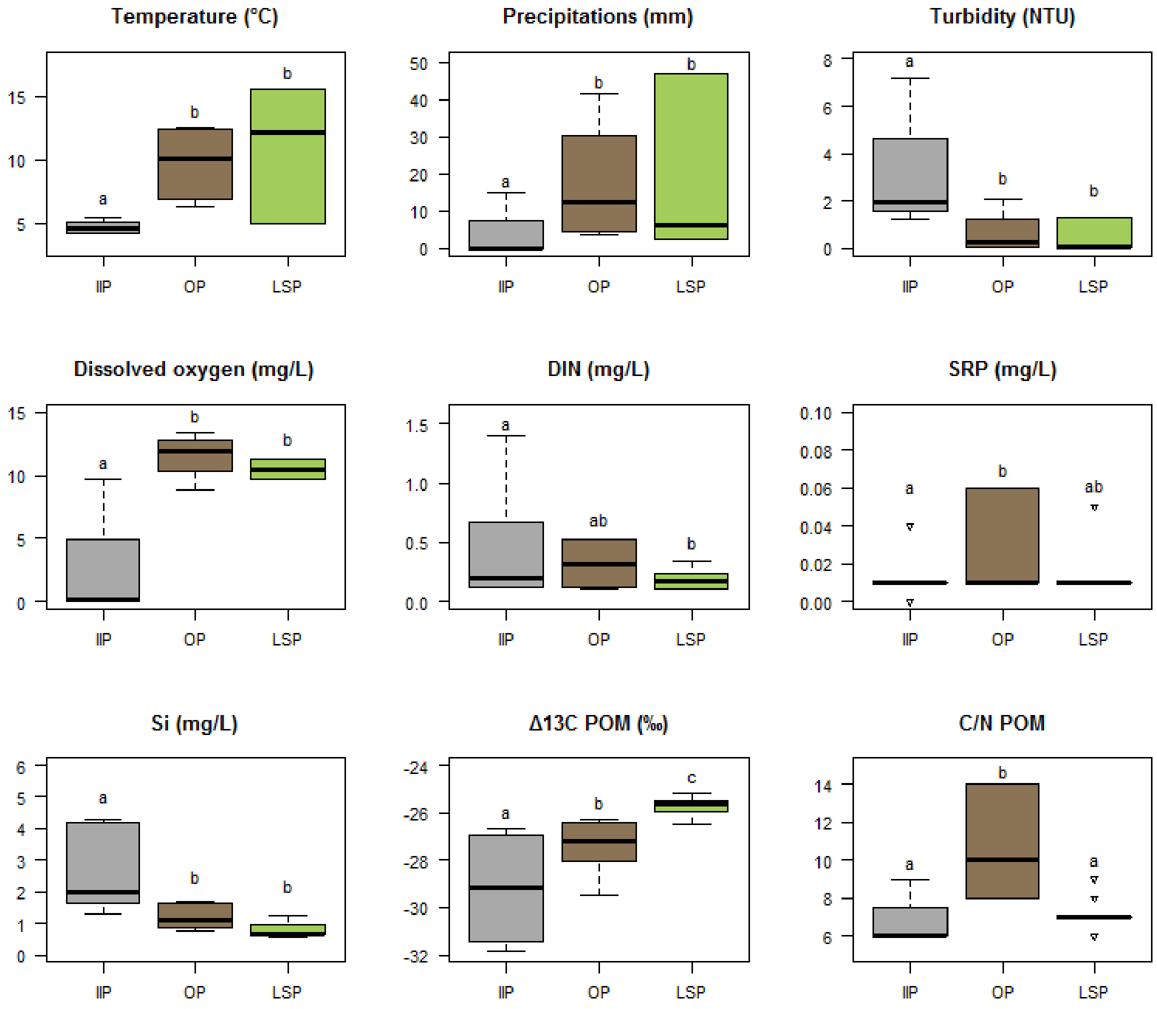

Figure 2.1. Abiotic and climate parameters measured in the three defined functional periods in Lake Cordes. DIN: dissolved inorganic nitrogen; SRP: soluble reactive phosphorus; Si: silica; POM: particulate organic matter.

3.2. Characterization and dynamics of DOM

Identification of the components by PARAFAC modeling

PARAFAC analysis of the EEM spectra of leachates and natural DOM samples identified four fluorescent components (C1, C2, C3, and C4) (**Figure 2.2**). 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).

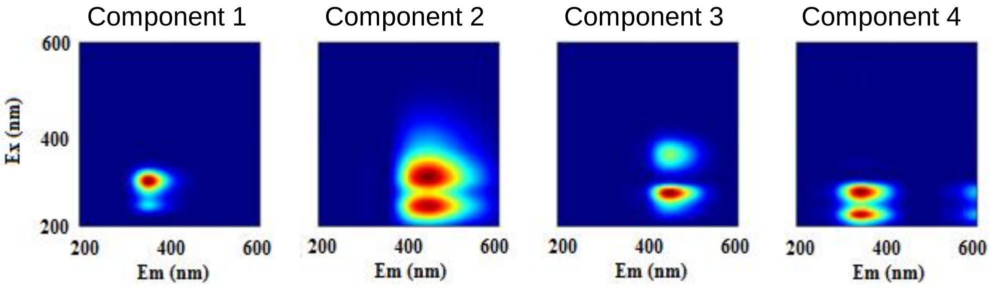

Figure 2.2. PARAFAC model output showing fluorescence signatures of the four fluorescent components identified in the four sources and in natural DOM.

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 2.3A**). 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 2.3B**). 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 2.3C**).

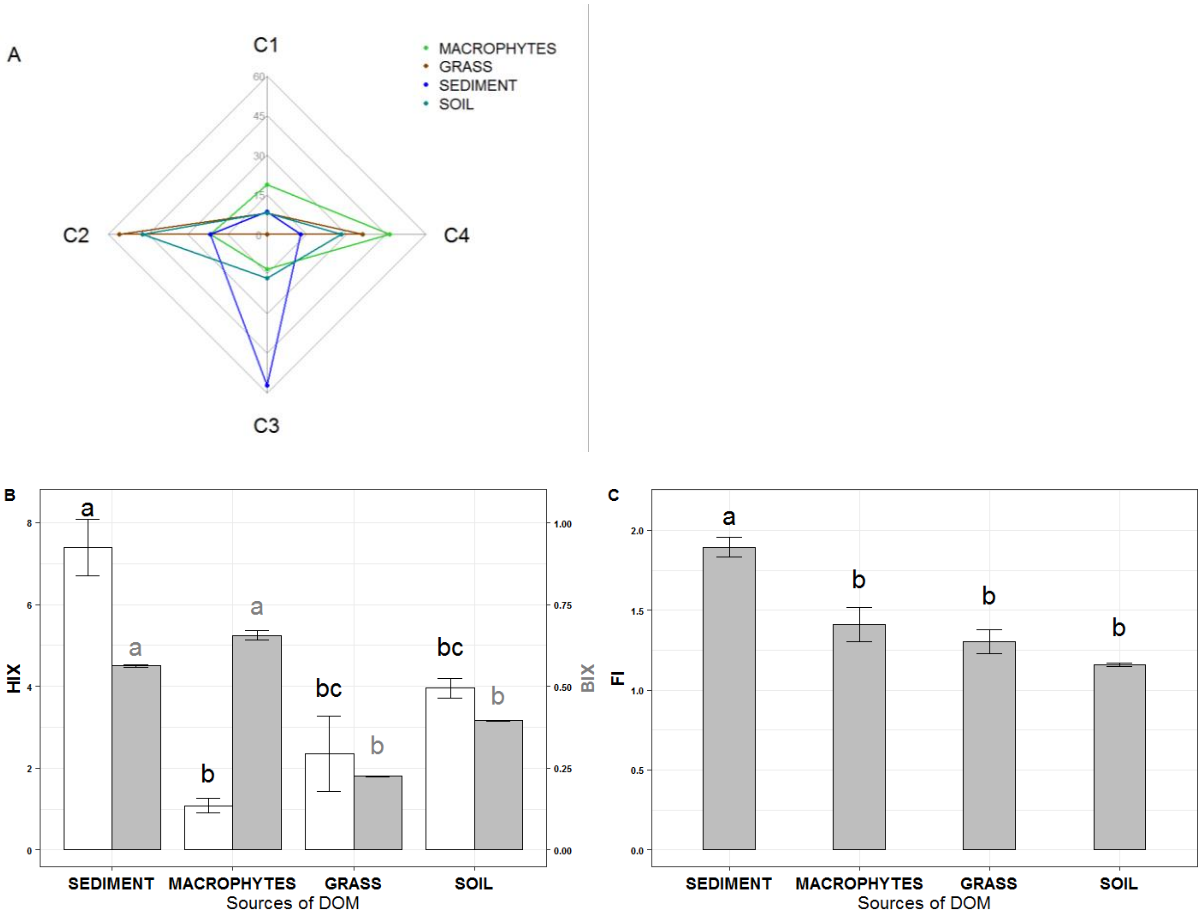

Figure 2.3. (A) Relative contribution of the four components for the four leachates of dissolved organic matter (DOM) sources; (B) Humification index (HIX) and freshness index (BIX) of the four leachates of DOM sources; (C) Fluorescence index of the four leachates of DOM sources

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 2.4**). 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.

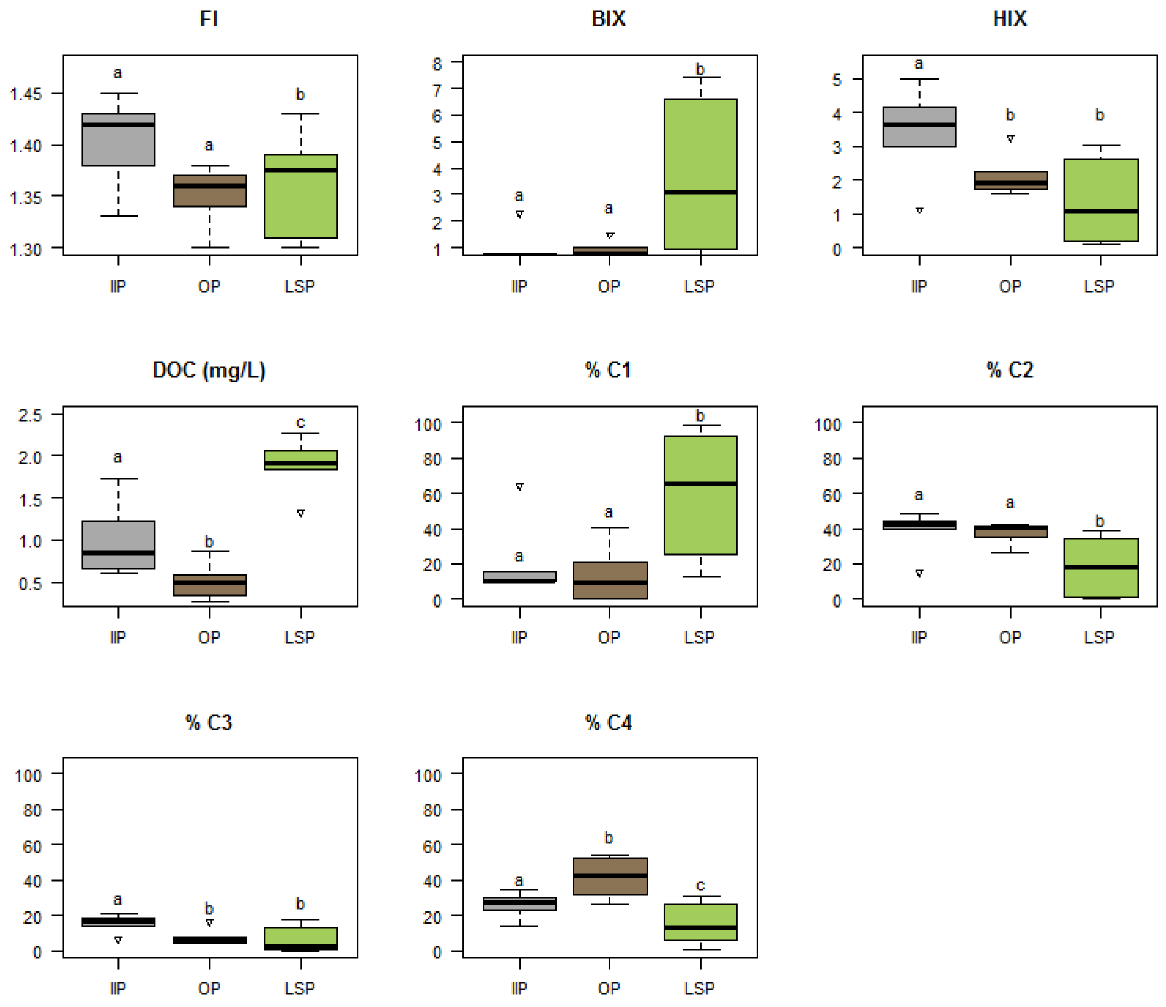

Figure 2.4. DOM characteristics measured in the three defined periods in Lake Cordes. DOC: dissolved organic carbon.

3.3. Planktonic community dynamics

Phytoplankton biomass was low during the ice-influenced period (IIP) and the overturn period (OP), increasing significantly during the late summer period (LSP) (**Figure 2.5**). While specific richness increased progressively from IIP to LSP, the Shannon diversity index was significantly higher in OP than during the two other periods. HP biomass increased progressively over the three periods. Ciliate abundance was low during IIP and OP, then significantly increased during LSP.

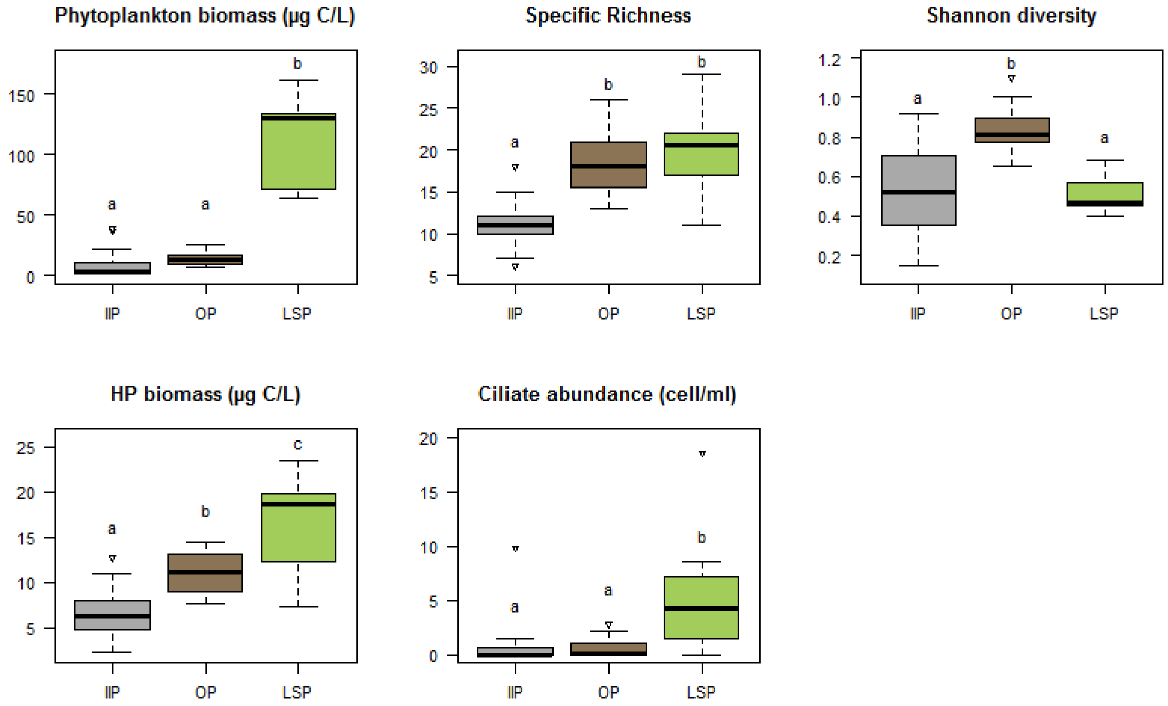

Figure 2.5. Biological parameters measured in the three defined periods in Lake Cordes.

Concerning taxonomical and functional changes in phytoplankton assemblages, a significant shift in phytoplankton community structure was observed over the three periods (**Figure A1.1,** Adonis, F = 29.362, r2 = 0.57, p-value < 0.001). Cyanobacteria dominated the other phytoplankton groups during IIP (**Figure 2.6**), which also showed a high proportion of cryptophytes, dinoflagellates, and phycoerythrin-rich picocyanobacteria (PE_Pcy). A relatively high proportion of constitutive mixotrophs was found during IIP (45%), as well as MBFG groups I (small cells with high surface/volume ratio) and V (flagellates of medium to large size) and FG groups K (shallow, nutrient-rich water column) and Y (small, enriched lakes, lentic ecosystems with low grazer pressure). OP was characterized by an increased proportion of diatoms (51%), which became dominant over the other phytoplankton groups. Dominance by the PE_rich PCy was replaced by that of the phycocyanin-rich picocyanobacteria (PC_Pcy). 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.

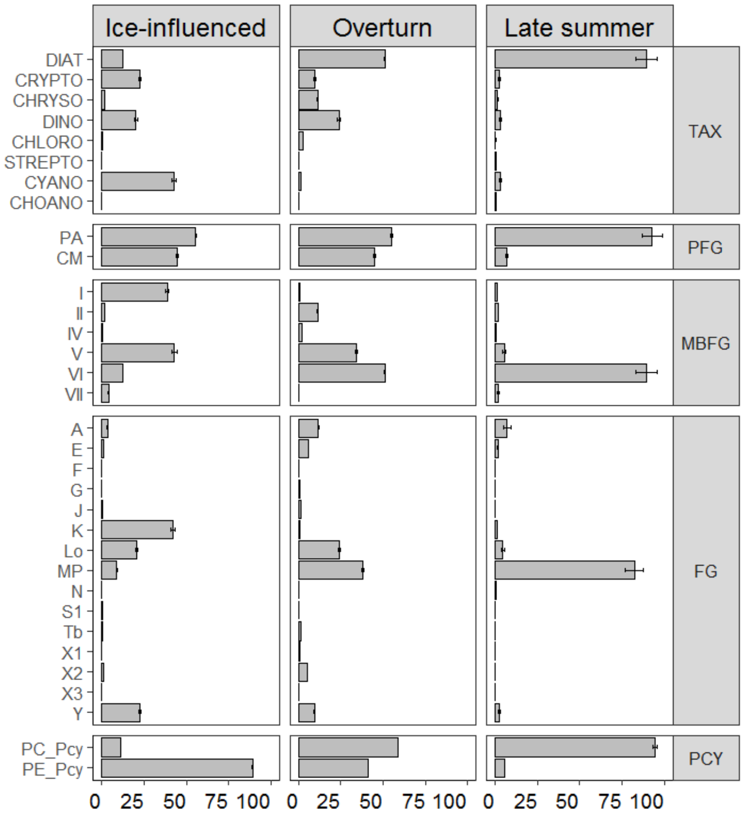

Figure 2. 6. Phytoplankton composition in each period (error bars correspond to standard error). DIAT = diatoms; CRYPTO = cryptophytes; CHRYSO = chrysophytes; DINO = dinoflagellates; CHLORO = chlorophytes; STREPTO = Streptophytes; CYANO = cyanobacteria; CHOANO = choanoflagellates; PA = photoautotrophic 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 and functional groups as response variables and abiotic parameters, and HP biomass and ciliate abundance as explanatory variables (**Figure 2.7**). The constrained variables explained 82% of inertia, and the eigenvalues for the first and second sorting axes were 0.194 and 0.077, respectively. The correlation coefficients between the environmental factors and the ranking axis of the species were 0.419 and 0.166, respectively. Dissolved oxygen, HP biomass, FI, temperature, and turbidity showed far higher correlation coefficients with the first axis (0.88, 0.64, 0.58, 0.57, and 0.56, 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. 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.

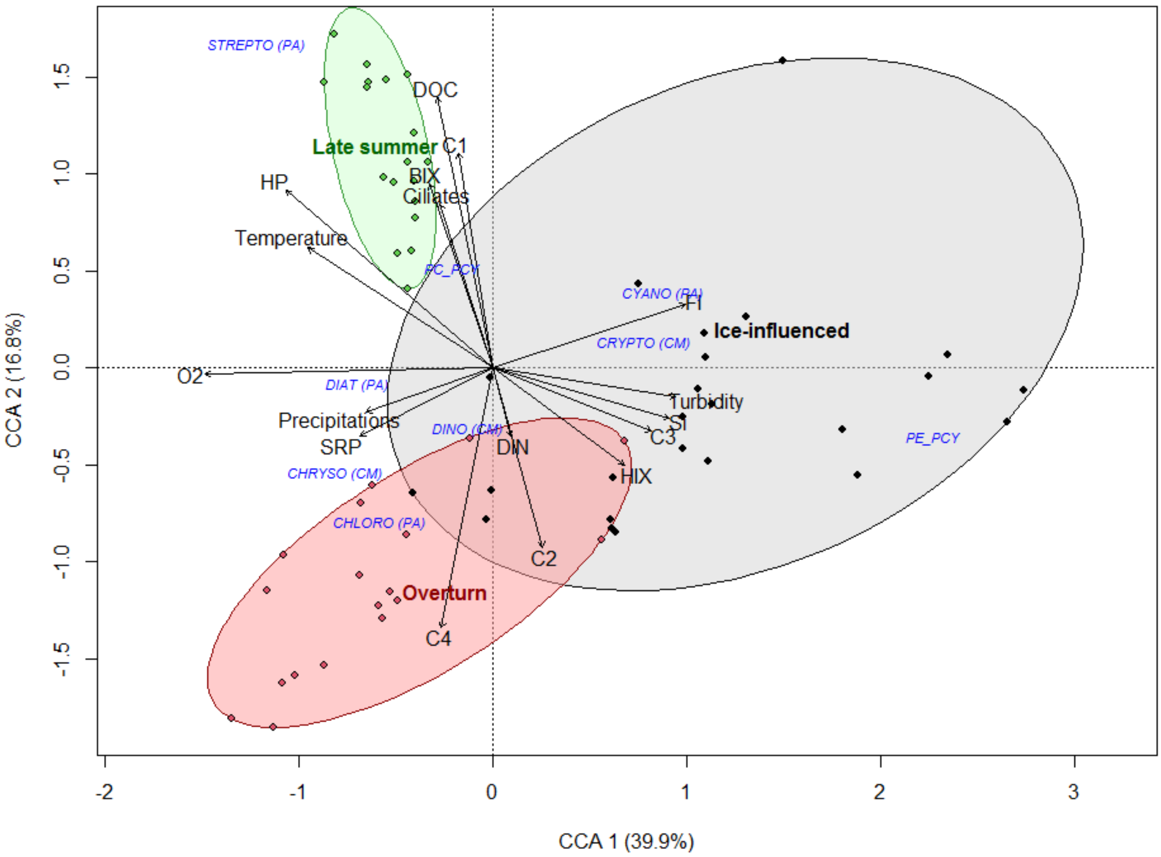

Figure 2.7. CCA performed on the taxonomic phytoplankton groups (and their corresponding PFG classification) and picophytoplankton groups (PCY) in relation to explanatory variables. DOC: dissolved organic carbon; HP: heterotrophic prokaryotes; O2: dissolved oxygen; SRP: soluble reactive phosphorus; DIN: 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.

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

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

4.2. Phytoplankton functional changes

Our results highlight the high sensitivity to environmental variations of phytoplankton assemblages, whose structure and composition changed according to shifts in lake functioning (**Figure 2.8**). 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., 2016b), 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 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 2.8**). 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.

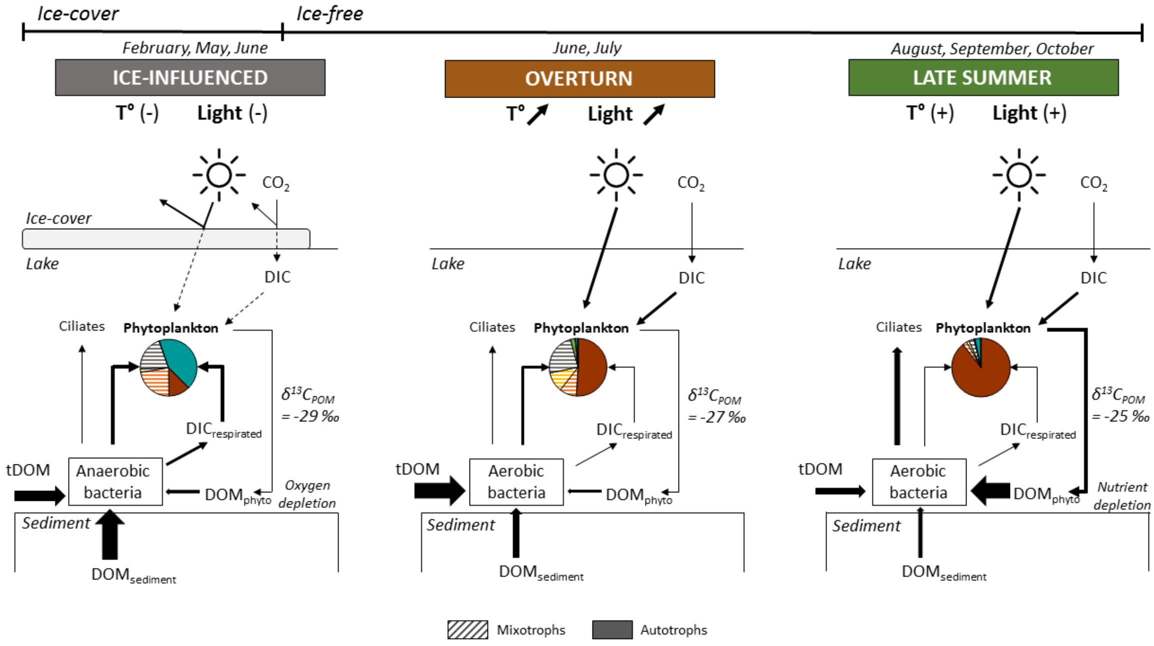

Figure 2.8. General outline reflecting shifts in phytoplankton control over bacteria in relation to dissolved organic matter properties in the Lake Cordes. tDOM: terrestrial allochthonous DOM; DOMphyto: autochthonous DOM derived from phytoplankton; DIC: dissolved inorganic carbon; T°: water temperature.

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Chapter 3

Chapter 3

Microbial consortia in an ice-covered high-altitude lake impacted by additions of dissolved organic carbon and nutrients

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Keywords

Glucose addition, Nutrient co-limitation, Heterotrophic bacteria, Mixotrophy, Trophic interactions

Abstract

1. High-altitude lakes are sentinels of change. Anticipated increases in inputs of dissolved organic carbon (DOC) from watersheds in high-altitude lakes could affect planktonic interactions, especially if inputs occur during the winter ablation phase. However, the responses of plankton communities to added DOC interacting with nutrients during this period are still not well understood.

2. We sampled under-ice water and used a multifactorial experimental design to investigate the DOC effect on planktonic organisms (phytoplankton and heterotrophic prokaryotic plankton) and their interactions during the winter ablation phase. The plankton community was subjected to DOC additions (0, 2, 4, and 6 mg C/L) under two nutrient-availability treatments (natural concentrations or enriched in N and P), two light conditions (dark or light), and two temperature conditions (10°C or 18°C).

3. We found heterotrophic prokaryotic plankton (HPP) to be co-limited by N, P, and C in our microcosms. Added glucose as available C-source for bacterioplankton was highly consumed at the end of the experiments. This consumption was not always related to an increase of the HPP biomass, due to a rising predatory control by ciliates and mixotrophic phytoplankton over bacteria. In dark conditions, the biomasses of both autotrophic and mixotrophic phytoplankton were substantially reduced, and the HPP biomass under DOC and nutrient additions principally depended on the predatory control exerted by ciliates. In light conditions, a diversification of top-down control over bacteria was observed, with the HPP response to DOC and nutrient additions depending on both mixotrophs and ciliates. Moreover, when heterotrophic ciliates and mixotrophs were present together in the experiments, the ciliates replaced the mixotrophs in phagotrophy over bacteria.

4. Our experimental results indicate that DOC inputs can rapidly alter the trophic interactions of the planktonic food web, depending on nutrient limitation in high-altitude lakes. We found decreased commensalistic interactions between bacteria and phytoplankton, but increased competition and predation, after DOC additions.

5. The expected future scenario of global change during the ablation phase is likely to change the balance between heterotrophic and autotrophic processes in planktonic food webs by

increasing heterotrophic processes. These changes could have major repercussions on energy transfer from the base to the top of the food chain in high-altitude lakes.

6. Exploring the planktonic response to rapid environmental changes during the ablation phase appears to be crucial in order to determine how vulnerable high-altitude lakes are to future change.

1. Introduction

Oligotrophic high-altitude lakes have been identified as sentinels of change (Moser et al., 2019; Williamson, Saros, Vincent, et al., 2009) and provide ideal ecosystems for studying the impacts of global change on biodiversity. Lakes in the alpine region are undergoing change driven by climate and anthropogenic factors (Smiatek et al., 2016). It has been found that atmospheric nutrient deposition of nitrogen (N) and phosphorus (P) is intercepted by mountain catchments (Battarbee et al., 2009), altering the nutrient limitations in alpine lakes (Bergström et al., 2008; Elser et al., 2009). Furthermore, the Alps have a particularly high warming rate: since the late 19th century, they have warmed twice as fast as the global or Northern Hemispheric average (Auer et al., 2007). Models of climate change also predict increasing extreme precipitation events in the alpine region during the current century (IPCC, 2013). Both the current upward shift of the treeline in alpine regions and the predicted increase in extreme precipitation events could substantially increase inputs to lakes from terrestrial runoff, including dissolved organic carbon (DOC) (Ejarque et al., 2018; Moser et al., 2019).

Increased allochthonous concentrations of DOC have been shown to seriously affect the planktonic food web functioning in aquatic systems. Inputs of allochthonous DOC fuel bacterial respiration and production (Soares et al., 2018), and reduce bacterial reliance on phytoplankton-produced carbon, particularly in oligotrophic, carbon-limiting ecosystems (Medina-Sánchez et al., 2004). High quantities of DOC modify bacterial limitation toward a primary limitation by nitrogen (N) and phosphorus (P) (Pinhassi et al., 2006), leading to direct competition with phytoplankton for these nutrients (Carney et al., 2016; Hitchcock & Mitrovic, 2013). Plankton communities dominated by bacteria are thus typically found in lakes with high inputs of allochthonous DOC (Jansson et al., 2000; Karlsson, 2001). Mixotrophic phytoplankton acquire carbon and/or mineral nutrients from bacterial prey, which allows them to eliminate their main competitors for these nutrients (Thingstad et al., 1996). Increased DOC concentrations therefore have the potential to directly alter the phytoplankton species assemblage by favoring mixotrophic flagellates relative to autotrophs when the plankton community is dominated by bacteria (Jansson et al., 1999).

Interactions between DOC and nutrients alter the net impact of DOC on aquatic systems (Hitchcock et al., 2010). Several studies reported a nutrient and carbon co-limitation in heterotrophic bacteria, leading to synergetic responses when resources were added concomitantly rather than separately (Dorado-García et al., 2014; Egli & Zinn, 2003). However, inorganic nutrients associated with dissolved organic matter may also stimulate primary productivity through enhanced nutrient availability (Deininger et al., 2017; Kissman et al., 2017). The generally low concentration of DOC in oligotrophic high-altitude lakes (commonly < 1 mg/L, see Laurion et al., 2000) makes these ecosystems highly sensitive to increased DOC inputs (Sadro & Melack, 2012). Thus, bacteria were found to depend strongly on photosynthetic labile carbon released by algae, i.e., commensalistic interactions, in high-altitude lakes (González-Olalla et al., 2018; Medina-Sánchez et al., 2004). However, little is known about the effects on planktonic organisms of a labile carbon source and its interactions with nutrients in high altitude lakes, especially during the ablation phase of the ice-cover period.

In alpine regions, ice cover is a recurrent and prolonged state for lakes, acting as a major physical control on ecosystems (Moser et al., 2019; Pernica et al., 2017). Felip et al. (2002) distinguish three phases of winter cover: formation, growth, and ablation. Ablation is influenced by water melting from the snowpack in the catchment, correlated with nutrient loading rates and availability in lakes (Leydecker et al., 2001). Dissolved organic matter of terrestrial origin and a predominantly heterotrophic epilimnion were thus observed immediately after snowmelt in a high-altitude lake of the Sierra Nevada (Sadro & Melack, 2012). Global change, by affecting the quantity of accumulated snow and the nutrient quality and quantity in the catchment snowpack, could have direct repercussions on ecosystem functioning in these lakes (Sadro et al., 2018).

The under-ice plankton communities of high-altitude lakes can tell us a lot about the effects of inputs of DOC and nutrients. Firstly, plankton communities experience the impact of inputs from the watershed and atmospheric deposition during snowmelt. Secondly, under-ice communities should be composed of taxa adapted to more heterotrophic conditions and should therefore show a clear response to DOC inputs. Finally, these communities constitute the “inoculum” of summer phytoplankton succession (Adrian et al., 1999), so that changes in community structure could significantly impact the biodiversity of high-altitude lakes. However, a clearer picture is needed of how increased allochthonous inputs during the ablation period alter the functioning of high mountain lakes, especially the trophic relationships between phytoplankton and bacterioplankton.

This study investigated a plankton community’s response to DOC inputs in interaction with inorganic nutrients during the ablation phase of the ice-covered period. We sampled the natural plankton assemblage under ice in a high-altitude lake and placed it under a dark: light cycle at 10°C in laboratory microcosms. Then we subjected it to DOC additions under two nutrient-availability conditions (ambient concentrations or enriched by N and P). Glucose was used as DOC because it's a readily available C-source for bacterioplankton. To clarify the phytoplankton-bacterioplankton relationship, a total darkness treatment was realized to target heterotrophic processes, and an 18°C treatment was realized to accelerate the metabolic processes of planktonic organisms. We hypothesized that (i) under dark conditions limiting the influence of autotrophs, the addition of DOC should favor heterotrophic bacteria and mixotrophic phytoplankton; (ii) under light conditions, the addition of DOC should have a negative effect on autotrophs but a positive effect on HPP and mixotrophs due to competition for nutrients.

2. Methods

2.1. Water sampling for experiments

Water for experiments was sampled in Lake Cordes, a high mountain lake located in the French Alps, adjacent to the Queyras regional nature park (France, 44°50'42" N, 6°47'20" E). Lake Cordes is situated above the treeline at 2 446 m a.s.l. The lake is small (1.8 ha) and shallow (maximum depth = 9 m). The catchment area is large (140 ha), consisting of developed soils and meadows with approximately 60% alpine grass cover. The catchment lithology is mainly metamorphic rock (blue Shales). This lake has been described as oligotrophic by Jacquemin et al. (2018), with strong limitation of phytoplankton by N and P. The lake is ice-covered for 7 to 8 months, from October to June.

Sampling was conducted on 26th April 2019, at the transition from the growth to the ablation phase, when ice cover is usually at maximum thickness (Catalan et al., 2006). The lake was reached by ski and the water sampled for experiments by drilling a hole in the snow and in the ice. Water samples totaling 30 L were collected using a Niskin Bottle every meter, so as to sample the entire water column. The sampled water was filtered through a 50 µm mesh to exclude larger potential grazers on food web components. This filtered water was then stored in sterilized opaque HDPE 8 l containers, placed in a cool box, and transported to the laboratory as soon as possible (between five and six hours later).

2.2. Experimental design

2.2.1. Factors and domain of interest

The aim of this study was to evaluate the effects of DOC and nutrient additions on a plankton community during the ablation phase in a high-altitude lake, particularly on interactions between heterotrophic bacteria and phytoplankton. To explore under-ice phytoplankton community responses, four factors were investigated:

- DOC additions (four treatments): 0, 2, 4, and 6 mg C/L, coded respectively [0C], [2C], [4C] and [6C].

For the DOC additions, glucose was chosen as the carbon source to ensure that only the influence of the available fraction of DOC inputs was tested. Glucose is commonly used as a source of DOC in other similar microcosm experiments (Hitchcock et al., 2010; Liao et al., 2019). It was added in relatively low concentrations (+2, +4 and +6 mg/L) consistent with theoretical values for the biodegradable DOC fraction transported into the lake during the ablation period, but sufficient to ensure that, gradually, higher than natural DOC concentrations were reached. The 0C treatments with no glucose addition were used as the DOC-control, for comparison with the DOC additions.

- Nutrient addition (two treatments): ambient-nutrient concentration [0NP] and nitrogen and phosphorus-enriched [NP].

For the nutrient addition, N was added as NH_4NO_3 to increase N concentrations to 335 µg/L and P was added as KH_2PO_4 to increase P concentrations to 35 µg/L. The aim was to avoid potential nutrient limitation throughout this experiment, while remaining consistent with natural concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) (Jacquemin et al., 2018).

- Temperature (two conditions): [10°C] and [18°C].

The 10°C temperature was chosen in order to mimic the conditions prevailing at the end of the ablation phase (a surface temperature of 2.79°C was measured on 9th June 2016 in Lake Cordes, subsequently reaching 10.27°C on 24th June 2016, pers.data). The 18°C temperature was chosen to enhance heterotrophy and accelerate the metabolic processes of organisms, without representing a threat to the planktonic organisms (Jacquemin, 2019).

- Light conditions (two conditions): total darkness [Dark] and light: dark cycle [Light].

The dark condition was intended to target heterotrophic processes while minimizing the influence of autotrophs. For the dark condition, microcosms were placed in the dark in growth chambers, surrounded by aluminum. The light condition was designed to mimic light conditions at the end of the ablation phase, after ice-out. For the light condition, microcosms were placed under a circadian cycle (Light: dark cycle = 14:10), at a fixed irradiance (45 ± 2 μmol m^{-2} s^{-1}). This irradiance was sufficient to avoid light limitation in the microcosms (Dubourg et al., 2015; Jacquemin et al., 2018).

2.2.2. Design of experiments

The experimental designs are mathematical and statistical techniques that optimize the relevance of the data obtained by quantifying the relationship between output and input variables (Box et al., 1978; Montgomery, 2017), while reducing the number of experiments required. Contrary to an OAT approach (one factor at the time), the experimental design involves simultaneous variation of all the factors, with a subset of combinations of experiments selected for modeling to estimate the effects of the four factors by considering the interactions between them. This approach, which is widely used in chemical studies, is relatively innovative in ecology.

The aim of this study was to evaluate the influence of DOC addition through interaction effects, i.e. quantifying a change in the effect of one factor according to the value of another factor. We considered the result of each experiment to be a linear combination of the main effects and interaction effects of each dimensionless factor, only taking into account interactions between two factors. This model could be written as follow:

$$Y = b_0 + b_{1A}X_{1A} + b_{1B}X1B + b_{1C}X_{1C} + b_{2A}X_{2A} + b_{3A}X_{3A} + b_{4A}X_{4A} + b_{1A-2A}X_{1A}X_{2A} + b_{1B-2A}X_{1B}X_{2A} + b_{1C-2A}X_{1C}X_{2A} + b_{1A-3A}X_{1A}X_{3A} + b_{1B-3A}X_{1B}X_{3A} + b_{1C-3A}X_{1C}X_{3A} + b_{1A-4A}X_{1A}X_{4A} + b_{1B-4A}X_{1B}X_{4A} + b_{1C-4A}X_{1C}X_{4A} + b_{2A-3A}X_{2A}X_{3A} + b_{2A-4A}X_{2A}X_{4A} + b_{3A-4A}X_{3A}X_{4A}$$

- X1 = DOC
- X2 = Nutrients
- X3 = Light
- X4 = Temperature
- b0 = model constant
- $b_{iA\ldots C}$ = coefficients for single and interactive effects

To estimate the coefficients of this linear model, we used a D-optimal design with 24 experiments, which is a subset of the experiments under the full 4*2*2*2 factorial design with 32 experiments. The linear model was also used to predict the output values for the 8 conditions not treated experimentally. The 32 experiments (24 treated experimentally and 8 modeled) are interpreted together in our study. All 24 experiments were realized in triplicate, for a total of 72 experiments (**Table A2.1**). Each experiment is a combination of one carbon treatment, one nutrient treatment, one temperature condition and one light condition. The experimental design made it possible to reduce the number of experiments experimentally realized, thereby reducing the quantity of water transported from the lake. All quantitative analyses were realized with the AZURAD software (version "Expert", AZURAD SAS, Marseille, France. 2019).

2.2.3. Experimental set-up

Plankton were first acclimatized overnight in three 10 l sterilized transparent polycarbonate plastic bottles (Nalgene®) at 10°C under the circadian cycle before the start of the experiments. Then, water from each polycarbonate plastic bottle was manually homogenized and transferred to the 72 microcosms. Each microcosm consisted of a 500 ml sterilized transparent polycarbonate plastic bottle (Nalgene®) filled with 350 ml of sampled water to allow gas exchanges, according to the protocol used by Jacquemin et al. (2018). Natural planktonic organisms were incubated under the different experimental conditions for 10 days. The microcosms were manually homogenized and randomly displaced inside the growth chamber every day.

2.3. Measurements

2.3.1. Dissolved organic carbon (DOC)

DOC was measured at the initial conditions and at the end of the experiments. Subsamples were filtered through precombusted 25 mm diameter glass filters (Whatman GF/F, 0.7 µm), then stored in 24 ml precombusted (4h at 450°C) glass tubes (Wheaton equipped with Teflon/silicone septa) and preserved with 30 µl of Sodium Azide solution (1 M NaN_3) at +4°C. DOC concentration was determined using a TOC-VCSH analyzer (Shimadzu, TOC-V). Consumed DOC was the difference between the initial DOC concentrations after carbon additions and the final DOC concentrations measured at the end of the experiments.

2.3.2. Anions and cations

Subsamples of water were filtered through precombusted 25 mm glass filters (Whatman GFF, 0.7 µm) for estimation of dissolved inorganic nitrogen (DIN = $NH4^+$ + NO_2^+ NO_3^{2-}) and soluble reactive phosphorus (SRP = PO_4^{3-}) concentrations at the initial conditions. The dissolved fraction was stored in a 125 ml HDPE bottle, placed in a cool box and frozen (−18°C) until analysis. Dissolved anions and cations were determined by ionic chromatography (Metrohm, 930 Compact IC Flex combined with 863 Compact Autosampler).

2.3.3. Chlorophyll a

Chlorophyll a concentration was measured at the initial conditions and at the end of the experiments to estimate chlorophyll biomass. Water was filtered through 47 mm glass filters (Whatman GF/C, 1.2 µm) placed in glass tubes filled with 10 ml of acetone solution (90%) for 24 hr at +4°C for chlorophyll a extraction. Chlorophyll a concentrations were measured spectrophotometrically (Jasco, V-630) by the Lorenzen method, with correction for pheophytin interference by acidification.

2.3.4. Phytoplankton

Our analyses distinguished between picophytoplankton (< 2 µm size) and larger phytoplankton (> 2 µm size), for a finer assessment of trophic functioning (Izaguirre et al., 2020).

Sub-samples of 1.5 ml were taken at the initial conditions and thereafter every 2 experimental days in the microcosms for picophytoplankton analyses by flow cytometry. Subsamples were fixed with glutaraldehyde (0.25% final concentration) and stored at -80°C until flow cytometry analysis. Picophytoplankton were characterized and enumerated using an Accuri C6 flow cytometer equipped with a blue laser (488 nm) and running 500 µl of sample at fast speed (66 µl min^{-1}). Data were acquired and analyzed using BD Accuri CFlow Plus Analysis software (BD-Biosciences, U.S.A). Picophytoplankton were identified according to size (FSC), complexity (SSC), and fluorescence (emission in the orange and red wavelength ranges, respectively 585 ± 20 nm and > 670 nm). Non-fluorescent polystyrene microspheres (Flow Cytometry Size Calibration Kit, ThermoFhischer Scientific) were used as a size standard. Flow cytometer analysis distinguished between two picophytoplankton groups. Small cells (< 2 µm) with high orange fluorescence and low granularity were classified as phycoerythrin-rich picocyanobacteria (picocyanobacteria). A picoeukaryote group was identified by its high red and low orange fluorescence, small size (< 2 µm) but higher granularity than the picocyanobacteria.

Phytoplankton > 2 µm in size were enumerated and identified via microscopy. Phytoplankton communities were analyzed before the start and at the end of the experiments. The samples for phytoplankton composition analysis were fixed with alkaline Lugol solution (0.5%) and stored in 150 ml HDPE bottles at +4°C. 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.

To ensure that picocyanobacteria and cyanobacteria > 2 µm were not double-counted, we calculated the average size of each of these groups. We estimated the mean size of picocyanobacteria from the average FSC value of their cytometric population. We then used the equation obtained from the linear regression between the size of polystyrene microspheres (Flow Cytometry Size Calibration Kit, ThermoFhischer Scientific) and their respective FSC values. For the unicellular cyanobacteria > 2 µm, we measured the length of each cell (n > 30) using NIS Element ® software, then calculated an average length for the group.

2.3.5. Heterotrophic prokaryotic plankton

Sub-samples of 1 ml were taken at the initial conditions and thereafter every 2 experimental days in the microcosms for heterotrophic prokaryotic plankton (HPP) analyses by flow cytometry. Subsamples were fixed with glutaraldehyde (0.25% final concentration) and stored at -80°C until flow cytometry analysis. After thawing, each sample was stained with 1:10000 (vol/vol) SYBR® Green II and incubated 20 min in darkness. HPP were characterized and enumerated using an Accuri C6 flow cytometer equipped with a blue laser (488 nm) and running 50 µl of stained sampled at medium speed (35 µl min^{-1}). Data were acquired and analyzed using BD Accuri CFlow Plus Analysis software (BD-Biosciences, U.S.A). HPP were identified by their small size (low FSC), low complexity (low SSC), high green fluorescence (530 ± 15 nm), and lack of red (> 670 nm) fluorescence.

2.3.6. Cellular carbon biomass calculation

To estimate the carbon biomass of the natural picoplankton organisms, abundance was transformed into carbon content based on the literature. Cell abundance (cell/mL) of heterotrophic prokaryotic plankton was converted to biomass (µg C/L) using as constant conversion factor 20 fg C/cell (Ducklow, 2000; Linacre et al., 2015). The conversion factor for picocyanobacteria was 237 fg C/cell (Gerea et al., 2019) and for picoeukaryotes it was 56 fg C/cell (Linacre et al., 2015). For phytoplankton > 2 µm, biovolume was estimated by shape assimilation to known geometric forms and direct measurement of the main cell dimensions. The mean biovolume was calculated for each class of phytoplankton, then the biovolume was converted into biomass using the particular carbon content defined for each class by Wetzel & Likens (2000).

2.3.7. Ciliate abundance estimations

We attempted here to minimize any possible predation by multicellular zooplankton and ciliates by filtering the water at 50 µm. However, to determine whether any predators had developed in our microcosms, ciliate abundances were estimated via the Utermöhl method (Felip et al., 2002), under initial conditions and at the end of the experiments.

2.4. Statistical analysis

2.4.1. Quantitative analysis

The effect of DOC additions in interaction with nutrients, with temperature, and with light was tested using the linear model estimated according to the experimental design. For each output variable, the coefficients of the model were calculated (version “Expert”, AZURAD SAS, Marseille, France. 2019) using multilinear regressions (see section 2.2.2). For the eight modeled experiments, data were calculated using the model and the error bars were calculated based on the mean standard errors of the 24 triplicates, with a 95% confidence interval.

2.4.2 Multivariate analysis

Multivariate analyses to detect changes in phytoplankton community structure were performed on the phytoplankton biomass at the end of the experiment. Multivariate analyses were performed with the “vegan” package on R software (v. 3.6.3). Because phytoplankton community structure differed strongly between the dark and the light conditions, all multivariate analyses were performed twice, under both dark and light conditions. To visualize the differences in phytoplankton community structure between treatments, we performed a non-metric multidimensional scaling (NMDS) analysis on the Bray-Curtis dissimilarity matrix to depict community structure patterns in two dimensions. To test the hypothesis that DOC addition structured the distribution of phytoplankton communities, permutational multivariate analysis of variance (PERMANOVA) was used with 999 permutations (p = 0.05) with the ADONIS function in R. We carried out a multivariate homogeneity of group dispersion analysis (beta diversity) to assess the homogeneity of phytoplankton assemblages within a group of samples (M. J. Anderson et al., 2006). A similarity percentage (SIMPER) analysis was performed to identify which phytoplankton groups govern community dynamics.

3. Results

3.1. Initial conditions

Chemical variables such as DOC, SRP and DIN were low at the start of the experiments, representative of typical oligotrophic lakes with C/N ratio = 2.5 (**Table 3.1**). Plankton biomass was dominated by HPP at the start of the experiments, but phytoplankton biomass showed a value relatively close to that of HPP biomass. Phytoplankton biomass was dominated by large diatoms such as *Fragilaria* sp. (32 %), picocyanobacteria (25 %), chrysophytes - mainly *Dinobryon* sp. - (19 %), and dinoflagellates (15 %). Unicellular cyanobacteria > 2 µm also represented a non-negligible proportion of the phytoplankton biomass.

Table 3.1. Mean values of the main chemical and biological variables measured in the initial experimental conditions. SRP = soluble reactive phosphorus; DIN = dissolved inorganic nitrogen; DOC = dissolved organic carbon; HPP = heterotrophic prokaryotic plankton.

Variable	Mean values ± SD
SRP (µg/L)	5 ± 2
DIN (mg/L)	0.27 ± 0.03
DOC (mg/L)	0.69 ± 0.12
HPP biomass (µg C/L)	17.73 ± 1.19
Chlorophyll biomass (µg/L)	1.01 ± 0.09
Phytoplankton biomass (µg C/L)	14.79 ± 2.82
Diatoms (µg C/L)	4.70 ± 0.60
Chlorophytes (µg C/L)	0.18 ± 0.07
Streptophytes (µg C/L)	0.00 ± 0.00
Chrysophytes (µg C/L)	2.83 ± 0.92
Cryptophytes (µg C/L)	0.39 ± 0.19
Dinoflagellates (µg C/L)	2.21 ± 0.60
Cyanobacteria > 2 µm (µg C/L)	0.69 ± 0.17
Picocyanobacteria (µg C/L)	3.77 ± 0.24
Picoeukaryotes (µg C/L)	0.01 ± 0.001
Ciliate abundance (cell/mL)	0.00 ± 0.00

3.2. Consumed dissolved organic carbon at the end of experiments

The DOC consumed at the end of the experiments varied according to DOC interaction with nutrients ($p<0.001$), with temperature ($p<0.001$), or with light ($p<0.001$). DOC consumed increased according to the glucose gradient in almost all the four conditions of temperature*light (**Figure 3.1**). In DOC-control treatments (0C), DOC was consumed in the dark but was in excess in the light at the end of the experiments, whatever the temperature. On the other hand, DOC was consumed more when glucose was added at 18°C than at 10°C, whatever the light condition. Finally, DOC was consumed more when glucose was added in combination with nutrient addition (NP) than in nutrient-ambient treatments (0NP).

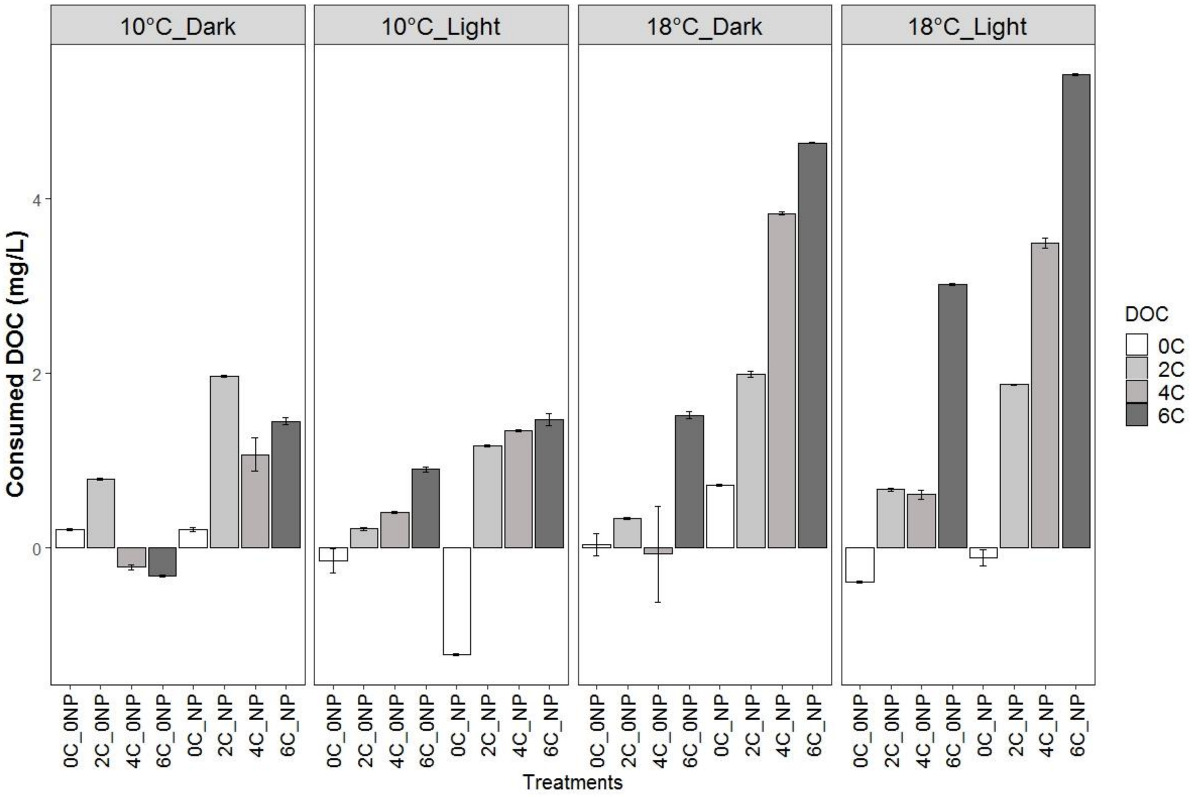

Figure 3.1. DOC consumed at the end of each experiment. Concentrations are shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3). High values indicate lower concentrations at the end of the experiments than at initial conditions, while negative values indicate higher concentrations at the end of the experiments than at initial conditions.

3.3. Heterotrophic prokaryotic plankton

Interactions between DOC and nutrients (p = 0.009), DOC and temperature ($p<0.001$), and DOC and light (p = 0.017) had significant effects on HPP biomass assessed at the end of the experiments. Under the 10°C_dark condition, addition of DOC increased HPP biomass compared to DOC-control treatments (**Figure 3.2**). Under the 10°C_light condition, addition of DOC increased HPP biomass compared to DOC-control in the ambient-nutrient treatments. HPP biomass decreased with DOC addition compared to 0C at 18°C_dark in the NP treatments, and at 18°C_light in both the 0NP and the NP treatments.

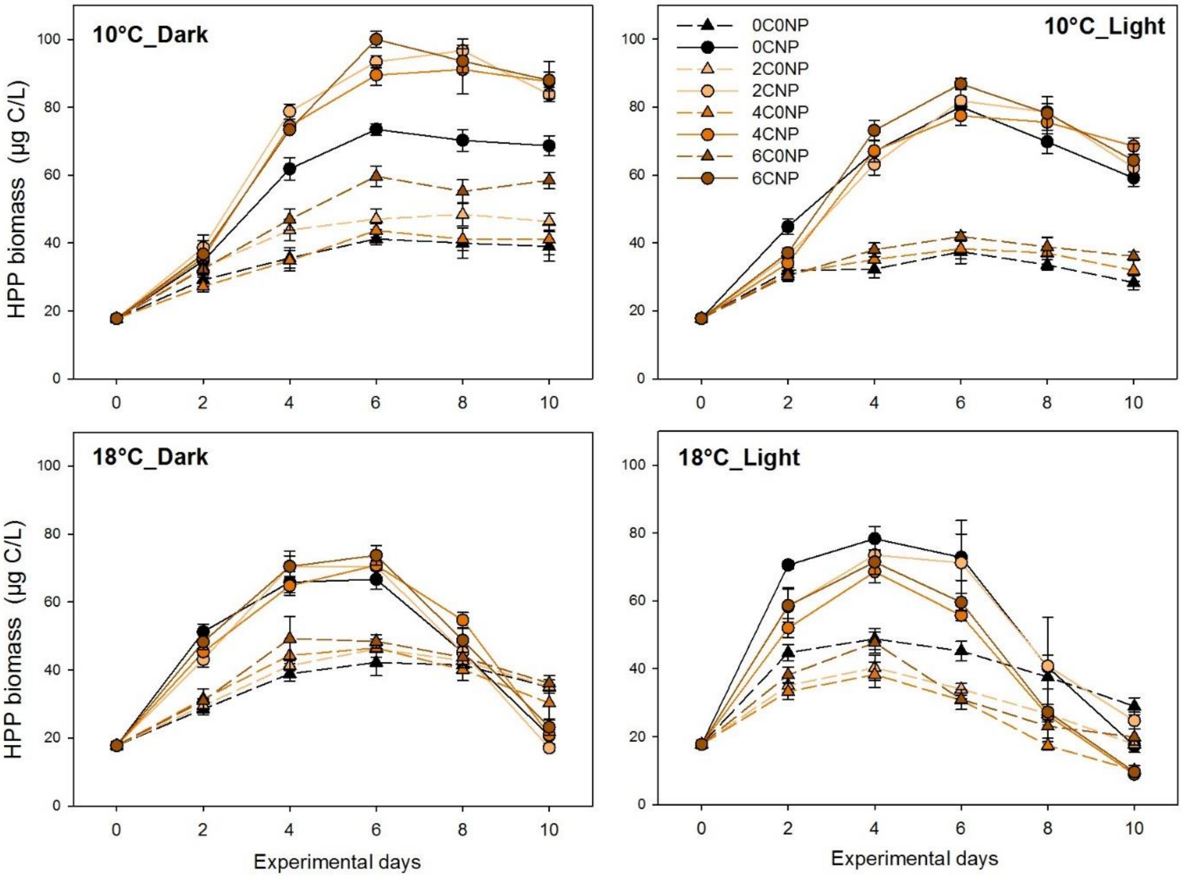

Figure 3.2. Heterotrophic prokaryotic plankton (HPP) biomass per experimental day. Biomass is shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

Moreover, interactions between nutrient addition and temperature affected HPP biomass (p < 0,001). At the end of the experiments, N and P additions increased HPP biomass at 10°C but not at 18°C. However, nutrient addition had a positive effect on HPP biomass at 18°C as well, until the 6th day of the experiments (**Figure 3.3 and Table A2.2**). This was followed by a strong decline in HPP biomass until the end of the experiments.

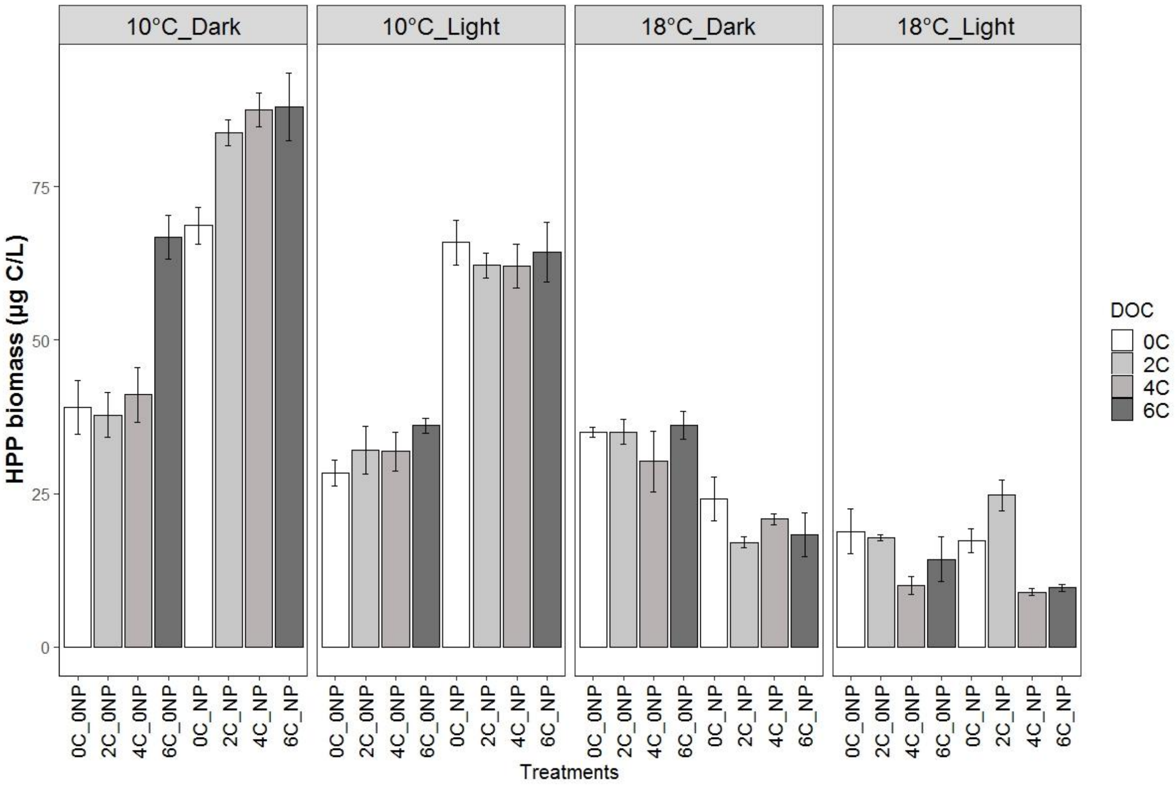

Figure 3.3. Heterotrophic prokaryotic plankton (HPP) biomass at the end of experiments. Biomass is shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

3.4. Ciliates

Interactions between DOC and nutrients (p = 0.003), and DOC and temperature ($p<0.001$) significantly affected ciliate abundance at the end of the experiments. Very few ciliates developed in the microcosms at 10°C, regardless of light condition (**Figure 3.4**). However, ciliates developed at 18°C when DOC was added either alone or in combination with nutrients. A particularly strong increase in ciliate abundance was observed when DOC was added at concentrations of 4 and 6 mg/L (4C and 6C). At 18°C, in the 4C_NP and 6C_NP treatments, ciliate abundance was higher in the dark than in the light at the end of the experiments.

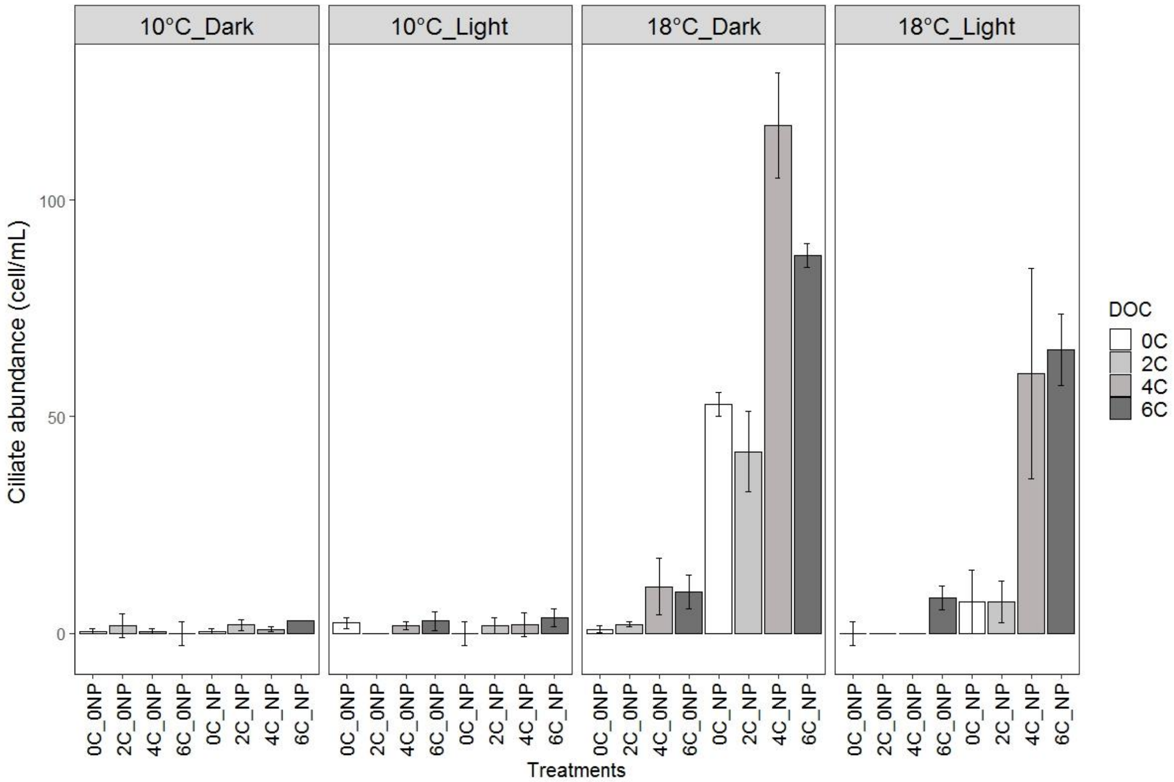

Figure 3.4. Ciliate abundances at the end of experiments. Abundances are shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

3.5. Chlorophyll biomass

There were significant effects from interactions between carbon and nutrients (p <0.001), carbon and temperature (p <0.001), and carbon and light (p <0.001) on chlorophyll biomass at the end of the experiments. In dark conditions, chlorophyll biomass was below the initial concentration of 1.01 µg/L in almost all treatments. The exceptions were the treatments at 10°C at 2C_0NP and 6C_0NP, where chlorophyll biomass reached 1.52 and 1.31 µg/L, and at 18°C at 0C_NP, where chlorophyll biomass reached 1.89 µg/L (**Figure 3.5**). In light conditions, chlorophyll biomass increased more strongly in DOC-controls than under DOC additions, except at 10°C in the 0NP treatments. This effect was more pronounced at 18°C in nutrient-enriched conditions, where chlorophyll biomass increased by 7.9 µg/L in the DOC-control but only between 4.5 and 5.1 µg/L after DOC additions.

Chlorophyll biomass was strongly correlated with final total phytoplankton biomass (Pearson's correlation, Cor=0.91, p<0.001).

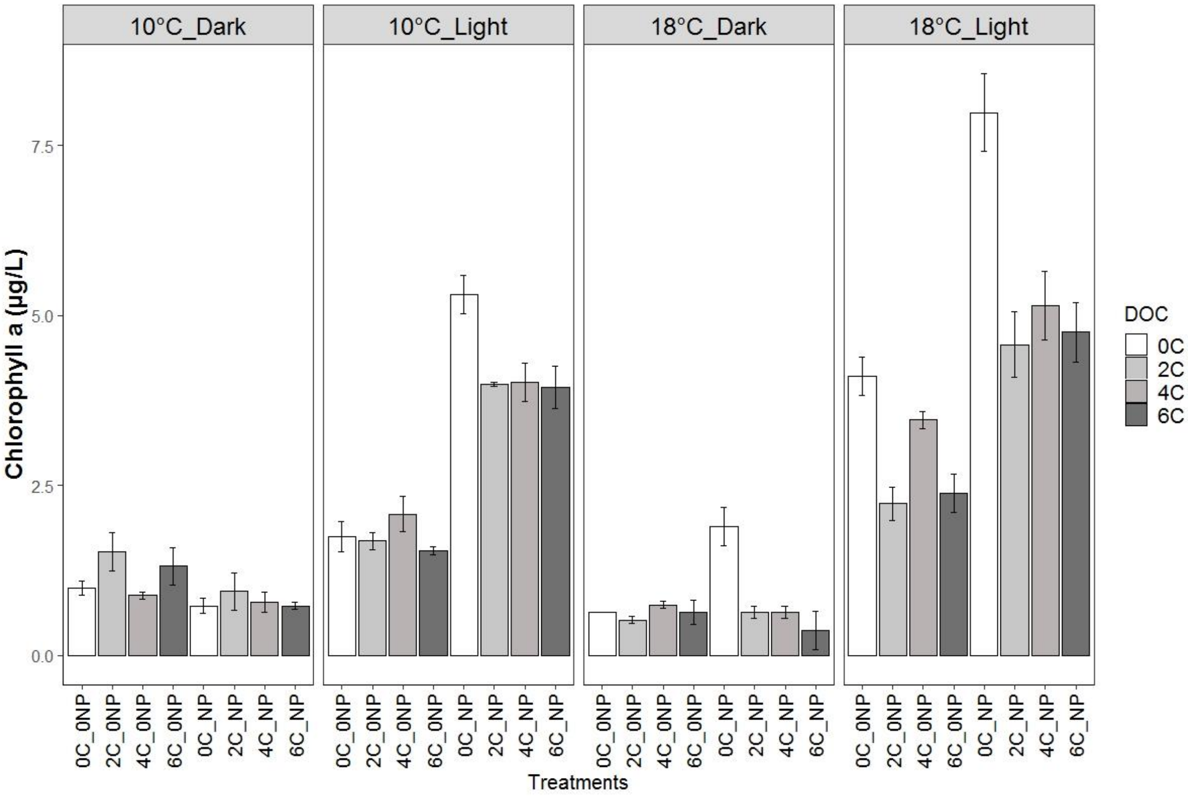

Figure 3.5. Chlorophyll biomass at the end of experiments. Concentrations are shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

3.6. Shifts in phytoplankton community structure

There were significant effects from interactions between carbon and nutrients (p = 0.003), carbon and temperature (p < 0.001), and carbon and light (p = 0.006) on total phytoplankton biomass at the end of the experiments. Permutational multivariate analysis of variance did not reveal any change in community composition under DOC addition alone, neither in the dark nor in the light.

However, ADONIS analyses showed that the interaction between DOC and nutrients led to significant differences in community structure under the different carbon*nutrients treatments, both in light ($r^2 = 0.54$, F = 5.4, p = 0.001) and in dark conditions ($r^2 = 0.30$, F = 1.9, p = 0.006). Moreover, significant differences in community structure were also detected under the different DOC*temperature conditions, both in light ($r^2 = 0.51$, F = 4.7, p = 0.001) and in dark conditions ($r^2 = 0.41$, F = 3.2, p = 0.001) (**Table A2.3**).

At the end of the experiments, five phytoplankton groups accounted for 94% of differences in community structure observed with increasing DOC (SIMPER analysis): chrysophytes (37 %), picocyanobacteria (27 %), diatoms (19 %), cyanobacteria > 2 µm (5 %), and dinoflagellates (6 %). Concerning the size of the two groups of cyanobacteria, the calculated and measured lengths were highly different between the groups: the mean size of the cyanobacteria > 2 µm was 5.04 ± 0.86 µm, and the mean size of the picocyanobacteria was 1.5 ± 0.03 µm (data not shown).

Interactions between carbon and nutrients ($p < 0.001$), carbon and temperature ($p < 0.001$), and carbon and light ($p < 0.001$) were shown at the end of the experiments to have significantly affected picophytoplankton biomass, mainly composed of picocyanobacteria (**Table A2.4 and figure A2.1**). Moreover, each interaction between DOC and temperature, light, and nutrients at the 10th experimental day was representative of the other experimental days. The addition of DOC had a negative effect on picocyanobacteria biomass in all conditions of temperature*light, except in the 10°C_dark condition (**Figure 3.6**). In the 10°C_light condition, picocyanobacteria biomass decreased with DOC addition only when nutrients were added to the microcosms (NP). In the 18°C_dark and 18°C_light conditions, picocyanobacteria biomass decreased with DOC addition at 4C and 6C in both NP and 0NP treatments.

For the mixotrophic taxa, DOC addition had a positive effect on the relative biomass of mixotrophic phytoplankton, depending on taxonomic groups, conditions of temperature*light, and nutrient addition. The relative biomass of chrysophytes increased with DOC addition in the 10°C_light condition in the NP treatments. The relative biomass of cryptophytes increased weakly with DOC addition in the 18°C_dark condition at 6C compared to DOC-controls, in both 0NP and NP treatments (**Figure A2.2**). The relative biomass of dinoflagellates increased weakly with DOC addition in the 18°C_dark condition in 0NP treatments. Finally, the relative biomass of cyanobacteria > 2 µm increased with DOC addition when nutrients were added to the microcosms, in all temperature*light conditions except the 10°C_dark condition.

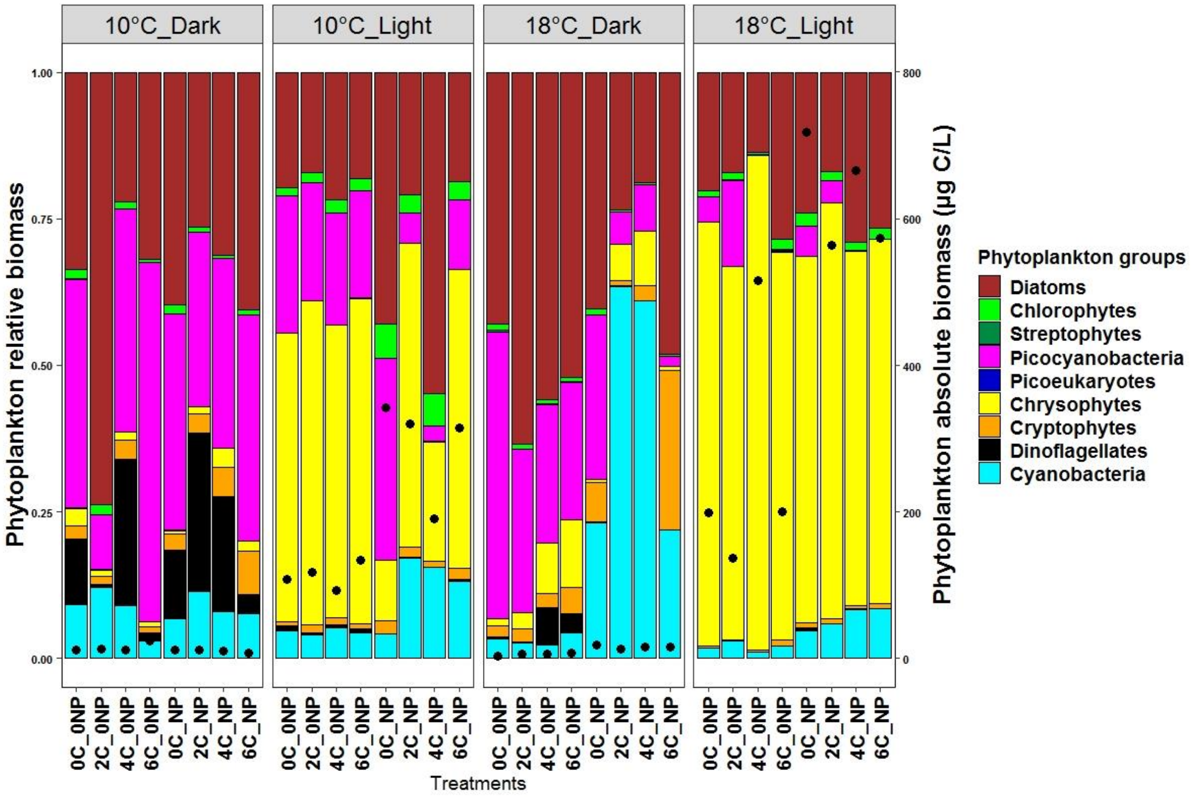

Figure 3.6. Phytoplankton groups' relative biomass and total absolute biomass (black points) at the end of experiments. Biomass is shown for each DOC*nutrient treatment under the four temperature*light conditions.

4. Discussion

The ablation phase is a crucial period in the functioning of high altitude lakes (Felip et al., 2002), because allochthonous inputs can directly affect the food web (Kissman et al., 2017) and therefore govern summer plankton communities. This study sought to assess how planktonic organisms respond to the joint addition of DOC and nutrients during the winter ablation phase in a high-altitude lake. We specifically used a multifactorial experimental design to focus on the phytoplankton – bacterioplankton relationship.

4.1. DOC and nutrient additions in heterotrophic dark conditions

HPP limitation and top-down control regulation

At the end of the experiments, glucose was consumed in the dark conditions, and more glucose was consumed when nutrients and glucose were added together. Moreover, we found an effect of interaction between DOC and nutrients on HPP biomass. At 10°C, this interactive effect resulted in a higher HPP biomass when nutrients and DOC were added together than under single additions of DOC or nutrients, evidence of co-limitation of HPP by C, N, and P. Several previous studies also deduced that there was co-limitation by nutrients and carbon in microbial communities of oligotrophic lakes (Vidal et al., 2011), finding stronger responses to combined resources than to single-nutrient additions. Particularly in arctic and alpine lakes, bacterial growth appears to be highly limited by low natural concentrations of inorganic nutrients and organic carbon (Bertoni et al., 2008; Granéli et al., 2004).

By contrast, we did not observe any positive effect of DOC additions on HPP biomass in our microcosms at 18°C in the dark conditions, neither when added alone nor when combined with nutrients. Ciliate abundance in our microcosms was stimulated by DOC addition at 18°C, especially under combined additions of DOC and nutrients. The strong decline in HPP biomass from the 6th day until the end of the experiments at 18°C further supports increased predatory pressure from ciliate development in our microcosms. Therefore, the observed HPP response to DOC additions at 18°C was likely the result of strong predation by ciliates. Ciliates are acknowledged to be major consumers of bacteria (Ameryk et al., 2005). In earlier mesocosm experiments, flagellates and ciliates were found to increase following DOC additions (Blomqvist et al., 2001) via a transfer of bacterial production up to these predators. Our findings support the idea that the presence of ciliates acts as a major control of HPP biomass. Moreover, the magnitude of the predatory control increased substantially with changing environmental conditions and overtook the bottom-up effect of DOC and nutrients on HPP biomass.

Phytoplankton response to DOC and nutrients in the dark

Both chlorophyll and phytoplankton biomass were substantially lower in the dark, indicating a strong reduction of phytoplanktonic influence in these experimental conditions. Due to this very low biomass, we did not observe a clear response to the DOC additions in the dark from either mixotroph or autotroph phytoplankton. There was only a slight tendency to increased biomass of cyanobacteria > 2 µm and of cryptophytes biomass under DOC and nutrient additions at 18°C. Although weak, this tendency confirms that cryptophytes are capable of prey ingestion in darkness (McKie-Krisberg et al., 2015), considered by several studies as a possible adaptation to permanent ice-cover in Antarctic lakes (Marshall & Laybourn-Parry, 2002). Moreover, even though cyanobacteria > 2 µm are considered strictly photoautotrophic in freshwater (Reynolds, 2006), glucose assimilation has been observed in some filamentous species in a deep mesotrophic perialpine lake (Zotina et al., 2003) and in several genera of cyanobacteria in a Siberian saline lake (Quesada et al., 2002). Our results suggest that unicellular cyanobacteria > 2 µm could also have played a role in glucose consumption in the dark in our microcosms.

Thus, contrary to our expectations, we found that mixotrophs were not particularly favored by DOC additions in the dark and that bacterivory by mixotrophs had only a minor impact on the HPP response to DOC addition. Mixotrophy is known to be an advantageous strategy in light-limiting and nutrient-limiting environments, despite a higher energetic cost compared to the obligate autotrophic or heterotrophic metabolism (Mitra et al., 2014). Acquiring carbon and/or mineral nutrients from bacterial prey allows mixotrophs to eliminate their main competitors for these nutrients, called the “Eating your competitor” strategy by Thingstad et al. (1996). However, it was also experimentally demonstrated that mixotrophic bacterivory on picoplankton was light-dependent (Fischer et al., 2017a). This dependency has been interpreted as a need for energetic photosynthesis to support phagotrophy (Li et al., 2000).

Overall, DOC additions stimulated HPP biomass in the dark, particularly when combined with addition of nutrients because of the co-limitation of HPP by C, N, and P. However, our results did not confirm our hypothesis regarding mixotrophic phytoplankton. When the influence of commensalistic and competitive interactions between phytoplankton and bacteria were reduced, ciliate predatory control was the major control of the HPP response to DOC addition.

4.2. DOC and nutrient additions in light conditions

Diversification of top-down controls in the light

As in the dark conditions, HPP biomass increased only at 10°C with DOC additions, but only when DOC was added alone. Moreover, the increase of ciliates at 18°C and the decrease of HPP biomass from the 6th day until the end of the experiments at 18°C suggested the same predatory control by ciliates over HPP in the light conditions. Multivariate analyses also revealed significant effects on the phytoplankton community from interactions between the DOC*nutrients treatments and between the DOC*temperature treatments. These interactive effects resulted in increased biomass of chrysophytes after combined DOC and nutrient addition at 10°C alone.

Our results reflected a diversification of top-down control in the light conditions, with potential ingestion of HPP by mixotrophs at 10°C under combined additions of DOC and nutrients. In the light conditions, mixotrophic phytoplankton and ciliates competed for the same prey in our microcosms. Previous studies reported competition between mixotroph and heterotrophic bacterivores for prey in the light (Fischer, Giebel, & Ptacnik, 2017b). Although temperature optima differ between species, most ciliates tolerate a relatively wide range of temperatures, with high growth rates at high temperatures (Weisse, 2006). Moreover, the metabolic theory of ecology predicts that the heterotrophic metabolism will respond more strongly to rising temperature than the autotrophic metabolism (Rose & Caron, 2007). Our results show that DOC additions stimulated the mixotrophic strategy of "eating your competitor" at 10°C when nutrients were added to the microcosms, while prey ingestion by ciliates replaced ingestion by mixotrophs at a higher temperature.

Competition processes with autotrophs

We found a strong decrease in picocyanobacteria biomass with DOC additions in the light, except at 10°C without nutrients. Previous studies reported a competitive advantage of heterotrophic bacteria over picocyanobacteria, especially after carbon inputs (Drakare, 2002; Liao et al., 2019). We therefore suggest that the observed decrease in picocyanobacteria could be due to competition between HPP and picocyanobacteria for nutrients. Added to the competition with HPP, picocyanobacteria can also suffer predation by mixotrophs and ciliates. In shallow oligotrophic lakes, ingestion of picocyanobacteria by mixotrophic flagellates was recently observed (Gerea, Queimalinos & Unrein 2019), notably by chrysophytes in subalpine lakes (Weisse & Moser, 2020). Unfortunately, our experiments did not enable us to determine the proportions of the decrease in picocyanobacteria explained by competition with HPP and by predation by bacterivores.

Contrary to the picocyanobacteria, the biomass of the cyanobacteria > 2 µm increased with combined additions of DOC and nutrients in the light. As in the dark, this increase indicates that the cyanobacteria > 2 µm were capable of carbon assimilation and depended on a sufficient stoichiometric C: NP ratio to develop in the light conditions.

Overall, DOC additions in the light stimulated the biomass of HPP and mixotrophs, and predation by mixotrophs acted as a strong top-down control of HPP biomass at 10°C. While DOC addition decreased autotrophic picocyanobacteria, the proportions of this decrease explained by competition with HPP and by predation by ciliates and mixotrophs could not be determined from our experiments. As in the dark, the predatory control exerted by ciliates on HPP biomass was the major control at the higher temperature.

4.3. Primarily nutrient limitation and commensalistic interaction

We found that DOC concentrations increased in the DOC-control treatments in the light conditions, but not in the dark, which indicates two indirect results: (i) phytoplankton-derived carbon was produced in the light; (ii) control by phytoplankton-derived carbon was severely limited in the dark at the end of the experiments. Moreover, HPP biomass in the dark conditions revealed that HPP were able to develop with added nutrients alone, evidence of limitation primarily by N and P of the HPP in our microcosms. Due to the generally low DOC concentrations in high-altitude lakes, heterotrophic bacteria are known to be primarily carbon-limited in these environments (Carrillo et al., 2002; Durán et al., 2016; Medina-Sánchez et al., 2010). Nevertheless, limitation primarily by nutrients and co-limitation by C of bacteria was previously observed in an oligotrophic mountain lake (Dorado-García et al., 2014). This pattern of co-limitation was explained by the autotrophic nature of the ecosystem, where carbon derived from phytoplankton was sufficient to support bacterial demands. In our experiments, while the initial phytoplankton biomass was slightly smaller than the HPP biomass, it may have been sufficient to generate limitation of HPP primarily by N and P and co-limitation by C. Despite stronger limitation by nutrients than by carbon, an increase in HPP biomass was observed in our microcosms after DOC additions. This could be interpreted as a release from HPP dependency on photosynthetic carbon excreted by phytoplankton, i.e., a decrease in commensalistic interactions between HPP and phytoplankton.

4.4. Ecological implications

Our results support previous findings that DOC inputs can greatly alter the food web in high-altitude lakes (Kissman et al., 2017), particularly if they occur during the ablation phase. Our results suggest that DOC inputs during the ablation phase, depending on nutrient limitation, may rapidly alter the trophic interactions between heterotrophic bacteria and phytoplankton. These changes are likely to involve decreased commensalistic interactions between bacteria and phytoplankton but increased competition with autotrophic picocyanobacteria, and an increase and diversification of predatory top-down controls over bacteria. Since the winter communities constitute the “inoculum” of the summer succession in high-altitude lakes (Adrian et al., 1999), rapid changes in trophic relationships between bacteria and phytoplankton can have major repercussions on the general functioning of lakes. While bacteria would benefit more than phytoplankton from labile DOC inputs in a future scenario of global change, higher predatory control over bacteria is also expected. Thus, our findings support a future scenario of global change involving modifications in the balance of heterotrophic vs autotrophic processes toward higher heterotrophy during the ablation phase in high-altitude lakes. The predicted change will vary according to: (i) the characteristics of the dissolved organic matter, i.e., nutrient content, lability of the carbon source, and proportion of humic colored substances; (ii) type of top-down control, such as predation by zooplankton or ciliates; (iii) the composition of phytoplankton assemblages, including the proportion of mixotrophic phytoplankton.

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Chapter 4

Chapter 4

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

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Keywords

Glucose additions, Phytoplankton-bacterioplankton relationship, Mountain lakes, Nutrient limitation, Co-limitation, Mixotrophy

Abstract

The predicted increase in allochthonous dissolved organic carbon (DOC) in high-altitude lakes is expected to alter the phytoplankton-bacterioplankton relationship. However, few studies address the influence of summer phytoplankton dynamics on microbial responses to DOC additions. We sampled natural plankton assemblages during two contrasting periods of summer in a high-altitude lake in the French Alps and subjected them to glucose and nutrient enrichments under two light conditions (dark or light) and two temperature conditions (10°C or 18°C). Our results indicate that glucose use by bacteria differs over the summer, depending on the availability of autochthonous DOC and the nutrient limitation. Glucose was consumed by bacteria more in early summer, however biomass increased with glucose addition more in late summer than in early summer. This pattern arose from the greater availability of phytoplankton-derived DOC in late summer, reducing the need for alternative carbon sources in late summer, when phytoplankton biomass was high. Mixotrophic taxa were stimulated after glucose additions both in early summer and in late summer. We found greater competition between bacteria and phytoplankton in late summer after glucose addition, linked to the summer nutrient limitation pattern. Our study thus highlights a differential response depending on the timing of summer DOC inputs. The global changes forecast for the French Alps should increase heterotrophic and mixotrophic processes in planktonic communities of shallow high-altitude lakes with vegetated catchments. This experimental study provides insights that will be useful in predicting ecological trajectories and in refining predictions of sentinel lakes' responses to global changes.

1. Introduction

Dissolved organic carbon (DOC) inputs associated with terrestrial dissolved organic matter (DOM) have substantially increased in recent decades in lake ecosystems, and will likely continue to evolve (Solomon et al., 2015). Dissolved organic carbon acts as a major regulator of lakes' functioning (Williamson et al., 1999). Dissolved organic carbon concentration and quality have been shown to alter water transparency and thermal stratification (Solomon et al., 2015), modify pH and alkalinity (Evans et al., 2005) and attenuate harmful ultraviolet radiation in lakes (Warner et al., 2020). By changing the lakes' pool of available nutrients, DOC acts as an important driver of bacterioplankton biomass (Schulhof et al., 2020), phytoplankton biomass (Hazukova et al., 2021; Isles et al., 2021) and their relationships in lake ecosystems. Allochthonous DOC inputs are consequently directly related to the degree of net heterotrophy in lakes (Jansson et al., 2008). Through its effects on planktonic processes, allochthonous DOC regulates energy transfer to higher trophic levels (Jansson et al., 2000), food web dynamics, and lake metabolism (Mitrovic & Baldwin, 2016; Sadro, Melack, et al., 2011a). However, its influence depends on the trophic nature of lake ecosystems (Dorado-García et al., 2014).

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

However, it has also been observed in temperate lakes that autochthonous DOC derived from phytoplankton is preferentially degraded over allochthonous DOC, owing to its greater accessibility and nutritional quality (Guillemette et al., 2013). The quantity of phytoplankton biomass and primary production, which together regulate the bioavailability of autochthonous organic carbon, condition the C-limitation of bacteria and thus bacterial abundance and production in lakes (Kritzberg et al., 2005; Morán et al., 2010). As an illustration, in oligotrophic ecosystems where phytoplankton-derived organic carbon was sufficient to support bacterial demands, bacteria did not experimentally respond to C additions like sucrose because of primary limitation by inorganic nutrients (Dorado-García et al., 2014). This nutrient limitation has also been shown to limit allochthonous DOC consumption by bacterioplankton in a high-altitude lake (Nelson & Carlson, 2011). The quantity of phytoplankton biomass, by regulating the quantity of autochthonous DOC bioavailable for bacteria, could therefore modify the microbial response to allochthonous DOC inputs. In addition, evidence suggests that the nature of the molecules liberated in the water differs among phytoplankton species (Romera-Castillo et al., 2010; Sarmento & Gasol, 2012) and the uptake of autochthonous DOC by bacteria varies according to the phytoplankton species composing the bulk of the community (Sarmento et al., 2013). Thus, phytoplankton biomass and composition, by exercising a dual bottom-up and top-down control over bacteria, could impact the microbial response to allochthonous DOC inputs in oligotrophic lakes. However, little is known about the mechanisms through which phytoplankton biomass and composition regulate the microbial response to DOC additions in oligotrophic ecosystems.

In high-altitude lakes, DOC concentrations vary seasonally (Nelson, 2009), but are generally low (< 1 mg C/L, see Laurion et al., 2000), and these ecosystems could respond significantly to increased flux from the surrounding catchments. In mountain lakes, most DOC is of autochthonous origin during summer (Reche et al., 2001; Sadro & Melack, 2012). However, the observed upward shift in the treeline (Harsch et al., 2009) and the predicted increase in extreme precipitation events (IPCC, 2013) are likely to increase allochthonous DOC fluxes to high-altitude lakes. Recent studies highlight the sensitivity of mountain lakes to climate changes (Sadro et al. 2019; Moser et al. 2019). Higher DOC values associated with wetter periods were observed between 2015 and 2017 in subalpine (Ejarque et al., 2018) and mountain lakes (Moser et al., 2019). This increased carbon flux may cause dramatic changes in mountain lakes' metabolism (Sadro & Melack, 2012).

In high-altitude lakes, rapid environmental changes usually induce rapid modifications of phytoplankton community composition over a short period (Tiberti et al., 2013; Ventura et al., 2000). For example, Jacquemin et al. (2018) observed changes in the structure and composition of phytoplankton communities, associated with an evolution of limitation patterns, during summer in alpine lakes. Due to poor light conditions, mixotrophic species dominate autotrophic ones during the ice-covered period (Leppäranta, 2015) and are a major component of phytoplankton assemblages of high-altitude lakes (Tolotti et al., 2003b, 2006), especially in early summer (Waibel et al., 2019). Rapid changes in chemical parameters are also conditioning biomass dynamics in high-altitude lakes (Bergström et al., 2020; Oleksy et al., 2020b). A bimodal pattern in phytoplankton biomass dynamics has been observed, with a first peak immediately after the snowmelt and a second one occurring at the end of the summer period (Tiberti et al., 2013). Since phytoplankton is an important regulator of bacteria in ecosystems receiving few allochthonous inputs, phytoplankton communities' seasonal dynamics in terms of biomass and composition should regulate the impact of DOC inputs on phytoplankton-bacterioplankton relationship in high-altitude lakes. However, few studies address the influence of summer phytoplankton dynamics on phytoplankton-bacterioplankton relationship in response to DOC additions in high-altitude lakes.

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

2. Methods

2.2. Study area and sampling

Water for experiments was sampled in Lake Cordes, an oligotrophic high-mountain lake located in the French Alps, adjacent to the Queyras regional natural park. The lake is located at an elevation of 2446 m a.s.l. It is shallow (maximum depth = 9 m) and small (1.8 ha) but has a large catchment area (140 ha) with developed soil and meadows (approximately 60% alpine grass cover). Water sampling campaigns were conducted twice over the summer, on 7th July 2019 (early summer) and on 28th August 2019 (late summer). Turbidity was high in early summer (average turbidity = 7 NTU), related to frequent previous storms, but low in late summer (average turbidity = 0.5 NTU). Water samples totalling 42 L were collected using a Niskin Bottle every metre from 1 to 8 meters to sample the entire water column and were 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.

2.3. Enrichment experiments under controlled conditions

2.3.1. Experimental design

Microbial community was acclimatized overnight in 10 L sterilised transparent polycarbonate plastic bottles (Nalgene®) at 10°C, which corresponded to an average summer temperature previously measured at the bottom of this lake during a previous study (Jacquemin et al., 2018). Physico-chemical and biological variables were measured in each bottle to define initial conditions before the start of the experiments. Then water from each bottle was manually homogenised and transferred to 96 microcosms. Transparent sterilised polycarbonate plastic bottles (Nalgene®) were filled with 350 ml of sampled water. In C treatments, glucose was added to the microcosms as an available DOC source at four different concentrations: no addition, + 2 mg $C.L^{-1}$ (+ 166 $\mu mol.L^{-1}$), + 4 mg $C.L^{-1}$ (+ 330 $\mu mol.L^{-1}$), and + 6 mg $C.L^{-1}$ (+ 500 $\mu mol.L^{-1}$) (**Figure 4.1**). In nutrient treatments, planktonic communities were either not enriched [0NP] or enriched with N and P [+N+P]. N was added as NH_4NO_3 sufficient to increase N concentrations to 335 µg $N.L^{-1}$ (23.9 $\mu mol.L^{-1}$) and P was added as KH_2PO_4 sufficient to increase P concentrations to 35 µg $P.L^{-1}$ (1.1 $\mu mol.L^{-1}$). The final DIN:SRP molar ratio of the nutrient enrichment was 21.72. In LIGHT conditions, the microcosms were placed under a circadian cycle (Light: dark cycle = 14:10), at a fixed irradiance (45 ± 2 μmol m^{-2} s^{-1}). In DARK conditions, the microcosms were placed in the dark in growth chambers, surrounded by aluminium, to target heterotrophic processes while minimising the influence of active and immediate photosynthesis. Finally, the microcosms were subjected to temperatures of either 10°C (no increase compared to acclimation, representing the average summer temperature), or 18°C (increase of 8°C compared to acclimation), sufficient to accelerate the metabolic processes of organisms without representing a threat to the planktonic organisms (Jacquemin et al., 2018). 18°C represents an increase of 2.5°C compared to the maximum temperature measured in this lake (15.5°C was measured on August 11th, 2019). The enrichments and the changes in temperature and light conditions were carried out just after the overnight acclimation and marked the beginning of the experiment. Thus, the experimental set-up consisted of a 4 x 2 x 2 x 2 factorial design (32 treatments in triplicate) implemented for 10 days, as in our previous study (Dory et al., 2021).

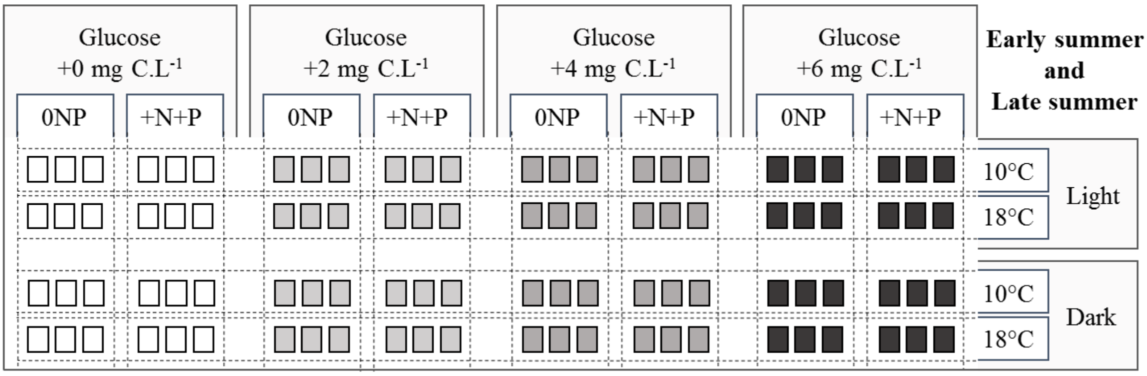

Figure 4.1. Experimental design performed in early summer and in late summer, with four carbon additions (0, 2, 4, 6 mg $C.L^{-1}$), two nutrient concentrations (0NP and +N+P), two light treatments (Light and Dark), and two temperatures (10°C and 18°C), in three replicates.

2.3.2. **Physico-chemical analysis**

Average in situ temperatures were calculated from the mean temperatures measured every metre on the water column during the sampling dates (hourly observations). Sub-samples for dissolved inorganic nitrogen (DIN = NH_4^+ + NO_2^- + NO_3^-), soluble reactive phosphorus (SRP = PO_4^{2-}), and dissolved organic carbon concentrations were filtered through pre-combusted 25 mm glass filters (Whatman GF/F, 0.7 mm) prior to launching the experiments. The dissolved fraction for DIN and SRP was stored in a 125 ml HDPE bottle, and frozen (-18°C) until analysis. DIN and SRP were determined by ionic chromatography (Metrohm, 930 Compact IC Flex combined with 863 Compact Autosampler). Dissolved organic carbon was measured under initial conditions and at the end of the experiments. Subsamples stored in 24 ml pre-combusted (4 hr at 450℃) glass tubes (Wheaton equipped with Teflon/silicone septa) and preserved in 30 µl of sodium azide solution (1 M N_aN_3) at +4℃. DOC concentration was determined using a TOC-VCSH analyzer (Shimadzu, TOC-V). We calculated consumed DOC as the difference between the initial DOC concentrations after glucose additions and the final DOC concentrations measured at the end of the experiments. Consumed DOC thus refers to the overall use of DOC, reflecting both respiration and assimilation.

2.3.3. **Biological variables**

Phytoplankton

Phytoplankton were enumerated and identified via microscopy. Phytoplankton communities were counted and analysed at the initial conditions and at the end of the experiments. The samples destined for phytoplankton composition analysis were fixed with alkaline Lugol solution (0.5%) and stored in 150 ml HDPE bottles at +4℃. 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.

In addition, we checked for any development of predators in our microcosms by estimating ciliate abundances via the Utermöhl method, under initial conditions and at the end of the experiments.

Picoplankton

Subsamples of 1.5 ml were taken under initial conditions and thereafter every 2 experimental days in the microcosms for picoplankton analyses by flow cytometry. Subsamples were fixed with glutaraldehyde (0.25% final concentration) and stored at −80℃ until flow cytometry analysis. Picoplankton was characterised and enumerated using an Accuri C6 flow cytometer equipped with a blue laser (488 nm) and using BD Accuri CFlow Plus Analysis software (BD-Biosciences). For picophytoplankton, 500 µl of sample were run at fast speed (66 $\mu l.min^{-1}$). Picophytoplankton were identified according to size (FSC), complexity (SSC) and fluorescence (emissions in the orange and red wavelength ranges, respectively 585 ± 20 and

>670 nm). Non-fluorescent polystyrene microspheres (Flow Cytometry Size Calibration Kit, Thermo Fisher Scientific) were used as a size standard. Flow cytometer analysis distinguished between two picophytoplanktonic groups. Small cells (<2 µm) with high orange fluorescence and low complexity were classified as phycoerythrin-rich picocyanobacteria (picocyanobacteria). A picoeukaryote group was identified by its high red and low orange fluorescence, small size (<2 µm) but higher complexity than the picocyanobacteria. We checked for any double-counting in cytometry and microscopy by calculating the average size of each picophytoplankton and phytoplankton group, as described in Dory et al. (2021). For heterotrophic prokaryotic plankton, samples were stained with 1:10,000 (vol/vol) SYBR® Green II and incubated 20 min in darkness. 50 µl of stained samples were run at medium speed (35 µl.min^{-1}). For the rest of the manuscript, prokaryotes will be used to designate heterotrophic prokaryotic plankton. Prokaryotes were identified by their small size (low FSC), low complexity (low SSC), high green fluorescence (530 ± 15 nm), and lack of red (> 670 nm) fluorescence. This counting method allowed us to exclude all autotrophs containing photosynthetic pigments, thus exclusively heterotrophic bacteria were counted (Gasol & Giorgio, 2000; Shapiro, 2005). Picoplankton data were acquired and analysed using BD Accuri CFlow Plus Analysis software (BD-Biosciences). Prokaryotes biomass was presented between 0 and 6 days to avoid uninterpretable results caused by grazing and viral lysis after few days in the bottle (Ammerman et al., 1984). Complete dynamics are showed in supplementary information (see section 3.2.2). Prokaryote biomass was analysed as start-end changes for days 2, 4 and 6, and statistical analyses were showed for the start-6-day change.

Cellular carbon biomass calculation

Abundances were transformed into carbon content based on the literature. Cell abundance (cells.mL^{-1}) of prokaryotes was converted to biomass (µg C.L^{-1}) using 20 fg C.cell^{-1} as constant conversion factor (Ducklow, 2000; Linacre et al., 2015). A conversion factor of 237 fg C.cell^{-1} was used for picocyanobacteria (Gerea et al., 2019) and a conversion factor of 183 fg C.µm^{-3} for picoeukaryotes (Lagaria et al., 2017). For phytoplankton, biovolume was estimated by shape assimilation to defined geometric forms and direct measurement of the main cell dimensions. Mean biovolume was calculated for each class of phytoplankton and then converted into biomass using the particular carbon content defined for each class by Wetzel & Likens (2000).

2.4. Data analysis

Quantitative analysis

For initial conditions, all biological and physico-chemical variables were checked for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Mean values were compared between early summer and late summer using Student or Welch t-tests.

For final conditions, we analysed DOC concentrations and prokaryote biomass in start-end change (delta). Phytoplankton biomass and composition, mixotrophs:autotrophs ratio, and ciliate abundance were analysed at the end of the experiments. Linear models were used to investigate how campaign, glucose, nutrients, light, and temperature affect DOC consumption, prokaryote biomass, phytoplankton biomass, mixotrophs:autotrophs ratio, and ciliate abundance. Biomass and abundance data were investigated using log-linear models. To avoid a violation of model assumptions, we fitted all the models with the five explanatory variables and their interactions, then we decided to drop explanatory variables and interactions that were not significant one by one. Thus a stepwise backward model selection based on the Chi squared statistic within maximum likelihood ratio tests was carried out for all models (Zuur et al., 2009). The models were refitted at each time and we graphically checked the model validation. Pairwise differences in glucose and nutrient additions between campaigns, light, and temperature were tested using post-hoc Tukey HSD tests. Quantitative analyses were performed with R 3.6.3 (R Core Team, 2018). The “emmeans” library was used for Tukey's tests.

Multivariate analysis

We performed multivariate analyses on the phytoplankton biomass to detect any changes in phytoplankton community structure between early summer and late summer under initial conditions, and between treatments at the end of the experiments. Multivariate analyses were performed with the vegan package on R 3.6.3 (R Core Team, 2018). Permutational multivariate analysis of variance (PERMANOVA) with 999 permutations ($p = 0.05$) on the Bray–Curtis dissimilarity matrix (ADONIS function in R) was used to test the hypothesis that the campaign (under initial conditions) or glucose additions (at the end of the experiments) structured the distribution of phytoplankton communities.

Results

3.1. Initial conditions

Initial concentrations of dissolved organic carbon (DOC), soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) were low in both campaigns (**Table 4.1**). Average DOC concentrations were significantly lower in early summer (0.61 ± 0.02 $mg.L^{-1}$) than in late summer (1.04 ± 0.09 $mg.L^{-1}$) (t = -9.4, df = 3.53, $p = 0.001$). Average SRP concentrations were low (below 10 $\mu g.L^{-1}$) and were not significantly different between campaigns (t = -0.44, df = 7.9, $p = 0.67$). Average DIN concentrations were significantly higher in early summer (0.60 ± 0.06 $mg.L^{-1}$) than in late summer (0.15 ± 0.04 $mg.L^{-1}$) (t = 16.22, df = 10.42, $p < 0.001$). Water temperature was significantly lower in early summer (8.9°C) than in late summer (14.6°C) (t = -99.56, df = 15.62, $p < 0.001$).

Concerning biotic parameters, the initial prokaryote biomass did not significantly differ between early summer and late summer (t = 1.61, df = 7.96, $p = 0.14$), being close to 17 $\mu g\ C.L^{-1}$ in both campaigns. Initial phytoplankton biomass was almost 7-fold greater in late summer than in early summer (t = -17.45, df = 6.19, $p < 0.001$). The initial phytoplankton community differed significantly between campaigns (permanova, $F_{1,9} = 214$, $p = 0.004$). In early summer, diatoms dominated the community (37%), followed by chrysophytes (23%), cryptophytes (17%), dinoflagellates (15%) and picocyanobacteria (6%). In late summer, diatoms were even more predominant (47%), followed by picocyanobacteria (22%), chrysophytes (16%) and dinoflagellates (13%). The mixotrophs:autotrophs (M:A) ratio was higher in early summer (1.13 ± 0.44) than in late summer (0.45 ± 0.05) (t = 3.04, df = 3, $p = 0.05$). Finally, ciliates were almost thirty-fold more abundant in late summer than in early summer (t = -3.00, df = 11.19, $p = 0.01$).

Table 4.1. Biotic and abiotic parameters measured under initial conditions before each experiment. Mean ± standard error. DOC: Dissolved organic carbon; SRP: Soluble reactive phosphorus; DIN: Dissolved inorganic nitrogen. Significant P-values: <0.001***; <0.01**; <0.05*.

	Early summer	**Late summer**
DOC (mg $C.L^{-1}$)	0,61 ± 0,02	1,04 ± 0,09**
SRP ($\mu g.L^{-1}$)	5 ± 0,14	3 ± 0,03
DIN ($mg.L^{-1}$)	0,60 ± 0,06	0,15 ± 0,04***
Temperature (°C)	8,98 ± 0,08	14,60 ± 0,18***
Prokaryote biomass (µg $C.L^{-1}$)	17,51 ± 1,39	16,56 ± 0,64
Phytoplankton biomass (µg $C.L^{-1}$)	16,25 ± 1,41	111,94 ± 14,55**
Mixotrophs:autotrophs ratio	1,13 ± 0,44	0,45 ± 0,05**
Ciliate abundance ($cells.mL^{-1}$)	0,12 ± 0,41	3,98 ± 4,43***

3.2. Plankton responses to experiment in microcosms

According to the linear models, the campaign effect significantly affected all response variables, phytoplankton biomass ($F_{1,158}$ = 2595.61, $p < 0.001$), prokaryote biomass ($F_{1,146}$ = 1153.15, $p < 0.001$), consumed DOC ($F1_{,153}$ = 113.15, $p < 0.001$), M:A ratio ($F_{1,143}$ = 19.69, $p < 0.001$), and ciliates ($F_{1,174}$ = 62.52, $p < 0.001$). Moreover, campaign effect interacted with at least one of the other factors for each variable, which means that the effects of glucose, nutrients, light, or temperature on planktonic communities varied between early summer and late summer.

3.2.1. Consumed DOC

At the end of the experiments, DOC was consumed more in treatments with glucose additions than in the control, in both early and late summer ($F_{3,153}$ = 113.16, $p < 0.001$) (**Figure 4.2**). The exceptions were in early summer, at 10°C in the light with glucose addition at 6 mg $C.L^{-1}$, and in late summer, at 10°C (dark and light) with glucose addition at 4 and 6 mg $C.L^{-1}$ (**Figure A3.1**). DOC was consumed more in early summer than in late summer with glucose added at 2 mg $C.L^{-1}$, 4 mg $C.L^{-1}$ and 6 mg $C.L^{-1}$ ($F_{3,153}$ = 31.42, $p < 0.001$) (**Figure 4.2**), the average consumed DOC in all glucose treatments reaching 49.2 % in early summer and 13.9 % in late summer. For example, in the treatment combining nutrients and glucose addition at 6 mg $C.L^{-1}$, the average consumed DOC reached 5.03 mg $C.L^{-1}$ in early summer but only 2.63 mg

C.L^{-1} in late summer. In both campaigns, the maximum values for consumed DOC were reached in the dark at 18°C (**Figure A3.1**).

In treatments receiving glucose, DOC was consumed more with nutrient additions than without nutrients ($F_{3,153} = 67.48$, $p < 0.001$) (**Figure 4.2**). In addition, DOC was consumed more at 18°C than at 10°C ($F_{3,153} = 18.66$, $p < 0.001$) (**Figure A3.1**). Finally, DOC was consumed more in the dark than in the light, and this effect was particularly higher in late summer than in early summer ($F_{3,153} = 8.20$, $p < 0.001$) (**Figure A3.1**).

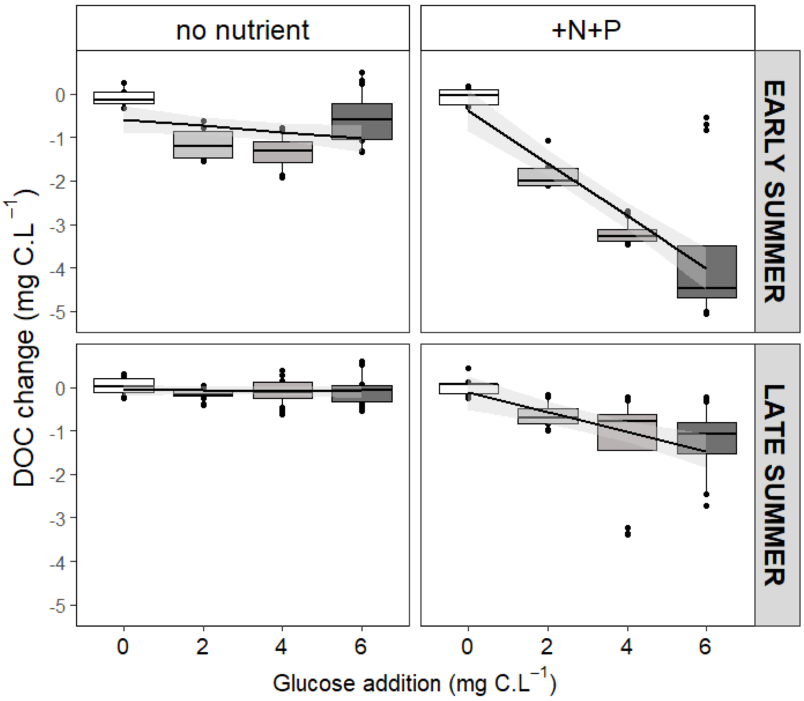

Figure 4.2. DOC change in the microcosms between initial conditions and end of experiments, in early summer and late summer. Concentrations are shown for each glucose addition (white for control; grey for glucose additions), without nutrients and with nutrients (+N+P). The boxplots show the median, the interquartile range and the tails of the distribution, including the two temperatures and light conditions. Regression lines show the glucose addition effect in each campaign and nutrient enrichment.

3.2.2. Prokaryote biomass

Glucose additions stimulated prokaryote biomass more in late summer than in early summer (**Figure 4.3**) ($F_{3,158} = 3.48$, $p = 0.01$). In addition, prokaryote biomass increased more with combined nutrient and glucose additions than with glucose addition alone and nutrient addition alone ($F_{3,158} = 20.17$, $p < 0.001$).

Prokaryote biomass dynamic differed between early and late summer: in early summer, prokaryote biomass rapidly decreased in almost all treatments except with both glucose and nutrient additions (**Figure 4.3 and Figure A3.2**). In late summer however, prokaryote biomass increased in almost all treatments, including controls without glucose, especially in the dark. A smaller and briefer increase in prokaryote biomass in the controls was observed in the light than in the dark (**Figure A3.2**).

In early summer, in the dark and in the light at 10°C, prokaryote biomass increased with combined nutrient and glucose additions until the 4th or 6th day compared to nutrient addition alone, then declined until the end of the experiment (**Figure A3.2**). In the light at 18°C, prokaryote biomass increased with combined nutrient and glucose additions until the 2nd day and then declined; however, biomass remained higher with combined nutrient and glucose additions than with nutrient addition alone.

In late summer, prokaryote biomass increased more with combined nutrient and glucose additions than with nutrient addition alone (**Figure 4.3).** In treatments receiving glucose alone, glucose additions stimulated prokaryote biomass only in the dark at 18°C (**Figure A3.2**).

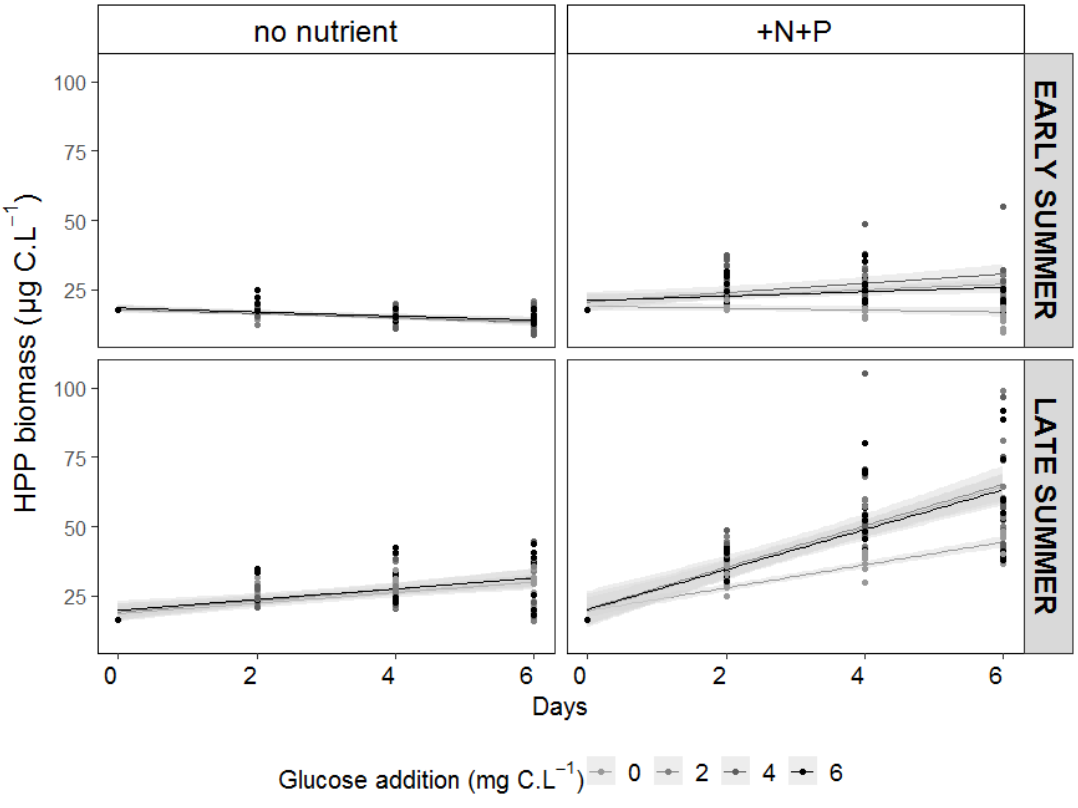

Figure 4.3. Heterotrophic prokaryotic plankton (HPP) biomass per experimental day, in early summer and late summer. Dynamics are supposed to be linear between the day 0 and the day 6. Biomass is shown for each glucose addition, without nutrients and with nutrients (+N+P). Points represent the HPP biomass in all conditions of temperature and light, and regression lines show the glucose addition effect in each campaign and nutrient enrichment.

3.2.3. Shifts in phytoplankton biomass and composition

Total phytoplankton biomass differed strongly between campaigns ($F_{1,158} = 2595.61$, $p < 0.001$) and was higher in late summer than in early summer in all conditions and treatments (**Figure 4.4**). Nutrient addition significantly stimulated phytoplankton biomass in both campaigns ($F_{1,158} = 615.70$, $p < 0.001$). However, glucose additions had almost no effect on phytoplankton biomass, neither alone nor in interaction.

At the end of the experiments, glucose additions significantly modified community composition in early summer, but glucose effect was weak (permanova, $F_{1,94} = 5.19$, $p = 0.004$). Glucose addition alone significantly didn't modify community composition in late summer (permanova, $F_{1,94} = 0.94$, $p = 0.41$). However, phytoplankton community composition differed significantly among conditions and treatments, both in early summer (permanova, $F_{31,64}$ = 10.83, $p < 0.001$) and in late summer (permanova, $F_{31,64}$ = 17.02, $p < 0.001$) (**Figure 4.4**).

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

Based on the linear model, we detected an effect of glucose addition alone on the ratio mixotrophs:autotrophs ($F_{3,143}$ = 18.02, $p < 0.001$). Moreover, glucose addition effect varied according to nutrients, light, and campaign ($F_{6,143}$ = 2.88, $p = 0.01$). In particular, glucose addition stimulated differently the mixotrophs between early summer and late summer depending on the light conditions ($F_{3,143}$ = 13.62, $p < 0.001$). In the dark, glucose additions stimulated more the mixotrophs in early summer than in late summer. At the opposite in the light, glucose additions stimulated more the mixotrophs in late summer than in early summer (**Figure A3.3**).

In early summer, the relative and absolute biomass of cryptophytes and dinoflagellates increased with glucose additions in the dark, both with and without nutrient additions (**Figure 4.4 and Figure A3.4**). Consequently, the proportion of mixotrophs increased in the dark conditions after glucose additions, with an accentuated effect with nutrient addition (**Figure A3.3**). In the light, the relative biomass of cryptophytes, chrysophytes and dinoflagellates increased with glucose addition alone at 10°C, and the relative and absolute biomass of chrysophytes increased with glucose addition alone at 18°C (**Figure 4.4 and Figure A3.4**). Consequently, the proportion of mixotrophs increased with glucose addition alone in the light (**Figure A3.3**).

In late summer, the relative and absolute biomass of choanoflagellates increased with glucose additions in the dark at 18°C, mainly with combined addition of nutrients (**Figure 4.4 and Figure A3.4**). The relative and absolute biomass of cryptophytes increased with glucose and nutrient additions in the dark at 18°C. Thus, the proportion of mixotrophs increased after glucose and nutrient additions in the dark at 18°C (**Figure A3.3**). In the light, the relative biomass of chrysophytes increased with glucose addition alone, and their absolute and relative biomass increased with glucose and nutrient additions at 18°C (**Figure A3.4**). The relative and absolute biomass of dinoflagellates increased with glucose and nutrients additions at 18°C. Thus, the proportion of mixotrophs increased in the light with glucose additions, without nutrients at 10°C and with nutrients at 18°C (**Figure A3.3**).

Finally, the biomass of autotrophs decreased with glucose in one condition in early summer (average decrease of 14.4 µg $C.L^{-1}$ compared to the control, data not shown) and in two conditions in late summer (average decrease of 45.8 µg $C.L^{-1}$ compared to the control). The biomass of mixotrophs increased with glucose addition in three conditions in both campaigns, but the average increase compared to the control without glucose was higher in late summer (31.6 µg $C.L^{-1}$) than in early summer (7.8 µg $C.L^{-1}$).

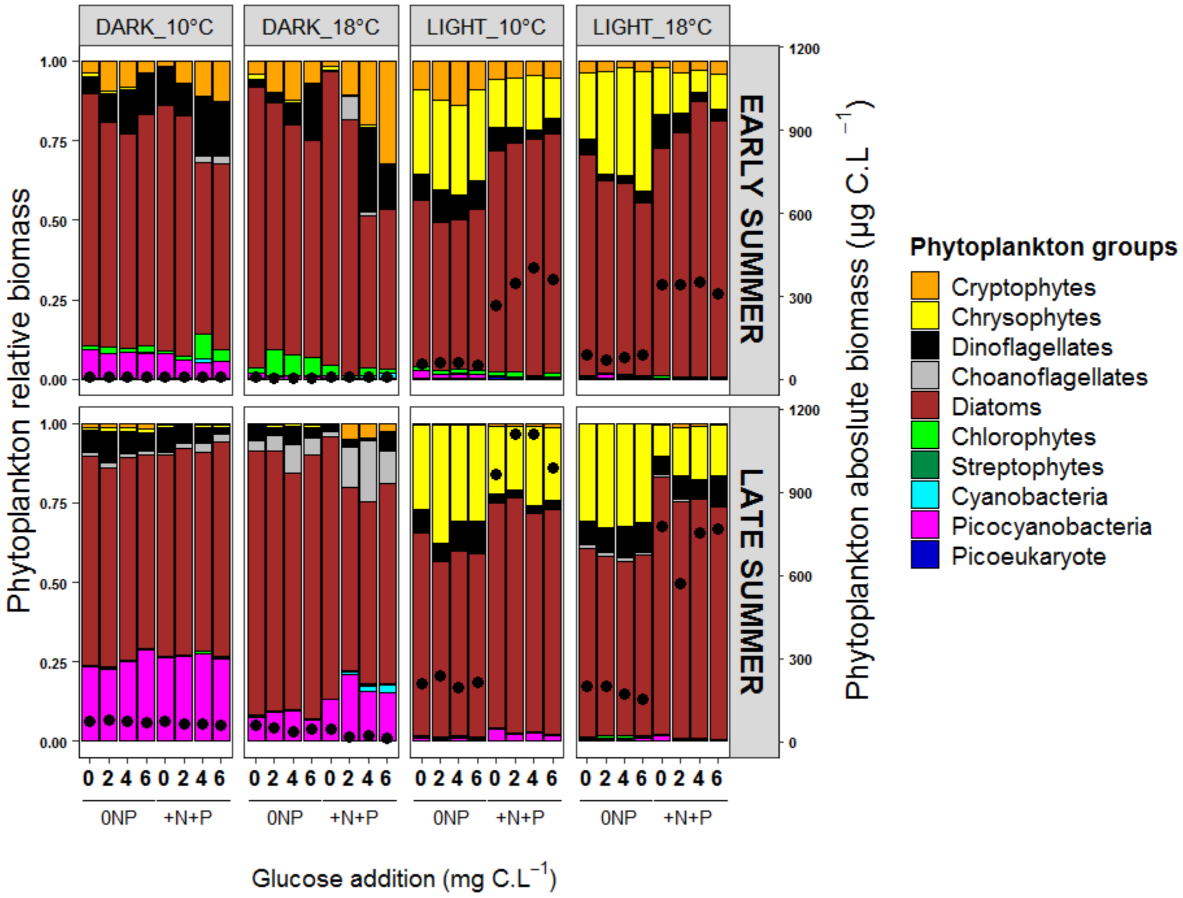

Figure 4.4. Phytoplankton groups' relative biomass and total absolute biomass (black points) at the end of experiments, in early summer and late summer. Biomass is shown for each glucose and nutrient treatments under the four temperature*light conditions.

3.2.4. Ciliate abundance

Ciliate abundance varied between early summer and late summer ($F_{1,174} = 62.52$, $p < 0.001$), and ciliate abundance increased with glucose addition ($F_{1,174} = 3.12$, $p = 0.02$), depending on light conditions ($F_{3,174} = 3.71$, $p = 0.01$) and nutrient enrichment ($F_{3,174} = 2.79$, $p = 0.04$) (**Figure A3.5**). In early summer, ciliate abundance increased with nutrient and glucose additions at 4 and 6 mg $C.L^{-1}$ in the dark at 10°C, and in all glucose concentrations in the dark at 18°C (**Figure A3.5**). In late summer, ciliate abundance increased in the light, with nutrient and glucose (4 mg $C.L^{-1}$) at 10°C, and with nutrient and glucose (6 mg $C.L^{-1}$) at 18°C (**Figure A3.5**).

Discussion

Dissolved organic carbon (DOC) acts as a major control of high-altitude lakes' functioning (Moser et al., 2019). Allochthonous DOC can be assimilated by microbial organisms, changing interactions between them. However, summer dynamics of phytoplankton biomass and composition could also impact microbial responses to DOC inputs. Our study aimed to assess how changes in the phytoplankton community between early summer and late summer modify these microbial responses. We used glucose as a simple carbohydrate source because glucose is generally assimilated more rapidly than other sugars by bacteria (Kirchman, 2003) and because it has been found to elicit a similar bacterial response to leachates of common riparian plants (Hitchcock et al., 2010). However, monosaccharide glucose have previously been shown to alter the composition of seawater bacterial communities by stimulating differently bacterial populations of diverging trophic strategies (Nelson & Carlson, 2012). Although our experimental conditions do not allow a perfect representativeness of the complexity of DOC inputs from the watershed, the experimental simplification is a necessity to evaluate the interactions effects. Experimental response to glucose additions is a first step for further investigations about more complex inputs of DOC.

4.1. Prokaryotic assimilation of glucose over the summer

In our microcosms, bacterial response was higher when glucose and nutrients were added jointly than after glucose addition alone. Moreover, prokaryotes were able to develop only when provided with inorganic nutrients, especially in late summer. These two results suggest limitation primarily by nutrients and co-limitation by C of bacteria. This pattern of co-limitation has already been observed in high-latitude lakes (Dorado-García et al., 2014) and in Lake Cordes in the ice-covered period (Dory et al., 2021). Both the present study and the previous one found evidence that bacterioplankton in Lake Cordes were co-limited by nutrients and C from April to August. This limitation pattern of heterotrophic bacteria by macronutrients have previously been shown in reservoirs, lakes (Grover, 2000; Ogbebo & Ochs, 2008), and in marine systems (Mills et al., 2008). These results highlighted a common pattern of bacterial co-limitation in oligotrophic systems depleted in dissolved inorganic nitrogen and phosphate.

We observed that glucose was consumed more in early summer than in late summer (**Figure 4.5**). This campaign effect on glucose consumption was robust regardless of variations in other experimental factors, especially temperature and light. Initial dissolved organic carbon concentrations were lower in early summer (0.61 mg.L^{-1}) than in late summer (1.04 mg.L^{-1}). As the magnitude of plankton response to nutrient additions depends on the nutrient limitation (O'Donnell et al., 2017), it could be that the greater glucose consumption observed in early summer resulted in greater C limitation of bacteria. Initial dissolved organic carbon concentrations measured in both campaigns varied with phytoplankton biomass, DOC concentrations being higher when the phytoplankton biomass was higher in late summer. The contrasting glucose consumption between campaigns could also be related to changes in DOC lability over the summer. Phytoplankton biomass was far lower in early summer (16.25 µg C.L^{-1}) than in late summer (111.94 µg C.L^{-1}), and the increased phytoplankton biomass could result from greater availability of autochthonous DOC in late summer (Ejarque et al., 2018). This interpretation of the phytoplankton biomass influence is also supported by the finding of greater differences in glucose consumed in dark versus light conditions in late summer than in early summer. Our findings are consistent with the conclusions of Kritzberg et al. (2006), namely that the bacterial growth efficiency and production were higher with amendments of algae leachates than with more refractory substrates in oligotrophic lakes. In addition, it has been established that phytoplankton-derived DOC is more quickly and efficiently consumed by bacteria than allochthonous DOC (Guillemette et al., 2013, 2016; Sadro, Nelson, et al., 2011b). To conclude, in early summer, prokaryotes actively consumed the glucose added to the microcosms likely because phytoplankton-derived DOC was low. In late summer, phytoplankton-derived DOC was more available and prokaryotes probably needed less glucose, which resulted in lower glucose consumption. Our results further demonstrated that DOC availability in natural conditions was a major factor regulating glucose consumption in the microcosms. Similar results have been observed in an estuary, where organic matter source and quality played an important role in regulating bacterial carbon metabolism and was more important than other factors such as nutrients availability in the regulation of bacterial growth (Apple & del Giorgio, 2007). Our work thus confirms that the expected changes of both quality and quantity of DOC in high-altitude lakes will lead to significant changes in carbon metabolism of bacterial communities.

Based on our linear model, glucose in interaction with nutrient additions was consumed more in early summer than in late summer, but glucose additions stimulated prokaryote biomass more in late summer than in early summer (**Figure 4.5**). This major result was mainly observed until the 6th day of the experiments, after what an increase of grazing rates by ciliates and mixotrophs, and possibly viral lysis (Riemann et al., 2009), was likely responsible of the change in the prokaryote dynamics. Seasonal modifications in bacterial communities' composition have previously been described in oligotrophic lakes, linked to qualitative and quantitative changes in allochthonous or autochthonous organic substrates (Kritzberg et al., 2006). In our study, lake conditions differed strongly between early summer and late summer, as demonstrated by the campaign effect detected by the linear model. In early summer, frequent storms associated with high turbidity likely constituted stressful environmental conditions for heterotrophic bacteria. Such environmental conditions have been linked to increased respiration of dissolved organic carbon by heterotrophic bacteria in high-altitude lakes (Sadro & Melack, 2012). Thus, in our experiments, the bacterial metabolism probably changed over the summer, related to the increased stability of environmental conditions and the more available autochthonous DOC in late summer. It is known that modified resource supply change microbial community composition by selecting species/groups with particular microbial resource utilization traits (Litchman et al., 2015). In support to our assumption, previous works have demonstrated phenological variation in the community composition of bacterioplankton in humic lakes (Paver et al., 2013) and high-elevation lake (Nelson, 2009). Unfortunately, our results do not permit us to determine whether the observed change was due to a taxonomic modification of the bacterial community, a functional modification of the bacterial community, or both. These results however underline the crucial interest to further describe bacterioplankton taxonomical and functional changes in these ecosystems in the light of DOC change.

At last, but not least, our experiments tell us about how increase temperature may modify the response of planktonic bacteria to DOC change. In our experiments, despite the temperature effect was not the larger effect compared to glucose and nutrient additions, temperature effect interacted with other experimental factors. For example, we showed that glucose consumption was higher at 18°C than at 10°C, and that prokaryote biomass increased more at 18°C. Interactive effect of temperature increase with resource availability and with ultraviolet exposition have been experimentally demonstrated on freshwater bacterioplankton and on phytoplankton-bacterioplankton relationships (Durán-Romero et al., 2020; Hall & Cotner, 2007). The later showed that the resource utilisation by bacteria was higher at higher temperature. In natural conditions in high-altitude lakes, an eight-degree difference can be easily reached after snowmelt, where the lake temperature usually shifts from about 2°C toward 10°C over a short period (about two weeks, pers. comm.). Additionally, 18°C represents an increase of 2.5°C compared to the maximum temperature measured in the lake Cordes, thus plausible in a scenario of warming. Our results thus corroborate the previous studies and suggest that temperature increase in natural conditions is expected to increase the complexity of communities' responses to allochthonous inputs.

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

4.2. Changes in competition and predation over the summer

In response to glucose additions, competitive processes for inorganic nutrients between bacteria and phytoplankton changed over the summer according to the light condition and nutrient addition. Mixotrophs increased more after glucose additions in early summer than in late summer in the dark. At the opposite, in the light, mixotrophs increased more after glucose additions in late summer than in early summer, especially with nutrients. In addition, we observed a decrease in initial DIN concentrations over the summer (**Figure 4.5**), consistent with the findings of Jacquemin et al. (2018) on the evolving nutrient limitation pattern in Lake Cordes during summer. Our results support the findings of Carney et al. (2016), who observed increased competition between bacteria and phytoplankton depending on limitation by inorganic nutrients in mesocosms. In our microcosms in the dark, it is therefore possible that the more pronounced increase in prokaryote biomass in late summer than in early summer results from a reduced predation by mixotrophs in the dark in late summer.

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

In our microcosms, the biomass of cryptophytes increased mainly in early summer in the dark after glucose additions. By contrast, in late summer cryptophytes increased only in relative biomass. It is therefore possible that a change in the nutritional preferences of cryptophytes occurs between early summer and late summer. Light availability has been shown to be a crucial factor in the proportions of mixotrophs in high-altitude lakes. In these ecosystems, the highest proportion of mixotrophs is usually observed after snowmelt, because the mixotrophic strategy is advantageous under ice-cover, when nutrients and light are limiting factors (Waibel et al., 2019). A recent microcosm study on Lake Cordes demonstrated that under-ice species of cryptophytes were able to develop in the dark, likely by ingesting bacterial prey (Dory et al., 2021). Conversely, other studies positioned cryptophytes towards the autotrophic end of an increasing trophic state gradient (Saad et al., 2016). These conclusions highlight the controversy surrounding the nutritional strategies of cryptophytes, which may differ in their degree of heterotrophy due to specific environmental limitations like light, nutrients, prey availability or a combination of these (Bergström et al., 2003). In addition, proportions of choanoflagellates in the dark were higher in late summer than in early summer. The observed differential response of cryptophytes to glucose additions in the dark could also result from evolving competition with other groups for prey. To sum up, in our study cryptophytes were only able to develop through prey ingestion in early summer, not in late summer, probably due to changes in environmental conditions or competition mechanisms.

Overall, we observed a stimulation of mixotrophic taxa after glucose additions in our experiments. The increase of the proportion of mixotrophs after glucose additions was dependent on nutrient addition and light conditions. Our hypothesis is partially validated, as mixotrophs increased more after glucose additions in early summer than in late summer only in the dark, resulting from more heterotrophic strategy after the ice-covered winter. In the light, the opposite pattern was observed, as mixotrophs increased more after glucose additions in late summer than in early summer. Although phytoplankton community was more autotrophic in late summer, in light conditions competition and predation over bacteria was higher in late summer after glucose addition than in early summer, due to an evolution of the nutrient limitation pattern through summer.

Conclusion

High-altitude lakes are particularly vulnerable to the combined action of dissolved organic carbon and nutrients. In our experimental study, glucose in combination with inorganic nutrients rapidly modified the relationship between bacterioplankton and phytoplankton. We show here that glucose was incorporated differently into the food web in early summer and in late summer, depending on the availability of autochthonous DOC and the nutrient limitation. In general, glucose additions promoted heterotrophic and mixotrophic pathways in the planktonic food web. Thus, our findings contribute information that can help predict the ecological trajectories of shallow high-altitude lakes with large, vegetated catchments: depending on the nutrient limitation, more heterotrophic and mixotrophic communities can be expected in the future under the environmental changes forecast for these ecosystems.

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

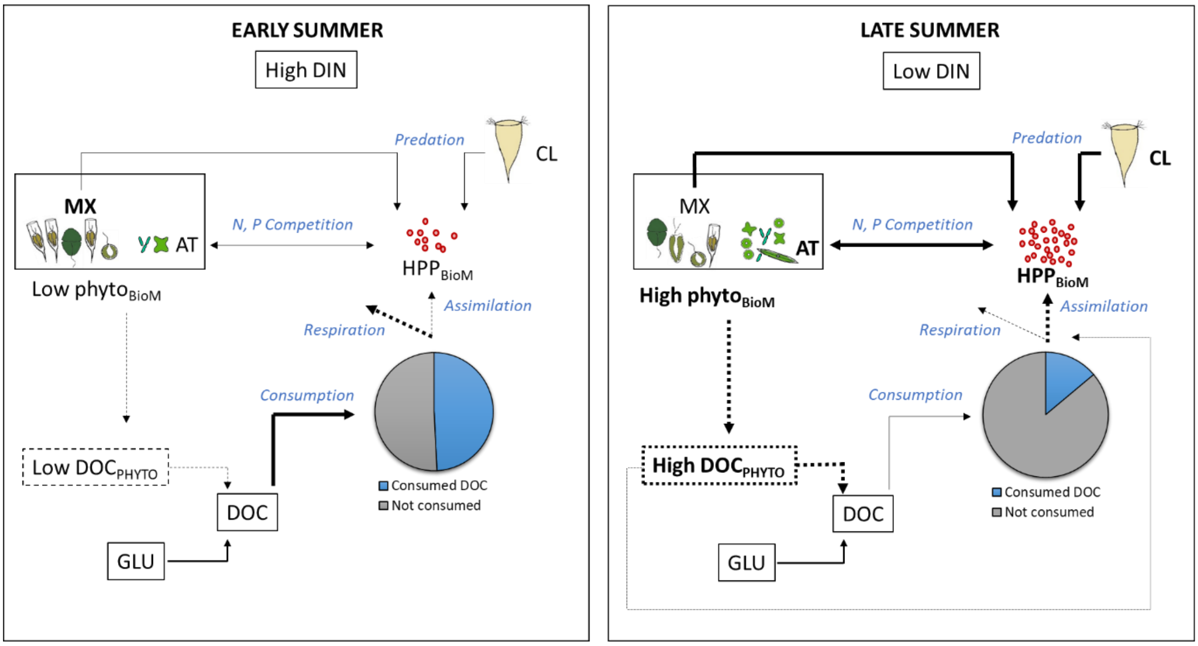

Figure 4.5. Scheme of the phytoplankton regulation of bacterial response to glucose addition in early summer and late summer. Black lines represent processes that are reinforced (heavy line) or weakened (light line). Dashed lines represent variables or processes not measured. GLU = glucose; DOC = dissolved organic carbon; CL = ciliates; MX = mixotrophs; AT = autotrophs; HPP$_{\text{BioM}}$ = Heterotrophic prokaryotic plankton biomass; Phyto$_{\text{BioM}}$ = phytoplankton biomass; DOC$_{\text{PHYTO}}$ = phytoplankton-derived DOC.

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Chapter 5

Chapter 5

Rising algal- and soil-derived dissolved organic matter modifies interactions between bacteria and phytoplankton in a high-altitude lake

Article in preparation

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Keywords

Laboratory experiments, Phytoplankton-bacterioplankton relationship, Mountain lakes, Nutrient co-limitation, Mixotrophy

Abstract

Both allochthonous and autochthonous dissolved organic matter (DOM) concentrations are expected to increase in high-altitude lakes with the forecast climate change. We studied the effect of allochthonous and autochthonous DOM increase in early summer on a planktonic community (bacterioplankton and phytoplankton) originating from a high-altitude lake. To investigate how DOM amendments modify interactions between bacterioplankton and phytoplankton, we performed laboratory experiments in microcosms amended with glucose, algal lysate, or soil extract, either enriched with inorganic nutrients or not. Planktonic community was incubated for 10 days, either in the dark or under artificial dark:light cycle. Both bacterioplankton and phytoplankton appeared to be highly limited by inorganic nutrients, since nutrient enrichment increased drastically their biomass and the proportion of autotrophs. Bacterioplankton consumed more dissolved organic carbon with algal lysate but bacterial biomass was higher after soil extract amendments. Rising the carbon source increased phytoplankton biomass when a top-down control by ciliates or mixotrophs regulated bacterial growth but decreased phytoplankton biomass when top-down regulation of bacteria was low. Mixotrophic phytoplankton taxa were stimulated with glucose, algal lysate, and soil extract when light or nutrient limitation occurred. Under non-limiting light conditions, the proportion of autotrophs increased more with algal lysate than with soil extract. Our results support the hypothesis that relationships between phytoplankton and bacterioplankton shift with DOM increase in high-altitude lakes. The expected climate-driven changes in DOM in high-altitude lakes may greatly alter aquatic food webs and the C transfer through the trophic chain.

1. Introduction

Dissolved organic matter (DOM) plays a fundamental role in freshwater ecosystems 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 bacterial metabolism. In response to increasing allochthonous sources of DOC, previous studies reported a reduction of the bacterial reliance on phytoplankton-produced carbon (Figueroa et al., 2016; Jansson et al., 2000), and an increase of competition with phytoplankton for inorganic nutrients (Carney et al., 2016; Hitchcock & Mitrovic, 2013). These competition processes often result in a decrease of the phytoplankton biomass (Drakare, 2002), but also benefit heterotrophs and mixotrophs, via grazing on bacteria (Deininger et al., 2017).

However, this pattern is not always observed and depends on the nutrient limitation of organisms. For example, the degree of heterotrophy of mixotrophic taxa may differ according to specific limitation by light, nutrient, or prey availability (Bergström et al., 2003) and some mixotrophs require both light or nutrient limitation and sufficient prey to develop (Livanou et al., 2020; Modenutti, 2014). In oligotrophic systems limited by nutrients, nutrient limitation has been shown to limit the bacterioplankton consumption of allochthonous DOC (Nelson & Carlson, 2011), sucrose (Dorado-García et al., 2014), and glucose (Dory et al., 2021). By providing nutrient subsidies besides fixed carbon, DOM inputs have been shown to increase bacterioplankton biomass (Pérez & Sommaruga, 2006), phytoplankton biomass (Kissman et al., 2017), or both (Hitchcock & Mitrovic, 2013). Assessing the limitation pattern of bacteria 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. DOM pool thus includes autochthonous DOM, mainly derived from primary production, and allochthonous DOM transported from catchments to aquatic environments. Depending of its origin, DOM differs in its optical and chemical characteristics: autochthonous DOM is usually mostly composed 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 bacterial degradation (Del Giorgio & Davis, 2003). These compositional differences are thought to influence the structure of natural bacterial assemblages (Kritzberg et al., 2006), as well as the allocation of substrates by bacteria to growth or respiration (Russell, 2007).Thus, it has recently been demonstrated that bacterial communities selectively allocate more autochthonous substrate for respiration, whereas terrestrial C is preferentially allocated for biosynthesis (Guillemette et al., 2016). The differential response of bacterial metabolism depending on the DOM source is likely to differentially influence the trophic interactions with phytoplankton. For example, an increase of bacterial mineralization of organic nutrients may increase nutrient availability for phytoplankton, resulting in a positive effect on phytoplankton biomass (Li et al., 2014). However, the effect of different DOM source on phytoplankton – bacterioplankton relationships and phytoplankton community structure is still poorly understood.

High-altitude lakes are oligotrophic (Catalan et al., 2006), are located above the treeline, and are characterized by small and poorly vegetated catchments. Consequently, both nutrients and dissolved organic carbon (DOC) concentrations are usually low and DOM exhibit a more autochthonous DOM fluorescence signature (Bastidas Navarro et al., 2014; Sommaruga & Augustin, 2006). The exception is during snowmelt or after extreme rain events, where DOM pool of the lakes is dominated by allochthonous DOM (Miller & McKnight, 2010; Perga et al., 2018; Sadro, Melack, et al., 2011a). The high-altitude lakes may thus respond greatly to a shift in the quantity and quality of DOM source. DOM is expected to change with the current global change in these ecosystems, especially in the alpine area (Ejarque et al., 2018). We expect an increase of terrestrial DOC export in the high-altitude lakes as a consequence of increase air temperature and increase frequency of extreme precipitation events (IPCC, 2021). On the other hand, higher temperature and associated reduction of the duration of the ice-cover period, as well as increase of atmospheric deposition of nitrogen (Kirchner et al., 2014; Kopáček et al., 2005) and phosphorus (Kopàček et al., 2011), may significantly increase the phytoplankton development (Bergström et al., 2005, 2008; Camarero & Catalan, 2012; Elser et al., 2009; Jacquemin et al., 2018), thus the production of autochthonous DOM.

To understand how DOM shift could influence phytoplankton – bacterioplankton relationships and phytoplankton community structure, we ran an experiment where a natural plankton assemblage of a high-altitude lake was supplied with DOM derived from algal lysate or soil extract. To highlight the effect of the nutrient associated to the DOM, a treatment with glucose addition served as a simple C-treatment, and all the C treatments (algal, soil, and glucose) were replicated with addition of inorganic nitrogen and phosphorus in excess. A treatment with inorganic nitrogen and phosphorus addition served as a simple nutrient-treatment. Finally, all treatments of C and nutrients were incubated either under artificial dark:light cycle or in the dark to minimize the direct influence of autotrophic phytoplankton. Because high-altitude lakes are traditionally oligotrophic, we suppose a co-limitation of bacterioplankton by C and nutrients. We expect that bacterioplankton preferentially consumes algal lysate but soil extract is more allocated for growth. Additionally, we suppose that phytoplankton response to a carbon source depends on both interactions with bacterioplankton and limitation patterns. This mean that (i) phytoplankton biomass is higher when nutrients are more available, by direct enrichments or interactions with bacterioplankton, and (ii) mixotrophs proportion is higher when prey are available and light or nutrients are limiting.

2. Methods

2.1. Study area and sampling

Water for experiment was sampled in Lake Cordes, an oligotrophic high mountain lake (2 446 m a.s.l) located in the French Alps, adjacent to the Queyras regional natural park. The lake is characterized by a shallow depth (maximum depth = 9 m) and a small area (1.8 ha), and the catchment is large (140 ha) with developed soil and meadows (approximately 60% alpine grass cover). Water for experiment (48 L) was sampled on 28th June 2021, collected using a Niskin Bottle every meter from 1 to 8 m to sample the entire water column, and were 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 the lake's temperature (10°C), water was distributed among 16 sets (4 x 2 x 2) of three replicates of 1-liter glass bottles. All glass were soaked in hydrochloric acid (10% final concentration) and sterilized. The first set of bottles (12 bottles) served as control of the experiment and did not receive DOM amendment. The second set of bottles (12 bottles) served as C_control and received glucose addition to reach DOC concentration at 2 mg L^{-1}. The concentration of 2 mg L^{-1} represents the maximal concentration measured in this lake during previous samplings. The third set of bottles (12 bottles) received a filtrate from an algal lysate to reach DOC concentration at 2 mg L^{-1}. The algal lysate was obtained from a natural phytoplankton community of the lake Cordes cultured in laboratory for several weeks that was mainly dominated by the green algae *Chlorella minutissima* after several days. The culture was rinsed, disrupted (One Shot Cell Disruptor, CellD) at 2 500 bars (40 ml min^{-1}) and filtrated through a 0.22-µm polycarbonate membrane to eliminate algal rests and bacteria. The fourth set of bottles (12 bottles) received a filtrate from a soil extract to reach DOC concentration at 2 mg L^{-1}. The soil extract was obtained from soil samples collected randomly in the watershed. 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), the suspensions were placed at 4°C and stirred manually within 24 h. The suspensions were then centrifuged (10 000 rpm, 20 min), filtered (0.2 µm), and subsequently stored at 4 °C until experiment.

For each of the four set of bottles, half (6 bottles) did not receive inorganic nutrients, and half (6 bottles) were enriched with NH_4NO_3 (2 mg L^{-1}) and KH_2PO_4 (0.6 mg L^{-1}) in excess. We chose these concentrations to approximate a DIN:SRP ratio previously measured in summer on this lake (unpublished data), while increasing concentrations in excess. All the 48 bottles were placed in growth chambers for 10 days, at the lake temperature measured at the sampling day (10°C). For each DOM and nutrients treatments, one set of bottles was placed under a circadian cycle at light: dark cycle = 14:10 (Light condition), at a fixed irradiance (45 ± 2 µmol m^{-2} s^{-1}) and the second set of bottles was 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. Nutrients (C, N, P)

Subsamples for nutrient analysis were collected at day 0 (beginning of the experiment) and day 10 (end of the experiment). Subsamples for dissolved inorganic nitrogen (DIN = NH_4^+ + NO_2^- + NO_3^-), soluble reactive phosphorus (SRP = PO_4^{2-}), and dissolved organic carbon (DOC) concentrations were filtered through pre-combusted (4h, 450°C) 25 mm glass filters (Whatman GF/F, 0.7 mm). 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 set of 24 ml acidified (HCl) and pre-combusted (4 h at 450°C) glass tubes (Wheaton equipped with Teflon/silicone septa). The samples were preserved in 25 µl of sodium azide solution (1 M N_aN_3) at +4℃. DOC concentrations were determined using a TOC-VCSH analyzer (Shimadzu, TOC-V).

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 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 & Mounier, 2018) in Matlab language.

2.5. Bacterioplankton biomass

Subsamples of 1.5 ml were taken at day 0 and thereafter every 2 experimental days in the microcosms for heterotrophic prokaryotes analyses by flow cytometry. Regarding of the low levels of archaea in oligotrophic waters (Wells & Deming, 2003), we hereafter refer heterotrophic prokaryotes to bacterioplankton. Subsamples were fixed with glutaraldehyde (0.25% final concentration) and stored at −80℃ until flow cytometry analysis. Subsamples were stained with 1:10,000 (vol/vol) SYBR® Green II and incubated 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. Bacterioplankton 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. Bacterioplankton production

Bacterioplankton production was measured every day from day 0 to day 4, then day 6, day 8, and day 10. Bacterioplankton production was estimated from rates of protein synthesis with ^{3}H-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-carbon conversion factor used was 1.5 kg C mol^{-1}. An estimation for bacterial growth efficiency (BGE) was calculated by integrating bacterioplankton production data from time zero (t0) to time 10 (t10), corresponding to the delta t where DOC decreases, as follow:

$$BGE\ (\%) = \frac{\mathrm{IBP}}{\Delta\mathrm{DOC}} * 100$$

where IBP (µM C) was time-integrated bacterioplankton production from t0 to the t10 with integration of discrete data, and assuming an exponential growth (or decay) between 2 time-points. ΔDOC was the difference in DOC concentrations (µM C) between concentrations measured at t10 and concentrations measured at t0.

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 according to the Utermöhl (1958) method, at 40-fold magnification under an inverted microscope (Olympus IX 70). 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 three groups (**Table A4.1**): (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 characterized according to two indexes: the specific richness (S), and the Pielou evenness (J).

To determine any predators development in our microcosms, ciliates abundances were estimated via the Utermöhl (1958) method.

2.8. Statistical analyses

Two-way analysis of variance (ANOVA) was used to compare changes in nutrient concentrations at the beginning of the experiment. Linear models were used to investigate how DOM source in combination with nutrient enrichment and light conditions affect abiotic and biological responses. Pairwise differences were tested using post-hoc Tukey HSD tests. The “emmeans” library was used for Tukey's tests. We analyzed changes in DOC concentrations between the beginning (t0) and the end (t10) of the experiment in the different treatments as delta of change. Multivariate regression trees (MRT) were constructed to display changes in the composition of phytoplankton community 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. It is a hierarchical method that aims to minimize the least sumsquares of the response data within a cluster by repeatedly splitting the data. A 1000 cross-validation process using the “1se” 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 phytoplankton community at the specific level. All statistical analyses were performed with R 3.6.3 (R Core Team, 2018).

3. Results

3.1. Initial conditions in the microcosms

The initial DOC concentrations in the control were low (< 1 mg L^{-1}), and the amendments of DOM (algal lysate and soil extract) and glucose significantly increased the DOC concentration to reach concentrations between 2 and 2.3 mg L^{-1} at the beginning of the experiment ($p < 0.001$) (**Table 5.1**). The initial DIN concentrations were low in the unenriched treatments (< 0.5 mg L^{-1}), and the amendments of algal lysate and soil extract didn’t significantly increase the DIN concentrations ($p = 0.99$ and $p = 0.98$ respectively). The nutrient enrichments significantly increased DIN concentrations to reach concentrations between 2.3 and 2.8 mg L^{-1} ($p < 0.001$). The initial SRP concentrations were below 10 µg L^{-1} in the unenriched treatments, and the amendments of algal lysate and soil extract didn’t significantly increase the SRP concentrations ($p = 0.99$). The nutrient enrichments significantly increased the SRP concentrations to reach concentrations between 0.5 and 0.7 mg L^{-1} ($p < 0.001$).

The PARAFAC analysis distinguished between four components at the beginning of the experiment. The 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. The component C3 (ex/em = 270/340 nm) have been associated 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. 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 characterized by the higher proportion of C4.

Table 5.1. Nutrient concentrations measured in the microcosms at the beginning of the experiment (t0), just after DOM and nutrient amendments. Means ± standard deviations are showed, each treatment was performed in triplicate except for the fluorescent components. DOC: dissolved organic carbon; DIN: dissolved inorganic nitrogen; SRP: soluble reactive phosphorus.

DOM	Nutrients	DOC (mg L^{-1})	DIN (mg L^{-1})	SRP (mg L^{-1})	C1 (%)	C2 (%)	C3 (%)	C4 (%)
Control	No nutrient	0.86 ± 0.08	0.42 ± 0.04	0.00 ± 0.01	45	25	19	11
Control	+N+P	0.86 ± 0.08	2.74 ± 0.53	0.71 ± 0.13	45	25	19	11
Glucose	No nutrient	2.03 ± 0.09	0.47 ± 0.06	0.00 ± 0.00	47	24	20	10
Glucose	+N+P	2.03 ± 0.09	2.30 ± 0.42	0.59 ± 0.12	47	24	20	10
Algae	No nutrient	2.28 ± 0.13	0.45 ± 0.4	0.00 ± 0.00	40	16	24	20
Algae	+N+P	2.28 ± 0.13	2.52 ± 0.57	0.70 ± 0.19	40	16	24	20
Soil	No nutrient	2.13 ± 0.04	0.45 ± 0.06	0.00 ± 0.00	43	22	24	10
Soil	+N+P	2.13 ± 0.04	2.39 ± 0.39	0.62 ± 0.12	43	22	24	10

Concerning biological parameters, initial phytoplankton biomass was very low in the microcosms (4,67 ± 0,68 µg C L^{-1}) and phytoplankton community was equally composed of mixotrophs (52,79 ± 4,9 %) and autotrophs (47,21 ± 4,9 %) (data not shown). Bacterioplankton biomass was also extremely low (1,47 ± 0,05 µg C L^{-1}) (data not shown).

3.2. Response to DOM, glucose, and nutrient enrichments

3.2.1. Change in quality and quantity of DOM

3.2.1.1. DOC quantity

In controls, DOC concentrations moderately decreased between the beginning and the end of the experiment (-38 ± 18%) (**Figure 5.1**) and DOC decreased more in the nutrient-enriched treatments (-42 ± 16%) than in unenriched treatments (-35 ± 20%) ($p < 0.001$), regardless of the light condition (**Figure A4.1**). In treatments receiving glucose, DOC decreased slightly in unenriched treatments (-11 ± 14%), whereas DOC decreased significantly more in nutrient-enriched treatments (-71 ± 7%) ($p < 0.001$). In the treatments receiving algal lysate and soil extract, DOC concentrations drastically decreased over time and the nutrient enrichment didn't modify the DOC change between the beginning and the end of the experiment ($p = 0.65$). DOC concentrations decreased more in treatments receiving algal lysate (-67 ± 6%) than soil extract (-59 ± 4%) ($p < 0.001$).

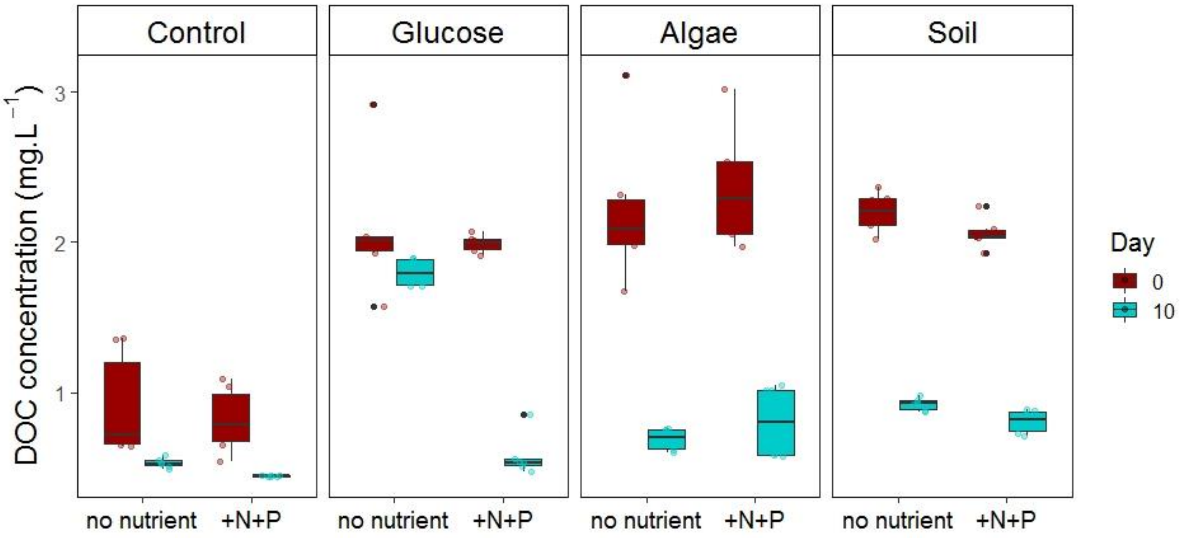

Figure 5.1. DOC concentrations at the beginning (t0, red) and the end (t10, blue) of the experiment in the different treatments. Data for light and dark conditions are plotted together.

3.2.1.2. DOM quality

There were few variations in DOM quality in controls between the beginning and the end of the experiment, and the percentage of variations of the four components were closed to 0 % (**Figure 5.2**). Similarly, there was little variation in the contribution of the 4 components in glucose treatments without nutrients (**Figure A4.2**). 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 glucose and nutrients together (**Figure 5.2 and Figure A4.2**). 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 algal lysate non-enriched with nutrients (**Figure 5.2 and Figure A4.2**).

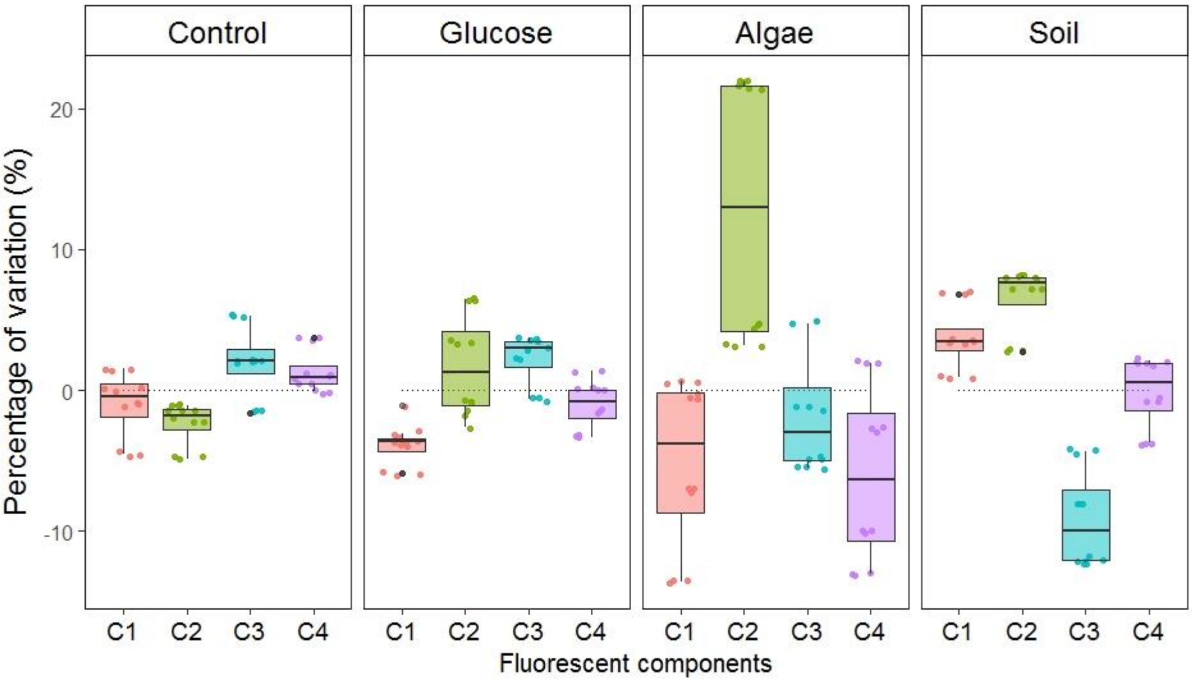

Figure 5.2. Percentage of variation of the contribution of the four fluorescent components between the beginning and the end of the experiment. Data are plotted together for both the nutrient-enriched and unenriched treatments, and for both the light and dark conditions.

3.2.2. Bacterioplankton biomass and production

Bacterioplankton biomass and production significantly differed between controls, glucose, algal, and soil treatments (8th day, $p = 0.001$), depending on the nutrient enrichment ($p = 0.01$) (**Figure 5.3**). In controls, bacterioplankton biomass slightly increased over the experiment, depending on the nutrient enrichment (8th day, $p < 0.001$). In unenriched treatments, bacterioplankton biomass reached its maximum at day 8 in the dark (84 ± 8 µg C L^{-1}). In nutrient-enriched treatments, bacterioplankton biomass reached its maximum at day 10 in the light (141 ± 37 µg C L^{-1}). In treatments receiving glucose, bacterioplankton biomass increased rapidly and was significantly higher in nutrient-enriched than in unenriched treatments (8th day, $p = 0.02$). In unenriched treatments, the peak of bacterioplankton biomass was reached after 6 days in the dark (92 ± 23 µg C L^{-1}), whereas in nutrient-enriched treatments, the peak of biomass was reached after 8 days in the light (155 ± 20 µg C L^{-1}). In treatments receiving algal lysate, bacterioplankton biomass drastically increased over the experiment and reached its maximum at day 6 in the dark. The magnitude of the peak depended of nutrient enrichment (8th day, $p = 0.01$): the biomass reached 166 ± 18 µg C L^{-1} in unenriched treatments and 218 ± 21 µg C L^{-1} in nutrient-enriched treatments. In the soil treatments, bacterioplankton biomass increased drastically over the experiment and reached its maximum at day 4 in the dark. Biomass reached 241 ± 12 µg C L^{-1} in unenriched treatments and 246 ± 21 µg C L^{-1} in nutrient-enriched treatments. In unenriched treatments, maximum bacterioplankton biomass was significantly higher with soil extract than with algal lysate ($p = 0,01$).

The C effect on bacterioplankton production varied according to the light conditions ($p < 0.001$) and the nutrient enrichment ($p < 0.001$) (**Figure 5.3**). Bacterioplankton production did not increase in controls, except at day 10 in the light (1647 ± 15 ng C L^{-1} h^{-1}). With glucose amendments, bacterioplankton production increased only in nutrient-enriched treatments, and reached its maximum at day 6 in the light (4682 ± 194 ng C L^{-1} h^{-1}). In algal treatments, bacterioplankton production increased drastically, depending on light conditions and nutrient enrichments. In unenriched treatments, bacterioplankton production reached its maximum at day 6 in the dark (8334 ± 80 ng C L^{-1} h^{-1}), whereas in nutrient-enriched treatments, the maximum was reached at day 4 in the dark (17307 ± 690 ng C L^{-1} h^{-1}). In soil treatments, bacterioplankton production increased over the experiment and reached its maximum after 4 days in the dark. The peak of production was 5245 ± 176 ng C L^{-1} h^{-1} in unenriched treatments and 7755 ± 555 ng C L^{-1} h^{-1} in nutrient-enriched treatments.

In unenriched treatments, BGE was higher with algal lysate and soil extract than control and glucose (**Figure A4.3**). In nutrient-enriched treatments, BGE was higher in all treatments and was maximum with algal lysate, with lower values in the control than in algal lysate, soil extract, and glucose amended treatments.

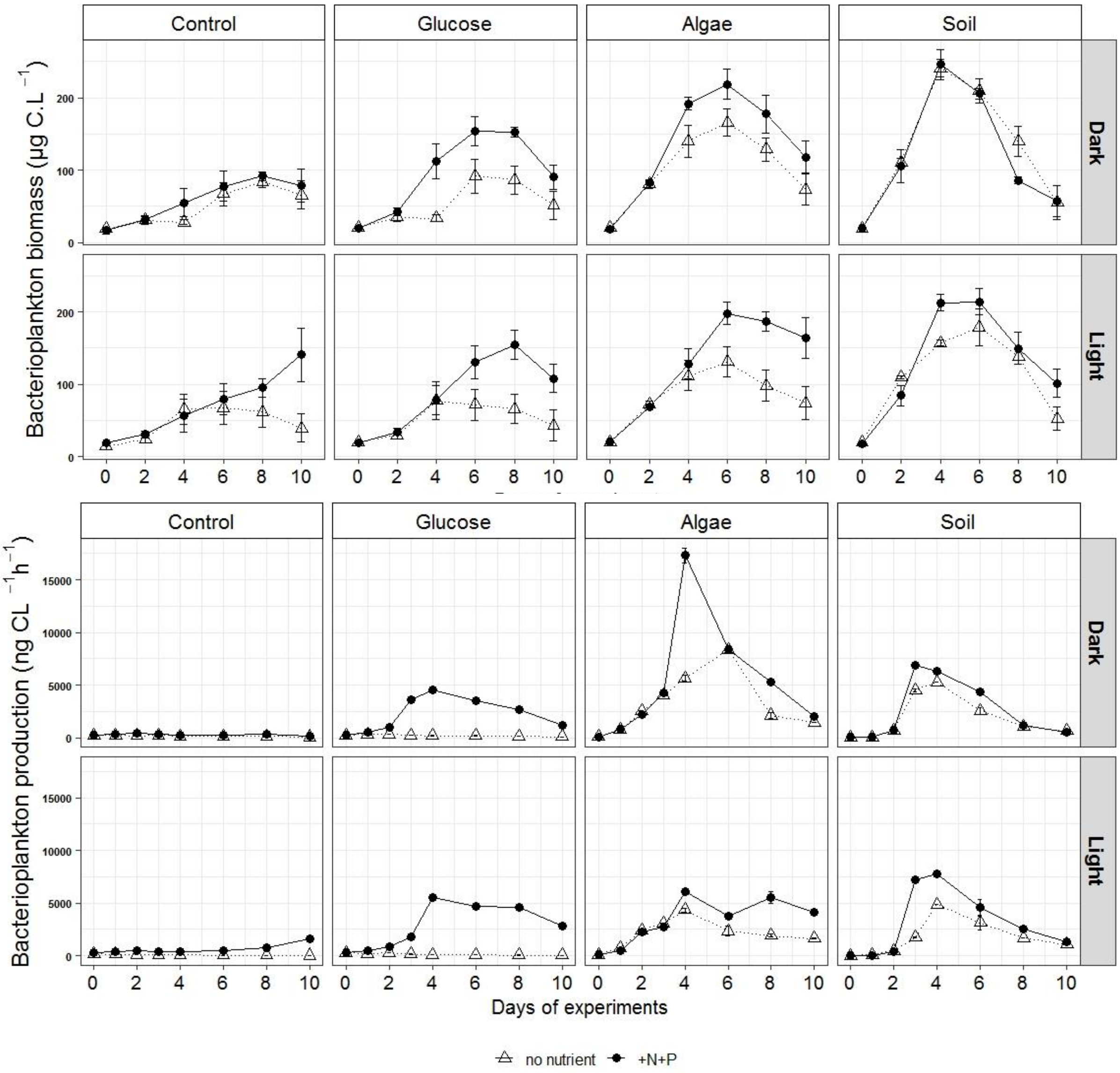

Figure 5.3. Temporal changes of bacterioplankton biomass and production in the different treatments. Error-bars represent standard error.

3.2.3. Phytoplankton changes

3.2.3.1. Phytoplankton biomass and diversity

Total phytoplankton biomass was lower in the dark (< 15.2 µg C L^{-1}) than in the light (> 26.2 µg C L^{-1}) (**Figure 5.4**). Under darkness conditions, phytoplankton biomass was higher in treatments receiving a carbon source (glucose, algal lysate, and soil extract) in comparison to controls, especially with soil extract in unenriched conditions ($p = 0.02$). In the light, nutrient enrichment highly increased phytoplankton biomass ($p < 0.001$) and interacted with C additions ($p = 0.01$). In unenriched treatments, phytoplankton biomass was low in the control (27 ± 2 µg C L^{-1}) and increased significantly with algal lysate (104 ± 24 µg C L^{-1}, $p = 0.002$) and soil extract (168 ± 45 µg C L^{-1}, $p < 0.001$). In nutrient-enriched treatments, phytoplankton biomass was high in the control (459 ± 87 µg C L^{-1}) and significantly increased with soil extract (765 ± 49 µg C L^{-1}, $p < 0.001$) compared to control. However, phytoplankton biomass significantly decreased with glucose (275 ± 57 µg C L^{-1}, p < 0.001) and algal lysate (316 ± 43 µg C L^{-1}, p = 0.003) compared to control.

Mixotrophs:autotrophs (M:A) ratio varied according C source amendment, light, and nutrient enrichment ($p = 0.04$). In the light, M:A decreased with C amendment, especially with nutrients ($p = 0.01$). The exception was with glucose addition in unenriched treatments where M:A increased compared to controls. In the dark, M:A increased with algal lysate ($p = 0.02$) and soil extract ($p = 0.002$) in unenriched treatments, and M:A increased with glucose ($p = 0.004$) algal lysate ($p = 0.001$), and soil extract ($p = 0.001$) in nutrient-enriched treatments.

Concerning the diversity indexes, specific richness was higher in the light conditions ($p < 0.001$) and with C amendments than in controls ($p = 0.01$). In the light, specific 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 addition alone (21.7 ± 0.6). At the opposite, evenness was higher in the dark than in the light ($p < 0.001$). The species evenness differed between DOM treatments ($p = 0.03$) and was lower in the control and glucose treatments than with algal lysate and soil extract (**Figure 5.4**).

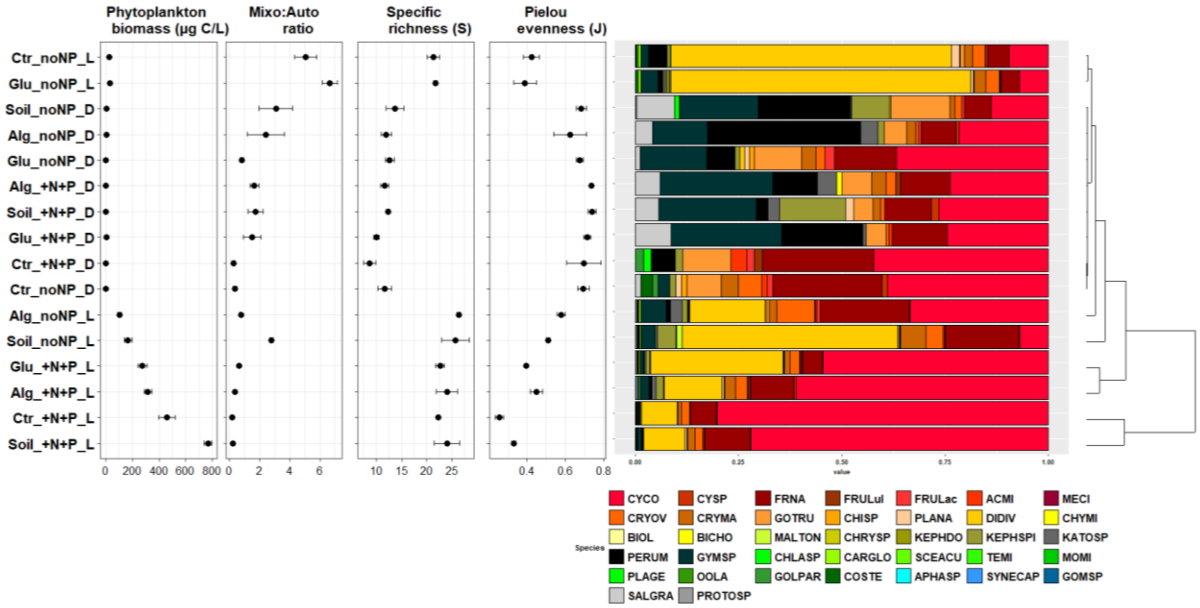

Figure 5.4. Total phytoplankton biomass, mixotrophs:autotrophs (Mixo:Auto) ratio, specific richness, Pielou evenness, and cluster analysis on species biomass controls (Ctr), glucose (Glu), algal (Alg), and soil (Soil) treatments, without nutrients (noNP) and with nutrients (+N+P), in the light (L) and dark (D). Species codes correspondences are showed in **Table A4.1.**

3.2.3.2. Community composition

Multivariate regression trees performed on the relative biomass of the MFG functional groups and phylogenetic affiliation (TAX) identified the light condition as the major factor structuring the community composition, regardless of the classification (**Figure 5.5** and **Figure A4.4**).

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

In the light, phytoplankton community was mainly structured by nutrient enrichment (**Figure 5.5**). In nutrient-enriched treatments, phytoplankton community consisted mainly of small centric diatoms (MFG, group 7a). In unenriched treatments, phytoplankton community differed according to the C treatment, with algal distinct from control, glucose, and soil treatments. The algal treatment consisted mainly in small centric diatoms, large pennate diatoms, and small chrysophytes (MFG, groups 7a, 6b, and 2a), as well as large cryptophytes and small dinoflagellates. In the control, glucose, and soil treatments, phytoplankton community was mainly composed of mixotrophic taxa, mostly chrysophytes, large cryptophytes and small dinoflagellates.

Nevertheless, at the species level, multivariate regression tree distinguished additionally between soil treatment and the two others (glucose and control) in the light without nutrients (**Figure A4.5**). This was mainly caused by the higher proportion of large pennate diatoms (*Fragilaria nanana*) in soil treatments than in glucose and control treatments, but the higher proportion of the large chrysophyte *Dinobryon divergens* than in algal treatments.

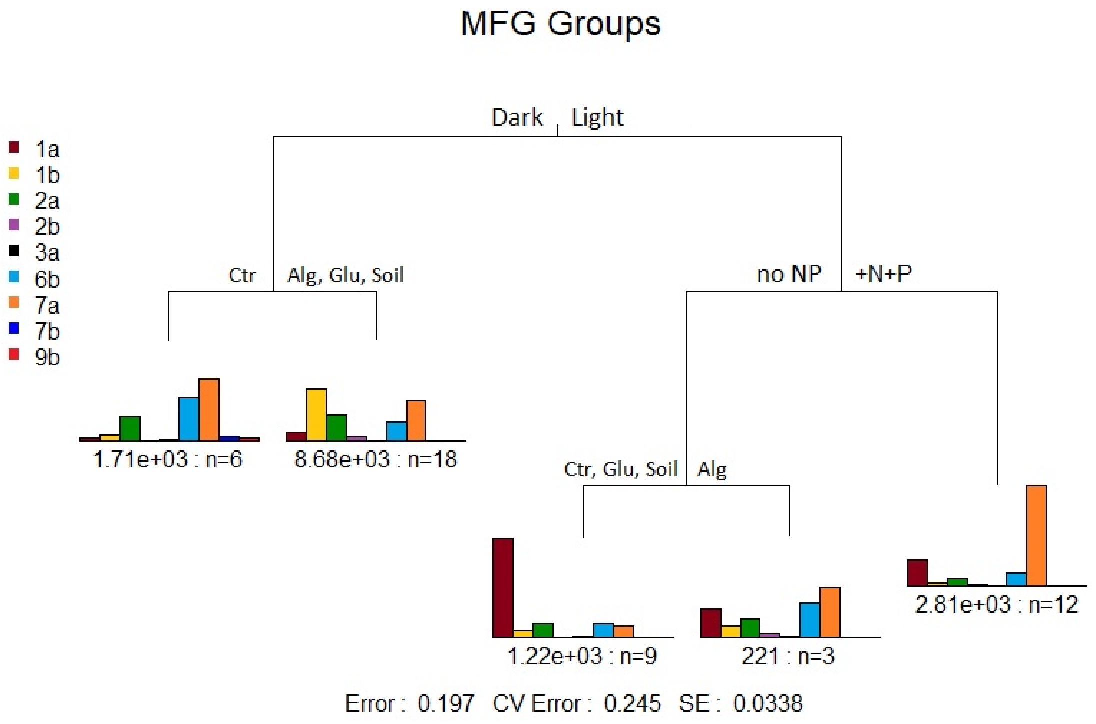

Figure 5.5. Multivariate regression tree analysis performed on relative biomass of phytoplankton MFG groups (> 5% of the total biomass). Ctr: control; Glu: glucose; Alg: algal-derived DOM; Soil: soil-derived DOM. +N+P: nutrient-enriched treatments; no NP: unenriched treatments. Dark: dark conditions; Light: light:dark cycle conditions.

3.2.4. Ciliate abundance

Ciliate abundance varied according to the C amendments and the light conditions (p = 0.001) (**Figure 5.6**). Ciliate abundance was low in the dark (1.8 ± 2.4 cells mL^{-1}) and did not significantly change according to the C source. In the light, under unenriched conditions, ciliate abundance increased with algal lysate (20.7 ± 8.2 cells mL^{-1}) and soil extract (25.1 ± 6.7 cells mL^{-1}) compared to the control (0.0 ± 0 cells mL^{-1}) and the glucose treatment (0.5 ± 0.8 cells mL^{-1}, p = 0.01). In the light under nutrient-enriched treatments, ciliate abundance significantly increased with soil extract (26.2 ± 14.3 cells mL^{-1}) compared to the control (0.0 ± 0 cells mL^{-1}, p = 0.006).

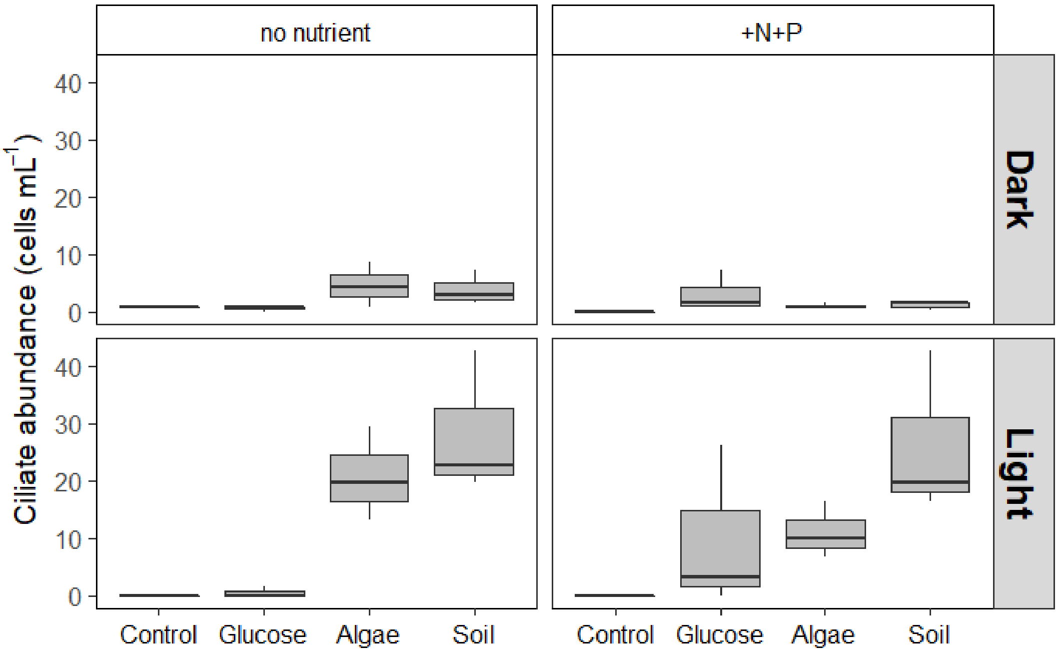

Figure 5.6. Ciliate abundance at the end of the experiment. Algae: algal-derived DOM; Soil: soil-derived DOM. +N+P: nutrient-enriched treatments; no nutrient: unenriched treatments. Dark: dark conditions; Light: light:dark cycle conditions.

4. Discussion

4.1. Bacterial co-limitation

Our findings demonstrate that bacterioplankton was co-limited by carbon and nutrients in the microcosms. The co-limitation pattern was reflected by the lower DOC decrease, and the lower bacterial growth observed with glucose addition alone compared to combined additions of glucose and inorganic nutrients, regardless of the light condition. High-altitude lakes are traditionally oligotrophic (Catalan et al., 2006) and co-limitation of bacteria by both inorganic nutrients and C have already been observed in these ecosystems (Dorado-García et al., 2014; Dory et al., 2022). Our results confirm the co-limitation pattern already observed in other systems depleted in nutrients, such as reservoirs, lakes (Grover, 2000; Ogbebo & Ochs, 2008), or marine systems (Mills et al., 2008). This co-limitation may influence the bacterioplankton utilization of dissolved organic matter: in nutrient-depleted systems like high-altitude lakes, increase phosphorus concentrations have been shown to enhance bacterial uptake of allochthonous DOM (Nelson & Carlson, 2011). Because DOM also contains nutrients, our results suggest that the nutrient content associated to DOM could influence the bacterial utilization of this DOM, as it has previously been suggested (Pérez & Sommaruga, 2006). In addition, atmospheric nutrients deposition have already been documented in this region of the Alps (Rogora et al., 2006), and our result suggest that atmospheric nutrient enrichment impacts may alter the heterotrophic metabolism of dissolved organic matter in these ecosystems.

4.2. Bacterioplankton response to DOM, glucose, and nutrients

Two major results in our experiment support the idea of differential bacterial consumption and transformation depending on the nature of DOM added in the microcosms. First, dissolved organic carbon concentration declined more with algal lysate (-67 ± 6 %) than with soil extract (-59 ± 4 %), glucose (-41 ± 10 %), and in controls (-38 ± 18 %). Second, DOM pool had different dynamics between treatments. The humic-like compound C2 increased in proportion in treatments receiving algal lysate, soil extract, and glucose combined with nutrient additions, and the proportion of humic-like compound C2 increased more with algal lysate (+13%) than in other C treatments. It has been previously demonstrated that biodegradation of organic matter could lead to the production of high molecular weight aromatic material, through alteration of existing compounds and/or production of new compounds by heterotrophic bacteria (Guillemette & del Giorgio, 2012; Jiao et al., 2010). Our study clearly confirmed the dual role of bacterioplankton in DOM dynamics, as consumers but also as producers of humic-like compounds, and further demonstrated that these patterns in bacterial DOM consumption and transformation vary according to the nature of DOM.

Regarding of the co-limitation of bacterioplankton, the higher bacterioplankton biomass and production observed with algal lysate and soil extract than in control, glucose addition, or nutrient addition, suggests that the two sources of DOM provided nutrients to bacterioplankton. In our study, we checked for DIN and SRP concentrations at the initial conditions, and neither DOM amendments increased the DIN and SRP concentrations in the microcosms. It was recognized that DON and DOP in organic matter are potentially very important sources of N and P to heterotrophic bacteria (Fuhrman, 1992), thus we suggest that organic nutrients were provided with both algal lysate and soil extract. Additionally, the peak of bacterioplankton biomass occurred earlier with algal lysate (6th day) and soil extract (4th day) than with glucose associated to inorganic nutrients (8th days). A previous study showed that bacteria preferred DON as amino acid to ammonium for biosynthesis (Kirchman et al., 1989). The observed difference in response dynamic in our microcosms supports the idea of higher bacterial efficiency to produce biomass with organic nutrients than with inorganic compounds. This assumption was confirmed by our BGE estimations, as estimated BGE was systematically higher with algal lysate and soil extract than with glucose additions, regardless of the nutrient enrichment and the light conditions.

Another striking feature was the observed discrepancy between DOC consumption, bacterioplankton biomass, and bacterioplankton production in response to algal lysate and soil extract amendments. Bacterioplankton biomass increased more after soil extract amendments whereas DOC was more consumed in treatments receiving algal lysate. Thus, we observed a higher DOC consumption and a higher bacterioplankton production with algal lysate, which resulted in lower bacterioplankton biomass, whereas the opposite pattern was observed with soil extract. A preferential consumption of algal DOC by lake bacteria has been previously observed (Guillemette et al., 2013; Kritzberg et al., 2004). Our results are totally in line with those of Pérez & Sommaruga (2006), which showed that algal lysate, presumably richer in labile compounds and nutrients, stimulated more the production of bacteria originated from a high-altitude lake than soil extract. More recently, Guillemette et al. (2016) demonstrated that bacterial communities preferentially remove algal C from the terrestrially dominated organic C pool of lakes, but selectively allocate this autochthonous substrate to respiration, whereas terrestrial C was preferentially allocated to biosynthesis. In our study, we thus argue that the observed uncoupling between DOC consumption and bacterioplankton biomass is due to a difference in accessibility and nutritional quality of the DOM.

4.3. Effects of DOM, glucose, and nutrients on phytoplankton biomass

In oligotrophic systems like 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 nitrogen and phosphorus enrichments, especially in the light, indicating a high limitation of phytoplankton by nutrients. This limitation was closely linked to the low concentrations of DIN and SRP of the water at initial conditions of the experiment. Our results confirm those of Jacquemin et al. (2018), who demonstrated that phytoplankton growth was co-limited by N and P in the Lake Cordes in early summer.

In freshwater environments, increasing allochthonous source of DOC may decrease phytoplankton biomass by competition effect between phytoplankton and bacterioplankton for nutrients (Carney et al., 2016), or shading effect (Seekell et al., 2015; Thrane et al., 2014). However, in clear lakes like arctic lakes in Sweden and Alaska or high-altitude lakes, DOM increase have been shown to support increase of phytoplankton biomass by increasing additional nutrients (Kissman et al., 2017; Seekell et al., 2015). In our study, phytoplankton biomass increased with C source amendments compared to controls, in most conditions of temperature and light. Phytoplankton biomass however decreased compared to controls when ciliate abundance was low in the microcosms, that is in the light in nutrient-enriched treatments, with glucose and algal lysate amendments. Ciliates are acknowledged to be major consumers of bacteria (Ameryk et al., 2005). DOC additions have been shown to increase the transfer of bacterial production up 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 prevailed competition between bacterioplankton and phytoplankton, allowing phytoplankton to take advantage of nutrients mineralization by bacterioplankton. At the opposite, strong competition between phytoplankton and bacterioplankton occurred in absence of any top-down regulation of bacterioplankton by ciliates, resulting in a decrease of phytoplankton biomass.

4.4. Effects of DOM, glucose, and nutrients on phytoplankton community composition

Increase of mixotrophy is classically reported with increasing carbon subsidies supplied by terrestrial DOC, in relation to increasing bacterial production (Bergström et al., 2003; Paczkowska et al., 2019). Prey ingestion by mixotrophs prevents the negative effect of competition and the bacterial dominance in the system (Thingstad et al., 1996). However, the nutritional behavior patterns of mixotrophs are very diverse and may vary according to factors at the organisms level, as well as environmental factors (Bergström et al., 2003; Flynn et al., 2019; Saad et al., 2016). Our results highlighted two response patterns of phytoplankton to increasing carbon, depending on the light or nutrient regime. In the dark, mixotrophs increased in the community with increasing glucose, algal lysate, and soil extract, regardless of the nutrient enrichment. In the light, carbon enrichment increased more the development of autotrophs, and the mixotrophs:autotrophs ratio decreased, except with glucose in unenriched treatments. Mixotrophy has been reported as an advantageous strategy under light-limiting conditions to acquire organic carbon, 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 favored by carbon amendments under light-limiting conditions or under nutrient-limiting conditions. We thus suggest that prey ingestion in our microcosms was an efficient strategy to acquire carbon and/or nutrients when light or nutrient limitation occurred. Osmotrophy, the ability to use dissolved organic compounds, is common in phytoplankton (Flynn et al., 2019). In our study, since carbon added in the microcosms was efficiently used by bacteria, we cannot exclude that osmotrophy also allowed to mixotrophs to acquire carbon and nutrients.

When phytoplankton was not limited by light or nutrients, that is in the light in nutrient-enriched conditions or with DOM amendments, the mixotrophs:autotrophs ratio decreased compared to controls. Interestingly, under unenriched conditions, algal lysate increased more the proportion of autotrophs than soil extract. In treatments receiving algal lysate, autotrophs dominated the community and were mainly represented by small centric diatoms. In treatments receiving soil extract, mixotrophs, although reduced, remained dominant in the community and autotrophs were mainly represented by large pennate diatoms. This result was consistent with the higher bacterial biomass observed with soil extract than with algal lysate amendments. Small phytoplankton cells possess advantages over larger cells for resource acquisition, growth rate and photosynthetic rate (Grover, 1989; Raven, 1998) and, because they grow faster, small size diatoms are usually more efficient for nutrient assimilation (Litchman et al., 2009, 2010). Some diatoms may even be able to outcompete bacteria under certain conditions (Havskum et al., 2003; Hitchcock & Mitrovic, 2013). By contrast, large diatoms have huge vacuoles relative to the cell volume, enhancing storage capacity and allowing them to keep the requirement of the limiting resource low (Litchman et al., 2009; Thingstad et al., 2005). We suggest that algal lysate, by providing more nutrients than soil extract, advantaged more the small centric diatoms over large diatoms or mixotrophs than soil extract.

Finally, in the light, specific richness was systematically higher in all treatments receiving nutrients as inorganic compounds or DOM compared to controls or glucose addition alone. An increase of phytoplankton richness has previously been observed with nutrient enrichments associated to resulting from autumn rainfalls (Spatharis et al., 2007) or from snowmelt inputs (Parker et al., 2008). Our results indicate that algal lysate and soil extracts amendments constituted a source of nutrients that had a positive effect on phytoplankton richness.

In summary, the effects of carbon amendments on planktonic communities varied according to the limitation patterns of organisms and the trophic interactions between bacteria and phytoplankton. Bacterial metabolism increased after carbon and nutrient enrichments, because of their co-limitation pattern by C and nutrients. Algal lysate was more consumed by bacteria but less allocated into biomass, whilst soil extract was less consumed but more allocated into biomass. Phytoplankton growth was NP-limited and phytoplankton biomass increased with nutrient enrichments. Increasing of the carbon source had a positive effect on phytoplankton biomass when a top-down control by ciliates or mixotrophs regulated bacterial growth, but a negative effect when top-down regulation of bacteria was low. In this sense, predation control over bacteria determined whether DOM increase could increase phytoplankton biomass by commensalism interactions between bacteria and phytoplankton or decrease phytoplankton biomass by competition with bacteria for nutrients. We further found that increasing a carbon source consistently stimulated mixotrophs under light or nutrient limitation. In the absence of limitation, increasing carbon promoted autotrophy in the community. The increase of autotrophy was more pronounced with algal lysate than with soil extract. This indicates that light and/or nutrient limitation is a major factor in controlling mixotrophy in high altitude lakes. Considering our results in connection to the global change forecasted in the Alps, we suggest that a quantitative and qualitative DOM shift in early summer may influence phytoplankton community and C transfer through the food chain through direct and indirect interactions with bacterioplankton in oligotrophic clear-water lakes with low DOC concentrations.

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General discussion

General discussion

Dissolved organic matter increase in the context of global changes is a major issue for the future of lake ecosystems (Ejarque et al., 2018; Solomon et al., 2015). High altitude lakes are sentinels of global changes (Moser et al., 2019) and should be particularly impacted by increase of dissolved organic matter concentrations. Their planktonic communities, in particular bacteria and phytoplankton, are at the base of the food web and play a determining role in the carbon transfer pathways and the metabolic functioning of these ecosystems (Medina- Sánchez et al., 2004). Dissolved organic matter, through its optical and chemical properties, and because it contains carbon and nutrients, can influence heterotrophic bacteria and phytoplankton differently (Creed et al., 2018). The objective of this thesis is to provide a better understanding of the sensitivity of planktonic communities to dissolved organic matter increase in high altitude lakes. Two main questions were asked and were addressed through four articles:

(iii) How do plankton communities (bacteria and phytoplankton) respond to seasonal variations in dissolved organic matter quality and quantity in a high-altitude lake?

(iv) How could the origin of organic matter, the limitation patterns and the nature of the phytoplankton community modulate the planktonic sensitivity to organic carbon and nutrient increase in high altitude-lakes?

This discussion relates the results of the four articles and aims to bring new elements to understand the response of planktonic communities to dissolved organic matter variations. From these elements also emerge questions and guidelines for reflection on new study perspectives.

First, we will discuss the context of carbon and nutrient limitation of organisms, then we will address the *sensitivity* of planktonic communities to the qualitative and quantitative variation of dissolved organic matter on a seasonal scale. Finally, we will discuss the *vulnerability* of high-altitude lakes to increased dissolved organic matter.

1. Limitation pattern

Sensitivity can be defined as the rapid and severe response of organisms to variations of environmental variations (Adrian et al., 2009). In oligotrophic systems such as high-altitude lakes, the nature of the limiting resource is a determining factor in communities' response to increased carbon or nutrients (Dorado-García et al., 2014).

High-altitude lakes are highly constrained by nutritive resources (Bergström et al., 2013; Lewis, 2011). The field survey of **chapter 2** provided a better understanding of the seasonal functioning of Lake Cordes and the dynamics of nutrient resources. During the ice-influenced period, Lake Cordes is characterized by a strong limitation by light under the ice, by cold temperatures, and hypoxia at the bottom of the water column. These conditions are classically observed under ice high-altitude lakes (Catalan et al., 2006). At the end of the ice-influenced period, the nutrient concentrations (N and P) are relatively low, as well as dissolved organic carbon concentrations (0,9 ± 0,3 $mg.L^{-1}$). The organic matter pool of Lake Cordes is essentially dominated by dissolved organic matter from sedimentary and allochthonous origin. The lability of organic matter decreases when isolated from external inputs (Del Giorgio & Davis, 2003), which suggests a refractory nature of organic matter on Lake Cordes before allochthonous inputs provided by snowmelt.

In early summer overturn, Lake Cordes is characterized by an increase in the light availability, temperature, and oxygen concentrations throughout the water column. These modifications are classically observed during the spring mixing period in high-altitude lakes (Catalan et al., 2006). The pool of organic matter in the lake is essentially from allochthonous origin, provided by catchment during snowmelt. DOC concentrations remain low (0,5 ± 0,2 $mg.L^{-1}$), but nutrient concentrations, particularly P, are higher during this period.

In late summer, Lake Cordes is characterized by an increase in water temperature but a decrease of in nutrient concentrations, especially dissolved inorganic nitrogen. At the opposite, dissolved organic carbon concentrations increase (1,9 ± 0,2 $mg.L^{-1}$) and dissolved organic matter mainly exhibit an autochthonous signature from phytoplankton.

In oligotrophic systems, heterotrophic bacteria can be limited by inorganic nutrients, mainly phosphorus (Bertoni et al., 2008), by organic carbon (Kritzberg et al., 2005),), or co-limited by nutrients and carbon (Vidal et al., 2011). The experimental results of **chapters 3 and 4** highlighted a co-limitation by carbon and nutrients of heterotrophic bacteria, whatever the season. However, in late summer, bacteria are more limited by inorganic nutrients in relation to a greater availability of phytoplankton-derived DOC in initial conditions.

In the field survey of **chapter 2**, the dynamic of limitation resulted in very low bacterial biomass during the ice-influenced period, and in a gradual increase of this biomass to reach the maximum values during late summer period. Patterns of bacterial limitation by inorganic nutrients (especially P) have already been observed throughout the summer in high altitude lakes in the Pyrenees (Zufiaurre, 2019). Our study confirms the co-limitation by C and by inorganic nutrients of heterotrophic bacteria in high-altitude lakes (Dorado-García et al., 2014) as well as the seasonal evolution of this limitation in relation to the increase of organic matter from phytoplankton excretions at the end of summer (Villar-Argaiz et al., 2002).

The experimental results of **chapters 3 and 4** highlighted a strong limitation by inorganic nutrients of the phytoplankton biomass, variable according to the seasons. Phytoplankton biomass was moderately limited by inorganic nutrients in under-ice communities, weakly limited in early summer communities, and strongly limited by nutrients in late summer communities. These results confirm the limitation of phytoplankton growth by inorganic nutrients in high altitude lakes (Bergström et al., 2013; Lewis, 2011) and the seasonal evolution of this limitation (Jacquemin et al., 2018).

In the field survey of **chapter 2**, this limitation dynamic resulted in low phytoplankton biomass during the ice-covered period and in early summer, and a maximum biomass in late summer. As has been shown in many studies, the type of nutrient limitation of phytoplankton growth is closely related to N and P concentrations in the water column (Leruste et al., 2019; Morris & Lewis, 1988).

The experiments of **chapters 3 and 4** highlighted a strong limitation by inorganic nutrients of heterotrophic bacteria and phytoplankton in Lake Cordes, whatever the season. The limitation by the same inorganic nutrients of bacteria and phytoplankton induces mechanisms of competition for the acquisition of these nutrients (Carney et al., 2016). Our results therefore confirm strong concurrence processes between bacteria and phytoplankton for nutrients, as has already been observed in a high-altitude lake of the Sierra Nevada (Spain) (González-Olalla et al., 2018). In addition, we show that this concurrence takes place during all the studied periods in Lake Cordes. In the field survey of **chapter 2**, we observed higher DOC concentrations from phytoplankton origin in late summer in Lake Cordes. Since commensalism occurs when bacteria use the organic carbon excreted by phytoplankton (Carrillo et al., 2015), our results suggest a strengthening of commensalistic interactions between bacteria and phytoplankton over the summer in Lake Cordes.

2. Sensitivity of planktonic communities to dissolved organic matter

2.1. Sensitivity of bacteria

In the experimentations of **chapters 3 and 4**, a seasonal response of heterotrophic bacteria to experimental increase of DOC and nutrients have been observed. In ice-covered communities, DOC is highly consumed by bacteria, and increased DOC and nutrients stimulate biomass production, although top-down regulation by ciliates and mixotrophs rapidly regulates bacterial growth. In early summer communities, DOC is highly consumed but increased DOC and nutrients lowly stimulate biomass. In late summer communities, DOC is lowly consumed but increase DOC and nutrients strongly stimulates bacterial biomass. It has previously been shown that autochthonous dissolved organic carbon is readily consumed by bacteria owing to its great accessibility and nutritional quality (Guillemette et al., 2013). Our results confirm that an increase in phytoplankton biomass in late summer can reduce the carbon limitation of bacteria, conditioning a lower DOC consumption under experimental conditions in late summer communities.

The rapid response of bacteria to a carbon source in high-altitude lakes is sometimes accompanied by a replacement of species such as copiotrophic taxa, i.e. taxa adapted to growth in nutrient-rich environments (Rofner et al., 2017). In a high-altitude lake, a taxonomic modification of bacterial communities has previously been observed after experimental additions of dissolved organic matter (Pérez & Sommaruga, 2006). The increase in organic substrates under experimental conditions also led to differences in structure, production, respiration and growth efficiency in a lake bacterial community (Kritzberg et al., 2006). In Lake Cordes, allochthonous inputs after snowmelt in early summer were able to select bacterial taxa with a nutritional strategy more focused on respiration than growth. We suggest that a taxonomic or functional change of bacteria between periods may have stimulated respiration more strongly with the controlled increase in DOC and nutrients in early summer communities.

- It would be interesting to better understand if the seasonal heterogeneity of the bacterial response to increase DOC and nutrients under experimental conditions is due to taxonomic, functional, or physiological modifications of the bacterial communities?

2.2. Sensitivity of phytoplankton

In high-altitude lakes, changes in environmental conditions induce seasonal dynamics in the biomass and composition of phytoplankton communities (McKnight et al., 1990; Tiberti et al., 2013). This dynamic in turn leads to variability in the response of communities to increased nutrient concentrations (Jacquemin et al., 2018). The experimental results of **chapters 3 and 4** highlighted a strong limitation by inorganic nutrients of the phytoplankton biomass, variable according to the seasons. In ice-covered communities in the light, we observed a trend of decreasing phytoplankton biomass with the joint increase of DOC and nutrients compared to nutrient additions alone. On the contrary, in early and late summer communities, the combined increase in DOC and nutrients does not modify the positive effect of nutrients (at 18°C) or trend toward an accentuation of the positive effect of nutrients (at 10°C) on phytoplankton biomass. Our results confirm that increasing DOC concentrations modifies the positive action of nutrients on phytoplankton biomass, as has already been shown in boreal lakes (Deininger et al., 2017) and underline the variability of this effect depending on the temperature and seasons.

Certain phytoplankton groups, such as cryptophytes, exhibited a strong seasonal response to the experimental increase of DOC and nutrients in interaction with light availability, despite a relatively similar species composition within this group over seasons. In under-ice and early-summer communities, cryptophytes were strongly favored by the experimental increase in organic carbon and nutrients in the dark. On the contrary, in late summer communities, these same species were only poorly favored in the dark with DOC and nutrients, and only at 18°C. Previous studies have shown that some species of cryptophytes were able to grow in the dark only through the ingestion of prey (McKie-Krisberg et al., 2015) and this group is often observed in under-ice environments where light is limiting (Laybourn-Parry & Marshall, 2003; Marshall & Laybourn- Parry, 2002; Rue et al., 2020). The life history of organisms, especially light availability, can induce seasonal variability in the planktonic response to environmental changes (Ji et al., 2010). Our studies confirm the growth potential of cryptophytes by phagotrophy under light-limiting conditions but go further by demonstrating a change in the physiology and in the nutritional strategy of taxa depending on environmental conditions. We hypothesize that the low light availability in winter in the Lake Cordes selects a predominantly mixotrophic strategy in cryptophytes in under-ice and early summer communities. The nutritional strategy of cryptophytes changes when light conditions become favorable for autotrophy again. The field survey of **chapter 2** showed that cryptophytes are particularly well represented in natural communities during the ice-influenced period and their proportion gradually decreases during the summer. These results support the idea that mixotrophy is a major competitive advantage for cryptophytes under light-limiting conditions in high-altitude lakes, as previously suggested (Rue et al., 2020). Cryptophytes seem to lose their competitive advantage against large pennate diatoms in relation to the increase of light availability in late summer in the natural community. The presence of vacuoles in large diatoms has been recognized as a competitive functional trait against mixotrophs (Cadier et al., 2020) and could explain the dominance of large pennate diatoms over cryptophytes at the end of summer in Lake Cordes. Previous studies found that large diatoms develop efficient nutrient acquisition strategies when nutrients are scarce (Endo et al., 2018; Litchman et al., 2009, 2010), and this strategy (K-strategy) allows large diatoms to dominate at the end of summer succession in oligotrophic lakes (Bürgi et al., 2003). In addition, large cells are more resistant to predation (Litchman et al., 2010; Salmaso et al., 2015), and the high abundance of ciliates observed during late summer period in Lake Cordes may have been a factor regulating mixotrophic phytoplankton (Haraguchi et al., 2018).

The experiments of **chapters 3 and 4** showed an increase of the proportion of large pennate diatoms in early summer communities with increase in controlled conditions of DOC and nutrients. A positive effect of experimental additions of dissolved organic matter on large pennate diatoms density has already been observed in a high-altitude lake (Kissman et al., 2013) and this response is explained by the positive effect of nutrients providing by organic matter additions. Our results confirm the autotrophic mode and the strong competitive potential of pennate diatoms in response to nutrient increase (Garrido et al., 2016). In addition, previous studies demonstrated that some diatoms were able to assimilate organic compounds (Tuchman et al., 2006) and are able to outcompete heterotrophic bacteria for inorganic nutrients when they are not limited by other resources, such as silica (Havskum et al., 2003). In high altitude lakes, early summer period is a less limiting period regarding on light and inorganic nutrient availability compared to ice-influenced or late summer period (Jacquemin et al., 2018; Rue et al., 2020). These results highlight that the competitive potential of diatoms varies seasonally with increasing DOC. We suggest that the reduction of natural constraints by light or nutrients in early summer may have favored autotrophic diatoms to the detriment of other phytoplankton groups after the increase in controlled conditions of DOC and nutrients.

Experimentation of **chapter 3 and 4** highlighted that chrysophytes are mainly favored by the experimental increase of DOC and nutrients when light is non-limiting. These chrysophytes are mostly represented by the genus *Dinobryon* sp. which has been reported in the literature as obligate phototroph, i.e. needing a sufficiently available light source to achieve mixotrophy (Rottberger et al., 2013). An exception could however be observed in under-ice communities at 18°C, where chrysophytes belonging to the genus *Ochromonas* sp. grew in the dark after DOC additions. This genus, whose phagotrophic capacities have been demonstrated in the dark in previous studies (Lie et al., 2017), was sparsely represented in the initial communities of early summer and late summer. Our work confirms the idea that chrysophytes of the genus *Dinobryon* sp. are obligate phototrophs, whatever the season. A variability in the phytoplankton response to DOC and nutrients can be observed depending on the initial composition of the community and the nutritive strategies of the organisms.

The field survey of **chapter 2** made it possible to highlight a strong representativeness of chrysophytes in the communities in early summer, which underlines the importance of light availability in the taxonomical and functional composition of phytoplankton communities in high-altitude lakes. As with cryptophytes, chrysophytes lost their competitive advantage against autotrophic diatoms in the natural late summer community.

Our results highlight a variability of nutritional strategies and functional traits depending on phytoplankton groups but also environmental conditions. In relation to the observed differences in response of planktonic communities, changes in the relationships between bacteria and phytoplankton are expected with variations in DOC and nutrient concentrations.

2.3. Interactions between bacteria and phytoplankton

The experimental results of **chapters 3 and 4** allowed to better understand to what extent the increase in DOC and nutrient concentrations could modify the existing interactions between bacteria and phytoplankton. We found an increase of the ratio between mixotrophic and autotrophic phytoplankton in the ice-covered and late summer communities with increasing DOC and nutrients, but a decrease of the proportion of mixotrophic phytoplankton in the early summer community. According to Modenutti (2014), mixotrophy is an advantageous strategy under light-limited or nutrient-limited conditions. In high-altitude lakes, light-limitation of phytoplankton is traditionally observed under ice during winter (Catalan et al., 2006), while nutrient limitation increases during summer to become more pronounced in late summer

(Jacquemin et al., 2018). We suggest that DOC and nutrients increase under experimental conditions favors the expression of mixotrophy in phytoplankton assemblages from under-ice and late summer periods, in relation with the initial limitation of these assemblages by light, or nutrients (**Figure 6.1**).

The experimental results of **chapter 4** support this hypothesis. Indeed, the supply of organic carbon increases the proportion of mixotrophs in phytoplankton assemblages in early summer but only under experimental conditions limited by light or by nutrients. Thus, the taxonomic and functional composition and the nutritional strategy of mixotrophic taxa, themselves determined by the environmental conditions, are key elements determining the seasonal expression of mixotrophy after DOC and nutrient inputs under controlled conditions.

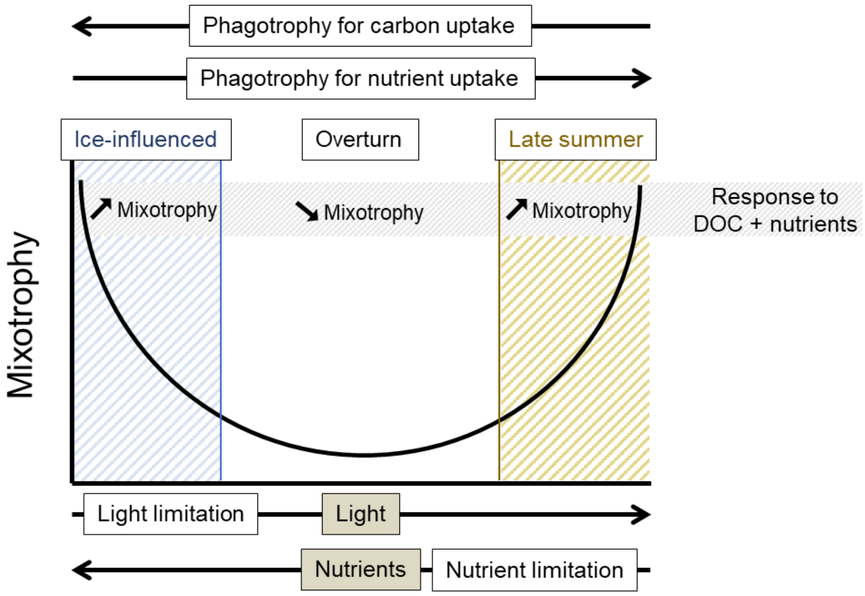


Figure 6.1. Replacement of the different periods of the Lake Cordes and experimental results observed at each period in the general model of factors influencing mixotrophy developed by Modenutti (2014).

The field survey of **chapter 2** did not reveal any increase of mixotrophy in late summer in relation to increase of DOC concentrations in the water column. During the late summer period, when DOC concentrations are at their maximum in the water column, autotrophy is favored with a very strong competitive advantage of large pennate diatoms. However, the nature of the organic matter could explain the differential response observed between the experimental and field survey results.

Indeed, in the experimentation of **chapter 5**, we highlighted a very strong decrease in the proportion of mixotrophic phytoplankton in early summer communities after experimental enrichments with autochthonous dissolved organic matter (algal lysate) compared to enrichments with allochthonous organic matter (soil extract). These phytoplankton modifications are closely linked to the bacterial utilization of organic matter: algal lysate is more consumed, but soil extract is more allocated into biomass. These results confirm that heterotrophic bacteria preferentially remove algal lysate in relation to the higher quality and lability of autochthonous dissolved organic matter (Guillemette et al., 2013), but allocate more allochthonous organic matter for biomass production (Guillemette et al., 2016). We suggest that the autochthonous nature of the dissolved organic matter at the end of summer in Lake Cordes may have favored the development of autotrophs thanks to (i) their strong competitive potential against mixotrophs for the acquisition of nutrients and (ii) their grazers resistance strategy (large size) when ciliates are more abundant in late summer.

The results of this thesis highlighted a strong sensitivity of bacteria and phytoplankton to dissolved organic carbon and nutrients increase, as well as to qualitative and quantitative variation of dissolved organic matter. These results made it possible to identify different response patterns of bacteria and phytoplankton depending on the season, the phytoplankton community composition, and the quality of the dissolved organic matter. Several questions merit further investigation in the future:

- Phytoplankton communities mirror the environmental fluctuations in high-altitude lakes (Kuefner et al., 2021). The metabolic theory of Wilken et al. (2013) predicts an increase in heterotrophy in mixotrophic taxa with increasing temperature. In some mixotrophs, effective mixotrophy is only expressed when prey and light availability in their environment reach sufficient quantities (Livanou et al., 2020). How could the nutritional strategy of the same organism evolve according to its environmental context? Will the response of mixotrophs to inorganic and organic inputs vary under different environmental constraints linked to global changes?

- An increase of the abundance of mixotrophic phytoplankton is not always followed by a proportional increase in prey ingestion rate (Gerea et al., 2016). How could ingestion rates of mixotrophs on bacteria (bacterivory) could vary with dissolved organic matter increase?

- Quantifying the proportion of organic carbon excreted by phytoplankton, and the proportion of this carbon used by bacteria would increase our understanding of the processes involved in the carbon transfer in response to increase of dissolved organic matter in high altitude lakes.

Both experimental and field survey results underlined the importance of the predation control exerted by ciliates in the bacterial and phytoplankton response. The complexity of food web dynamics and structure can create alternative pathways of energy flow toward the highest trophic levels (Hulot et al., 2014; Vadeboncoeur et al., 2005). Increased dissolved organic carbon can increase or decrease the efficiency of energy transfer in the food web depending on the presence of zooplankton or fish (Degerman et al., 2018). Most of the high-altitude lakes in the Southern Alps are subject to fish introduction, which directly affects the structure of zooplankton assemblages (Cavalli et al., 2001; Tiberti et al., 2014). In perialpine lakes, zooplankton has been shown to be a good indicator of the lake functioning (Jacquet et al., 2014).

- It would be interesting to integrate the entire food web in future studies to know how top-down control by ciliates, zooplankton and fish regulates the response of bacteria and phytoplankton to increased DOM.

3. Vulnerability of Lake Cordes

The vulnerability to global changes refers to the level of threats that global changes pose to ecosystems and results in the degree of exposition to climatic and anthropogenic impacts, the sensitivity of organisms, and the adaptive capacity of the ecosystem (Kling et al., 2020). Within the Southern Alps, high-altitude lakes are subject to landscape filters (geographical position, catchments characteristics, lake morphology) and to internal filters (abiotic and biotic interactions) which condition the response patterns of the ecosystem to environmental variations (Blenckner, 2005).

Lake Cordes is located in a geographical area with low exposure to atmospheric nitrogen and phosphorus deposition (Fagerli et al., 2015). In this sense, it is not highly exposed to an increase in inorganic nutrient concentrations related to atmospheric deposition. In this lake, a strong limitation of phytoplankton growth by inorganic nutrients has been shown during the summer period, accentuated during the growing season with resources depletion (Jacquemin et al., 2018). In relation to the strong limitation of organisms, the increase in nutrient concentrations should lead to a high magnitude response from planktonic communities on this lake (O'Donnell et al., 2017). Lake Cordes is characterized by a large catchment area, mainly covered with alpine meadow. The area of the catchment and the percentage of vegetation condition the quantity of material transferred to the lakes (Rose et al., 2015). The amount of allochthonous organic matter transferred to the lakes should be greater in lakes with large, vegetated catchments. The main contributions of the thesis results to the assessment of the sensitivity of planktonic communities and the vulnerability of Lake Cordes to the increase of organic matter are summarized in **Figure 6.2**.

High-altitude lakes of the Southern Alps are expected to experience major environmental changes with the forecasted global changes (Gobiet et al., 2014; Moser et al., 2019). More intense and frequent extreme rainfall events are expected to induce an increase of dissolved organic matter pulse into the lakes (Perga et al., 2018) although dilution effects could also lead to a reduction in the quantity of organic and inorganic nutrients (Anderson & Stedmon, 2007). Temperature increase will lead to catchments afforestation in altitude and to increase of catchment productivity (Elliott & Kipfmueller, 2011) and therefore the quantity of allochthonous organic matter transferred from the catchments to the lakes (Ejarque et al., 2018). A lengthening of the summer season will lead to an increase in the temporal window of non-limiting light and temperature increase (De Senerpont Domis et al., 2013). Shorter winter periods are expected to increase lake primary productivity (Oleksy et al., 2020b; Roberts et al., 2017) and increase the proportion of diatoms in phytoplankton communities (Rühland et al., 2008; Rühland et al., 2015). However, a lower snow cover in winter related to higher temperature could also reduce the amount of allochthonous inputs transferred to the lake during snowmelt.

In the context of global changes, increasing concentration of organic matter in Lake Cordes should increase growth and/or respiration of heterotrophic bacteria. The increase of bacterial biomass could increase the probability of development of mixotrophic phytoplankton and heterotrophic ciliates during winter and late summer communities. A better availability of inorganic nutrients associated to dissolved organic matter inputs should increase phytoplankton biomass. In early summer, an increase of autochthonous dissolved organic matter should significantly decrease the proportion of mixotrophic phytoplankton in communities. An increase of allochthonous dissolved organic matter should induce a slight decline of mixotrophic taxa in early summer phytoplankton communities. The effects of organic matter increase should interact with water temperature increase and light availability and should induce a complexification of planktonic communities' response and interactions between phytoplankton and bacterioplankton on Lake Cordes. However, if caused by storm events, the increase of organic matter concentrations should be associated with a decrease in water temperature (Doubek et al., 2021), which could attenuate the interaction effect of increasing temperature with increasing DOM concentrations.

The expected modifications of bacteria and phytoplankton communities should affect the whole functioning of Lake Cordes and the services it provides. If inorganic nutrient concentrations increase associated with dissolved organic matter exceeds the nutritional requirements of both bacteria and phytoplankton, the increase in phytoplankton biomass could lead to eutrophication processes, as has already been observed in lakes in the Southern Alps in relation to pastoralism or tourist activity (Jacquemin, 2019). A decrease of the ratio between mixotrophic and autotrophic phytoplankton in early summer should increase the number of trophic links between microbial loop and higher trophic levels (zooplankton, fishes). Consequently, we expect a decrease of carbon transfer efficiency through the trophic chain (González-Olalla et al., 2018; Medina-Sánchez et al., 2004). These consequences should not be observed in winter or in late summer. The seasonal dynamic of dissolved organic matter should unbalance the equilibrium between heterotrophy and autotrophy and therefore the overall metabolism of the lake at a seasonal scale.

4. Vulnerability of high-altitude lakes

The increase of dissolved organic matter concentration in high-altitude lakes should induce (i) a taxonomical and functional modification of phytoplankton communities and (ii) a modification of interactions, with bacteria with variable effects at a seasonal scale and with organic matter quality. However, differences in the vulnerability of lakes to dissolved organic matter variations are expected according to the contexts of atmospheric nutrients deposition and the catchment characteristics.

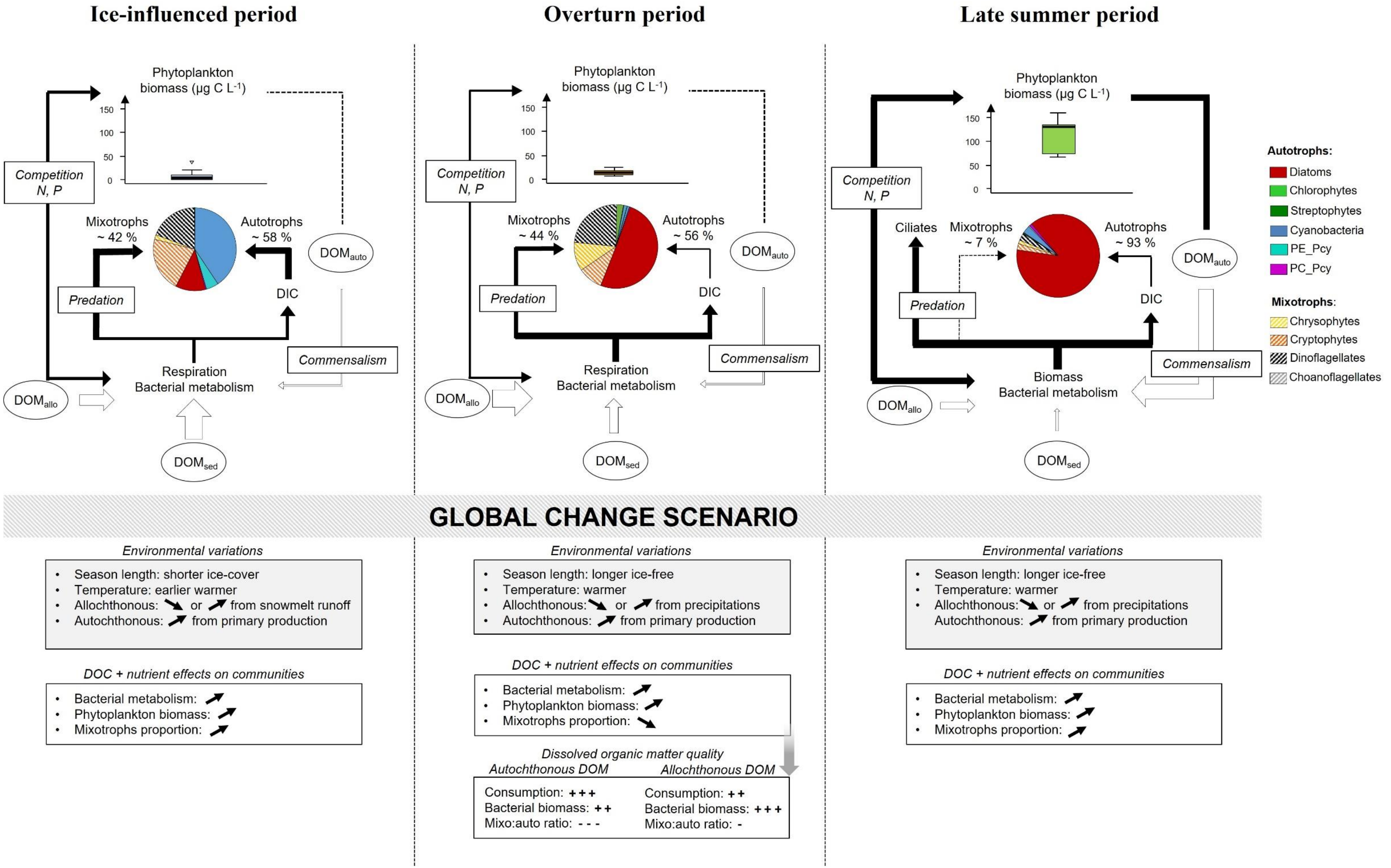
In lakes subjected to elevated atmospheric deposition of N and P, heterotrophic bacteria are expected to be limited primarily by organic carbon. We therefore expect less important competition processes between bacteria and phytoplankton for inorganic nutrients after DOC inputs. In turn, this could induce an uncoupling between the bacterial and phytoplankton response (Nelson & Carlson, 2012) and a weakening of interactions between phytoplankton and bacteria.

Since the characteristics of the catchments determine the proportion of mixotrophs and the composition of phytoplankton communities (Jacquemin et al., 2019; Tolotti et al., 2003, 2006), catchments with lower area and vegetation cover could reduce the top-down control of predation by mixotrophs on heterotrophic bacteria after additions of organic matter. Assuming that, the top-down control over bacteria could be mainly performed by ciliates and nanoflagellate heterotrophs in such lakes.

Depending on the lakes and catchment characteristics, late summer phytoplankton communities may be dominated by other types of phytoplankton assemblages in high altitude lakes (McKnight et al., 1990; Tiberti et al., 2013), such as mixotrophs or chlorophytes. Heterotrophic bacteria could be more impacted by increased competition for inorganic nutrients if phytoplankton assemblages are dominated by chlorophytes, which could have stronger competitive potentials than diatoms (Oleksy et al., 2020a).

It would be interesting to understand more precisely the influence of catchments characteristics and the atmospheric deposition context of nitrogen and phosphorus on the evolution of interactions between bacteria and phytoplankton in relation to the increase in dissolved organic matter.

The magnitude of changes in planktonic food webs induced by variations in dissolved organic matter will vary depending on (i) the context of lake exposure to atmospheric deposition of nitrogen and phosphorus (Camarero & Catalan, 2012; Jacquemin et al., 2019); (ii) the morphology of lakes and the characteristics of their watersheds (Blenckner, 2005; Jacquemin et al., 2019); (iii) biotic interactions of bacteria and phytoplankton with other trophic compartments, such as the benthic compartment, macrophytes, predatory zooplankton, or fish (Degerman et al., 2018; Kissman et al., 2017); (vi) interactions of organic matter with other environmental factors, such as temperature, light, stratification, or the length of the summer period (Cabrerizo et al., 2017; Carrillo et al., 2015; Durán et al., 2016).



Environmental variations

- Season length: shorter ice-cover
- Temperature: earlier warmer
- Allochthonous: ↘ or ↗ from snowmelt runoff
- Autochthonous: ↗ from primary production

DOC + nutrient effects on communities

- Bacterial metabolism: ↗
- Phytoplankton biomass: ↗
- Mixotrophs proportion: ↗

Environmental variations

- Season length: longer ice-free
- Temperature: warmer
- Allochthonous: ↘ or ↗ from precipitations
- Autochthonous: ↗ from primary production

DOC + nutrient effects on communities

- Bacterial metabolism: ↗
- Phytoplankton biomass: ↗
- Mixotrophs proportion: ↘

Dissolved organic matter quality

Autochthonous DOM	*Allochthonous DOM*
Consumption: + + +	Consumption: + +
Bacterial biomass: + +	Bacterial biomass: + + +
Mixo:auto ratio: - - -	Mixo:auto ratio: -

Environmental variations

- Season length: longer ice-free
- Temperature: warmer
- Allochthonous: ↘ or ↗ from precipitations

Autochthonous: ↗ from primary production

DOC + nutrient effects on communities

- Bacterial metabolism: ↗
- Phytoplankton biomass: ↗
- Mixotrophs proportion: ↗

Figure 6.2. Synthetic diagram of the relationships between dissolved organic matter and planktonic communities observed on Lake Cordes during the different periods, and sensitivity of these communities and their interactions to increase of dissolved organic matter in a global change scenario.

Conclusions and perspectives

The main conclusions of this thesis are as follows:

- The origin and the concentration of dissolved organic matter is very dynamic at an annual scale. This variability can be related to the phytoplankton community structure. Dissolved organic matter characteristics interact with other environmental factors such as light, temperature and nutrients and determine the seasonal variation of bacteria – phytoplankton relationships.
- The quality of dissolved organic matter influences the bacterial and phytoplankton response to increase DOM concentrations. Autochthonous dissolved organic matter is more consumed by bacteria and the proportion of mixotrophs decreased drastically in non-limiting light conditions. Allochthonous dissolved organic matter is less consumed by bacteria but stimulates more the biomass production, and the proportion of mixotrophic phytoplankton decreases slightly in the phytoplankton community in non-limiting light conditions.
- On Lake Cordes, the contrasted environmental conditions influence the phytoplankton community composition, which itself determines the response of bacteria and phytoplankton to qualitative and quantitative variations in dissolved organic matter. The observed response is the result of the organisms' intrinsic response and fluctuating environmental conditions. Our thesis work highlights the importance of integrating dissolved organic matter in the assessment of the vulnerability of high-altitude lakes. This evaluation of organic matter must be associated with the environmental context which acts as a modulator of the response of organisms.

The results of this thesis raise new perspectives:

- The quantification of the interactions between bacteria and phytoplankton is interesting to assess the hypothesis that the increase of dissolved organic matter in high altitude lakes should alter the carbon transfer pathways to higher trophic levels. This quantification of interactions involves measurements of the rate of ingestion of bacteria by mixotrophic taxa (bacterivory), the amount of organic carbon excreted by phytoplankton, and the proportion of this carbon used by heterotrophic bacteria.
- To improve our understanding of the processes influencing the interactions between bacteria and phytoplankton, it would be interesting to know to what extent the taxonomic and functional modifications of bacteria can in turn influence the phytoplankton response to dissolved organic matter variations.
- Extending the results obtained on Lake Cordes is crucial for an accurate assessment of the vulnerability of high-altitude lakes to dissolved organic matter variations. It could be interesting to characterize the seasonal dynamics of organic matter in relation to planktonic communities in lakes subject to different contexts of exposure to atmospheric deposition and characteristics of watersheds, and therefore different nutrient limitation contexts. Assessing the response of taxonomically and functionally contrasting phytoplankton communities would provide a better understanding of the role of the phytoplankton community in the response of organisms to dissolved organic matter.

Appendices

1

Appendix 1: supplementary information of chapter 2

Table A1.1. Abiotic and biotic parameters measured in Lake Cordes over the sampling periods. Mean ± standard errors. DOC: dissolved organic carbon; P (rain): precipitation excluding snowfall; T: temperature; O2: dissolved oxygen; Turbi: turbidity; DIN: dissolved inorganic nitrogen; SRP: soluble reactive phosphorus; Si: silica; Phyto: phytoplankton biomass; HP: bacterioplankton biomass; PCY: picocyanobacteria biomass. IIP: ice-influenced period; OP: overturn period; LSP: Late summer period.

Period	IIP	IIP	IIP	IIP	OP	OP	OP	OP	LSP	LSP	LSP
Date	**12/02/20**	**16/03/21**	**04/05/21**	**16/06/21**	**17/06/20**	**16/07/20**	**29/06/21**	**22/07/21**	**25/08/20**	**18/09/20**	**10/10/20**
DOC (mg/L)	1,18 ± 0,22	0,66 ± 0,00	0,88 ± 0,20	1,17 ± 0,61	0,44 ± 0,03	0,51 ± 0,00	0,28 ± 0,00	0,77 ± 0,11	1,62 ± 0,32	1,87 ± 0,05	2,16 ± 0,11
HIX		2,26 ± 1,23	3,46 ± 0,53	4,58 ± 0,47	2,15 ± 0,11	1,77 ± 0,02	2,43 ± 0,90		0,27 ± 0,11	0,95 ± 0,96	2,83 ± 0,25
BIX		1,53 ± 0,83	0,72 ± 0,00	0,64 ± 0,00	0,89 ± 0,12	0,72 ± 0,00	1,09 ± 0,42		5,88 ± 0,79	4,22 ± 3,47	0,84 ± 0,13
FI		1,42 ± 0,01	1,40 ± 0,03	1,39 ± 0,07	1,35 ± 0,01	1,36 ± 0,01	1,34 ± 0,04		1,41 ± 0,02	1,37 ± 0,02	1,31 ± 0,01
P (rain) (mm)	0,00	0,00	0,00	15,00	41,60	3,60	5,60	19,00	2,40	6,40	46,80
T (°C)	4,78 ±	4,25 ±	4,30 ±	5,41 ±	6,25 ±	12,42 ±	7,68 ±	12,51 ±	15,61 ±	12,20 ±	4,99 ±
O2 (mg/L)	0,04 ±	0,11 ±	0,14 ±	9,66 ±	12,15 ±	11,74 ±	8,83 ±	13,39 ±	11,26 ±	9,73 ±	10,48 ±
Turbi (NTU)	1,87 ±	2,08 ±	7,16 ±	1,26 ±	0,45 ±	0,10 ±	2,08 ±	0,07 ±	0,04 ±	0,04 ±	1,31 ±
DIN (mg/L)	0,67 ± 0,06	0,14 ± 0,03	0,17 ± 0,05	0,76 ± 0,71	0,52 ± 0,00	0,31 ± 0,02	0,12 ± 0,00		0,22 ± 0,12	0,12 ± 0,01	0,24 ± 0,00
SRP (mg/L)	0,02 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0,06 ± 0,00	0,01 ± 0,00	0,01 ± 0,00		0,01 ± 0,00	0,03 ± 0,00	0,01 ± 0,00
C:N$_{POM}$		6,00 ± 0,00	7,50 ± 1,29			12,33 ± 6,1			7,33 ± 1,03	7,00 ± 0,00	
Si (mg/L)		1,75 ± 0,50	2,91 ± 1,41	3,04 ± 1,35	1,67 ± 0,02	0,80 ±0,05	1,12 ± 0,01		0,61 ± 0,03	0,68 ± 0,01	1,10 ± 0,15
δ^{13}C$_{POM}$ (‰)			-29,2 ± 2,6		-28,2 ± 0,8	-26,6 ± 0,4			-25,7 ± 0,2	-25,8 ± 0,6	
Richness	5,59 ± 2,14	3,71 ±1,47	3,50 ± 2,10	4,24 ± 2,81	5,68 ± 1,97	7,56 ± 2,43	6,32 ± 1,90	9,13 ± 2,73	6,55 ± 3,39	5,67 ± 1,64	4,36 ± 3,58
Diversity	0,79 ± 0,11	0,48 ± 0,12	0,34 ± 0,19	0,50 ± 0,20	0,73 ± 0,07	0,84 ± 0,06	0,83 ± 0,08	0,93 ± 0,11	0,61 ± 0,05	0,45 ± 0,02	0,46 ± 0,04
Phyto (µg C/L)	4,26 ± 1,67	1,70 ± 0,70	9,93 ± 8,66	20,4 ± 18,4	12,79 ± 0,8	20,70 ± 3,6	11,87 ± 6,3	9,50 ± 2,4	138,2 ± 14,1	130,6 ± 9,7	69,9 ± 5,3
HP (µg C/L)	10,50 ± 1,4	4,00 ±1,5	6,58 ± 0,4	5,02 ± 1,2	8,17 ± 0,8	13,78 ± 0,7	11,20 ± 0,9		20,12 ± 2,6	19,33 ± 0,7	10,31 ± 3,2
PCY (µg C/L)	0,42 ± 0,0	0,38 ± 0,1	0,08 ± 0,1	0,06 ± 0,1	0,06 ± 0,0	0,02 ± 0,0	0,14 ± 0,0		0,16 ± 0,1	0,26 ± 0,1	1,29 ± 0,2

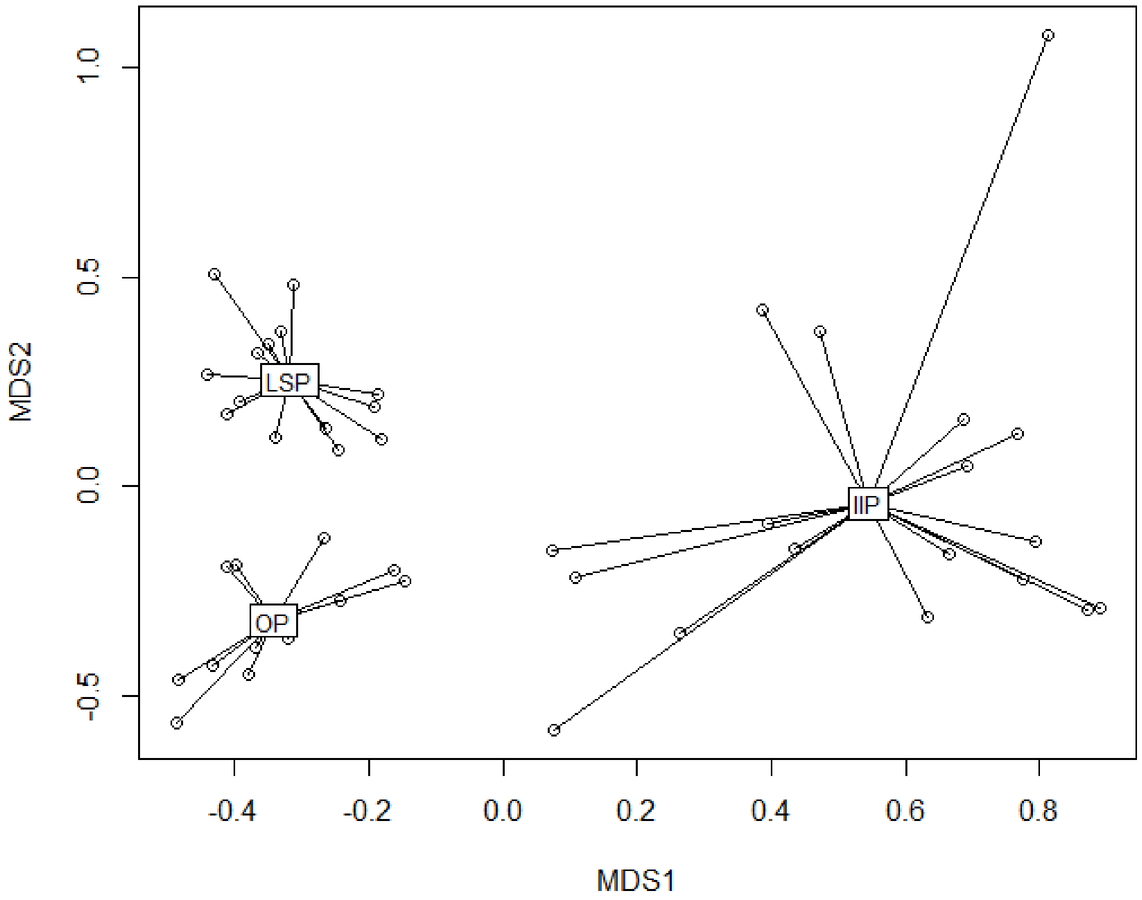


Figure A1.1. Non-metric multidimensional scaling (NMDS) plots of changes in phytoplankton community composition in the three identified periods. IIP: ice-influenced period; OP: overturn period; LSP: late summer period.

Appendix 2: supplementary information of chapter 3

Table A2.1. D-optimal design with the four factors. Each experiment was replicated in triplicate for a total of 72 experiments. 0NP: natural nutrient conditions; NP: nitrogen- and phosphorus-enriched; D: dark conditions; L: light conditions.

Experiment	DOC additions (mg C L^{-1})	Nutrient addition	Temperature (°C)	Light conditions
1	0	0NP	10	D
2	4	0NP	10	D
3	0	NP	10	D
4	2	NP	10	D
5	4	NP	10	D
6	6	NP	10	D
7	0	0NP	10	L
8	2	0NP	10	L
9	4	0NP	10	L
10	6	0NP	10	L
11	2	NP	10	L
12	6	NP	10	L
13	0	0NP	18	D
14	2	0NP	18	D
15	4	0NP	18	D
16	6	0NP	18	D
17	0	NP	18	D
18	2	NP	18	D
19	2	0NP	18	L
20	4	0NP	18	L
21	0	NP	18	L
22	2	NP	18	L
23	4	NP	18	L
24	6	NP	18	L

Table A2.2. Model results for each condition and double interaction effects on HPP biomass. P-values are shown for the biomass every second day of the experiments. C_added: carbon-addition effect; N_added: nutrient-addition effect; T+: temperature increase effect; Light: light conditions effect; Significant p-values: <0.001*** ;< 0.01** ;< 0.05*. Carbon-addition effect was considered significant when a significant effect of at least one level of carbon addition was detected by the model.

		Day 2	**Day 4**	**Day 6**	**Day 8**	**Day 10**
HPP	C_added	0.008**	0.12	0.07	0.16	0.10
	NP_added	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	T+	<0.001***	0.07	<0.001***	<0.001***	<0.001***
	Light	<0.001***	0.56	<0.001***	<0.001***	<0.001***
	C_added x NP_added	0.10	0.51	0.23	0.16	0.009**
	C_added x T+	0.09	0.051	0.01*	0.03**	<0.001***
	C_added x Light	0.006**	0.01*	0.01*	0.02*	0.02*
	Light x T+	0.001**	0.03*	0.91	0.36	0.03*
	Light x NP_added	0.26	0.47	0.41	0.85	0.36
	NP_added x T+	<0.001***	0.14	<0.001***	<0.001***	<0.001***

Table A2.3. Results of Permutational multivariate analysis of variance (adonis function) and multivariate homogeneity of group dispersions analysis (betadisper function) performed on phytoplankton community between the different conditions of DOC*nutrients and DOC*temperature: A) in the light condition; B) in the dark condition. Tests are based on Bray-Curtis dissimilarity distances and 999 permutations.

A) Light condition

Permutational multivariate analysis of variance

	Df	Sums Sq	Mean Sq	F Model	R2	Pr(>F)
DOC*nutrients	7	2.72	0.38	5.45	0.54	0.001***
Residuals	32	2.28	0.07		0.45	
Total	39	5.01			1.00	
DOC*Temperature	7	2.56	0.36	4.78	0.51	0.001***
Residuals	32	2.44	0.07		0.48	
Total	39	5.00			1.00	

Multivariate homogeneity of group dispersions analysis

		Df	Sums Sq	Mean Sq	F Value	Pr(>F)
DOC*nutrients	Groups	7	0.25	0.03	1.85	0.11
	Residuals	32	2.28	0.07		
DOC*Temperature	Groups	7	0.11	0.016	0.66	0.69
	Residuals	32	0.81	0.025		

B) Dark condition

Permutational multivariate analysis of variance

	Df	Sums Sq	Mean Sq	F Model	R2	Pr(>F)
DOC*nutrients	7	1.17	0.16	1.99	0.30	0.006**
Residuals	32	2.68	0.08		0.69	
Total	39	3.95			1.00	
DOC*Temperature	7	1.58	0.22	3.19	0.41	0.001***
Residuals	32	2.27	0.07		0.58	
Total	39	3.85			1.00	

Multivariate homogeneity of group dispersions analysis

		Df	Sums Sq	Mean Sq	F Value	Pr(>F)
DOC*nutrients	Groups	7	0.14	0.020	0.85	0.55
	Residuals	32	0.76	0.023		
DOC*Temperature	Groups	7	0.28	0.041	1.80	0.12
	Residuals	32	0.73	0.022		

Table A2.4. Model results for each condition and double interaction effects on picophytoplankton biomass. P-values are shown for the biomass every second day of the experiments. C_added: carbon addition effect; N_added: nutrient addition effect; T+: temperature increase effect; Light: light conditions effect; Significant p-values: <0.001***;<0.01**;<0.05*. Carbon addition effect was considered significant when a significant effect of at least one level of carbon addition was detected by the model.

		Day 2	**Day 4**	**Day 6**	**Day 8**	**Day 10**
Pico-phyto	C_added	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	NP_added	0.001**	0.003**	0.14	0.21	0.15
	T+	<0.001***	0.27	0.77	0.06	<0.001***
	Light	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	C_added x NP_added	0.009**	0.003**	<0.001***	<0.001***	<0.001***
	C_added x T+	0.02*	<0.001***	<0.001***	<0.001***	<0.001***
	C_added x Light	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	Light x T+	<0.001***	0.32	0.50	0.42	0.27
	Light x NP_added	<0.001***	<0.001***	0.15	0.12	0.81
	NP_added x T+	0.07	0.69	0.13	0.70	0.02*

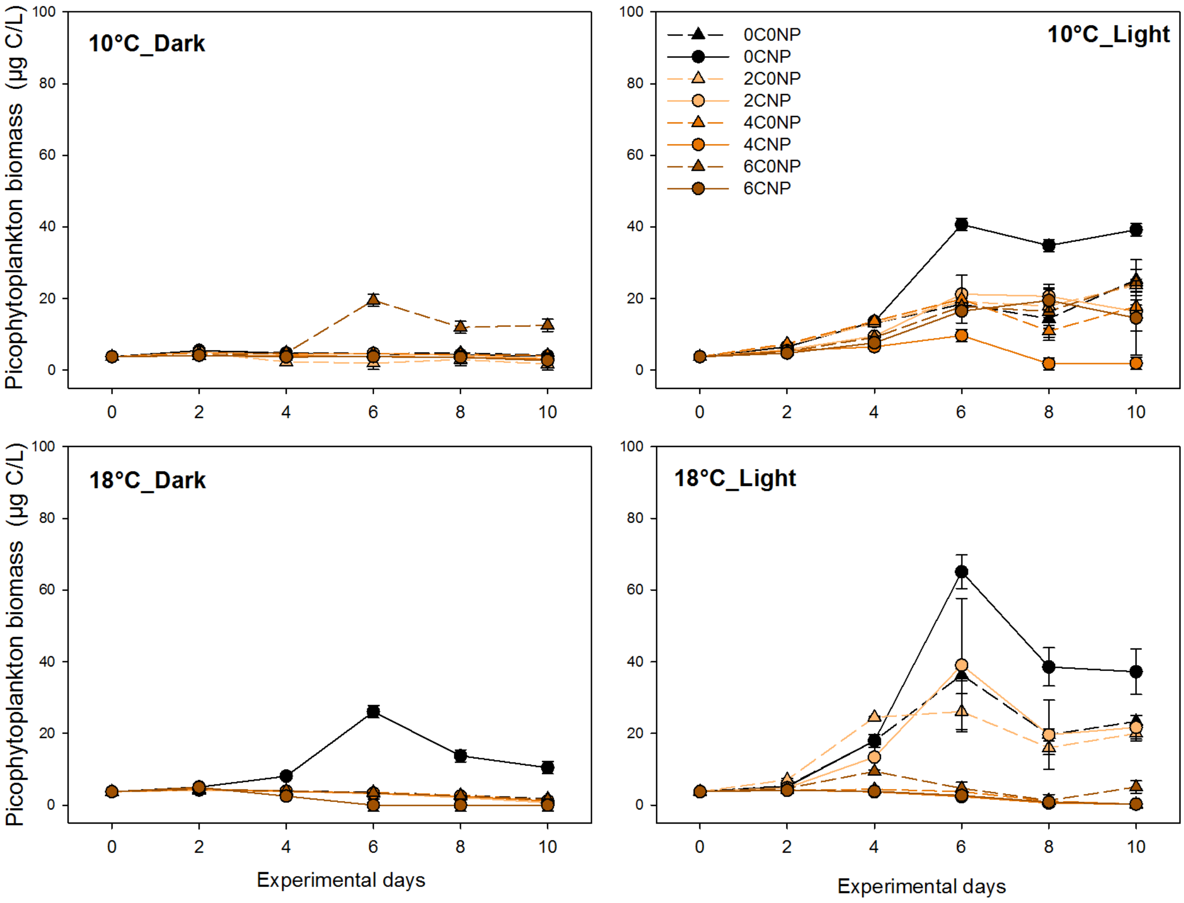


Figure A2.1. Picophytoplankton biomass per experimental day. Biomass is shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

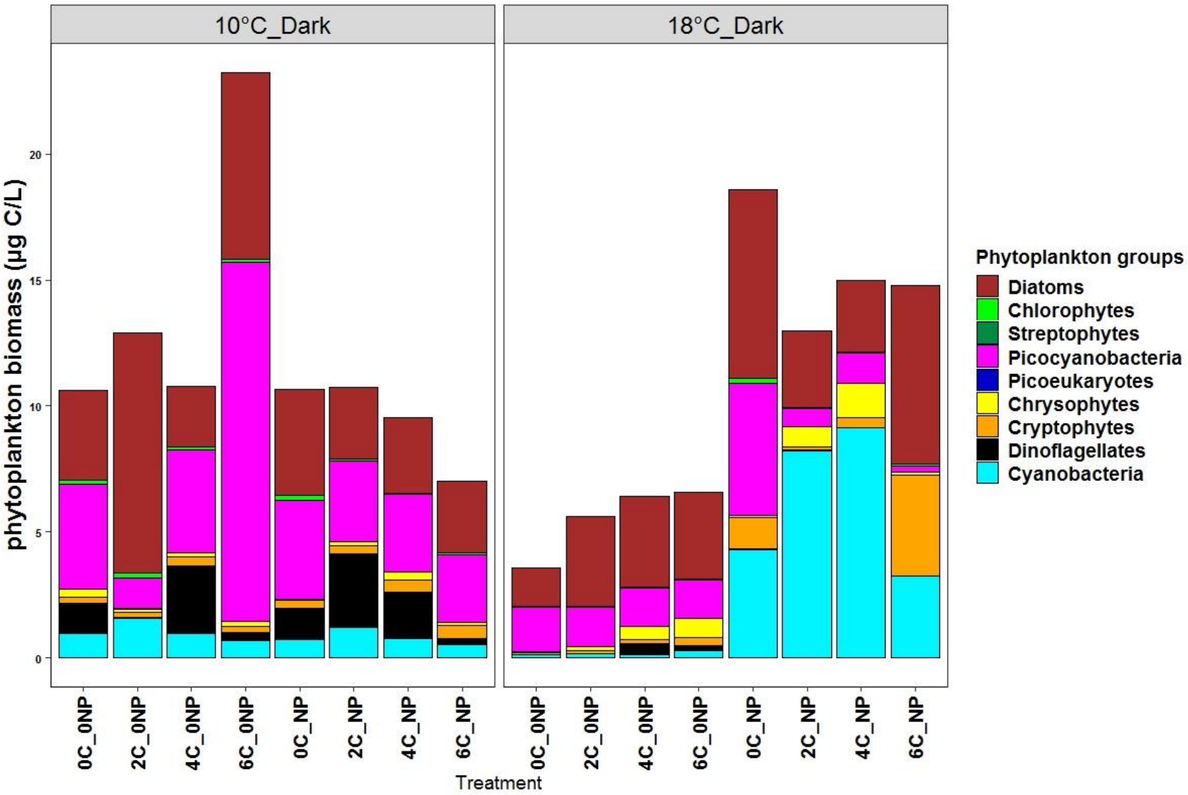


Figure A2.2. Phytoplankton biomass at the end of the experiment. Biomasses are shown for each DOC*nutrient treatment under the two temperature*dark conditions.

Appendix 3: supplementary information of chapter 4

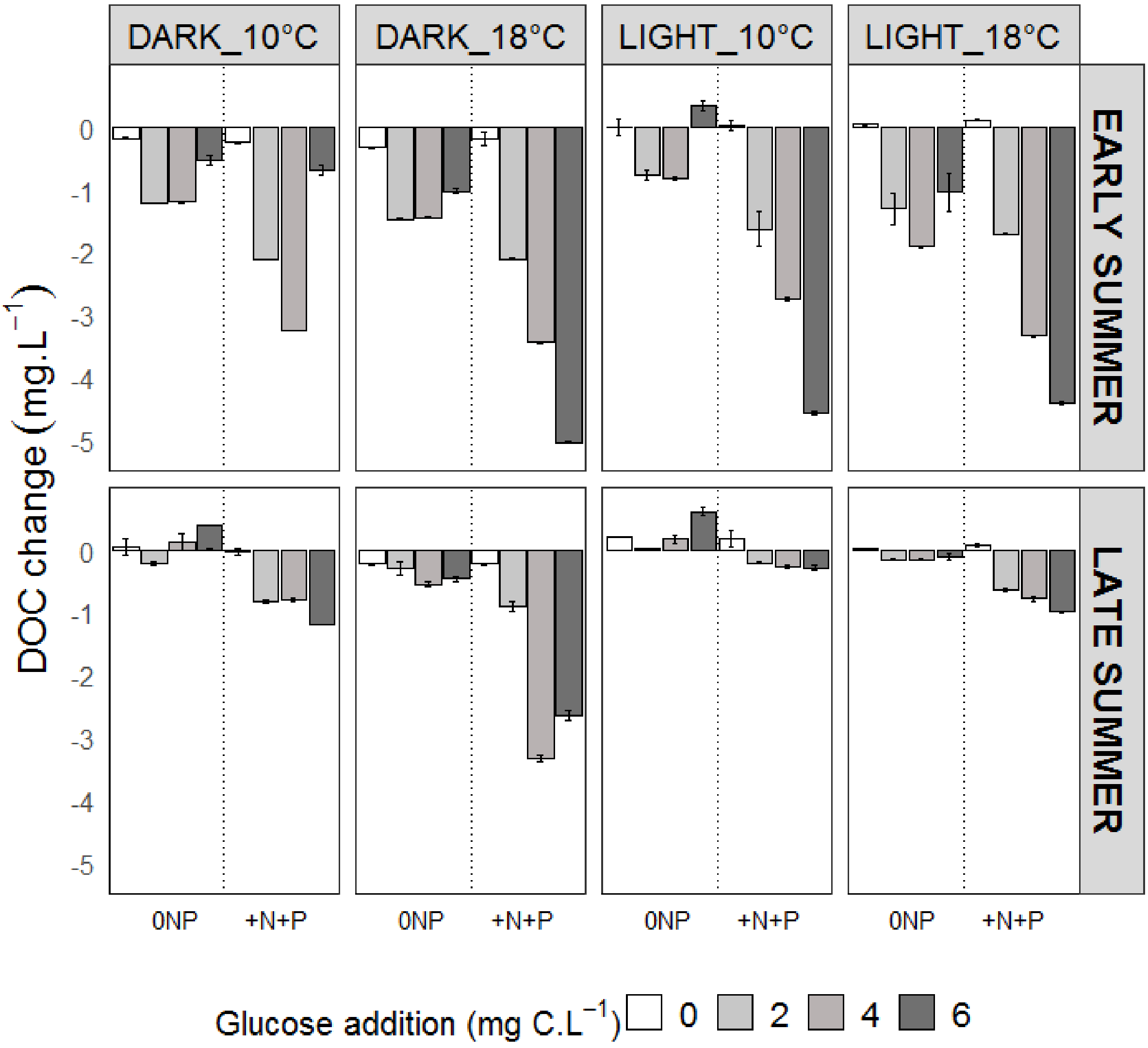


Figure A3.1. Dissolved organic carbon concentration change in the microcosms between initial conditions and end of experiments, in early summer and late summer. Concentrations are shown for each glucose and nutrient treatment. Error-bars represent standard error (n = 3).

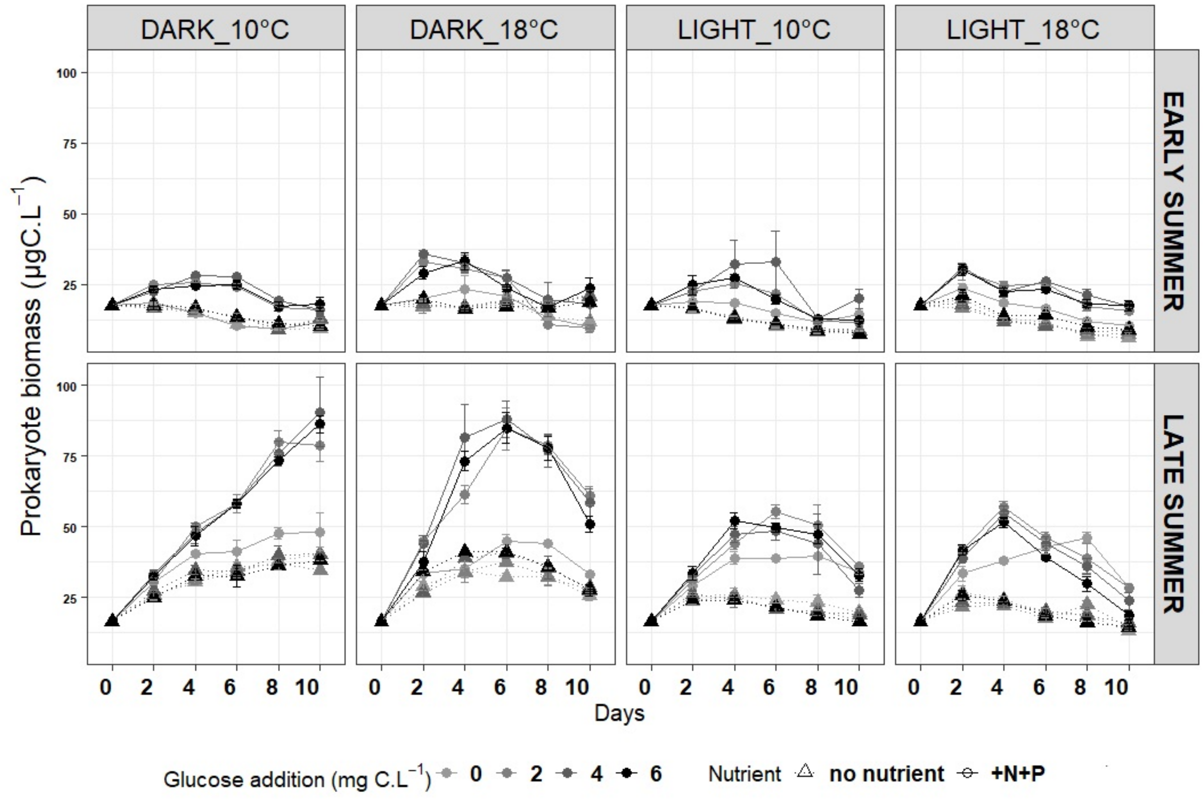


Figure A3.2. Prokaryote biomass per experimental day, in early summer and late summer. Biomass is shown for each glucose and nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

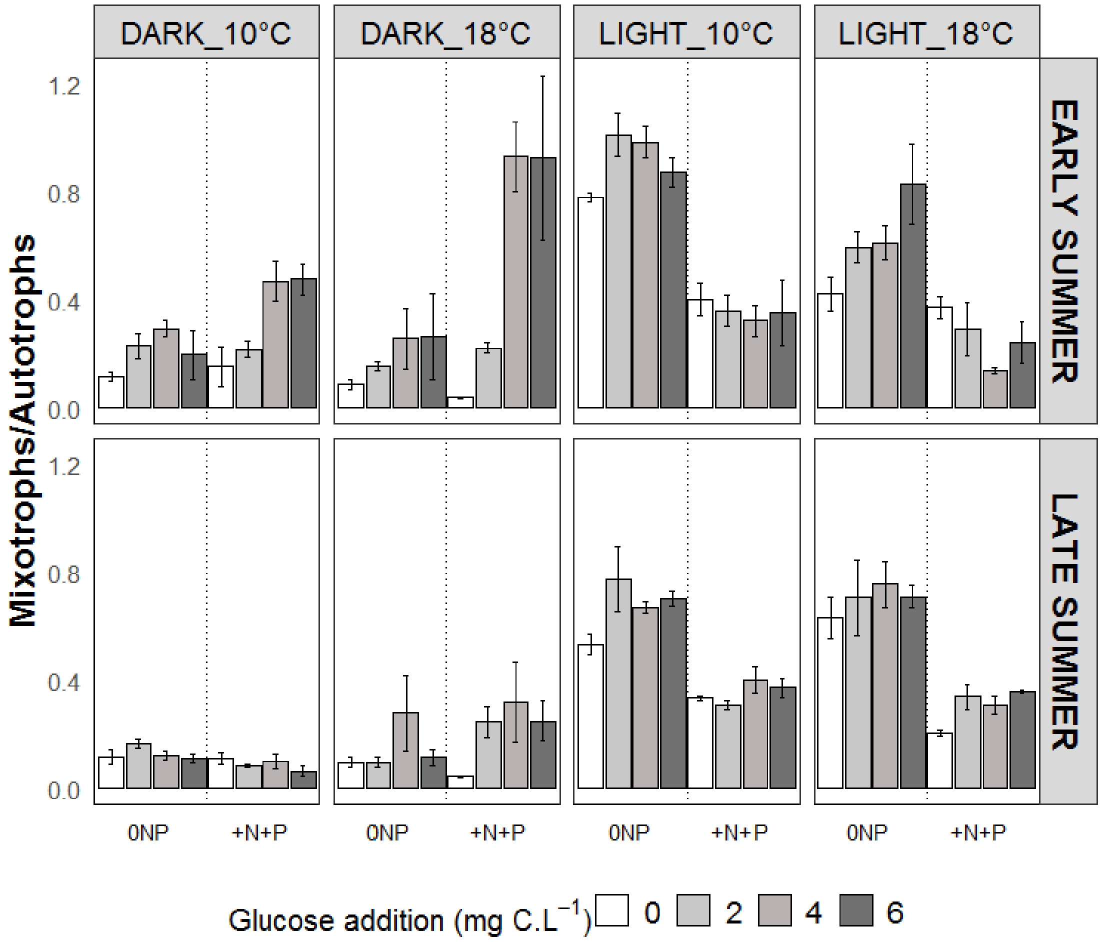


Figure A3.3. Mixotrophs:autotrophs ratio at the end of the experiments, in early summer and late summer. Ratio is shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

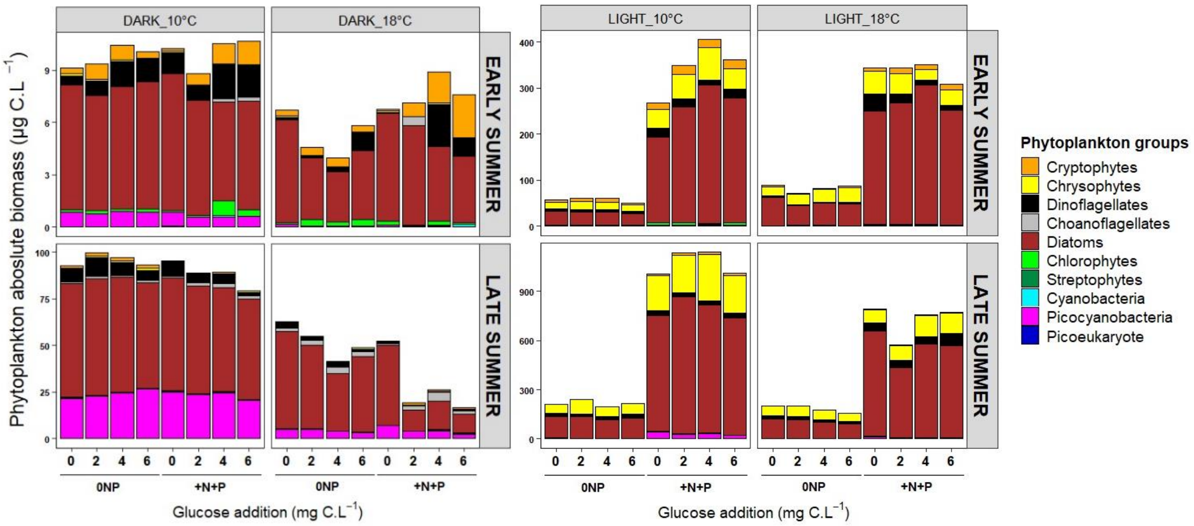


Figure A3.4. Phytoplankton biomass in the light and in the dark at the end of experiments, in early summer and late summer. Abundances are shown for each DOC*nutrient treatment under the four temperature*light conditions.

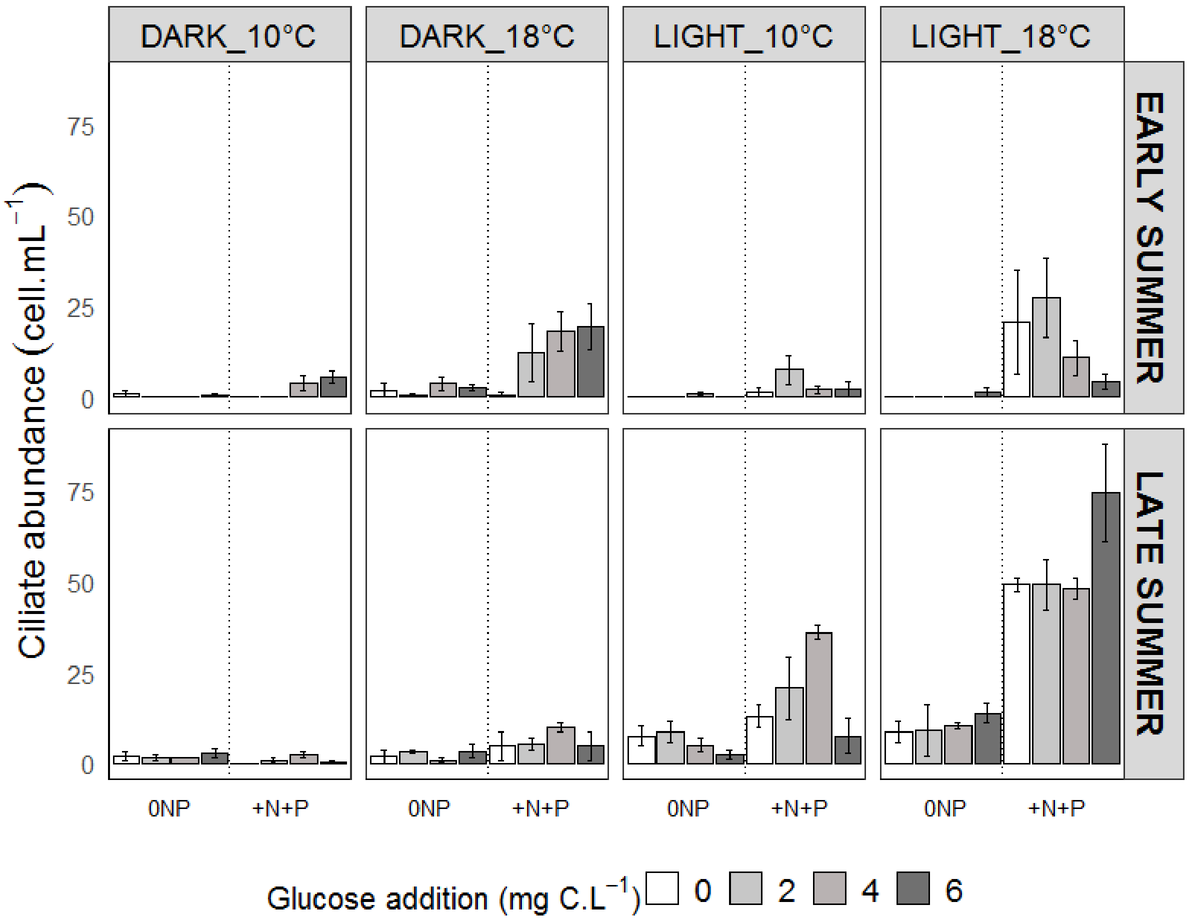


Figure A3.5. Ciliate abundances at the end of experiments, in early summer and late summer. Abundances are shown for each DOC*nutrient treatment under the four temperature*light conditions. Error-bars represent standard error (n = 3).

Appendix 4: supplementary information of chapter 5

Table A4.1. Functional groups and phylogenetic affiliation of phytoplankton taxa identified in the studied lake.

Species	Code	MFG	PFG	TAX
Cyclotella comensis	CYCO	7a	Autotrophic	Diatoms
Cyclotella sp.	CYSP	7a	Autotrophic	Diatoms
Fragilaria nanana	FRNA	6b	Autotrophic	Diatoms
Fragilaria ulna var ulna	FRULul	6b	Autotrophic	Diatoms
Fragilaria ulna var acus	FRULac	6b	Autotrophic	Diatoms
Achnanthidium minutissimum	ACMI	7b	Autotrophic	Diatoms
Meridion circulare	MECI	7b	Autotrophic	Diatoms
Cryptomonas ovata	CRYOV	2a	Mixotrophic	Cryptophytes
Cryptomonas marsonii	CRYMA	2a	Mixotrophic	Cryptophytes
Goniomonas truncata	GOTRU	2a	Mixotrophic	Cryptophytes
Chilomonas sp	CHISP	2a	Mixotrophic	Cryptophytes
Plagioselmis nannoplanctica	PLANA	2a	Mixotrophic	Cryptophytes
Dinobryon divergens	DIDIV	1a	Mixotrophic	Chrysophytes
Chrysococcus minutus	CHYMI	2a	Mixotrophic	Chrysophytes
Bitrichia ollula	BIOL	1a	Mixotrophic	Chrysophytes
Bitrichia chodatii	BICHO	1a	Mixotrophic	Chrysophytes
Mallomonas tonsurata var alpina	MALTON	1a	Mixotrophic	Chrysophytes
Chrysolykos sp	CHRYSP	2a	Mixotrophic	Chrysophytes
Kephyrion doliolum	KEPHDO	1a	Mixotrophic	Chrysophytes
Kephyrion spirale	KEPHSPI	1a	Mixotrophic	Chrysophytes
Katodinium sp	KATOSP	2b	Mixotrophic	Dinoflagellates
Peridinium umbonatum	PERUM	1b	Mixotrophic	Dinoflagellates
Gymnodinium sp	GYMSP	1b	Mixotrophic	Dinoflagellates
Chlamydomonas pyriformis	CHLASP	3a	Autotrophic	Chlorophytes
Carteria globosa	CARGLO	3a	Autotrophic	Chlorophytes
Scenedesmus aculeolatus	SCEACU	11a	Autotrophic	Chlorophytes
Tetraedron minimum	TEMI	9b	Autotrophic	Chlorophytes
Monoraphidium minutum	MOMI	9b	Autotrophic	Chlorophytes
Planktosphaeria gelatinosa	PLAGE	8a	Autotrophic	Chlorophytes
Oocytis lacustris	OOLA	11b	Autotrophic	Chlorophytes
Golenkiniopsis parvula	GOLPAR	9b	Autotrophic	Chlorophytes
Cosmarium tenue	COSTE	9a	Autotrophic	Zygnematophyceae
Aphanothece sp.	APHASP	5d	Autotrophic	Cyanobacteria
Synechococcus capitatus	SYNECAP	4	Autotrophic	Cyanobacteria
Gomphosphaeria sp.	GOMSP	5c	Autotrophic	Cyanobacteria
Salpingoeca gracilis	SALGRA	2a	Mixotrophic	Choanoflagellates
Protospongia Haeckelii	PROTOSP	2a	Mixotrophic	Choanoflagellates

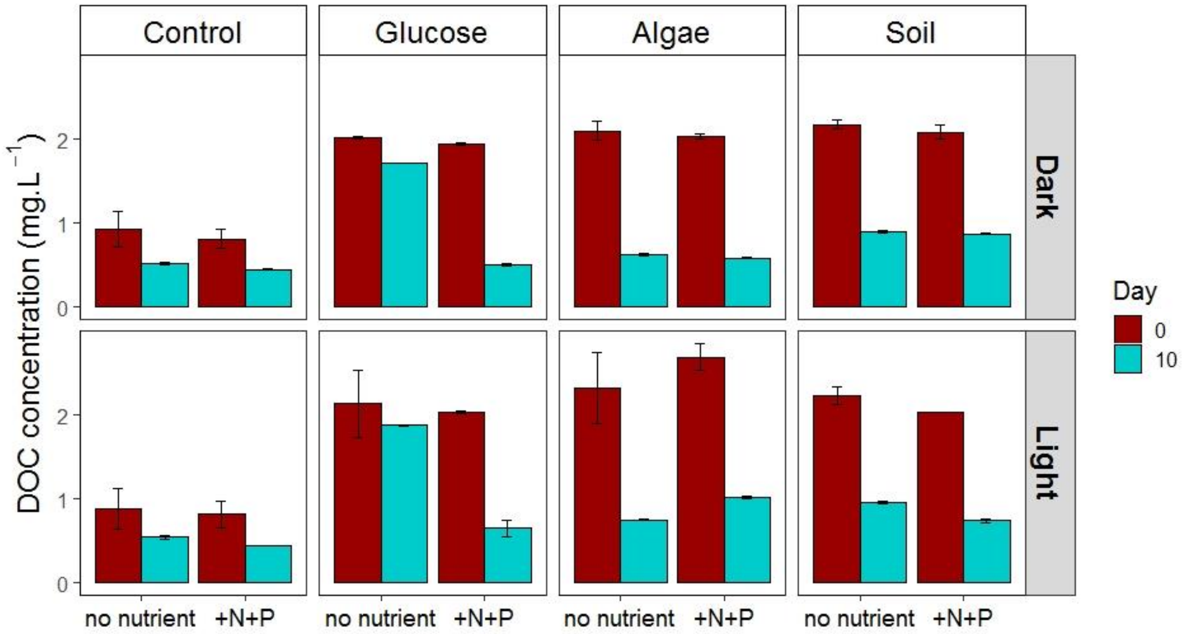


Figure A4.1. Average DOC concentrations at the beginning (t0) and the end (t10) of the experiment in the different treatments. Error-bars represent standard error.

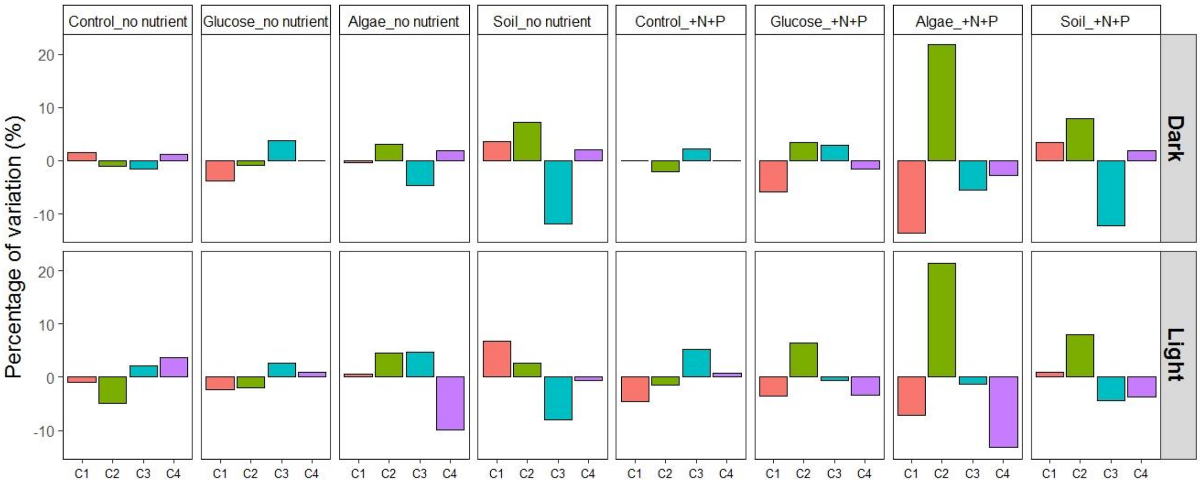


Figure A4.2. Percentage of variation of the contribution of the four fluorescent components between the beginning and the end of the experiment.

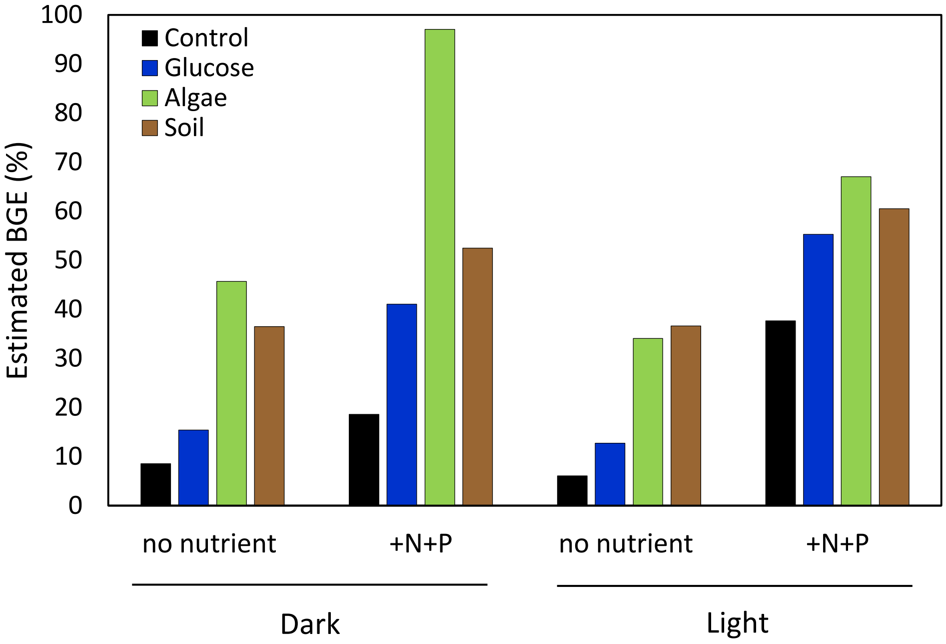


Figure A4.3. BGE estimation in the different treatments.

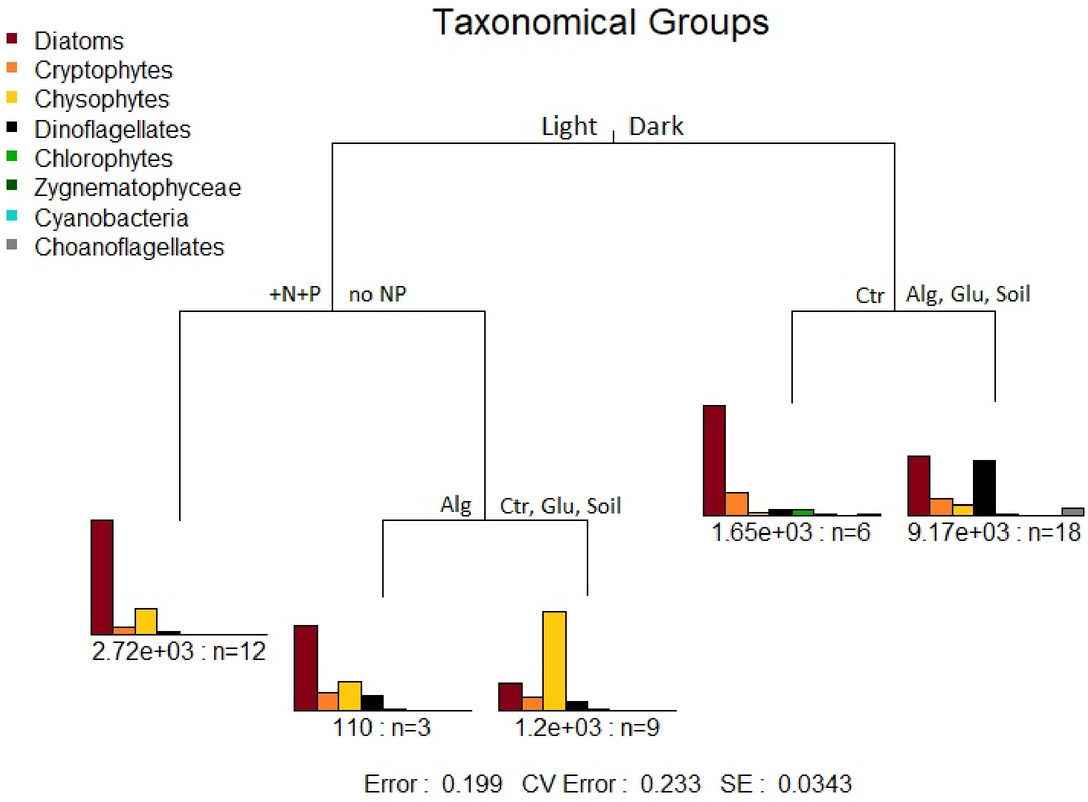


Figure A4.4. Multivariate regression tree analysis performed on relative biomass of phytoplankton taxonomical groups (TAX).

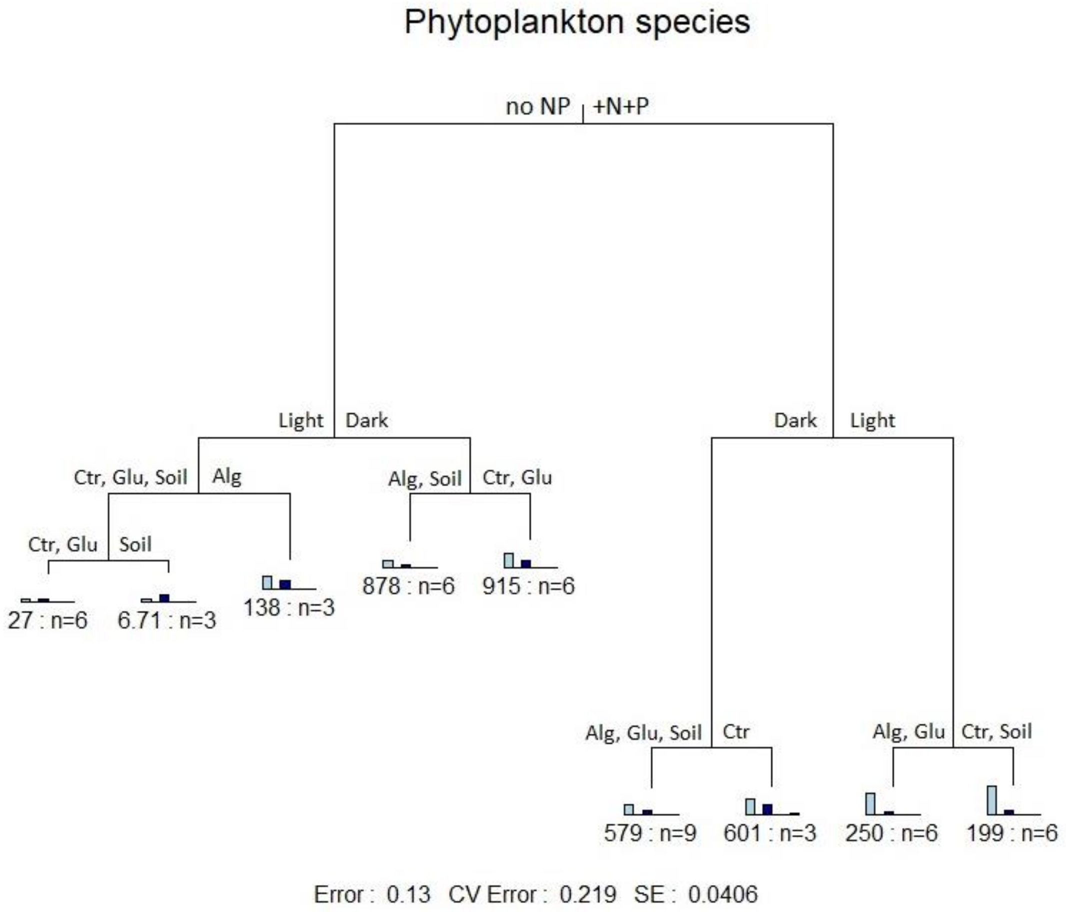


Figure A4.5. Multivariate regression tree analysis performed on relative biomass of phytoplankton species.

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Résumé

Les lacs de haute altitude sont des écosystèmes sentinelles, vulnérables aux variations des facteurs environnementaux. Dans le cadre des changements globaux, l'augmentation de la matière organique dissoute a été pointée comme un élément clé, capable de modifier les réseaux trophiques planctoniques à la base du fonctionnement des lacs. Ce travail de thèse a pour but de mieux comprendre les effets de la variation quantitative et qualitative de la matière organique dissoute sur les interactions entre bactéries hétérotrophes et phytoplancton.

La sensibilité des communautés planctoniques a été abordée à travers deux approches, expérimentale et *in situ*. Le suivi *in situ* a été réalisé sur le lac des Cordes, un lac de haute altitude oligotrophe peu profond à grand bassin versant. A travers une série d'expérimentations en laboratoire, les communautés de ce lac ont été exposées à différents enrichissements en carbone organique dissous (glucose), nutriments (azote, phosphore), et en matière organique dissoute.

Les résultats mettent en évidence une composition taxonomique et fonctionnelle du phytoplancton et une biomasse bactérienne fortement régulées par la dynamique saisonnière de la matière organique dissoute et par le contexte de limitation en carbone et en nutriments. En conditions expérimentales, l'augmentation de la concentration en carbone organique et en nutriments stimule le métabolisme bactérien et favorise les taxa mixotrophes dans les communautés hivernales et de fin d'été. En revanche, dans ces conditions d'enrichissement, la communauté phytoplanctonique de début d'été est dominée par les autotrophes. En lien avec l'utilisation de la matière organique dissoute par les bactéries, un apport autochtone entraîne un déclin plus important des mixotrophes qu'un apport allochtone.

Ce travail fournit des éléments de discussion sur l'évolution des relations entre bactéries et phytoplancton et démontre le rôle clé de la matière organique dissoute dans le fonctionnement des communautés planctoniques en lac de haute altitude. Cette thèse ouvre des perspectives sur les trajectoires évolutives potentielles des lacs de haute altitude au regard des changements globaux.

Mots clés : Phytoplancton, Changements fonctionnels, Matière organique dissoute, Dynamique saisonnière, Interactions trophiques

Abstract

High-altitude lakes are sentinel ecosystems, vulnerable to environmental variations. The increase of dissolved organic matter forecasted with global changes has been recognized as a key factor regulating the planktonic food web at the base of the functioning of the lakes. This thesis aims to better understand how qualitative and quantitative variations of dissolved organic matter could affect interactions between bacterioplankton and phytoplankton.

The sensitivity of planktonic communities has been addressed through an *in-situ* survey and an experimental approach. The *in-situ* survey was carried out on the Lake Cordes, a shallow oligotrophic high-altitude lake with a large catchment. Through laboratory experiments, the communities of this lake were exposed to different enrichments in dissolved organic carbon (glucose), nutrients (nitrogen, phosphorus), and dissolved organic matter.

Our results showed that the taxonomic and functional composition of phytoplankton and bacterial biomass are highly regulated by the seasonal dynamics of dissolved organic matter and by the context of carbon and nutrient limitation. In experimental conditions, increase concentrations of organic carbon and nutrients stimulates the bacterial metabolism and benefit mixotrophic taxa in winter and late summer communities. By contrast, the early summer phytoplankton community is dominated by autotrophs under these enrichment conditions. In connection with the use of dissolved organic matter by bacterioplankton, an autochthonous input induces a greater decline of mixotrophs than an allochthonous input.

Our work provides insights on the relationships between bacterioplankton and phytoplankton and demonstrates the key role of dissolved organic matter in the functioning of planktonic communities of high-altitude lakes. This thesis opens perspectives on the potential trajectories of high-altitude lakes regarding global change.

Keywords: Phytoplankton, Functional changes, Dissolved organic matter, Seasonal dynamics, Trophic interactions