Phytoplankton community responses to extreme climate events investigated by mesocosm approach

Louise Campione, Frédéric Rimet, Serena Rasconi

Phytoplankton diversity and functional traits under global change scenarios simulated by a pelagic mesocosm experiment

A mesocosm on Lake Geneva

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INRAE-CARRTEL
75bis avenue de Corzent
74 200 Thonon-les-bains

Degrees: Agronomic engineer, M2 Ecosystem & Anthropisation

Author: Louise Campione

ENSAT supervisor: Séverine Jean

INRAE-CARRTEL supervisor: Serena Rasconi

INRAE-CARRTEL co-supervisor: Frédéric Rimet

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Table of content

List of figures

List of tables

List of annexes

Abbreviations

ANOVA: ANalysis Of VAriance

CA: Correspondence Analysis

CARRTEL: Centre Alpin de Recherche sur les Réseaux Trophiques des Ecosystèmes Limniques

CCA: Constrained Correspondence Analysis

CEMAGREF: CEntre national du Machinisme Agricole du Génie Rural, des Eaux et des Forêts

CEN: Comité Européen de Normalisation

CIPEL: Commission Internationale pour la Protection des Eaux du Léman

DCM: De Ceuster Meststoffen

DOC: Dissolved Organic Carbon (fr.: COD)

IDH: Intermediate Disturbance Hypothesis

INRAE: Institut National de Recherche pour l'Agriculture, l'alimentation et l'Environnement

IPCC: Intergovernmental Panel on Climate Change (fr.: GIEC)

NMDS: Non-metric Multi-Dimensional Scaling

OLA: Observatory on LAkes (fr.: Observatoire des Lacs Alpins)

PAR: Photosynthetic Active Radiation

PCA: Principal Component Analysis (fr.: ACP)

PERMANOVA: PERmutational Multivariate ANalysis Of VAriance

TOC: Total Organic Carbon (fr.: COT)

UMR: Unité Mixte de Recherche

USMB: Université Savoie Mont-Blanc

WFD: Water Framework Directive (fr.: DCE)

Summary

This report presents my Master 2 internship at the mixed research unit INRAE-CARRTEL in Thonon-les-bains (France). Under the supervision of the researcher Serena Rasconi, I analysed samples collected during an *in situ* mesocosm experiment conducted in July 2019 in Lake Geneva. This experimentation aimed to reproduce global disturbances (storm, flood events) in order to better understand the dynamics of plankton communities, playing keys roles in the lakes (by storing CO_2, providing O_2 and food for the rest of the aquatic food web). Two treatments, one intermediate (M) and one intensive (H), and one control treatment were applied in 9 pelagic mesocosms with water column mixing, light reduction and inputs of dissolved organic carbon. The H treatment had the strongest effect on phytoplankton communities with the decrease of the Shannon's diversity index and community evenness. In particular, heterotrophs, represented by *Desmarella brachycalyx*, spread along with shifts of physico-chemical parameters (reduction of O_2, increases of assimilable phosphorus and NO_3^-) for a short time. Biotic and abiotic conditions recovered within a couple of days suggesting that lake ecosystems are resilient. The M treatment implied a maximum diversity supporting the Intermediate Disturbance Hypothesis (Connell, 1978) but had less effects on phytoplankton assemblages. All the treatments were also affected by the weather. It was thus challenging to unravel the effects of the treatments from the ones of the seasonality. Nevertheless, lake monitoring remains essential to forecast global changes consequences. Lakes provide indeed important ecosystem services (fish production, drinking water, habitats, aesthetic values…) that have to be maintained with a sustainable management. Phytoplankton communities are good bioindicators demonstrating the current state of the lake and their fast turnover is an important asset to estimate biodiversity feedbacks to climate change in the further decades.

Résumé

Ce rapport présente les résultats de mon stage de Master 2 effectué à l'UMR INRAE-CARRTEL de Thonon-les-bains (France). Sous la supervision de la chargée de recherche Serena Rasconi, j'ai analysé les échantillons collectés en juillet 2019 durant l'expérience en mésocosmes *in situ* au lac Léman. Le projet avait pour but de simuler les effets d'évènements extrêmes (tempêtes, inondations) afin de mieux comprendre les dynamiques des communautés de phytoplanctons qui jouent des rôles clés dans les écosystèmes lacustres (stockant du CO_2, fournissant de l'O_2 ainsi que de la nourriture pour le reste du réseau trophique aquatique). Deux traitements, l'un intermédiaire (M) et l'autre intensif (H), et des témoins ont été incorporés dans 9 mésocosmes pélagiques avec des ajouts de carbone organique dissous, du mixage de la colonne d'eau et de la réduction de luminosité. Le traitement H eut un effet marqué sur les communautés de phytoplancton avec une baisse de la diversité de Shannon et de l'équité. En particulier, les hétérotrophes représentés par *Desmarella brachycalyx* se sont répandus, tandis que des paramètres physico-chimiques ont été également impactés (baisse d'O_2 et hausses de phosphore assimilable et de NO_3^-) sur une courte période. Le rétablissement des paramètres biotiques et abiotiques appuie l'hypothèse de résilience des écosystèmes. Le traitement M suggéra une diversité maximale en lien avec l'hypothèse de perturbation intermédiaire (IDH) (Connell, 1978). Tous les mésocosmes ont été affectés par la météo, ce qui fut difficile à interpréter des effets des traitements. Néanmoins, le suivi des lacs reste essentiel afin de prévoir les conséquences des changements climatiques. Les lacs fournissent d'importants services écosystémiques (pêche, eau potable, habitats, valeurs esthétiques…) qui doivent être maintenus par une gestion durable. Les communautés de phytoplanctons sont de bons bioindicateurs de l'état actuel du lac. Leur turnover rapide est un atout avéré pour estimer les réactions de la biodiversité face aux changements climatiques dans les prochaines décennies.

Introduction

General environment of the study

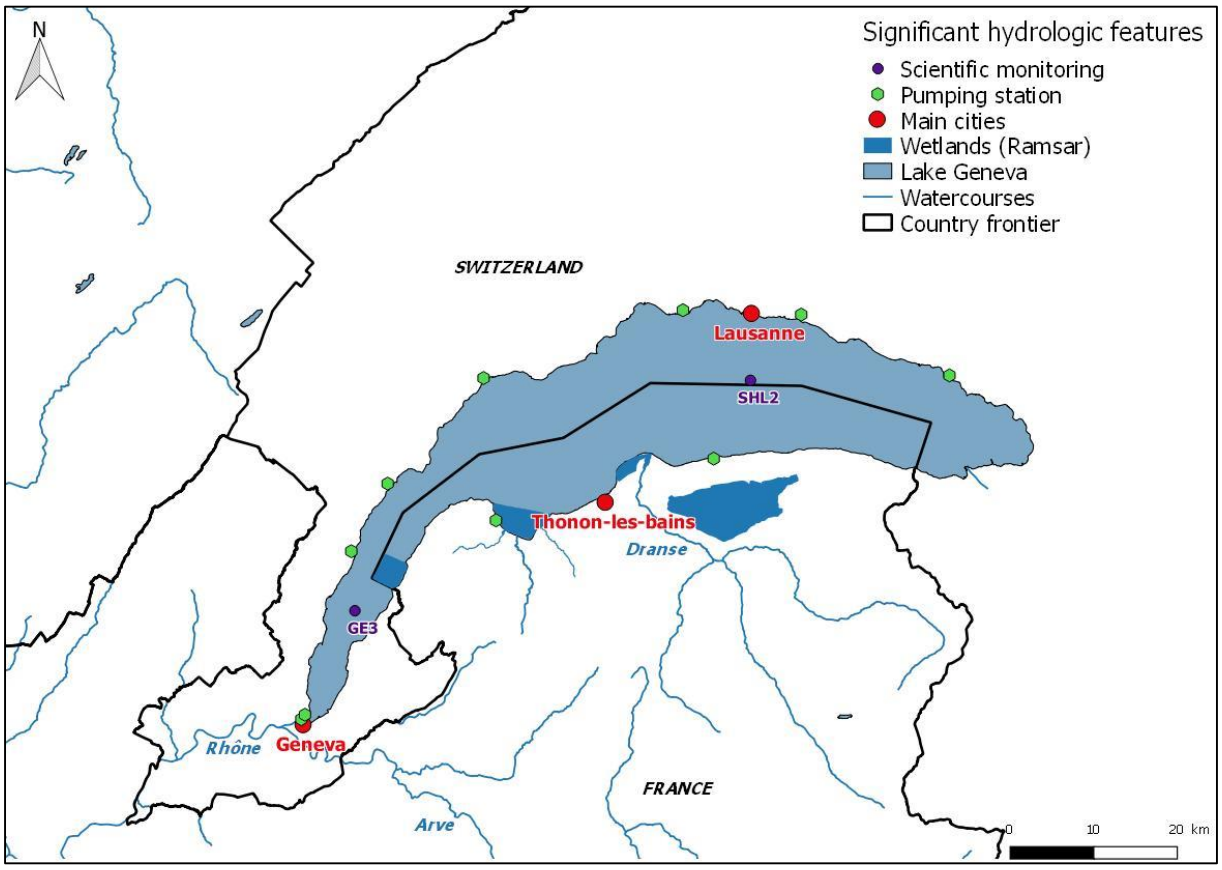

Figure 1: Lake Geneva and main hydrologic features

Lake Geneva (Figure 1) is a deep and large monomict lake (surface temperature always up to 4°C and a complete water mixing once a year) located at the frontier between France and Switzerland and connecting two mountain ranges: The Alps and the Jura massif. It is the greatest alpine lake of Europe with an overall surface of 580 km² and containing 89 km^3 of water. Its main affluent is the Rhône river bringing 70% of its surface inputs, and the rest is divided into 39 other tributaries including the Dranse river (Druart & Balvay, 2007). The watershed is about 13 times bigger than the lake. About 20% of the watershed is used for farmlands (mainly grasslands), there are also 23% of pastures, 22% of forests and 34.5% of uncultivated lands. Moreover, the riverbanks are largely urbanized: 60% of the riverbanks are artificial (roads and houses) and only 26% are still natural (reed beds, forests…). On the French side, there are three wetlands (Ramsar sites) of 1915 ha since 1991 that host many migratory birds (the black kite, the white wagtail…) and others (tufted duck, common pochard, Eurasian coot, cormorant…) as well as a mosaic of habitats, macrophytes and amphibians. In addition to

its regulating and cultural services, Lake Geneva provides drinking water and about 1000 t of fish per year (1241 t in 2013 (Hofmann & Raymond, 2014)). There are about 140 professional fishermen and 8000 amateur fishermen fishing mainly the perch, the arctic char and the European whitefish. There are also about 20 other fishes in the lake (pike, roach, trout…). Concerning drinking water, about 81 million of m^3 are served to around 900 000 inhabitants thanks to 10 pumping stations.

The internship took place at the UMR INRAE-USMB CARRTEL, in Thonon-les-Bains. It is a mixed research unit formed in 1999 between INRAE and the university Savoie Mont-Blanc (based in Chambéry). The UMR is part of the OLA (Observatory on Lakes, Rimet et al 2020), an observation system focusing on long term monitoring of abiotic and biotic parameters, mainly of alpine and peri-alpine lakes including Lake Geneva. Technicians go every two weeks at the point SHL2 at the deeper part of the lake (310 m, Figure 1) collecting data to monitor the ecological status of the lake in the current context of global and local perturbation (e.g. climatic or anthropic). The collaboration among scientists and lake managers (CIPEL, "Commission internationale pour la protection des eaux du Léman") ensures the provision of ecosystem services (fish production, drinking water, habitats, tourism…) in the context of global changes.

An important parameter regularly monitored is the phytoplankton and part of the work is dedicated to the use of phytoplankton as bioindicator or to prevent toxic blooms of cyanobacteria and preserve fishing resources. Frédéric Rimet writes every year a report concerning the state of phytoplankton communities in the Lake Geneva for the CIPEL. The long-term monitoring indicated an improvement of the water quality since 1974 from an eutrophic state of the lake to a mesotrophic state with a concentration of phosphorus of 19 μgL^{-1} in 2016 (Mercier et al., 2016).

Despite of the recent reoligotrophication, algal species more typical of eutrophic water are still encountered from the time of higher concentration of nutrients. Since 1963, there are occasionally blooms of *Mougetia gracillima* making fishing nets ineffective, which seems due mainly to the high concentration of phosphorus (Druart & Balvay, 2007). Over the last years, attention has been drawn to the presence of *Planktothrix rubescens* that is a potential toxic cyanobacteria spreading at the end of summer after the domination of Zygophycea. Furthermore, there is a recent rise of species indicative for shallow, enriched and turbid water such as the diatom *Ulnaria acus* and *Fragilaria sp.* (Rimet, 2019). The objective of the CIPEL for 2020 is to reach an oligo-mesotrophic state and a phosphorous concentration between 10

and 15 µg to securely limit the development of harmful algae (Action Plan 2011-2020 of the CIPEL, 2010). The CIPEL aims also to keep the total phytoplankton biomass under 1000 $\mu g.L^{-1}$. An index evaluating the state of the lake is the Brettum index, which is based on phytoplankton taxa classification according to their preferences for total phosphorus into seven classes. The higher the index is, the more oligotrophic the lake is. In 2018, the index was equal to 3.14 and the objective of the CIPEL is to reach an oligo-mesotrophic state equal to an index of 4.

These observations of the phytoplankton community of Lake Geneva confirm the strong link between shifts in environmental parameters (anthropically driven or not) and the evolution of biological communities such as phytoplankton. Therefore, it is essential to continue monitoring the related metrics (primary production, chlorophyll a, total phosphorus, temperature…) in order to better forecast the consequences of such stressors and reach a good management of the lake resources.

Global changes affecting lake services

Lakes provide through their processes fundamental ecosystem services to humankind: resource provisioning (drinking water, fishery), cultural (leisure activities, tourism), regulating services (water regulation, carbon sequestration, substrate for biodiversity). Understanding better the underpinning biodiversity and processes in the lakes is essential to forecast and manage these ecosystems under pressure.

Local and global forcings are causing changes of abiotic and biotic features of the lakes and pose risks for the services the lakes provide.

Temperature rises affect directly meteorological events such as heatwaves and climate extremes (IPCC, 2012). Depletion of oxygen has been seen in lakes because of temperature rises and releases of phosphorus from sediments in the hypolimnion (Wilhelm & Adrian, 2008). In 2003, high heat peaks caused a decrease of oxygen in Swiss lakes (Jankowski et al, 2006). The scientists argue that heatwaves implying anoxic conditions in deep water is a major issue of non-anthropogenic eutrophication. Temperature rises cause also a thermal stratification of the water column preventing nutrients to reach higher parts of the water column, which has a further impact on the biodiversity.

Moreover, the higher intensity and frequency of extreme events (IPCC, 2014) can change strongly the lake conditions. In fact, record-breaking raining events have been studied over the past century showing a higher frequency in Europe: +31% from 1980 to 2010

compared to expectations of rainy events in a steady climate (Lehmann et al., 2015). In another part of the world, extreme precipitation tends to intensify, as for Lake Victoria, where extreme rainfalls are predicted to double over the lake by the end of the century (Thiery et al., 2016). Storms, as the merger of heavy rains and heavy winds (Easterling et al., 2000), alter the physical parameters of lakes such as light availability, nutrient mixing, temperature distribution in the water column. In particular, heavy rains strengthen the inputs of organic matter and heavy winds intensify water column mixing and thus affect the turbidity of water (Stockwell et al., 2020).

These shifts on the abiotic features of the lakes have consequences on the aquatic trophic networks. From a rise of heterotrophic bacteria (Rasconi et al., 2015) to the collapse of cold-water fishes along with overfishing pressure (Jenny et al., 2020), monitoring lakes is crucial in order to better manage them.

Phytoplankton as bioindicators of the global changes

Phytoplankton constitutes the basis of the trophic chain and plays a major role in lake ecosystem services as absorbing carbon dioxide and providing dioxygen for other organisms. As having a short life cycle and a fast turnover, phytoplankton reacts rapidly to external forcing and altered ecosystem processes. It is thus studied as an indicator of the ecological quality of lakes at large scale according to the European Water Framework Directive (WFD) and for instance its main application is as an indicator of eutrophication (Thackeray et al., 2013).

The rise of temperature causing a depletion of nutrients impacts directly on phytoplankton communities. Larger phytoplankton such as diatoms, that require higher amount of nutrients, tend to be disadvantaged, contrary to smaller ones such as cyanobacteria (Bopp et al, 2005).

Storm events cause a decrease of light availability and nutrient mixing that are key determinants for phytoplankton growth (Stockwell et al., 2020). In this quoted review, it has been demonstrated that windy and rainy events lead to a decrease of phytoplankton biomass, whereas chlorophyll a remains stable. Thus, there are changes in phytoplankton assemblages. According to the CSR strategy (Reynolds, 1988), ruderal species would survive storm events due to their higher intrinsic growth rate, whereas competitive species are dominating in stable environment (high nutrient loads and light) and coming later in ecological succession (Altermatt et al., 2011). According to the intermediate disturbance hypothesis (IDH) (Connell, 1978), it is assumed that the maximum diversity would be reached at a medium stage of disturbance. Species with efficient uptake ratio would outcompete other species at low-level storm event,

whereas ruderal species would exploit the internal and external nutriment loads and dominate the ecosystem.

Despite the observed shifts due to storms, it has been demonstrated that phytoplankton communities are resilient. A study focusing on the Lake Okeechobee in the United States, which was affected by several hurricanes, showed that most of phytoplankton species did not recover even five years after the storms, while the physico-chemical conditions returned to pre-storm conditions (less turbid and light-limited water). In fact, on a long-term scale, most of the communities were resilient regarding their functional traits which were found to be the same and represented at the same proportion as before the hurricanes. However, where nitrogen concentration was very low, half of the biodiversity got lost in the pelagic area (Ji et al, 2018). Scientists argue that the capacity of biodiversity to recover strongly depends on the nature of carted organic matter and sediment loads on short-term responses. At a mid-term scale, some species might be fostered by the resuspended nutrients (such as cyanobacteria *Microcystis* in the previous study). Heterotrophs could be promoted by the inputs of organic matter coming from watershed due to storms (especially carbon) (Drakare et al., 2002), whereas autothrophs are disadvantaged by the reduction of light (Jennings et al., 2012). Another example of flood event occurred in the lake Lough Feeagh in Ireland and showed that abiotic parameters were resilient and so were phytoplankton assemblages (De Eyto et al., 2016).

Phytoplankton assemblages are not only affected by global changes, but also by seasonality and lake's own condition. This is indeed an issue to unravel global changes consequences from spatio-temporal framework. For instance, storm events with the same characteristics may reduce phytoplankton diversity in meso-eutrophic lakes, whereas it could be increased in deep stratified lakes as consequence of higher heterogeneity of resources. Moreover, a same storm event in autumn would lead to the dominance of diatoms and small chlorophytes because of sufficient light and disruption of stratification, while this pattern would not appear during spring. Cyanobacteria dominate indeed during summer, while diatoms and chlorophytes are more represented during the other seasons (Stockwell et al., 2020). If there is not sufficient light but favourable turbulence conditions, large-celled diatoms would be able to replace colonial cyanobacteria as more competitive for light absorption (Stockwell et al., 2020). To conclude, the effect of storms on phytoplankton dynamics not only depend on the intensity and frequency of storms, but also on the timing and the location of these extreme events.

Functional groups as a simplified way to assess phytoplankton communities

There are several ways to categorize phytoplankton because they have different morphologies (filamentous, circular, colonies) and characteristics that confer them many functions (N-fixing for some cyanobacteria, flagella and gas-vesicles for buoyancy regulators and motile species…). An interesting way to study the ecology of phytoplankton communities is to classify them into functional groups. Over the past decades, scientists proposed different classifications. For instance, Reynolds suggested first in the late eighties the CSR concept (Competitor, Stress-tolerant and Ruderal) that is close to the 'r' and 'K' strategists classification of MacArthur and Wilson (MacArthur & Wilson, 1967). Some species are more adapted to spread fast and colonize new environment ('r' and ruderal species proliferating in meso-eutrophic lakes), whereas some other species are more specialized and competitive to grow in stable environments, for example having structures for efficient uptake of nutrients ('K', as competitor species adapted to oligotrophic lakes). In parallel, Reynolds proposed a classification in 31 functional groups representative of different habitats (eutrophic state, mixing water…) and range of tolerance (nutrient deficiency, level of light, pH…) (Reynolds et al., 2002). This classification has then been revised by three scientists into 41 functional groups improving accuracy of descriptions and misplacements of some species by other authors (Padisák et al., 2009). Classification into functional groups has simplified the monitoring by environmental agencies (Padisák et al., 2009) and constitutes a helpful tool for the Water Framework Directive.

Project MESOLAC

The aim of the internship was to understand the potential effects of high and middle intensity weather events (such as storms and floods) on the functional traits of phytoplankton communities living in the lake using an experimental approach.

In situ mesocosms have been used for climate change research since 1995 (Stewart et al., 2013). This approach enables to isolate some parts of the lake and manipulate directly some parameters of the ecosystem. It is thus a good compromise between laboratory experiments and field surveys. The mesocosm size (i.e. > 1000 L) enables to experiment at a broader scale compared to microcosms. For instance, it allows investigating at the community level the direct effects of climate change, such as changes of species phenology and the indirect effect such as trophic and non-trophic interactions (Jenny et al., 2020).

The main questions of this internship were to understand *to what extent extreme weather events (as storms, floods) impact on the compositions of phytoplankton communities in a large pre-alpine lake.*

The main hypotheses were:

1. The biodiversity of phytoplankton would be reduced with storms of higher frequency and intensity and be replaced by generalist organisms.
2. The impacts would be more visible on storms of higher intensity within a short time than on storms with higher frequency on a longer time.
3. It is assumed that ecosystems can be resilient. Therefore, we expected the effects of different storms lasting for a short time.

The first part of this report focuses on the description of the mesocosm experiment that was run in 2019 and the methodology to assess phytoplankton communities. In the results, the main trends concerning physico-chemical parameters and phytoplankton communities are presented, followed by the interpretation of the results and a final part discussing the limits of the study.

1 Material and method

1.1 Mesocosm experimental design

The mesocosm experiment was conducted during July 2019 to simulate the effect of predicted scenarios of extreme climate events on natural plankton communities. Nine pelagic mesocosms (about 3000 L, 3 m depth) were deployed near the shore of Thonon-les-Bains (France) in Lake Geneva.

The design of the experiment consisted of three treatments each replicated three times: a control treatment (named C – no treatment applied) and two different treatments simulating different intensities of weather events. A medium intensity treatment (M) aiming at reproducing less intense and more prolonged events and a high intensity treatment (H) aiming at reproducing short and intense weather events such as violent storms.

In order to simulate these weather conditions, three main parameters were modified:

- *Dissolved Organic Carbon*: DOC concentration was increased by adding a solution prepared by extracting commercially available bio-peat soil (bought from the Belgian company DCM). 150 g of peat soil was mixed with 1.5 L of distilled water and autoclaved for one hour at 120 °C. This solution was then centrifuged for 15 min at

3500 r/min and the supernatant filtered through a 0.7 µm glass fiber filter. The filtrate was again autoclaved for sterilization before it was added to the mesocosms. Different volumes of the solution were added to the different treatments as described in Table 1.

- *Light*: incident irradiance was reduced using vinyl filters (bought from the American company LEE Filters). Those filters were placed at the top of the mesocosms (Figure 2) with different opacity as described in Table 1.
- *Mixing:* a top-bottom current in the mesocosm water column was created by manual mixing performed by lowering and lifting a three-meter-long stick with a drilled disk.

Table 1: Summary of parameters manipulated in the different treatments

Control	Treatment M – 2 weeks	Treatment H – 5 days
• **DOC**: Lake concentration, i.e. total DOC ~1.3 mgL^{-1} • **Transmitted light**: 95% • **Mixing**: No	• **DOC**: 1.5 x increased concentration, i.e. ~ 2 mgL^{-1} • **Transmitted light**: 70% • **Mixing**: 5 mins daily	• **DOC**: 5 x increased concentration, i.e. ~ 6 mgL^{-1} • **Transmitted light**: 15% • **Mixing**: 15 mins daily

The mesocosms were arranged randomly forming a Latin square (Figure 3). They consisted of polypropylene reinforced bags (produced by Insinööritoimisto Haikonen Oy, Finland) of about three meters depth and one-meter wide ending as a cone (about 3000 L volume). All the mesocosm bags were filled passively with lake water the same day within few hours and left to acclimate for three days before the start of the experiment. The experiment lasted in total four weeks (July 4 to 30), the high intensity treatment (H) consisted of a short-term intense stress applied for 5 days during the first week (from July 4 to 8). After this period, the H treatment mesocosms were exposed to the control conditions (covered with a 95% transmitted light filter, no further DOC increase and no mixing). The medium intensity treatment (M) was maintained for 4 weeks. In this work, we present the results from the first 2 weeks of the experiment (July 4 to 16, 4 samplings), to focus more on the effect and responses of the phytoplankton community to the high intensity treatment.

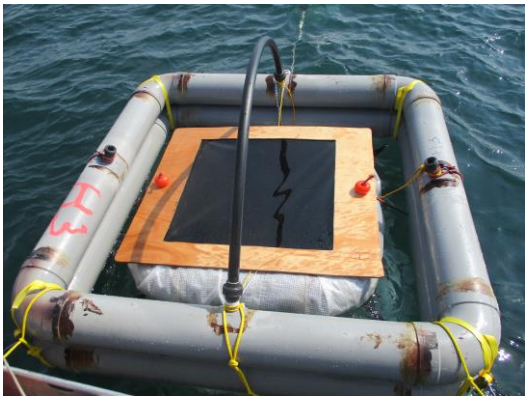

Figure 2: Mesocosm H3 immersed in Lake Geneva (Léman) with the light filter

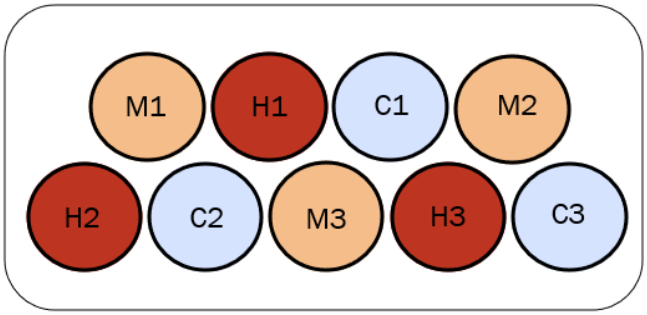

Figure 3: Mesocosms design (top view)

Table 2: Schedule of application of treatments and samplings. H: high intensity treatment (red); M: medium intensity treatment (orange); Si: sample n°i

July 2019		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DOC added (mgL^{-1})	H				1.92		1.92		1.92								
	M				0.64						0.64						0.64
Mixing ($'d^{-1}$)	H				15	15	15	15	15	-	-	-	-	-	-	-	-
	M				5	5	5	5	5	5	5	5	5	5	5	5	5
Transmitted Light (%)	H				15	15	15	15	15	95	95	95	95	95	95	95	95
	M				70	70	70	70	70	70	70	70	70	70	70	70	70
Sampling					S1				S2			S3					S4

1.2 Sample analysis

All the mesocosms were sampled twice a week according to the plan presented in Table 2. The schedule was adapted to fit the meteorological conditions.

1.2.1 Physico-chemical parameters

Physico-chemical characterisation of each mesocosm included *in situ* measures of temperature, pH, conductivity, oxygen, redox potential and turbidity using a multiparameter probe (YSI EXO1) and light spectral measurements using a RAMSES-ASC-VIS irradiance sensor. Organic matter (total organic carbon, TOC), and nutrients (P, N, Si) were measured by laboratory analysis (all the physico-chemical parameters are presented in Annex 3).

1.2.2 Phytoplankton community

Samples for phytoplankton community characterization were taken in every mesocosm at 2 m depth using a Niskin bottle. 100 ml of raw sample were immediately fixed by adding 5 ml of Lugol (iodine solution) according to the INRAE protocol (Druart & Rimet, 2008), which is also in agreement with the protocol used in the context of the Water Framework Directive (CEMAGREF & INRA, 2009) and follows the Utermöhl technique (Utermöhl, 1958) which

has been standardised and European level (CEN, 2006). The amber color given by Lugol, enables certain organisms to be more visible. The samples were labelled with the corresponding day of the sample (S1, S2, S3 and S4) and the corresponding mesocosm (C1, C2, C3, H1, H2, H3, M1, M2, M3). The samples were preserved in the dark at 4 °C and analysed within few months.

In February 2020, I took the samples out. Each sample was gently shaken to be homogenised and then poured into a 25 mL sedimentation chamber (or Utermöhl chamber) superposed on a slide with a depression. After 12 hours, the sedimentation chamber was removed and was replaced by a cover slip. Then, the slide was examined under an inverted microscope (Figure 4, Figure 5).

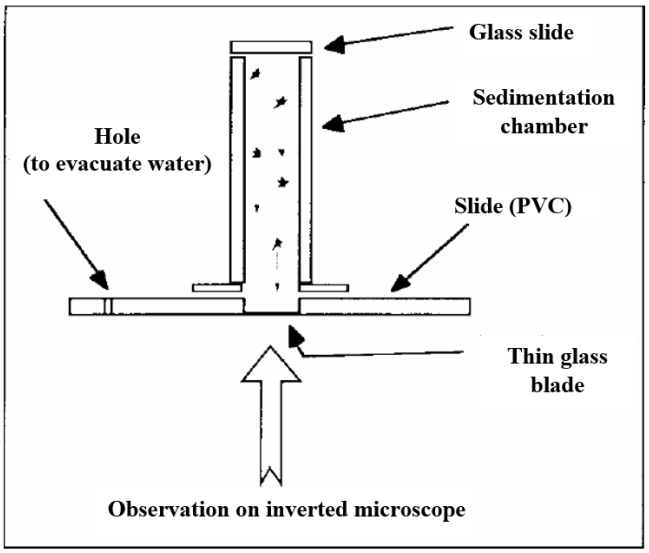

Figure 4: Sedimentation chamber (translated from Druart & Rimet, 2008)

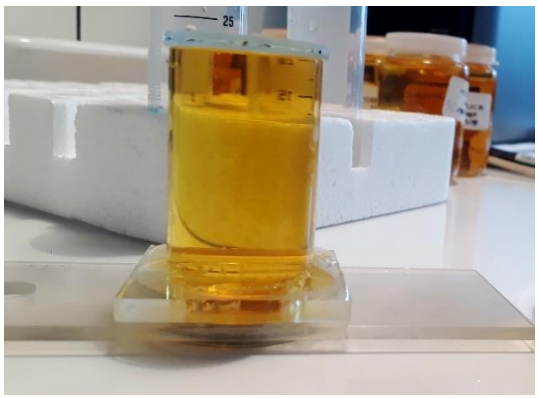

Figure 5: Picture of the sedimentation chamber

1.2.3 Counting of phytoplankton

A Zeiss Axiovert 135 microscope was used at 40*1.6 magnification. The interference contrast (DIC 5.1A) was used for the phytoplankton identifications (Figure 6), but the phase contrast could be also used (Figure 7). The Cell_P software (Olympus Soft Imaging Solutions, Germany) with an Olympus DP71 (camera) was used to visualize phytoplankton on a computer screen and take pictures.

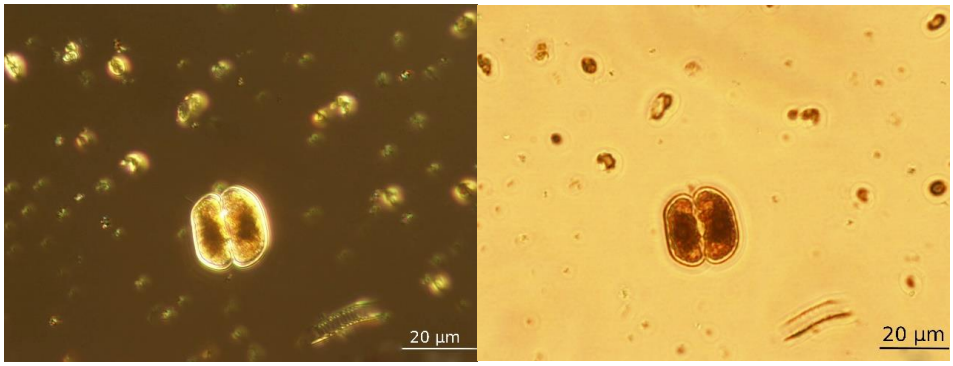

Figure 6: Cosmarium depressum under differential interference contrast (left picture) or without any contrast (right picture)

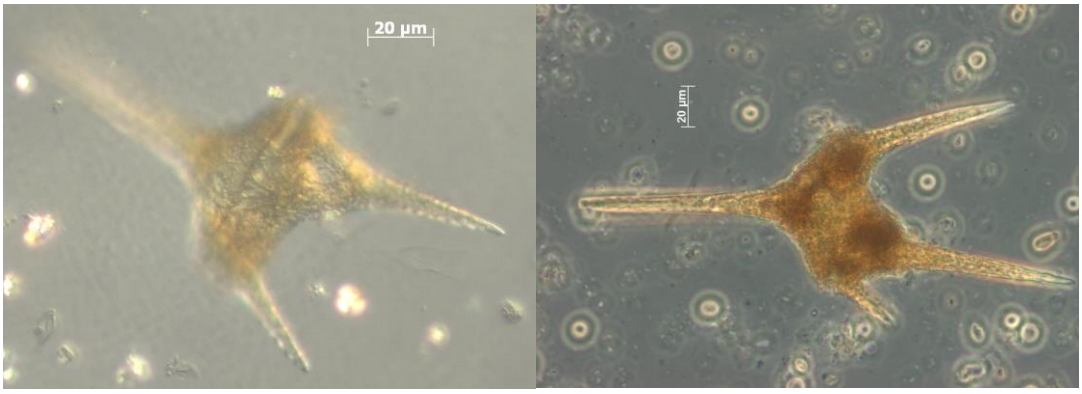

Figure 7: Ceratium hirundinella under differential interference contrast (left picture) or phase contrast (right picture), source: F. Rimet

For phytoplankton counting I followed the standard procedure (NF EN 15.204 from 2006) congruent with Utermöhl's methodology. The method consists in counting at least 400 individuals or "algal object". It is defined as one or several cells forming an independent group regarding other particles of the sample. The definition depends on the organism shape: whether it is filamentous or unicellular. Some common rules have been established (Annex 1). For instance, a filamentous individual is accounted every 100µm, that is to say a quarter of the ocular field whereas colonies such as *Aphanocapsa delicatissima* are accounted as one individual from 10 µm. Only viable cells (i.e. concerning diatoms the frustules containing plastids) were counted, except for the Dinobryon for which the lorica were counted.

It has been sometimes laborious to count until 400 individuals requiring up to five times more ocular fields than usual. There are few solutions to avoid this issue such as lowering the number of individuals to count or using a bigger sedimentation chamber (one of 50 mL for example that enables a larger amount of phytoplankton to sediment). The last solution has some drawbacks regarding mainly the volume of the collected sample (100 mL) that reduces the number of slides to be observed under the microscope in case of leaks. Besides, a larger tube increases the instability of the chamber on the slide.

1.2.4 Conversion from algal object counts to biovolume

Phytoplankton abundance data were sorted on an Excel sheet (provided by Frédéric Rimet) containing characteristics on each species and allowing conversion of the counted species abundance into biovolumes ($\mu m^3 mL^{-1}$) according to the previous standard of AFNOR (Rimet & Druart, 2018). In order to follow the calculation process (Table 3), it is needed to know some variables for each species counted under the microscope:

N = number of counted ocular fields for one sample;

n = abundance of one species within one ocular field;

V = sedimented volume (volume of the sedimentation tube in mL);

Bs = specific biovolume of species (μm^3).

Table 3: Procedure for species biovolume calculation from microscope counts

Parameter	Formula
1. Surface of one ocular field (40*1.6) (mm^2) **(So)**	$\pi . r^2 = (0.18)^2 . \pi \approx 0.10 mm^2$
2. Surface of the basis of the Utermöhl chamber (mm^2) **(Sc)**	$\pi . r^2 = (13)^2 . \pi \approx 531 mm^2$
3. Counting ratio **(R)**	$\frac{Sc}{So . N} = \frac{531}{0.1 . N} = \frac{5310}{N}$
4. Concentration (number of objects mL^{-1}) **(C)**	$\frac{n . R}{V} = \frac{5310 . n}{N . V}$
5. Biovolume ($\mu m^3 mL^{-1}$) **(B)**	$Bs . C$

The specific biovolume has been determined using standardized geometric shapes of phytoplankton (examples in Annex 2) with a methodology used and developed for 50 years at INRAE-CARRTEL (Rimet & Druart, 2018). However, sometimes some algal objects were not determined to species level or some historical attributed specific biovolumes did not match with the current species. Therefore, some specific biovolumes were adjusted by taking measures (length, diameter, width) using the Cell_P software.

1.2.5 Phytoplankton diversity and functional groups

To assess species distribution at a community-level, two different diversity indexes were calculated according the formula presented in Figure 8.

The Shannon's index (H) was calculated as the sum of the species richness *S* and the proportion of individuals occurrence belonging to the i^{th} species in the dataset (p_i, expressed as abundance or biovolume according to S) (Hill, 1973). The H value is high when species are equally distributed (*pi = pj*). The evenness (J) was calculated as the ratio between the Shannon's index and the species richness (S). It is thus more efficient for comparisons between

communities with different total number of species and varies between 0 and 1 (the more it is close to 1, the more even the communities are).

$$H = -\sum_{i=1}^{S} p_i \ln(p_i)$$

$$J = H/\ln(S)$$

Figure 8: Shannon's index (H) and evenness (J) formula, based on Hill, 1973

The taxonomically identified phytoplankton biovolumes have been grouped in 20 functional groups according to Padisák (Annex 4) (Padisák et al., 2009). This classification is based on functional traits (morphology, growth rate, nutrient uptake…), and is representative of habitat conditions (hydrology, nutrient loads, turbidity…) (Reynolds et al., 2002).

We also defined three main groups based on their trophic strategy. We distinguished the phytoplankton community among pure autotrophs, mixotrophs (organisms that can exploit autotroph and heterotroph strategy) and we could also recognize some pure heterotrophs based on the microscope determination.

As proxy for community turnover we calculated Bray-Curtis dissimilarity (D_{BC}) (Figure 9). It compares shared species between two groups (*j* and *k*) using the information of species richness and abundance. Values vary between 0 (the groups are similar) and 1 (the groups have no common species).

$$D_{BC} = \frac{\sum_{i=1}^{S} |p_{ij} - p_{ik}|}{\sum_{i=1}^{S} p_{ij} + p_{ik}}$$

Figure 9: Bray-Curtis dissimilarity (DBC) formula based on Bray & Curtis, 1957

1.3 Data analyses

Data analysis was performed using the software R studio. Biovolume data were analysed using the relative frequency values expressed as percentage and data were log transformed prior to statistical analysis to improve normality when ANOVA's assumptions were not met. The statistically significant difference value was set at $p < 0.05$.

The decision tree conducting the workflow for data analysis is presented in Annex 5. First, I evaluated differences in the phytoplankton community at the beginning of the experiment (S1) using analyses of variances (ANOVA and PERMANOVA). Dependent variables were phytoplankton biovolume, diversity indexes, functional groups and trophic

strategies. Independent variables were time and treatment parameters: total organic carbon (TOC), light, mixing. Then, I evaluated whether phytoplankton communities were different during the experiment (S2 to S4). When significant differences were found, I checked for single-step multiple comparisons (Tukey HSD test, multipatt test and simper test) and then, for significant differences among treatments at specific dates. The final step was to relate the observed differences to manipulated environmental data. I used co-inertia combining both matrices of environmental (all the investigated physico-chemical parameters) and biological parameters (phytoplankton functional groups) to assess a co-construction of the variables (symmetric approach). Co-inertia maximized the covariance of both tables on a common space of projection. Therefore, mesocosms are projected both by the environmental and biological variables. I also used constrained correspondence analysis (CCA) to discriminate and represent the phytoplankton functional groups against the environmental parameters. This function consists by projecting the mesocosms with multiple regressions. The correspondence analysis (CA) of the functional group's matrix with relative data is restricted by linear combinations of physico-chemical variables (asymmetric approach). The followed procedure to choose the displayed physico-chemical variables involved the best correlations (Spearman's correlation >0.5) between either the Axis 1 or Axis 2 of the CA and each relevant physico-chemical parameter considering the treatment applications.

2 Results

2.1 Physico-chemical parameters

At the beginning of the experiment (S1) there was no significant difference in TOC concentration (Figure 10), the average was 1.3 $mgCL^{-1}$ ± 0.11 in all the treatments. In the C treatment, TOC remained stable (1.33 $mgCL^{-1}$ ± 0.12) during the entire experiment. In the treatment M, TOC concentration increased during the experiment (S2 and S3: 1.73 $mgCL^{-1}$ ± 0.04 and 1.9 $mgCL^{-1}$ ± 0.14 respectively) and was lower at the end (S4: 1.21 $mgCL^{-1}$ ± 0.06). In the H treatment, there was a peak in the TOC concentration (S2: 3.97 $mgCL^{-1}$ ± 0.08 i.e. 2.5 times more than in the other treatments), followed by a decrease (S3 and S4: 1.73 $mgCL^{-1}$ ± 0.08 and 1.16 $mgCL^{-1}$ ± 0.01 respectively).

Light (Figure 11) at the beginning of the experiment was lower in the H treatment (38.7 $\mu molm^{-2}s^{-1}$) compared to M (51.4 $\mu molm^{-2}s^{-1}$) and C (57.2 $\mu molm^{-2}s^{-1}$). During the experiment, light was highly variable and lowest in H (S2: 40.8 $\mu molm^{-2}s^{-1}$ ± 15.8), compared to C (63.1 $\mu molm^{-2}s^{-1}$ ± 36.2) and M (84 $\mu molm^{-2}s^{-1}$ ± 32.3). Following, a decrease was observed in all treatments and again values were lowest in H (S3: 25.6 $\mu molm^{-2}s^{-1}$ ± 2.2), lower in M (30.9

µmolm^{-2}s^{-1} ± 6.9) and slightly higher in C (32.9 µmolm^{-2}s^{-1} ± 4). At the end of the experiment, light was lowest in M (S4: 53.9 µmolm^{-2}s^{-1} ± 12.7), intermediate in H (56.7 µmolm^{-2}s^{-1} ± 13.5) and highest in C (59 µmolm^{-2}s^{-1} ± 7.6).

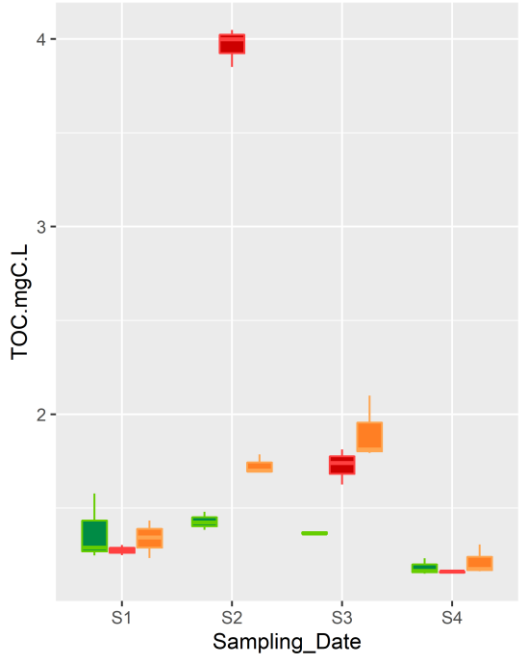

Figure 10: Evolution of TOC durinig the experiment

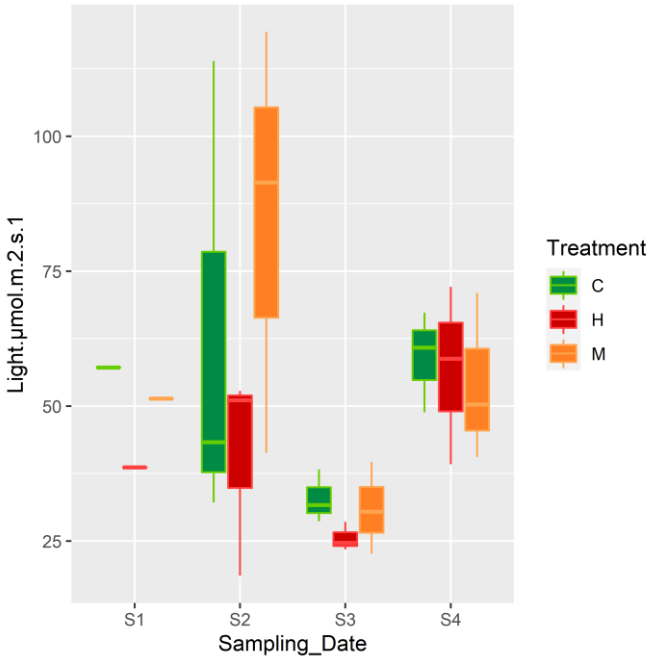

Figure 11: Evolution of light during the experiment

In total, 17 physico-chemical parameters have been assessed during the experiment (Annex 3). All these measured parameters together explained 58.7% of the variability observed in the treatments (Annex 6) and the significant drivers for the observed variance are illustrated in Figure 12. Dissolved oxygen (O_2) was correlated with nitrate (NO_3^-) (ρ = 0.84, pvalue = 1.7E-10[1]) and both together were mostly drivers for variance at S1 and S4. They were anticorrelated with TOC (ρ = -0.86, pvalue = 6.8E-11[2]), total phosphorous (Ptot) (ρ = -0.41, pvalue = 0.014), sulphate (SO_4^{2-}) (ρ = -0.84, pvalue = 4.7E-10) and, to less extent, to phosphate (PO_4^{3-}) (ρ = -0.47, pvalue = 0.0038) and particulate phosphorous (Ppart) (ρ = -0.58, pvalue = 7.1E-04), which were mostly drivers for S2. Significant parameters were also silicon dioxide (SiO_2) and ammonium (NH_4^+), characterising S3.

[1] Spearman's correlation (covariance of variable 1 and variable 2 divided by the product of their standard deviation)

[2] Mean of O_2 and NO_3^- ρ and pvalue

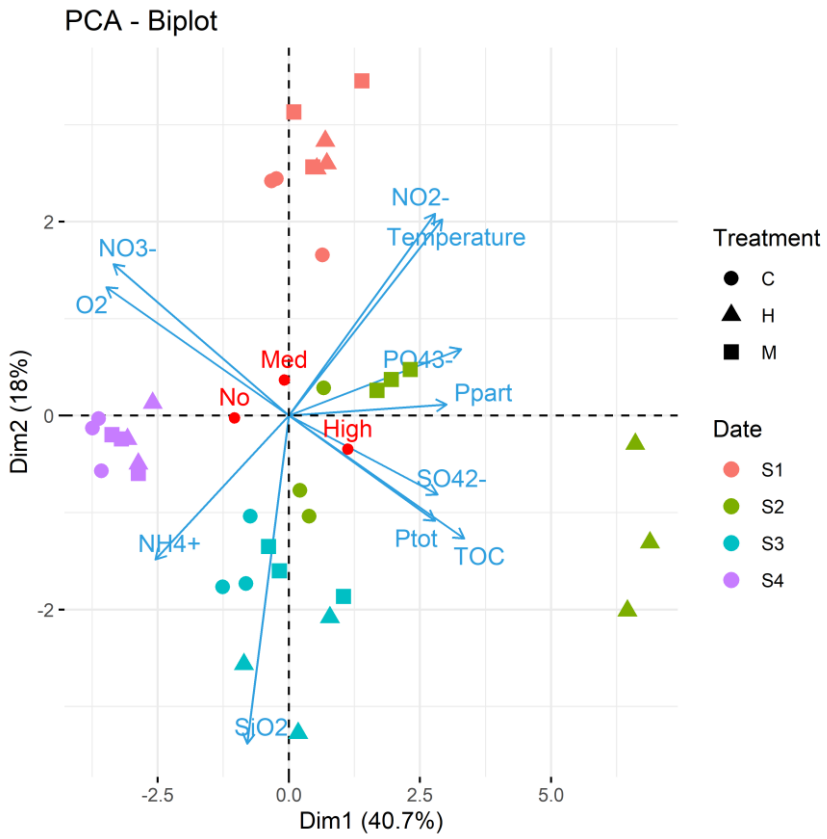

Figure 12: Distribution and correlation of physico-chemical parameters as drivers for treatments during the experiment (PCA), red dots are representative of the mixing parameter: No= Control, Med = medium intensity treatment, High = high intensity treatment.

2.2 Phytoplankton diversity

2.2.1 Diversity indexes

Phytoplankton biovolume at the first date (S1) was significantly higher (pvalue = 0.00925, ANOVA)[3] in C compared to M and H. During the experiment the treatments were not significantly different (pvalue = 0.093063, ANOVA) and followed the same general dynamic. From S2 to S3 there was a decrease of phytoplankton biovolume in all the treatments, followed by an increase at S4.

Shannon's and evenness indexes (Figure 13) at S1 were not significantly different among treatments (respectively pvalue = 0.537, pvalue = 0.1, ANOVA). From S2 both these indexes were significantly lower in H (1.9 ± 0.42 and 0.62 ± 0.14; respectively Shannon's and evenness) (HSD.test, pvalue = 0.0107, pvalue = 0.0302, ANOVA; respectively Shannon's and evenness) and similar in C and M (2.39 ± 0.26 and 0.75 ± 0.07; respectively Shannon's and evenness).

[3] See the summary table in Annex 8 for complete statistical test results

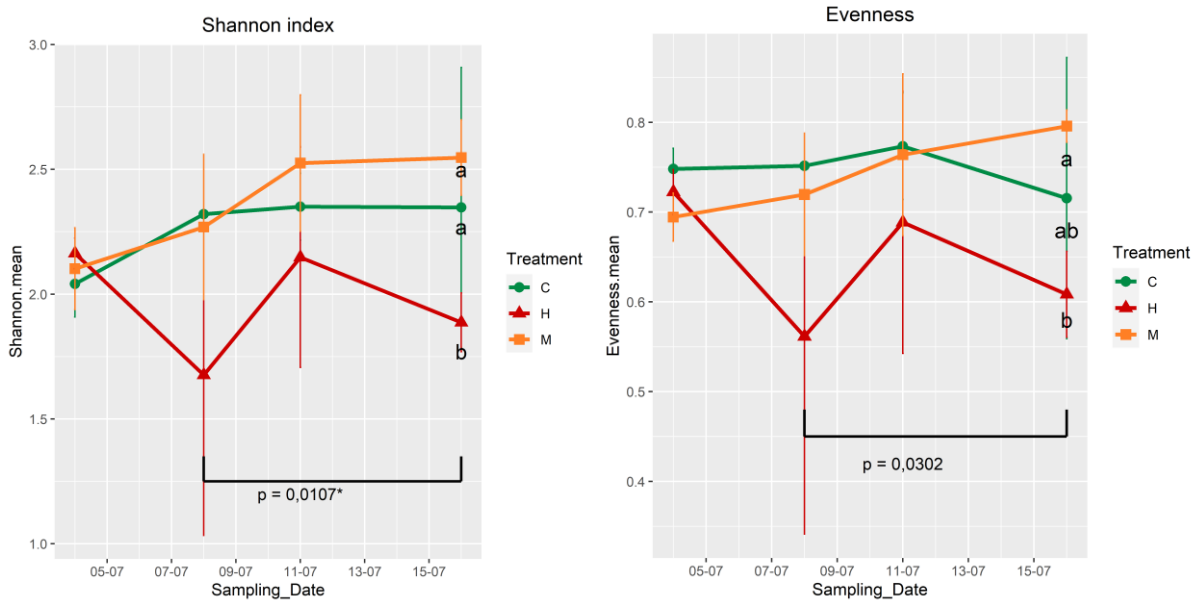

Figure 13: Shannon index and evenness in the treatments during the experiment (groups a, b given by HSD.test)

2.2.2 Phytoplankton functional groups

At S1, the main functional group in all the treatments was the "E" group (27% ± 8) mainly composed by the species *Dinobryon*. At S2, the treatment H was principally constituted by the "Unclassified" group (30% ± 13), while C and M were still represented by the "E" group (respectively 39% ± 5 and 45% ± 9). At S3, C, H and M were constituted mainly by the "X1" group (23% ± 7) and the species *Monoraphidium*. The "E" group was still present in the M treatment (14% ± 5). At S4, H was principally composed by the "Lo" group (51% ± 8). C was represented by the "P" group (13% ± 4) composed of diatoms such as *Diatoma elongatum* and *Fragilaria crotonensis* and the "X1" group (13% ± 5). M was composed by the "Y" group (15% ±3) represented by the species *Cryptomonas* and *Gymnodinium*, and the "X1" group (17% ± 4).

The NMDS (Figure 14) shows the distribution of the phytoplankton functional groups during the experiment. At S1, the phytoplankton community was significantly different between treatments (pvalue ≤ 0.01, PERMANOVA). Separated from the core cluster were the replicates H1 and H2 at S2. At this moment, due to the effect of the treatments, the phytoplankton community in H was significantly different from M and C (pvalue ≤ 0.01, PERMANOVA). It was mainly composed by the group "Unclassified", constituted only by the species *Desmarella brachycalyx* from the class of Choanoflagellatea (even if they do not contain any chlorophyll, most of Choanoflagellatea are traditionally counted in the phytoplankton analyses). Significant differences in the phytoplankton community between S2

and S4 were due to the interaction between time and treatment (pvalue ≤ 0.001, PERMANOVA). The "Y" functional group was indicative for the M treatment (pvalue ≤ 0.01, multipatt) and differentiated M-H (pvalue ≤ 0.001, simper) and M-C (pvalue ≤ 0.01, simper). "Lo" group and "Unclassified" group were indicative for the H treatment (pvalue ≤ 0.01, multipatt), but only the "Unclassified" group significantly contributed to differences between M-H (respectively pvalue ≤ 0.01 and pvalue ≤ 0.01, simper) and C-H (respectively pvalue ≤ 0.01 and pvalue ≤ 0.01, simper).

The community turnover (Figure 15) between dates was significantly higher in H compared to C and M (pvalue = 0.000371, ANOVA), and the highest turnover was between S2-S3 (0.77 ± 0.06). In the C treatment, the community turnover was similar between S1-S2 and S2-S3 and lower between S3-S4. In the M treatment, the community turnover had the same pattern as in treatment H. The highest turnover was between S2-S3 (0.56 ± 0.05), while it was lower and similar between S1-S2 and S3-S4.

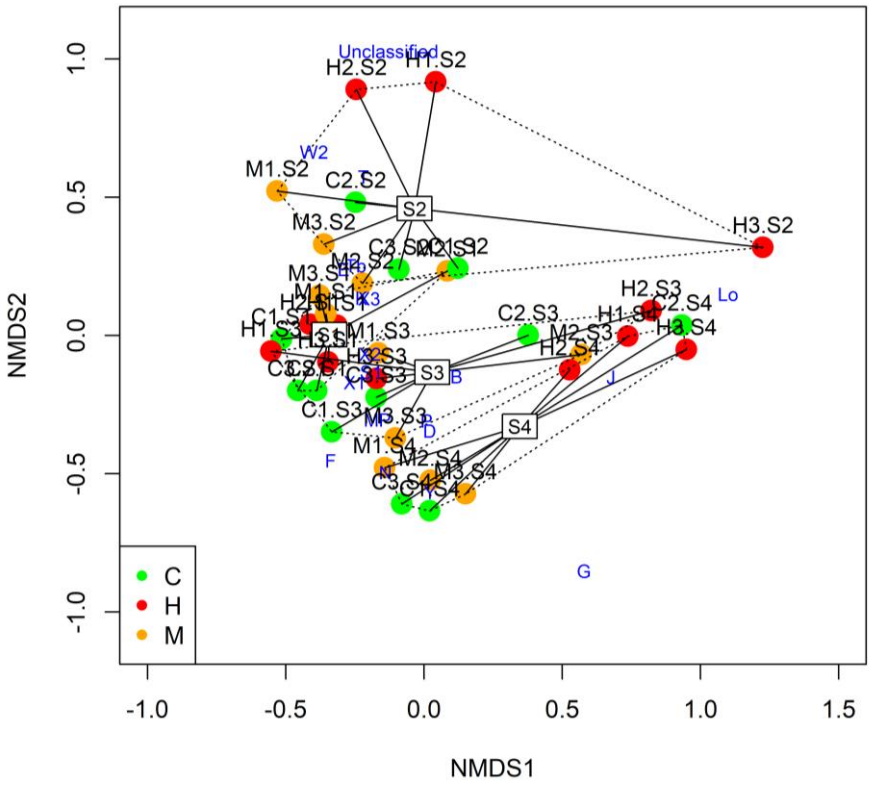

Figure 14: Non-Metric Dimensional Scaling (NMDS) showing the functional groups distribution in the mesocosms at the different samplings (stress= 0.15)

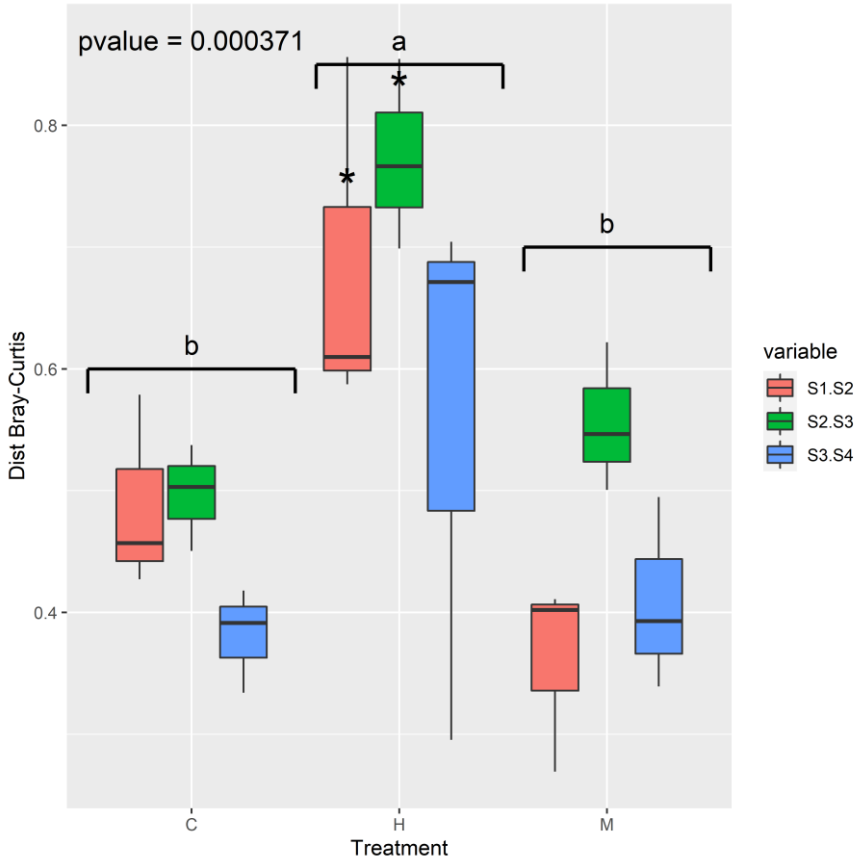

*Figure 15: Community turnover between treatments and samplings (groups a, b given by HSD.test ; * p < 0.05)*

2.2.3 Trophic strategy of functional groups

Mixotrophs were the most represented trophic group during the experiment (50.7% ± 12.9), followed by autotrophs (43.7% ± 13), whereas heterotrophs were poorly represented (5.6% ± 12.2). However, a significantly higher proportion of heterotrophs was found at S2 in the H treatment (pvalue = 0.02732, Kruskal-Wallis) (Figure 16). At this time, *Desmarella brachycalyx* represented almost half (45% ± 7.9) of the total community biovolume, whereas it was poorly represented in C (0.37% ± 0.39) and M (4.47% ± 2.19).

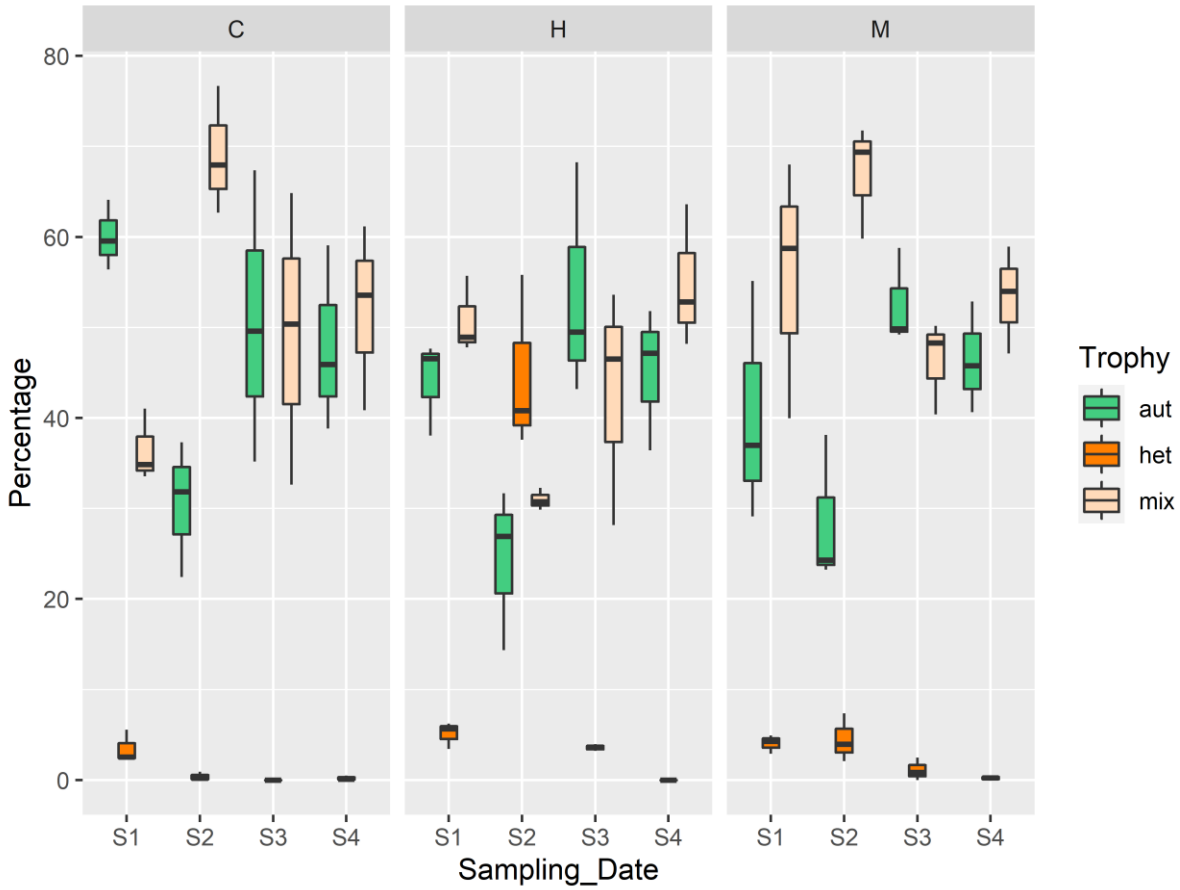

Figure 16: Distribution of trophic strategy among treatments during the experiment (aut: autotrophs, het: heterotrophs, mix: mixotrophs)

2.3 Effect of the treatments on phytoplankton community

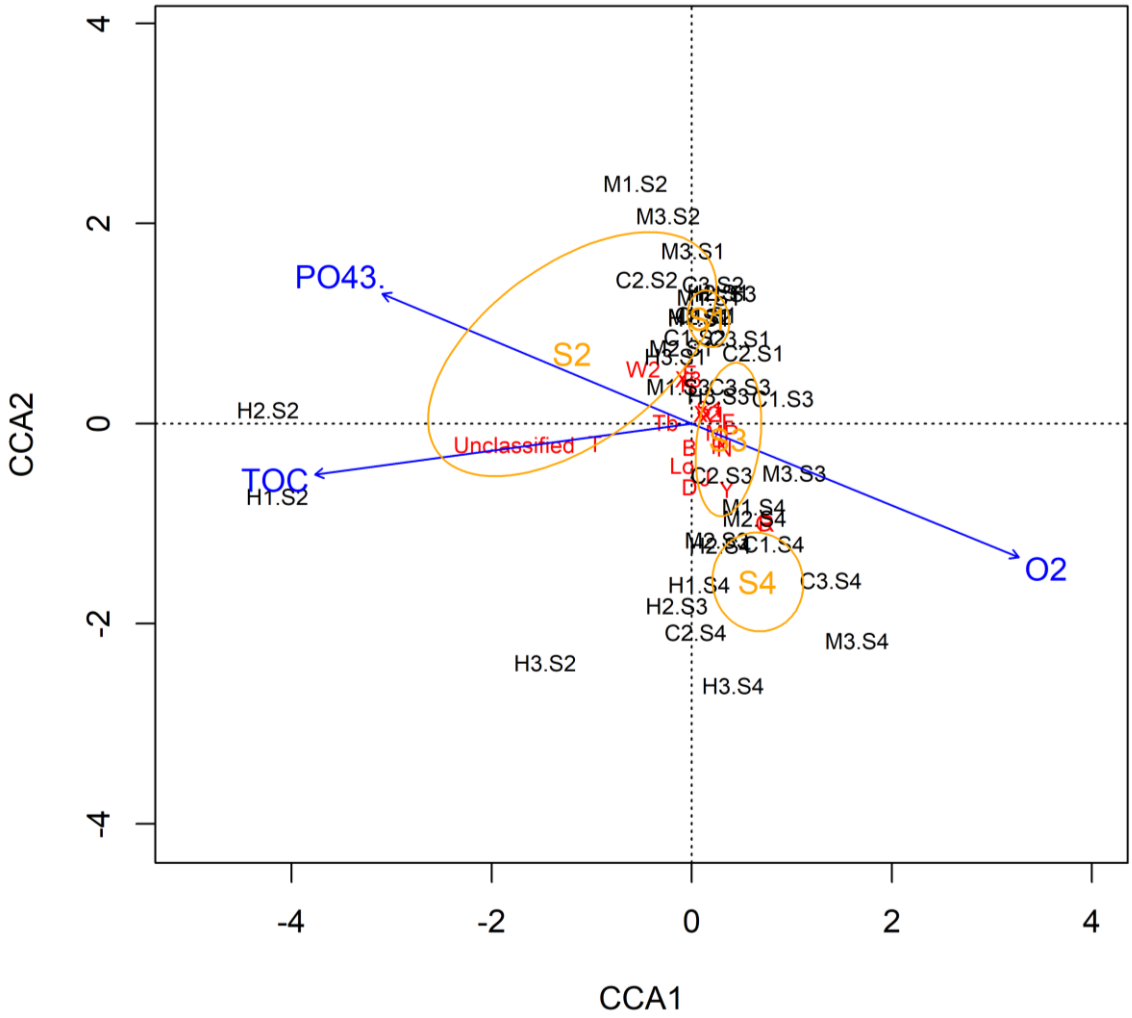

Figure 17: CCA of phytoplankton functional groups and three selected environmental variables: TOC (Total Organic Carbon), PO_4^{3-} (orthophosphates), O_2 (dioxygen) (pvalue = 0.001)

The CCA ordination significantly (pvalue = 0.001, ANOVA) explained 93% of the constraint inertia on the first two axes. Three environmental parameters were retained as significant for the distribution of the phytoplankton functional groups between the treatments (Figure 17). TOC and PO_4^{3-} mostly explained the variance on the left side of Axis 1 while O_2, explained the variance on the opposite side of Axis 1. Specifically, the phytoplankton community in replicates H1 and H2 was represented by the undetermined group formed by *Desmarella brachycalyx* and was driven by total organic carbon concentration. The phytoplankton community in the other mesocosms was mainly distributed along Axis 2, which separated on the top part the phytoplankton communities at S1 and S2, while at the bottom were the communities at S3 and S4. Co-inertia (Annex 7) confirmed that TOC and "Unclassified" group were the best drivers for variance among treatments during the experiment with a reliable

representation of the mesocosm co-structure as vectors were not superposed (pvalue ≤ 0.05, RV.test).

3 Discussion

3.1 Physico-chemical parameters

The total organic carbon dynamic reflected the manipulated inputs of dissolved organic carbon and was the main parameter characterising the H treatment. The highest peak was indeed observed in this treatment at S2, which aimed at reproducing extreme events such as violent storms. Other parameters correlated to TOC at S2 were reactive phosphorus (orthophosphate PO_4^{3-}) and particulate phosphorus, whereas NO_3^-, O_2 and NH_4^+ to some extent had an inverse dynamic. Particulate phosphorus represents the form fixed by phytoplankton and thus testify their metabolism dynamic (annual report of the scientific council of the CIPEL, 2018), that is more intense because of the inputs of nutrients occurred at that time. However, O_2 does not increase and it is anticorrelated to phosphorus, suggesting a higher metabolic activity from non-autotrophic organisms. The M treatment did not have a similar dynamic regarding these parameters. The slight increase of TOC between S1 and S3 was not significantly different from the control, neither were the dynamic of total phosphorus nor O_2. Therefore, the M treatment had little impact on the physico-chemical parameters.

Light dynamic was more difficult to assess and the effect of the filters remained lower compared to TOC concentration during the experiment. The H treatment had less light at S2 and S3 than the other treatments (around four times less) and this effect was more accentuated at the depth 0-50cm. In general, the filters had the highest effect at the surface, however these data were not taken into account as phytoplankton identification was done at two-meter depth. Moreover, the surface layer was more likely to be disturbed by the weather and the interaction with the atmosphere. Weather monitoring (data from the platform INRAE CLIMATIK) revealed that no specific pattern was related to light dynamic. Raining events were weak (< 2 mm of rain per day for 2 days during the experiment). However, photosynthetic active radiation (PAR) maximum decreased of 178 Jcm^{-2} between S1 and S3 and increased of 58 Jcm^{-2} between S3 and S4. PAR maximum dynamic was thus similar to light dynamic at two-meter depth. The scattered light throughout water column could be explained by the presence of inorganic particles, which have a high refractive index (Deyong et al., 2009). Scattering by particles is the main regulator of transparency (or Secchi depth) in lakes: the higher the coefficient, the higher the transparency is at deeper layers (Peng & Effler, 2013). Moreover, the water can be particularly transparent at this period of the year in Lake Geneva because of the annual

maximum peak of grazing between June and July (Druart & Balvay, 2007) allowing light to infiltrate deeply.

Other parameters followed a temporal dynamic such as temperature, SiO_2, Cl^- and SO_4^{2}. About 40% of treatment distribution remained unexplained by the PCA. The third axis, mainly driven by pH, represented 13.5% of total inertia. There was indeed an increase of pH only in the H treatment at S3 (pH > 8.4). However, the peak of higher pH appeared after the development of heterotrophic species, which may suggest an effect of the heterotrophic metabolism in increasing the pH.

The PCA-biplot showed also a resilience of the physico-chemical parameters as treatments were grouped at S4 after the split at S2. Lake abiotic parameters may not change on a long-term basis even if storm events can be intense or with higher frequency (Perga et al., 2018). In this study focusing on an alpine lake, it has been shown that "clear storms" with increasing water instability had no consequences on seasonal patterns. However, "turbid storms" with deep water mixing could change hydrodynamic features through slow sedimentation, less light penetration and thus impact metabolisms (low decay rate, shifts of trophic strategy). The environment resilience capacity strongly depends on lake's own antecedent conditions (Jennings et al., 2012). A study conducted in different lakes at a broader scale (Europe, North America and Taiwan) showed that storms impacted widely on abiotic conditions but the recovery period lasted from several days to an entire year depending on the lake, but also on the scarcity of these extreme events for some areas. In our study, we can suggest that experimental conditions simulating storms were not as strong considering the trophic state of Lake Geneva. Therefore, the resilience period was short.

To conclude on physico-chemical parameters, TOC was the best driver representing the H treatment, followed by nutrients (phosphorus, nitrogen) and O_2, whereas light and mixing did not characterise well the differences between treatments. These parameters had indeed a temporal dynamic, along with other physico-chemical variables. Regarding our hypotheses, physico-chemical parameters confirmed the second hypothesis stating that the effects of the H treatment would be higher than the effects of the M treatment. They also partly confirmed the third hypothesis claiming that the ecosystem is resilient.

3.2 . Phytoplankton community

3.2.1 Phytoplankton biovolume

Total phytoplankton biovolume was not retained as significant measure as differences were present before the application of treatments (S1) and did not appear during the experiment (S2 to S4). Even if phytoplankton total abundance constitutes a good proxy for the phytoplankton dynamic, it was not explicative regarding our hypotheses about the treatment effects. Total biovolume indeed decreased in all the treatments between S1 and S2. This decrease could be explained by the stress caused by the artificial bag constriction even if the experiment started after three days of acclimation of the mesocosms. As organisms are concentrated in a mesocosm, it could promote competitions. Another explanation could be a predator effect. *Daphnia* species (mainly *Daphnia hyalina*) are particularly abundant in July in the epilimnion of Lake Geneva (Druart & Balvay, 2007). It has been demonstrated that, as microfiltrators, cladocerans prefer non-filamentous and non-colonial nanoplankton (< 5µm). *Monoraphidium convolutum* was dominant in the initial samples (Druart & Balvay, 2007; Horn, 1985) and could constitute a preferential prey. To answer our hypotheses about shifts of phytoplankton communities due to the treatments, other parameters at the community-level have to be assessed such as the Shannon's index or the evenness.

3.2.2 Diversity indexes

The Shannon's index and evenness values confirmed our hypothesis that biodiversity of phytoplankton community would be reduced during intense weather events as simulated in the intensive treatment and that species evenness would be affected. The Intermediate Disturbance Hypothesis (Connell, 1978) can explain the dynamic observed in this experiment. In fact, the M treatment had the highest diversity index and evenness during the experiment, even though the outcomes were not significantly different from the control. Intermediate disturbance such as gentle wind mixing stimulate diversity by promoting nutrient resuspension (Holzmann, 1993), which could explain why the diversity was higher in treatment M.

Diversity indices such as Shannon and evenness are limited by the counting methodology as they are estimated on the species richness. In fact, if a species predominates in the sample, fewer ocular fields are required in order to count 400 individuals. Thus, some species were only seen on the slide outside of the counted ocular fields. For instance, in the replicate C3 at S1, *Cosmarium depressum*, *Fragilaria crotonensis*, *Peridinium sp* and *Cryptomonas sp.* were identified outside of the accounted ocular field. On the contrary, if species abundance is quite even and low, higher species richness is calculated because more

ocular fields are required. Although these indices still constitute a simplification of the reality, we find this counting methodology the most effective in terms of representation of the biodiversity dynamic during the experiment.

3.2.3 Phytoplankton functional groups and turnover

Phytoplankton functional groups strongly differed in the H treatment because of the emergence of the "Unclassified group" composed by strictly heterotrophic organisms at S2, which was representative for replicates H1 and H2. Our hypothesis that the biodiversity of phytoplankton would be reduced with storms of higher frequency and intensity and be replaced by generalist organism (such as 'r' strategists) is confirmed by the development of heterotrophs and replacement in phytoplankton functional group repartitions. The second hypothesis of stronger impacts of storms of higher intensity is also supported by the emergence of heterotrophs only in the H treatment and not in the M treatment. At the end of the experiment, the composition in this treatment was also different due to the presence of the "Lo" group that composed about the half of the phytoplankton community. It is constituted of big *Peridinium* species and *Ceratium hirundinella*, which are indeed the species with the highest specific biovolume among identified phytoplankton and are typical of summer epilimnion in mesotrophic lakes.

Phytoplankton functional group dynamic was similar in the M treatment and in the control. The samples ordination suggested a time effect due to the weather with the separation between sampling dates with a small cluster for S1 (homogeneity of conditions) and separated clusters for other dates. The "E" group indeed dominated at S1 in all treatments. This group is typical of habitats with oligotrophic conditions and is able to tolerate low nutrient conditions (Reynolds, 2002), as species in this group (*Dinobryon sp.*) are efficient to assimilate CO_2, but are also able to feed on bacteria. The presence of the "E" group in all mesocosms reflects the summer habitat condition: the rise of temperatures caused a thermal stratification leading to the decrease of nutrients in the epilimnion. Then, the "X1" group dominated, characterising mixed habitats and enriched conditions, which is surprising for the control. Although, between S2 and S3, there was a moderate wind (with a daily average around 12 kmh^{-1}) that could explain the mixed conditions even in the C treatment. Additionally, the "P" group gained some importance in the C treatment and represented species from eutrophic epilimnion with a tolerance for carbon depletion and a sensitivity for stratification. It could also be related to moderate wind the day before with the highest daily peak of the month (23 kmh^{-1}). These natural wind events suggest a weather effect acting in all mesocosms in addition to the treatment effect.

The turnover dynamic was useful to show differences in community transitions between treatments. The H treatment had the highest turnover compared to the other treatments. This high turnover at the date S2 confirmed the fast change induced by the environmental conditions simulated in the high intensity treatment. The following reduced turnover rate between S3 and S4 showed a resilience trend, supporting the hypothesis that the effects of the treatments do not persist over time and that phytoplankton community would recover after the disturbance. The M treatment had a similar pattern at a smaller scale, as the turnover rates were not significantly different from the control.

In general, the functional group approach is a good way to characterize community samples. Padisák et al. review enabled to accurately assign each phytoplankton species to the corresponding group, which reflect habitat conditions and is more informative on the ecological state than considering the presence of each species independently. Some morphological or physiological traits are similar among species that do not share the same phylogenetic group (Reynolds et al., 2002). An environment constrained by either nutrients or light would be more likely to be dominated by species in the same functional group. Moreover, functional groups should reflect processes within the habitat such as sedimentation or consumption by grazers using relevant functional traits (ability to get nutrients, to grow…) (Kruk et al., 2010). There are yet some limits to apply this method on a large-scale as it is still complex to assign a functional group to a species. The differences among groups are not always clear for non-specialists as different levels of accuracy are described (Padisák et al., 2009). Functional groups are described by either abiotic factors (light, water column mixing, silicate concentration…) and/or biotic factors (surface/volume ratio, trophic strategy, skeletal silicon) that make each group's definition more complex. Moreover, precise identification under microscope is sometimes impossible, whereas species could belong to different groups: *Cyclotella sp.* belong either to group A or B. Finally, functional trait approach is missing lake antecedent conditions and seasonality that play a major role on phytoplankton assemblages (Stockwell et al., 2020).

3.2.4 Trophic strategy of functional groups

The analysis of the trophic strategies showed the emergence of heterotrophs in the H treatment at S2 as was suggested also by the decrease of O_2. The large input of dissolved organic carbon increased the ratio of organic carbon over inorganic carbon in the epilimnion, which fostered organisms using organic carbon as a source of energy such as the heterotrophs. The concentration of heterotroph organisms, represented by *Desmarella brachycalyx*, increased up to about 15 times more compared to the other dates within the treatment. These microorganisms

are small and unicellular and adapted to low light condition and their fast nutrient uptake is also adapted in oligotrophic condition (with a quite large Surface/Volume ratio) (Naselli-Flores, Padisák & Albay, 2007).

The spread of heterotrophs testifies a growth of 'r' species. They are also referred as 'C-strategists' characterized by an efficiency in colonising environments and a fast uptake rate (Reynolds, 1987). However, there are not competitive enough when nutrient concentrations are lower, as the 'K' species or 'S-strategists' are tolerant to this condition because of a better storage capacity. Species such as *Ceratium hirundinella*, *Peridinium sp.* and *Gymnodinium sp*. are indeed more represented at the lower nutrient concentrations at S4 in treatment H.

Even if the dominance of heterotrophs in the H treatment was consequent, it lasted only a couple of days, which testified the capacity of resilience of the phytoplankton community. With increases in organic carbon, mixing and less photosynthetic active radiation, heterotrophs dominated. The recovery of autotrophs depends on within-lake processes (intrinsic characteristics of the lake catchment) (McGowan et al., 2008) and the period without extreme events (Jennings et al., 2012). In the example of the lake Lough Feeagh studied in the previous paper but also further by the GLEON, phytoplankton assemblages recovered after extreme flood events within 3 months (De Eyto et al., 2016). A warning should remain as ecosystem processes manage extreme events as long as biotic and abiotic factors function within their own law of toleration (Shelford, 1931).

Trophic strategy analysis was similar between the M and C treatments. Mixotrophs predominated, especially in the M treatment. These organisms have the advantage to use either organic or inorganic carbon as energy source. We can conclude that the experimental conditions of the M treatment were not extreme enough to cause a high stress among the phytoplankton community.

3.3 Effect of the manipulated environmental parameters on the phytoplankton community

The intensive storm treatment (H treatment) confirmed a rapid and short-term impact on certain physico-chemical parameters and on the phytoplankton community. First, the decrease of the evenness and Shannon's index supported the expectation of a decrease of species abundance and diversity caused by disturbance events (Grover & Chrzanowski , 2004). The emergence in the H treatment of the heterotroph *Desmarella brachycalyx,* which is able to spread fast and uses strictly organic carbon, demonstrated a shift on the trophic strategy of the

species, supporting further hypothesis. Some physico-chemical parameters (TOC, PO_4^{3-}, O_2) and some biotic parameters (diversity indexes, phytoplankton functional traits, trophic strategy and community turnover) showed stronger changes in the H treatment, confirming that the most intensive storm would have a stronger impact than the intermediate event. Finally, the observed recovery of the manipulated environmental parameters, together with the decrease of the turnover and the recovery rate of the autotrophic strategy, supported our hypothesis of the resilience of the phytoplankton community after disturbance.

However, the recurrence and dominance of heterotrophs in the H treatment could have weakened the resilience capacity of the community. The decrease of diversity relates to the functional diversity and may reduce the spectra of ecological feedbacks when facing disturbances. There is indeed a consensus stating that "biodiversity increases the stability of ecosystem functions through time" (Cardinale et al., 2012). Moreover, there is a need to maintain species performing similar functions facing global changes in order to compensate the decline of single species (Science for Environment Policy, 2015).

In this study, the overtaking of heterotrophs on autotrophs might alter the lake's own processes. Autotrophs play key roles in the lake ecosystem services by trapping inorganic carbon, which balances the constant increase of greenhouse gases. Moreover, these organisms provide O_2 for multiple species. Therefore, the decline of autotrophs could lead to the decline of many species that need O_2 at different trophic levels. It could thus impact on fish production. *Desmarella brachycalyx* is not a toxic species, however, it spreads fast similarly to toxic cyanobacteria, such as *Planktothrix rubescens*, although different environmental conditions (e.g. higher nutrients concentration) are required for cyanobacteria proliferation. In a near alpine lake, Lake Bourget, climate change promote harmful algal bloom since the late nineties (Jacquet et al., 2005).

Conclusion

The project Mesolac aimed at understanding the impacts of storm events of different intensity on the biocenosis in the context of climate change. My internship focused on the effects on the phytoplankton communities. It was assumed that the shift of physico-chemical parameters (increase of DOC, reduction of light, mixing) would impact the phytoplankton assemblages. According to the hypotheses, we have seen that Shannon's diversity index and evenness decreased in the treatment simulating the intensive storm (H treatment) demonstrating a change in phytoplankton diversity and functional traits. The functional group analysis showed

that this shift in the community was related to the growth of *Desmarella brachycalyx*. This species was the only heterotroph in the samples, and thus testifies a shift concerning species functions within the water column. The organic carbon / inorganic carbon ratio is a key indicator to estimate carbon sinks (reservoirs) or carbon sources related to the increase of greenhouse gases, and especially in this case to estimate the ratio between photosynthetic rate and respiration rate. The short-term shift of species composition due to the treatments shows that ecosystems are resilient as long as the extreme events are included in the tolerance interval (Shelford, 1931). Both the physico-chemical parameters and the phytoplankton communities were similar at the end of the experiment in all treatments confirming the resilience of the ecosystems in the mesocosms. The physio-chemical parameters and the phytoplankton community were submitted as well to the seasonal dynamic as expected at this latitude. We can conclude that extreme events as storms affected the composition of phytoplankton communities in addition to the seasonal successions. The main change was due to a switch of the community metabolism from autotrophic to heterotrophic. We could also observe these effects lasting for a couple of days and then the community returned at the same composition and diversity values observed at the beginning of the experiment.

Further research is needed to be done at different levels. On one hand, we can expand the project with a study starting from the early blooms of phytoplankton in spring to the end of summer in order to integrate longer seasonal shifts. Moreover, storms are especially frequent in summer with the rise of warm air masses. A coupling of the rise of storm events and temperature could be considered for a study. The combination between temperature rises and "brownification" has been analysed on other lakes. For instance, this combination was tested in a mesocosm experiment near Lake Lunz and the lowest evenness was calculated in the combined temperature x brownification treatment over time (Rasconi, 2015). Even if temperature rise alone had a stronger effect on planktonic food web in this quoted study, different scenarios have to be considered depending on each lake.

The forecasts of the ecological consequences of climate change are particularly difficult to disentangle, especially if synergies with stressors prevent ecosystems from recovering. Other combinations could thus be explored with simulations of storms and a higher trophic state (e.g. with local fishes) or with anthropogenic inputs (e.g. with heavy metals and toxic pollution) (Stewart et al., 2013).

Personal experience

This six-month internship was rewarding on different aspects. I gained knowledge on phytoplankton taxonomy and the relationships between their structures and their functions (e.g. filamentous algae that are more efficient to use light and are not easily eaten by zooplankton). I also learned the commonly used method for phytoplankton monitoring and quantification (Utermöhl, 1958). During my teleworking period due to the health crisis, I analysed data using R. I gained autonomy and organisation in order to learn the use of several packages including the package *ggplot2* for graphic illustrations or the package *vegan* especially used in ecology. Moreover, I gained research methodology by building a tree decision to disentangle research questions. I had to do short presentations during the internship to present intermediate results that helped summing relevant questions and outcomes. I had a good communication with my supervisors that was essential, especially during teleworking, and their remarks were always helpful to expand the analytical perspectives. Finally, I improved my English writing on a research report.

I experienced the ins and outs to work on the second year of this innovative project. In fact, mesocosms are more used as ponds (Stewart et al., 2013) outside the lake. These mesocosm devices using polypropylene bags within the water enables the water column to be impacted by currents that is more representative of the reality. However, as they are not totally hermetic (with an aperture at the surface) and require a boat for their access, their use is limited to the weather (storms, wind…). Therefore, it was really interesting to me to experience this issue in regard to the experimental conditions. It was thus challenging to understand how the fieldwork was conducted and which potential impacts exist. I learned the importance to label and store the samples and to choose an adequate volume, as it was done correctly and enabled anyone to understand the experiment. This experience makes me want to be involved in a project from the beginning to the end. I gained skills concerning the analysing and writing parts and I understood the stakes of the experimenting part that I would like to improve.

My double degree coupling an engineering formation (named quality of the environment and resource management) and a master (ecosystem and anthropisation) enabled me to better connect the study and the context in which it was realized. The engineering formation permits to understand more the human issues considering a region. The stakes are particularly high in the region around Lake Geneva concerning water and climate change. For instance, a conference was held in 2017 in the Canton of Valais about tourism and water issues: water as a resource for tourism, the impacts of tourism on water management, water and climate change

(https://www.unil.ch/igd/fr/home/menuinst/colloques--conferences/colloques/2017/eau-et-tourisme--water-and-tourism.html). The diverse trade-offs between actors are thus important to understand the stakes of these kind of studies. In fact, Lake Geneva provides diverse services (fish, drinking water, water purification…) involving a multitude of stakeholders (fishermen, researchers, managers, inhabitants, tourists) who have to get along while protecting the surrounding ecosystem (especially the wetlands). The formation enables me to see the perspectives of this study on the local scale: with an increase of storm events and thus matter inputs, what are the relationships between physico-chemical properties of these inputs and the land use of the lake catchment? What are the ecological consequences? I thereby assume that agricultural lands would impact differently from urban lands. The master formation offered me the possibility to interact with researchers whose projects were focused on the ecological effects of global changes. The issue is to better understand processes involved when the ecosystems are facing perturbations due to climate change. I improved my knowledge concerning the biological responses to these events (e.g. increase of exotic species). At a further step, it is interesting to take part to the ecosystem managing in order to act consistently with the outcomes of research studies. The scientific council of the lake managers is a good way to conciliate monitoring and decision-making.

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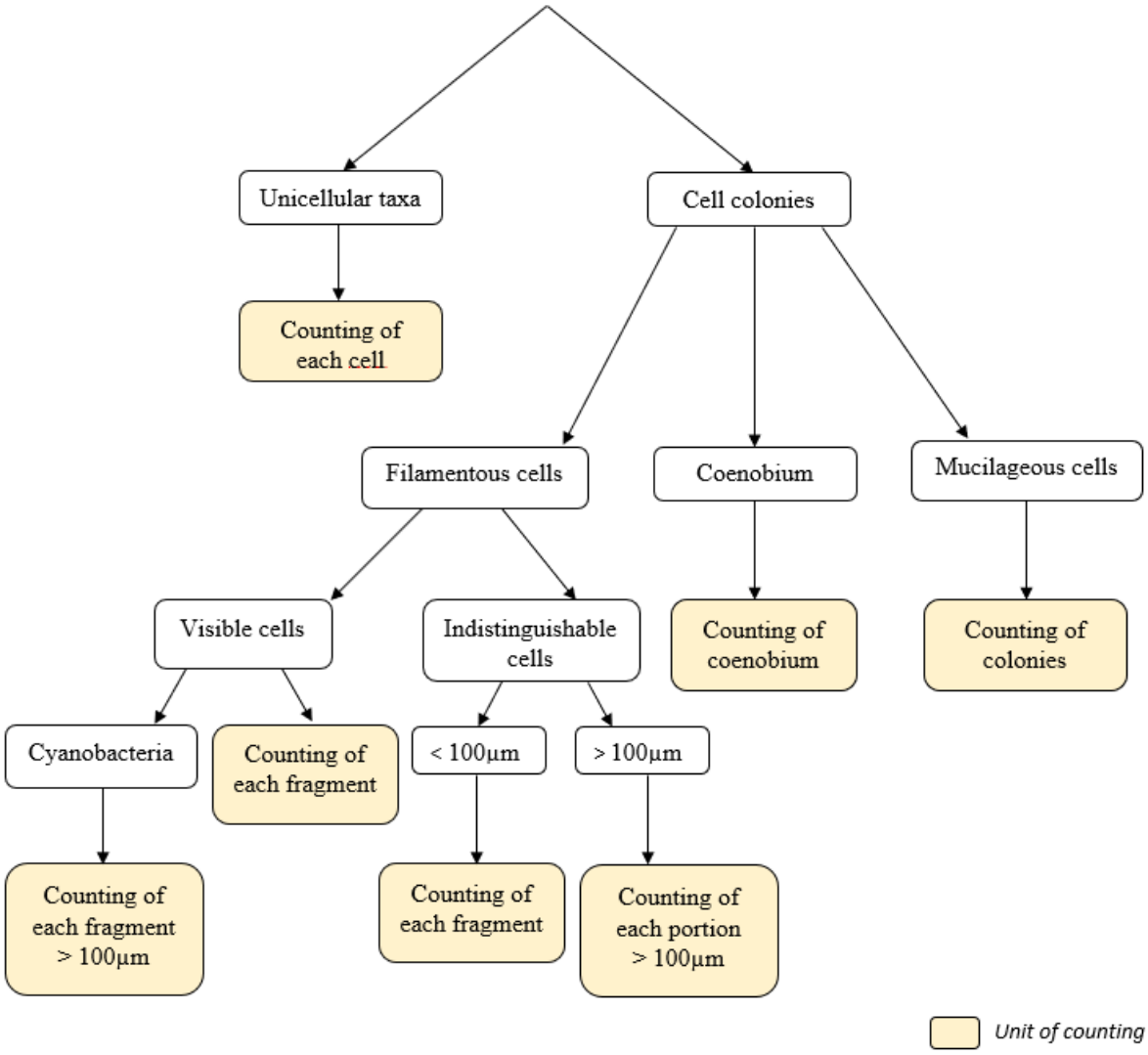
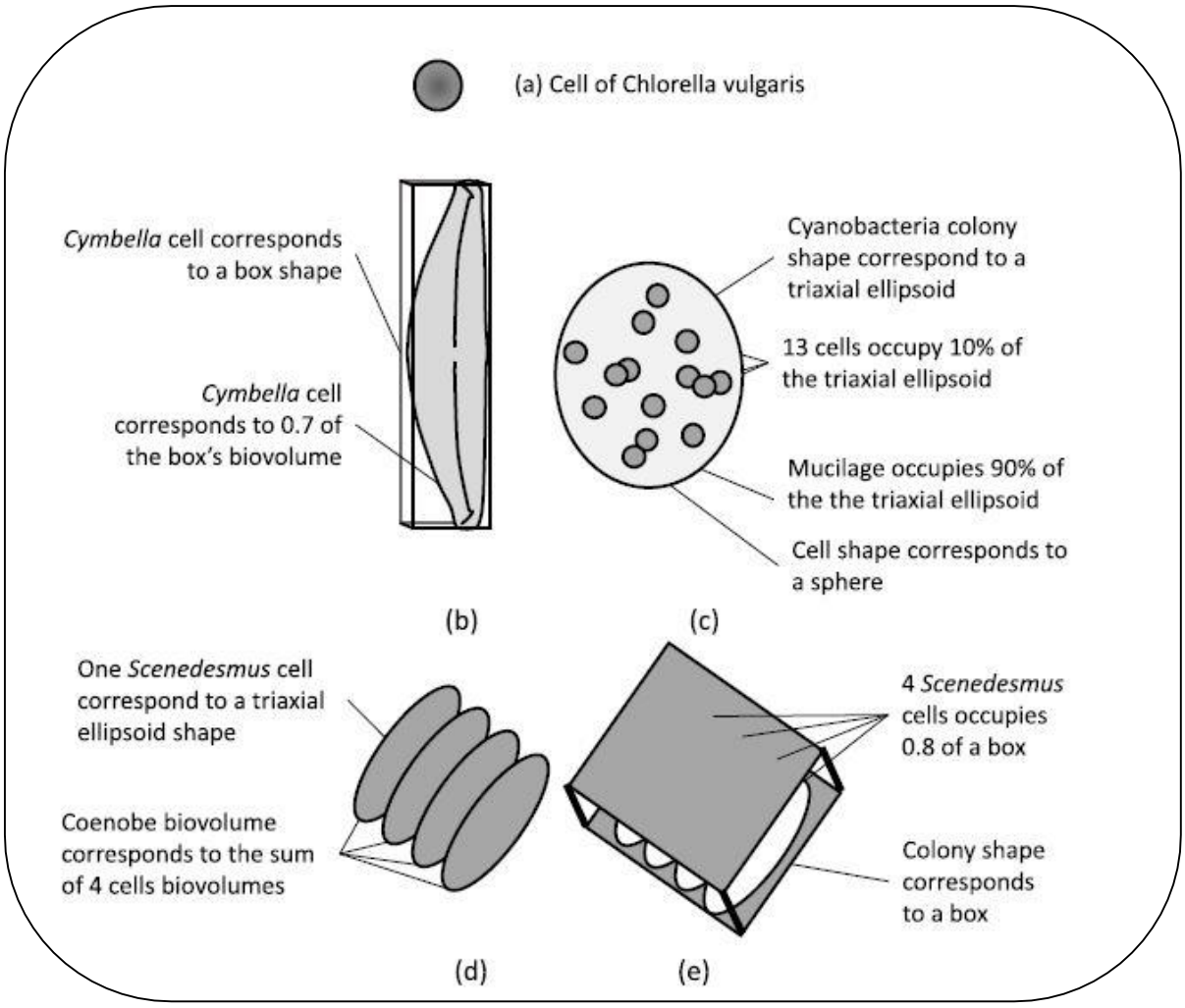
Annexes



Annex 1: Method to count phytoplankton (adapted from Druart & Rimet, 2008)



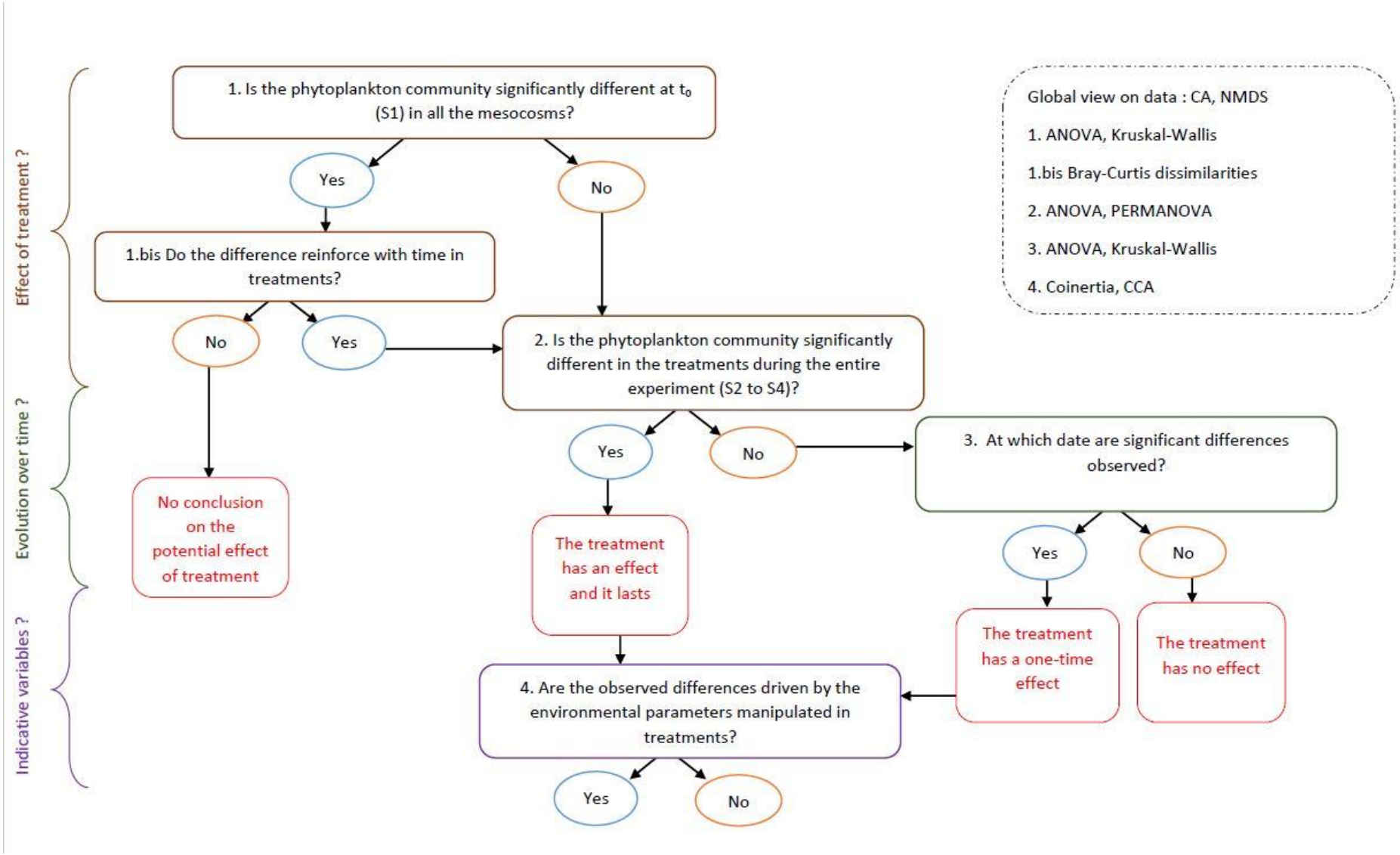
Annex 2: Examples to calculate the specific biovolume of phytoplankton (Rimet & Druart, 2018)

Parameter	EC	pH	TAC	NH_4^+	NO_2^-	NO_3^-	Ntot	PO_4^{3-}	Ptot	Ppart	TOC	Cl^-	SO_4^{2-}	SiO_2	Light	O_2	Temperature
Unit	µSc m^{-1}		meqL^{-1}	mgN L^{-1}	mgN L^{-1}	mgN L^{-1}	mgN L^{-1}	mgP L^{-1}	mgP L^{-1}	mgP L^{-1}	mgC L^{-1}	mg Cl^- L^{-1}	gSO_4^{2-} L^{-1}	mgSi O_2 L^{-1}	µmol m^{-2} s^{-1}	mg L^{-1}	°C
Name	Electroconductivity		complete alkalimetric title (Concentration of Ca^{2+} and Mg^{2+} bound to CO_3^{2-})	Ammonium	Nitrite	Nitrate	Total-N	Phosphates	Total-P	Particulate phosphorus	Dissolved organic carbon	Chloride	Sulphate	Silica		Disssolved oxygen	

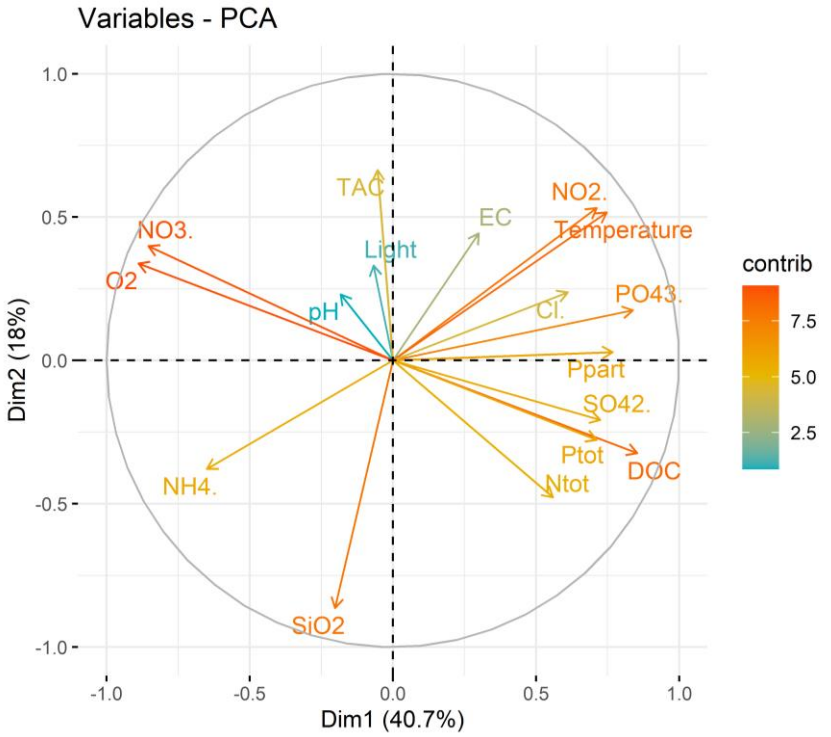
Annex 3: Physico-chemical parameters

Functional group	B	D	E	F	G	J	K	Lo	Mp	N	P	R	S1	T	Tb	W2	X1	X2	X3	Y
Habitat	Mesotrophic lakes with species sensitive to the onset of stratification	Shallow turbid waters	Small, shallow, base poor lakes or heterotrophic ponds	Clear, deeply mixed meso-eutrophic lakes	Nutrient-rich conditions in stagnating water columns	Shallow, mixed, highly enriched	Shallow, nutrient-rich water columns	Deep and shallow, oligo to eutrophic	Frequently stirred up, inorganically turbid shallow lakes (lots of diatoms)	Mixed layer of 2–3 m in thickness	As N, at higher trophic states	Under stratification, in the metalimnion or upper hypolimnion of deep oligomesotrophic lakes	Turbid mixed, only shade-adapted cyanoprokaryotes	mixed layers, light = constraint	Highly lotic	Meso-eutrophic ponds	Shallow, eu-hypertrophic	Shallow, meso-eutrophic	Shallow, well mixed oligotrophic	Lentic, low grazing

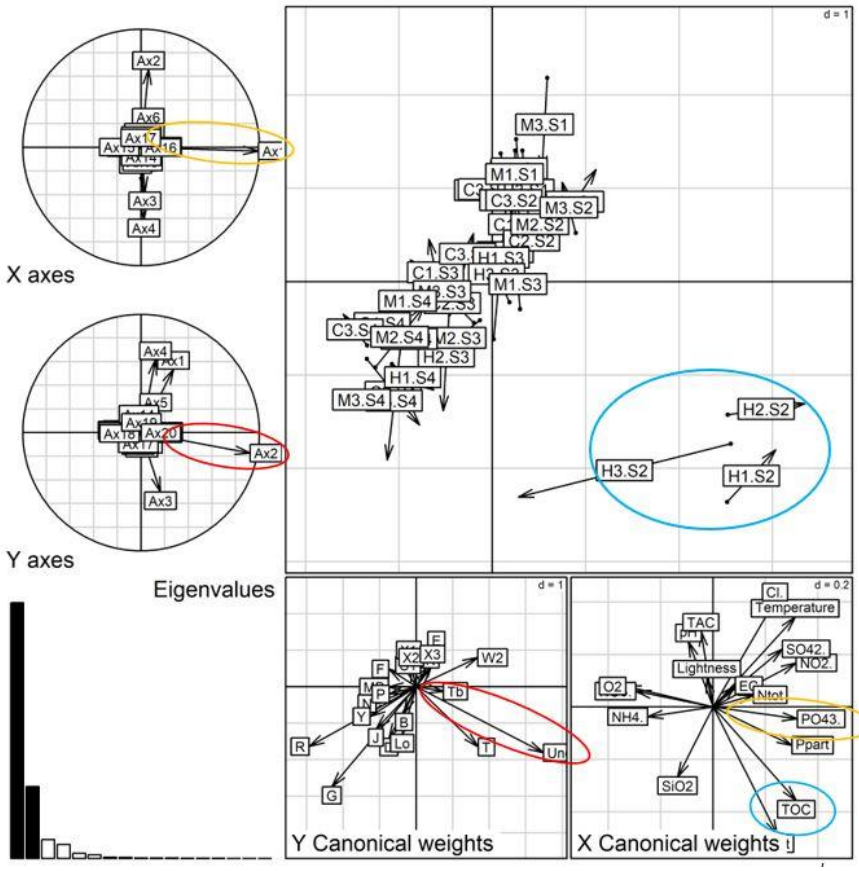
Annex 4: Functional groups present in the samples (adapted from Padisák, 2009)



Annex 5: Decision tree regarding potential effect of treatments with phytoplankton communities



Annex 6: Distribution of physico-chemical parameters throughout the experiment (PCA)



Annex 7: Co-inertia plot of environmental parameters (PCA X) and biological parameters (CA Y)

	1. Differences among phytoplankton communities in treatents at S1	2. Differences among phytoplankton communities in treatments during the experiment	3. Differences among phytoplankton communities in treatments at specific dates	4. Differences in phytoplankton communities related to environmental parameters at S2-S4
Total biovolume ($\mu m^3 ml^{-1}$)	ANOVA (pvalue=0.00925, F = 11.29, df=2) Biovolume groups C 418191.1 a M 342974.0 ab H 212795.8 b	Treatments: ANOVA (pvalue=0.093063, F=2.717, df=2) Time: ANOVA (pvalue=0.000884, F=10.658, df=2) *Data transformed ^-0.5050505* Biovolume2 groups S2 0.002258482 a S3 0.002185474 a S4 0.001459379 b	/	/
Shannon	ANOVA (pvalue=0.537, F=0.692, df=2)	Treatments: ANOVA (pvalue=0.0107, F=5.895, df=2) Shannon groups M 2.446703 a C 2.339164 a H 1.903375 b Time: ANOVA (pvalue=0.3283, F=1.186, df=2)	S2: ANOVA (pvalue=0.18, F=2.315, df=2) S3: ANOVA (pvalue=0.387, F=1.115, df=2) S4: ANOVA (pvalue=0.129, F=2.936, df=2)	/
Evenness	ANOVA (pvalue=0.0994, F=3.475, df=2)	Treatment: ANOVA (pvalue=0.0302, F= 4.278, df=2) Evenness groups M 0.7597265 a C 0.7468057 ab H 0.6194436 b Time: ANOVA (pvalue=0.4912, F=0.740 df=2)	S2: ANOVA (pvalue=0.254, F=1.734, df=2) S3: ANOVA (pvalue=0.583, F=0.591, df=2) S4: ANOVA (pvalue=0.129, F=2.932, df=2)	/

	1. Differences among phytoplankton communities in treatents at S1	2. Differences among phytoplankton communities in treatments during the experiment	3. Differences among phytoplankton communities in treatments at specific dates	4. Differences in phytoplankton communities related to environmental parameters at S2-S4
Trophy	ANOVA (pvalue=0.544, F=0.630, df=2) *Log transformed relative data*	Treatments: aut: Kruskal-Wallis (pvalue=0.9385, chi2=0.12698, df=2) het: Kruskal-Wallis (pvalue=0.06007, chi2=5.6246, df=2) mix: Krukal-Wallis (pvalue=0.05713, chi2=5.7249, df=2) Time: aut: Kruskal-Wallis (pvalue=0.0002981, chi2=16.236, df=2) het: Kruskal-Wallis (pvalue=0.01205, chi2=8.8375, df=2) mix: Krukal-Wallis (pvalue=0.2392, chi2= 2.8607, df=2) *Log transformed relative data*	S2: aut: Kruskal-Wallis (pvalue=0.7326, chi2=0.62222, df=2) het: Kruskal-Wallis (pvalue=0.02732, chi2=7.2, df=2)) mix: Krukal-Wallis (pvalue=0.06646 , chi2=5.4222, df=2)) S3: aut: Kruskal-Wallis (pvalue=0.9565, chi2=0.088889, df=2)) het: Kruskal-Wallis (pvalue=0.03399, chi2=6.7636, df=2)) mix: Krukal-Wallis (pvalue=0.7326, chi2=0.62222, df=2)) S4: aut: Kruskal-Wallis (pvalue=0.9565, chi2= 0.088889, df=2)) het: Kruskal-Wallis (pvalue=0.2369, chi2= 2.88, df=2)) mix: Krukal-Wallis (pvalue=0.9565, chi2=0.088889, df=2) *Relative data*	/

	1. Differences among phytoplankton communities in treatents at S1	2. Differences among phytoplankton communities in treatments during the experiment	3. Differences among phytoplankton communities in treatments at specific dates	4. Differences in phytoplankton communities related to environmental parameters at S2-S4

Functional groups	PERMANOVA (pvalue≈0.028, F=2.2616, df=2)
	Relative data
		Treatments: ANOVA on Bray-Curtis distances (pvalue=0.000371, F=12.646, df=2)
		`value groups`
		`H 0.6715799 a`
		`C 0.4552754 b`
		`M 0.4420424 b`
		Turnover dates: ANOVA on Bray-Curtis distances (pvalue=0.019465, F= 4.942, df=2)
		`value groups`
		`S2.S3 0.6088634 a`
		`S1.S2 0.5109518 ab`
		`S3.S4 0.4490825 b`
		Treatments: PERMANOVA (pvalue≈0.04, F=2.175, df=2)
		Interaction time/treatment: PERMANOVA (pvalue≈10^{-4}, F=2.9186, df=8)
		Time: PERMANOVA (pvalue≈10^{-4}, F=4.2559, df=2)
		Relative data
			S2: PERMANOVA (pvalue≈0.016, F= 3.2324, df=2)
			S3: PERMANOVA (pvalue≈0.729, F=0.50753, df=2)
			S4: PERMANOVA (pvalue≈0.096, F=2.9823, df=2)
			Relative data
				Coinertia, RV.test (pvalue≈0.045)
				CCA, ANOVA (pvalue=0.001, F=3.0738, df=3)

Annex 8: Summary table of statistical results following questions of the decision tree (significant results are colored in red)