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Interactions between green and brown food webs in aquatic ecosystems: a mesocosm experiment

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Key words

Nutrient cycling, microorganisms, zooplankton, fish, mixotrophy, light, allochthonous dissolved organic carbon, top-down, bottom up, trophic cascade

Abstract

The classical concepts of top-down and bottom-up cascading effects in ecosystems are challenged by the interactions between green and brown food webs. The green food web relies on photosynthesis and the brown food web relies on the mineralisation of dead organic matter. Mutualistic interactions between these two food webs are crucial for ecosystem functioning because a major fraction of carbon is produced by the green food web while mineral nutrients are mainly recycled through the brown one. However, green and brown food webs can compete for mineral nutrients if decomposers are nutrient limited. Thus, bottom-up or top-down effects on one food web can have major cascading effects on the other one. In our freshwater mesocosm experiment, we tested cascading bottom-up effects targeting the green food web through sunlight filtering and the brown food web through organic carbon addition, and cascading top-down effects mediated by fish presence. Our main finding is a positive effect of fish on phytoplankton density in mesocosms where daylight was reduced. The absence of a significant decrease in zooplankton biomass in the presence of fish suggests that fish had a positive bottom-up effect on phytoplankton mediated by nutrient cycling, which would decrease the sequestration of nutrients and increase their availability for phytoplankton. This enhanced recycling by fish was visible through positive effects on both sediment production and DOC accumulation, which also had a positive effect on the brown food web with increased abundances of heterotrophic prokaryotes and microbial eukaryotes. We did not observe any cascading effect of light treatment on the brown food web, and of organic carbon treatment on the green food web. Our organic carbon treatment had nearly no effect on the brown food web, probably because of the moderate quantity of added carbon and of the low bioavailability of some of the added molecules. Our experiment shows that fish can strongly stimulate phytoplankton through bottom-up effects due to nutrient cycling, and not only through the classic trophic cascade due to the top-down control on large herbivorous zooplankton.

Introduction

The relative importance of bottom-up and top-down controls on ecosystem functioning has received a lot of attention in food web ecology (*e.g.* Hunter and Price (1992) and Hulot et al. (2014)). However, most experimental and theoretical studies on this topic have ignored a fundamental aspect of ecosystem functioning which is the division of flows of matter and energy into two pathways : the pathway that relies on photosynthesis and constitutes the green food web, and the pathway that relies on detritus and constitutes the brown food web (Moore et al., 2004). The tight interactions between these two pathways are crucial for ecosystem functioning because a major fraction of carbon enters through the green pathway while mineral nutrients are mainly produced by the brown pathway.

Green and brown food webs have often been studied independently (Rosemond et al., 2001; Jones and Sayer, 2003; Jardillier et al., 2004), and most studies have focused on green food webs, in particular in aquatic ecosystems (Carpenter et al., 1985; Kagata and Ohgushi, 2006; Heath et al., 2014). However, green and brown food webs strongly interact and these complex interactions are expected to mediate top-down and bottom-up cascading effects.

First, green and brown food webs interact at the base of the food webs through mutualistic interactions via nutrient recycling or competition between decomposers and primary producers for nutrients. Thus, primary producers and decomposers can be either mutualists or competitors, depending on whether decomposers are limited by carbon or by nutrients such as nitrogen or phosphorus (Daufresne and Loreau, 2001; Danger et al., 2007b). For instance, Joint et al. (2002) observed a decrease in phytoplankton biomass and an increase in prokaryote (heterotrophic bacteria and archaea) biomass following glucose addition in a mesocosm experiment. The theoretical study of Zou et al. (2016) showed that the nutrient/carbon limitation of decomposers was likely a key determinant of the cascading effects of the green food web on decomposer production and associated bottom-up effects in the brown food web. When decomposers are carbon-limited, cascading effects of the green food web on decomposer production are expected to parallel those observed on primary production, while these effects can be opposite when decomposers are nutrient-limited (Zou et al., 2016). However, mixotrophic phytoplankton is able to bypass nutrient limitation by preying on prokaryotes (phagotrophic mixotrophy) or by consuming other organic compounds (osmotrophic mixotrophy). Mixotrophy is a versatile metabolism that allows some phytoplanktonic cells to shift metabolism triggered by environmental conditions (nutrient and light availability). Such a feature is key for ecosystem stability because primary producers are at the base of trophic food webs. For instance, Faithfull et al. (2011) did not observe a reduction in phytoplankton abundance in response to glucose addition thanks to the dominance of mixotrophic species in the phytoplankton community of the lake in which they run their mesocosm experiment.

Second, green and brown food webs also interact at higher trophic levels via generalist predators that consume prey in both green and brown webs (Rooney et al., 2006; Wolkovich et al., 2014). For instance, crustacean zooplankton can feed both on phytoplankton in the green food web and on predators of the brown microbial food web such as ciliates (Urbano et al., 2022). A few theoretical studies have shown that the coupling of distinct energy pathways by predators can have strong consequences on ecosystem structure and stability (Rooney et al., 2006; Wollrab et al., 2012; Mougi, 2020). These predators can have a stabilising effect by preferentially grazing the most abundant prey and balancing biomass distribution among prey, however they might also lead to apparent competition between the coupled food webs (Vadeboncoeur et al., 2005). Decomposers and primary producers are expected to respond in the same or in opposite directions to enrichment depending on the way predators couple the green and brown energy pathways (Wollrab et al., 2012; Ward et al., 2015).

Finally, interactions between green and brown webs at both the base and top of food webs are not independent. For example, ecosystems dominated by planktivorous fish as top predators produce more degradable detritus than ecosystems dominated by large herbivorous zooplankton (Harrault et al., 2012). These differences in detritus degradability can have bottom-up cascading effects on the brown food web and consequently modify the type of interactions between primary producers and decomposers (Harrault et al., 2014). Predators also strongly modify the composition and size structure of food webs (Lazzaro et al., 2009; Gauzens et al., 2016), which could affect the interactions at the bottom of the food webs.

Despite the importance of these interactions for ecosystem functioning, very few experimental studies have investigated top-down and bottom-up effects in food webs by manipulating explicitly the green and the brown food webs at the same time (Daufresne et al., 2008; Faithfull et al., 2011; Degerman et al., 2018). The few existing studies have mostly focused either on interactions between decomposers and primary producers in response to nutrient enrichment (Danger et al., 2007a; Daufresne et al., 2008) or on consequences of allochthonous carbon inputs as a bottom-up perturbation of the brown food web (Faithfull et al., 2011; Degerman et al., 2018). The aim of this study is to investigate further the

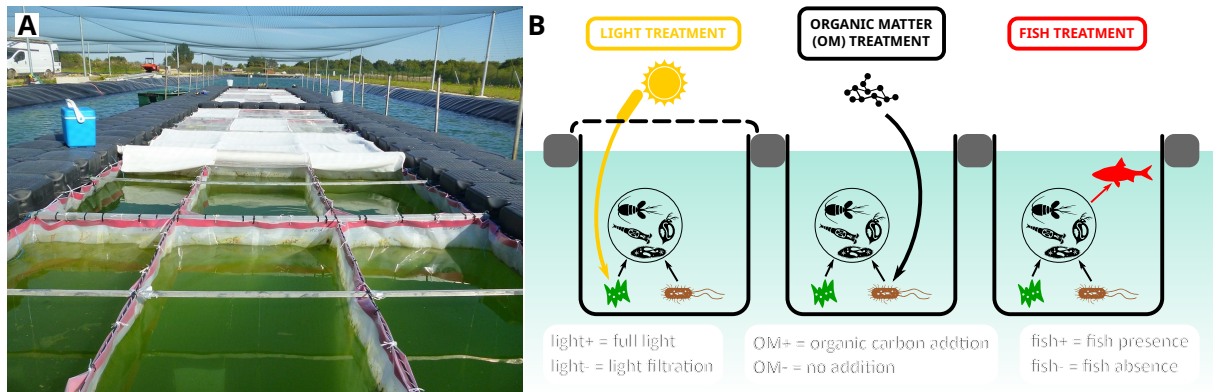


Figure 1: **A)** Photo of the experimental setting during a sampling session. The six mesocosms at the front are uncovered to allow sampling. **B)** Experimental design consists of three crossed treatments: light filtration (Light+ = no light filtration), soluble organic carbon addition (OM+ = carbon addition) and fish addition (Fish+ = four Leuscicidae fish). Light filtration (-50% of light intensity) is expected to decrease the photosynthetic activity of phytoplankton to target the green food web. OM addition is expected to promote bacterial production and target the brown food web. Fish presence, which affects food-web structure, is expected to modify the coupling between green and brown food webs. Organisms in circles represent heterotrophic microbial eukaryotes and zooplankton, which are the consumers in freshwater planktonic food webs.

cascading effects between green and brown food webs and their consequences on ecosystem functioning by manipulating in a factorial design light availability (*i.e.* bottom-up perturbation on the green food web), level of added carbon organic matter (*i.e.* bottom-up perturbation on the brown food web), and fish presence (*i.e.* top-down control of the food web) in freshwater mesocosms. By combining these three treatments, we aim to highlight the complex interactions resulting from the interplay between bottom-up and top-down effects.

We expect the reduction of light availability to reduce primary production and to lead to a carbon limitation of the brown food web, thus decreasing the biomass of organisms from both green and brown food webs. Organic matter addition should promote the growth of heterotrophic prokaryotes and increase their limitation by nutrients such as nitrogen and phosphorus. As a consequence, we expect an increased competition between heterotrophic prokaryotes and phytoplankton for nutrients, which would lead to a decrease in the biomass of the organisms of the green food web. However, such a competition could be mitigated by the stimulation of the heterotrophic metabolism pathway of mixotrophic phytoplankton able to prey on heterotrophic prokaryotes. Therefore, mixotrophic phytoplankton should be more abundant when light is depleted or organic carbon is added. Finally, we expect fish to increase phytoplankton and heterotrophic prokaryotes biomass through the top-down control of zooplankton or a positive bottom-up effect triggered by easily degradable faeces and the release of mineral nutrients.

Material and methods

Experimentation site and experimental design

The experiment was set in the Experimental Pond Platform (EPP, N48°16'57", E2°40'20") of the PLANAQUA facility ("PLAteforme Nationale expérimentale en écologie AQUAtique") near to Paris, France. The EPP includes 16 artificial ponds with an individual volume of $\sim 750\text{ m}^3$, in addition to a storage pond and an overflow lake of 4000 m^3 each (Mougin et al., 2015). Ponds are isolated from the ground by two successive sets of geotextiles and two HDPE geomembranes. In summer 2014, they received 30 cm of washed sand originating from the Loire River and they were filled with a mixture of rainwater and drinking water. Subsequently, they were naturally colonised by aquatic organisms.

We installed a floating pontoon ($27 \times 5\text{ m}$) in the water storage pond ($125 \times 15 \times 3\text{ m}$ deep). The pontoon delimited two blocks of $12 \times 3\text{ m}$ on which enclosures can be suspended (Fig. 1A). The mesocosm study was performed from the end of June 2015 to the beginning of November 2015 and thus began one year after the beginning of pond colonisation. Thirty-six translucent polyethylene enclosures ($2 \times 1 \times 2.75\text{ m}$, 5 m^3), sealed at the bottom, were attached to the pontoon 750 cm above the lake surface. The pontoon was divided into two blocks of 3×6 enclosures. The different enclosures with the same combination of treatment were uniformly distributed to avoid potential confounding edge effects (Fig. S2-1 in the supporting information).

The enclosures were filled with water from the storage pond hosting the pontoon on the 29th and 30th of June. All enclosures were successively filled for two minutes to avoid any bias due to variations in water composition during the day. Then, the water was enriched at the beginning of July with nitrogen (N) and phosphorus (P) to sustain the development of the community (nutrient concentrations in the storage water were initially very low). We twice added (3rd and 10th of July) 1.27 g NH₄NO₃, 0.65 g KH₂PO₄ and 0.39 g K₂HPO₄·3H₂O in each enclosure. Such addition corresponded to 140 µg_N L⁻¹ and 56 µg_P L⁻¹ (*i.e.* P:N ratio of 2.5). Zooplankton was collected with a net (140 µm mesh size) in the experimental ponds of the platform, pooled and introduced to the enclosures to initialise the community on the 6th of July. We noticed later that large cladocerans such as daphnia were absent in the collected community. Three treatments (with two modalities) were crossed in each enclosure: light, organic carbon and fish, leading to eight different combinations (Fig. 1B). Each combination with fishes had five replicates and each combination without fishes had four replicates. All treatments aimed to specifically target phytoplankton (green food web), heterotrophic prokaryotes (brown food web) and food web structure in order to alter the coupling between the two sub-food webs.

Experimental treatments

Light treatment

The filtration of daylight to limit photosynthesis was performed by two types of shade meshes used for crops, filtering 10 % (Diatex FOM20B200CR) and 30 % (Diatex F1038T-200BLANC) of daylight without changing the spectra quality. All enclosures were covered by shade meshes in order to avoid differential access by flying insects: the 10 % shade mesh acted as a null treatment, while the light-depleted enclosures were covered by one layer of 30 % and two layers of 10 % shade mesh for a total reduction of 50 % of light intensity in water (confirmed by a Li Cor light sensor). The shade mesh was attached to parallel aluminium bars, making a structure that could be rolled up for sampling (Fig. 1A).

Organic matter treatment

Dissolved organic carbon (DOC) was added each week from the end of July to the beginning of November (for a total of 15 weeks) to stimulate bacterial activity. Each week, a total of 1.8 g C was added in half of the enclosures as a mixture of three organic molecules providing one third of the carbon each: glucose (C₆H₁₂O₆), cellobiose (C₁₂H₂₂O₁₁) and α-cyclodextrine (C₃₆H₆₀O₃₀) (SigmaAldrich). These molecules are considered to vary in biodegradability from high to low, respectively, and are also present as substrates in the Biolog Ecoplate 96-well microplates (see the *Functional diversity of bacteria* section). A total of 5.4 mg C L⁻¹ was added to each treated enclosure, more than doubling the dissolved carbon stock of the system (initial DOC of the water: 3.96 mg C L⁻¹).

Fish treatment

Fish were used as top predators for the last treatment and were added in the enclosures on the 16th of July. A set of randomly selected enclosures received four planktivorous fishes belonging to Leuciscidae, mainly rudds (*Scardinius erythrophthalmus*) and a few roach individuals (*Rutilus rutilus*) because all the fish we received did not belong to the same species, with an average length of 8 cm and an average weight of 6.76 ± 1.44 g. Fish were anaesthetised before manipulation with MS222 at 0.3 g mL⁻¹ and weighed to the nearest mg. Fish were marked individually using a nanotech pit-tag (Lutronic International, 1 × 6 mm) injected in the peritoneal cavity. Three fish died during the experiment and were immediately replaced. At the end of the experiment, fish were again weighted to the nearest mg and euthanized with MS222. All the procedures were conducted in accordance with appropriate European (Directive, 2010/63/EU) and French national guidelines, permits, and regulations regarding animal care and experimental use (Agreement n° B77-431-1). Individual relative growth rate g (g g⁻¹ d⁻¹) was calculated assuming an exponential growth over the time spent in the enclosures Δt ($mass_{final} = mass_{initial} \exp(g \Delta t)$). This implies that g was assumed to be constant and that fish biomass production at time t was proportional to fish biomass.

Sampling and measurements

Phytoplankton

Phytoplankton was sampled weekly from the end of July to November with a 2 L sampling bottle (Uwitec). 2 L of water were sampled at three different depths for a total of 6 L (0–50 cm, 50–100 cm, 100–150 cm) and mixed together. 3 mL were added to 150 µL of 20 % paraformaldehyde and incubated

at 4 °C for one hour before liquid nitrogen freezing, then samples were kept at –80 °C for later FACS analysis (see the *Flow cytometry analysis of microorganisms* section). Moreover, 50 mL were directly analysed after sampling by a BBE fluoroprobe (Moldaenke, Kiel, Germany), which gives a proxy of the abundance of four phytoplankton groups: Chlorophyceae, Cyanobacteria, Bacillariophyceae/Dinophyceae and Cryptophyceae. At last, Lugol solution was added to 200 mL of water at the end of September for later identification of phytoplankton taxa (Table S2-11 in the supporting information) by an independent consultancy society (Bi-Eau, Angers, France). The Utermöhl (1958) method was used to quantify phytoplankton, with an inverted microscope. Phytoplankton biovolume was estimated on the basis of cell measurements and assignment of organism forms to geometric shapes. At the beginning of October, the same protocol was applied to collect the seston (particulate matter between 0.7 and 50 µm). Sampled water was first filtered through a 50 µm nylon filter to remove zooplankton and was then filtered through a pre-weighted and carbonised Whatman GF/F glass-fibre filter (nominal cut-off 0.7 µm). Filters were dried overnight at 60 °C and weighed to determine seston mass.

Stoichiometry

The filters were then used to quantify the percentage of carbon and nitrogen contained in the seston organic matter (*i.e.* phytoplankton and particulate organic matter (POM) < 50 µm) using a CHN elemental analyser (Carlo Erba NA2100, Thermo Quest CE International, Milan, Italy). Organic phosphorus content was quantified after sodium persulfate digestion and spectrophotometry. Subsamples were weighed to the nearest 0.001 mg (Perkin Elmer AD6 Autobalance). All elemental ratios are expressed in molar units.

Zooplankton

Zooplankton was sampled weekly from the end of July to November with a 2 L sampling bottle (Uwitec). A total of 24 L was sampled at four different locations in the enclosures and at three different depths (0–50 cm, 50–100 cm, 100–150 cm). Then, the water was pooled and filtered through a 50 µm nylon filter and zooplankton was stored in 96 % ethanol for later estimation of the main taxa abundance. At the beginning of October, the same protocol was applied to collect zooplankton that was then dried for 24 hours at 60 °C just after sampling. The dry biomass of zooplankton was assessed by weighting the nylon filter before and after the sampling.

Flow cytometry analysis of microorganisms

The frozen samples collected (see the Phytoplankton section) were analysed by Fluorescence Activated Cell Sorting (FACS) through flow cytometry (Accuri C6 Plus, Becton Dickinson). Pigmented cells were identified based on the autofluorescence of the chlorophyll *a* (orange fluorescence) (FL2, 585 ± 40 nm) vs red fluorescence (FL3, 670 nm). Cells containing chlorophyll *a* were distinguished using side scatter (SSC) vs FL3 and distinct size classes were defined to discriminate cyanobacteria from pico- and nano-eukaryotes. A subsample of each sample was stained with the nucleic acid stain SYBr Green I (Zubkov et al., 2007) to distinguish heterotrophic organisms using SSC vs green fluorescence (FL1, 533 ± 30 nm). Chlorophyll *a*-containing organisms were gated off plots of SSC vs FL1, having been identified in plots of FL3 vs FL1.

Water physico-chemistry

The main water characteristics were measured weekly with a multiparameter probe (Kor Exo) at three different depths (0–50 cm, 50–100 cm, 100–150 cm). Chlorophyll *a*, pH, dissolved O₂ concentration, turbidity and temperature were thus measured. In addition, water filtered through pre-weighted and carbonised Whatman GF/F glass-fibre filters (nominal cut-off 0.7 µm) was used to determine water chemistry. Two 200 mL samples were collected at two different dates in October and were frozen at –20 °C for later P and N analysis with a continuous segmented flow analyser (SEAL AA3 HR). Every two weeks, duplicate 30 mL samples for the measurement of DOC were collected. Samples were acidified with 35 µL of 85 % phosphoric acid and stored in the dark at room temperature in carbonised glass tubes sealed with Teflon lids. Dissolved organic carbon (DOC) concentration was determined using a total organic carbon analyser (TOCV-CSH; Shimadzu, Kyoto, Japan).

Sediments

Sediments were collected in each enclosure by 5 cm diameter and 30 cm height PVC tubes suspended at 1.5 m below the water surface (see Danger et al. (2012)). Tubes were put in place in the middle of

Table 1: Summary of the statistical models. Our three treatments and their interactions are considered as fixed effects. The date column represents the number of dates at which each variable has been sampled.

Variable	Dates	Additional effects	Random effects	Distribution	Test
BBE data (<i>e.g.</i> chlorophyll <i>a</i>)	13	-	mesocosm+date	Gaussian	Chi ²
Multiparameter data (<i>e.g.</i> pH)	13	depth	mesocosm+date	Gaussian	Chi ²
Fish growth rate	-	-	-	Gaussian	F
Seston	1	-	-	Gaussian	F
C:N and C:P of seston	1	-	-	Gaussian	F
Zooplankton dry biomass	1	-	-	Gaussian	F
Sediment	1	-	-	Gaussian	F
Cytometry data (<i>e.g.</i> bacteria)	4	-	mesocosm+date	Poisson	Chi ²
DOC	7	-	mesocosm	Gaussian	Chi ²
Phytoplankton (count)	1	-	-	Gaussian	F
Zooplankton (count)	2	date	mesocosm	quasi-Poisson	F
EcoPlates Biolog	2	substrate+date	-	Gaussian	F

July and were removed in the middle of November, after a total of four months. The supernatant water in the tube was removed and the sediments were collected in 200 mL plastic jars and dried at 60 °C for several days.

Functional diversity of heterotrophic prokaryotes

The catabolic capacity of each aquatic microbial community was determined using Biolog Ecoplate™ 96-well microplates as performed by Pommier et al. (2014). Two different heterotrophic prokaryote communities were sampled: the pelagic community was sampled as was phytoplankton (see Phytoplankton section) at the end of September and at the end of October (with a total of two measures for each enclosure). The benthic community was sampled at the end of November in the supernatant of the sediment collection tubes (with two replicates per enclosure). The microplates include, in triplicate, 31 different carbon-based substrates and one water control. Each well, which contains growth media and a tetrazolium violet dye becoming purple when the substrate is oxidised, was inoculated with 150 µL of sample. The plates were incubated in the dark at lake temperature for seven days. Colour development (OD at 590 nm) was measured using a Bio-Rad Laboratories, Model 680 Microplate Reader every 24 h just after inoculation (d_0) and at $d_0 + 1$, $d_0 + 2$, $d_0 + 3$, $d_0 + 4$ and $d_0 + 7$. We calculated the average colour development for each group of substrates (Supporting Information) after subtraction of the appropriate water blanks.

The catabolic capacity of each aquatic microbial community was determined using Biolog Ecoplate™ 96-well microplates as performed by Pommier et al. (2014). Two different bacterial communities were sampled: the pelagic community was sampled as was phytoplankton (see Phytoplankton section) at the end of September and at the end of October (with a total of two measures for each enclosure), and the benthic community was sampled at the end of November in the supernatant of the tubes collecting the sediments (with two replicates per enclosure). The microplates include 31 different carbon-based substrates and one water control in triplicates. Each well, which contained growth media and a tetrazolium violet dye becoming purple when the substrate is oxidised, was inoculated with 150 µL of sample. The plates were incubated in the dark at lake temperature for seven days. Colour development (OD at 590 nm) was measured using a Bio-Rad Laboratories, model 680 Microplate Reader every 24 h just after inoculation (d_0) and at $d_0 + 1$, $d_0 + 2$, $d_0 + 3$, $d_0 + 4$ and $d_0 + 7$. We calculated the average colour development for each group of substrates after subtraction of the appropriate water blanks.

Statistical analysis

Statistical analyses were performed with *R* version 4.3.1 (R Core Team, 2023) and are summarised in Table 1. We log-transformed response variables when necessary to guarantee the normality of residuals. Our three treatments were considered as qualitative variables, with two modalities each, whose interactions were considered in the model. Additional qualitative variables were considered to account for repeated measurements or depth (see *Additional effects* column in Table 1). We used linear mixed effect models with the function `lmer()` of the package *nlme* to set mesocosm and sampling date as random effects when the response variable was repeatedly measured in time (more than two repeated measures). Sampling date was converted into a continuous variable to model DOC because of its linear accumulation

in water over time. We used generalised linear models with random effects with the function `glmer()` of the package *lme4* for flow cytometry data with a Poisson distribution (count data) and offset equal to the logarithm of the analysed volume to account for variation in the analysed volume with this method across samples. Zooplankton counting data were modelled with the function `glmPQL()` of the *MASS* package to implement a quasi-Poisson distribution of residuals to account for the over-representation of zeros in data because of the absence of several taxa in some enclosures. The statistical significance of our three crossed treatments and their interactions, and corresponding p-values, were obtained with type II ANOVA implemented by the function `Anova()` of the package *car*, with Chi² tests for Poisson distributions and mixed models, and F tests for Gaussian and quasi-Poisson distributions. *Post hoc* Tukey's tests were performed by the function `emmeans()` of the package *emmeans* to compare the different modalities of treatment interactions, which were extracted with the `cld()` function of the package *lsmeans*. A Principal Components Analysis (PCA) of the functional diversity zooplankton was performed with the `PCA()` function of the package *FactoMineR*, then, the significance of our treatments on functional diversity was tested with a PERMANOVA implemented by the function `adonis2()` of the package *vegan*, permutations being restricted to data points with the same sampling date. The data and R code used to perform these statistical analyses and plot figures are available on GitHub at the following link: https://github.com/PierreQuevreur/stat_mesocosm_green_brown_CEREEP.

Results

Overall response of the food web

Chlorophyll *a* concentration (Fig. 2A) (and dry concentration of seston, see Fig. S1-4A in the supporting information) responded positively to fish presence and light exposure. We also noticed a significant effect of the interaction between fish and light treatment (Table 2: shading significantly reduced chlorophyll *a* and seston concentration only when fish were absent. The total dry mass concentration of zooplankton (Fig. 2B) did not respond significantly to any treatment. Fish in enclosures fully exposed to daylight had a significantly higher growth rate ($+0.0011 \pm 0.0003 \text{ g g}^{-1} \text{ d}^{-1}$, (mean+SE)) than fishes in light depleted enclosures ($-0.0006 \pm 0.0002 \text{ g g}^{-1} \text{ d}^{-1}$, Fig. 2C). Heterotrophic protists (Fig. 2D) and heterotrophic prokaryotes (Fig. 2E) were significantly more abundant in enclosures with fish. Sediment mass (Fig. 2F) and DOC accumulation (Fig. 2G) were significantly higher in enclosures with fish (respectively $+0.508 \pm 0.122 \text{ mg}$ and $+0.0030 \pm 0.0002 \text{ mg L}^{-1} \text{ d}^{-1}$). DOC accumulation also responded positively to full daylight exposition ($+0.0020 \pm 0.0002 \text{ mg L}^{-1} \text{ d}^{-1}$) and to organic carbon addition ($+0.0011 \pm 0.0002 \text{ mg L}^{-1} \text{ d}^{-1}$), and displayed complex responses to interactions between treatments. On the one hand, daylight exposition and organic carbon addition had positive independent effects on DOC accumulation in the absence of fish. On the other hand, in the presence of fish, light exposure only had a positive effect when organic carbon was added.

Functional diversity and community composition

The different taxonomic classes of phytoplankton had contrasted responses to our treatments (Fig. 3A, Table 2). Chlorophyceae were not abundant compared to the two other taxa and only responded significantly to a fish×light interaction (Fig. 3B). Fish had a negative effect on Dinophyceae abundance (Fig. 3C), while Zygnematomyceae were affected by both fish and light treatments, and their interaction (Fig. 3D). Zygnematomyceae had a response similar to chlorophyll *a* concentration (2A) and were clearly the taxonomic group responsible for its variations. Although fish did not impact zooplankton biomass (2B), they strongly altered their community composition (Fig. 3E and Fig. S1-8 and Table S1-7 in the supporting information for more details on the PCA) with a positive effect on small cladocerans (large cladocerans such as *Daphnia* being absent) and copepods, and a negative effect on *Chaoborus*. Fish did not affect significantly rotifers (see Fig. S1-9, S1-11 and S1-12 and Table S1-8 for the detailed response of the different taxa). Finally, fish presence significantly decreased the degradation capacity of the benthic community for all substrate families present in the EcoPlates™ Biolog except polymers (Fig. 3F and statistics summarised in Table S1-9 in the supporting information), but not in the pelagic community. The light and the organic carbon addition treatments significantly affected a few substrate families but no general trend could be identified (detailed results per substrate family are given in Fig. S1-13 and S1-14 in the supporting information).

Physiological response of phytoplankton

The chlorophyll *a* concentration per seston mass unit did not respond significantly to any treatment (Fig. 4A, Table 2), which indicates that the production of chlorophyll *a* by phytoplankton did not respond

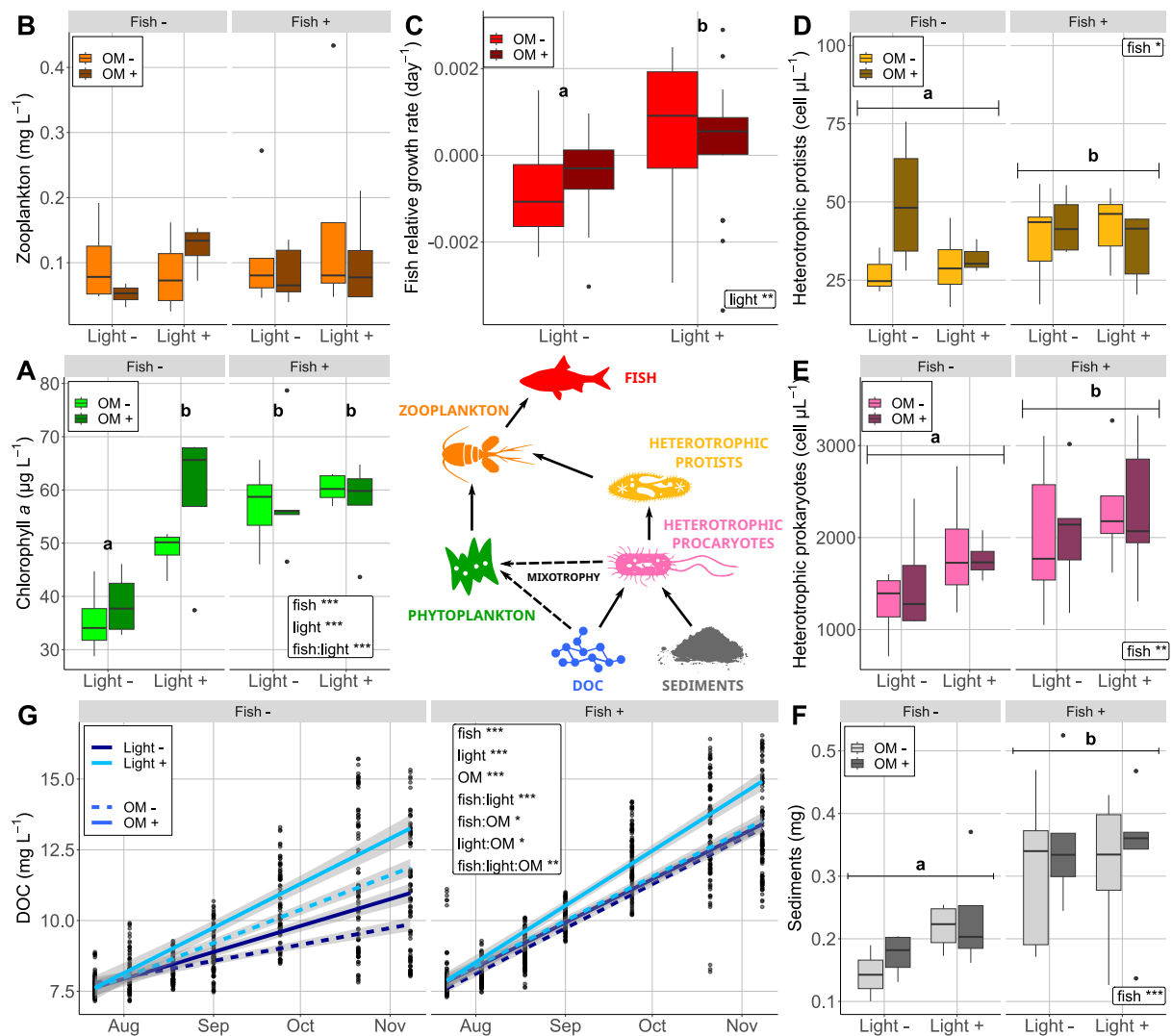


Figure 2: Response of the main compartments of the food web to the eight combinations of our three treatments. **A)** Chlorophyll *a* concentration ($\mu\text{g L}^{-1}$) measured by the BBE probe. **B)** Zooplankton dry biomass concentration (mg L^{-1}). **C)** Individual growth rate of fish ($\text{g g}^{-1} \text{d}^{-1}$) calculated over 133 days on average. **D)** Heterotrophic protist (heterotrophic microbial eukaryote) concentration measured by FACS ($\text{cell } \mu\text{L}^{-1}$). **E)** Heterotrophic prokaryotes (bacteria and archaea) concentration measured by FACS ($\text{cell } \mu\text{L}^{-1}$). **F)** Sediment dry mass collected at the end of the experiment (mg). **G)** Dissolved organic carbon (DOC) concentration over time (mg L^{-1}). Statistically significant treatments and interactions are notified on each graph and letters represent treatment combinations leading to significantly different responses of measured variables according to *post hoc* Tukey's tests.

to our treatments. Mixotrophic phytoplankton abundance, which was determined by identification at the species level, was negatively affected by fish (Fig. 4B) and had a response similar to Dinophyceae (Fig. 3D). Dinophyceae species present in our mesocosm are known to be mixotrophic phytoplankton contrary to Zygnematophyceae (according to the current knowledge) and, given their high abundance, were clearly the main mixotrophic taxa. The C:N ratio of seston was higher in mesocosms receiving less daylight (Fig. 4C), while the C:P ratio was significantly higher in enclosures with fish ($+187 \pm 48$) than in enclosures without fish (Fig. 4D).

Discussion

The results of our experiment can be summarised in a few major points (Fig. 5). First, we observed consistent top-down effects of fish presence on components of the green food web (phytoplankton abundance and composition), the brown food web (planktonic heterotrophic prokaryotes and protists abundance and catabolic activity of their benthic counterparts), zooplankton composition and on water physico-chemistry variables (DOC). This top-down effect of fish also interacts with the bottom-up effect of light

Table 2: Significance of treatments (p-values). Each sub-part of the table corresponds to a figure panel. We only provide the significance of the treatments on the slope of the linear regression model between DOC concentration and time, time being considered as a quantitative variable here. Similarly, we provide the significance of the intercept of the regression between C:N and C:P ratios, and chlorophyll *a* concentration.

Variable		Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM
Chlorophyll	p-value	<.001	<.001	0.581	0.001	0.128	0.972	0.775
	χ^2	32.98	15.83	0.305	11.01	2.322	0.001	0.082
	Df	1	1	1	1	1	1	1
Zooplankton	p-value	0.424	0.287	0.554	0.733	0.529	0.295	0.174
	F	0.658	1.179	0.359	0.118	0.406	1.137	1.942
	Df	1	1	1	1	1	1	1
Fish growth	p-value	-	<.001	0.85	-	-	0.239	-
	F	-	14.6	0.036	-	-	1.408	-
	Df	-	1	1	-	-	1	-
Heterotrophic protists	p-value	0.024	0.328	0.556	0.84	0.213	0.044	0.354
	χ^2	5.118	0.957	0.346	0.041	1.553	4.052	0.86
	Df	1	1	1	1	1	1	1
Heterotrophic procaryotes	p-value	0.006	0.064	0.949	0.322	0.876	0.415	0.959
	χ^2	7.633	3.438	0.004	0.979	0.024	0.665	0.003
	Df	1	1	1	1	1	1	1
Sediments	p-value	<.001	0.303	0.323	0.125	0.975	0.589	0.884
	F	16.89	1.101	1.012	2.496	0.001	0.299	0.022
	Df	1	1	1	1	1	1	1
DOC	p-value	<.001	<.001	<.001	<.001	0.015	0.047	0.002
	χ^2	195.69	69.1	27.97	36.63	5.883	3.959	9.32
	Df	1	1	1	1	1	1	1
Chlorophyceae	p-value	0.9	0.262	0.165	0.023	0.923	0.728	0.176
	F	0.016	1.311	2.029	5.749	0.009	0.123	1.923
	Df	1	1	1	1	1	1	1
Zygnematophyceae	p-value	0.022	0.022	0.924	0.012	0.451	0.794	0.975
	F	5.894	5.897	0.009	7.287	0.584	0.069	0.001
	Df	1	1	1	1	1	1	1
Dinophyceae	p-value	<.001	0.059	0.756	0.456	0.192	0.888	0.327
	F	19.71	3.881	0.099	0.572	1.79	0.02	0.996
	Df	1	1	1	1	1	1	1
Diversity zoo	p-value	0.001	0.502	0.792	0.934	0.671	0.906	0.817
	F	4.938	0.792	0.541	0.386	0.629	0.412	0.518
	Df	1	1	1	1	1	1	1
Chloro:seston	p-value	0.826	0.296	0.304	0.912	0.841	0.73	0.813
	F	0.049	1.136	1.1	0.012	0.041	0.122	0.057
	Df	1	1	1	1	1	1	1
Mixotrophs	p-value	0.025	0.122	0.552	0.282	0.814	0.798	0.901
	F	5.61	2.539	0.362	1.204	0.056	0.067	0.016
	Df	1	1	1	1	1	1	1
C:N-chloro	p-value	0.713	0.041	0.78	0.81	0.99	0.502	0.692
	F	0.139	4.747	0.08	0.059	0	0.467	0.162
	Df	1	1	1	1	1	1	1
C:P-chloro	p-value	0.029	0.555	0.934	0.906	0.924	0.437	0.346
	F	5.522	0.361	0.007	0.014	0.009	0.628	0.931
	Df	1	1	1	1	1	1	1

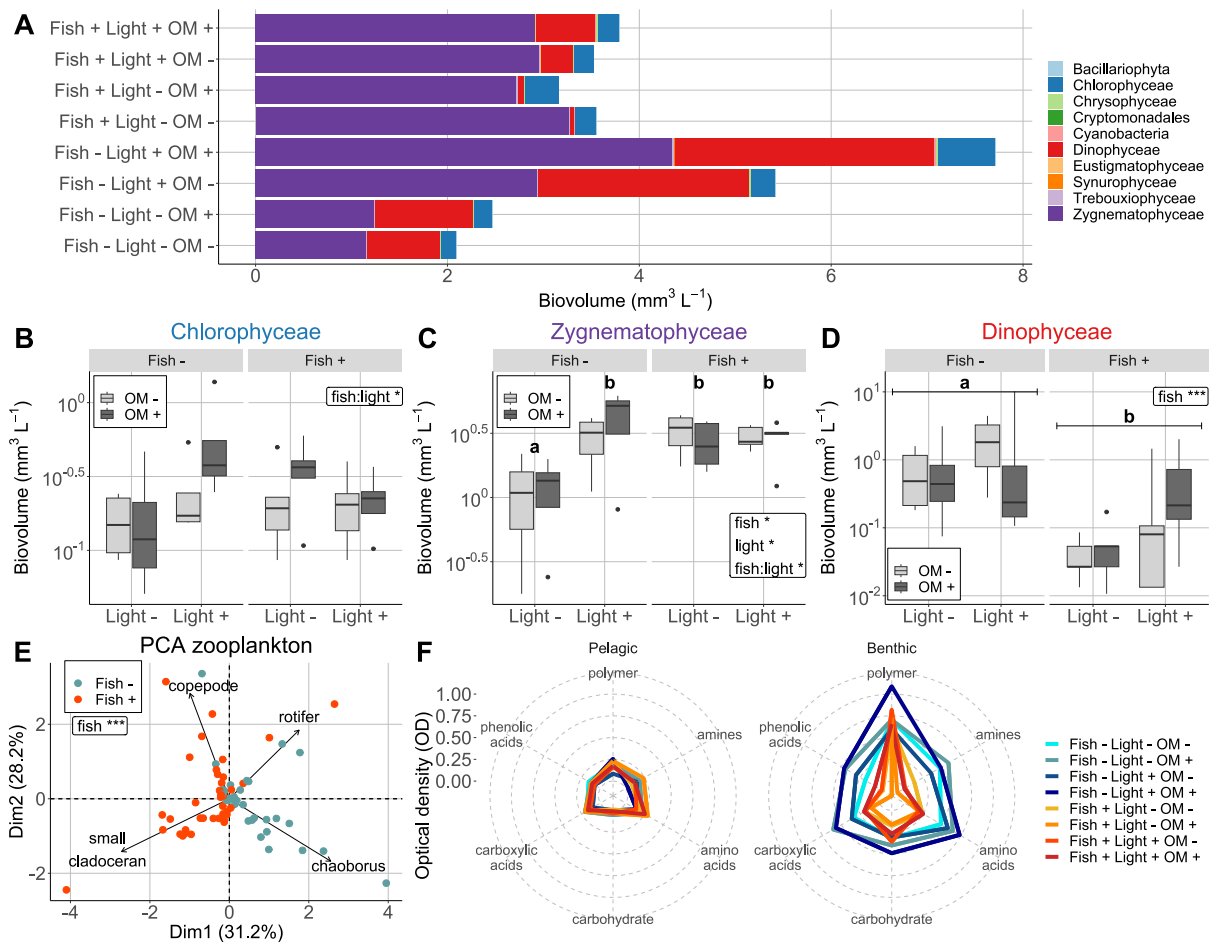


Figure 3: Response of the composition of phytoplankton (including cyanobacteria), zooplankton and functional diversity of heterotrophic prokaryotes. **A**) Biomass distribution (assessed by the biovolume) among the main phytoplankton taxonomic classes. Biovolume concentration of the three dominant taxa: **B**) Chlorophyceae, **C**) Zygnematophyceae and **D**) Dinophyceae. Statistically significant treatments and interactions are notified on each graph and letters represent treatment combinations leading to significantly different responses of measured variables according to *post hoc* Tukey's tests. **E**) Biplot of the principal components analysis (PCA) showing the response of the main zooplankton groups to treatments. Additional details on the PCA are given in the supporting information. The statistical analysis (PERMANOVA, Table 2) has been performed at the lowest taxonomic levels (species and genus levels) but we did the PCA at higher levels for the sake of readability. Significant effects of treatments and interactions on species composition of phytoplankton and zooplankton are notified in each panel. **F**) Metabolic profile of the heterotrophic prokaryote community in the pelagic and benthic compartments (measured by EcoPlates™ Biolog). The position on each of the six axes indicates the average absorbance (or optical density OD) for that group of substrates after 7 days of incubation.

to change phytoplankton abundance and composition, suggesting a reduction of nutrient limitation of phytoplankton by fish. Second, our results suggest only weak cascading effects between the green and the brown food webs, since light filtration did not clearly affect measured components of the brown food web (abundance and catabolic activity of microbial communities) and the addition of organic carbon had no clear effect on the main components of the green food web (no effect on fish growth, zooplankton and phytoplankton).

Top-down effects of fish on green food web: importance of direct nutrient cycling through fish excretion?

Fish occurrence induced a positive effect on phytoplankton abundance, but only under the low-light treatment (Light-). This fish \times light effect was reflected by estimates of total chlorophyll *a* (BBE probe in Fig. 2A and multi-parameter probe in Fig. S1-3B and C in the supporting information), seston biomass (Fig. S1-4A in the supporting information), and turbidity (S1-2D in the supporting information). A positive effect of fish on phytoplankton has been very frequently observed and has been interpreted as

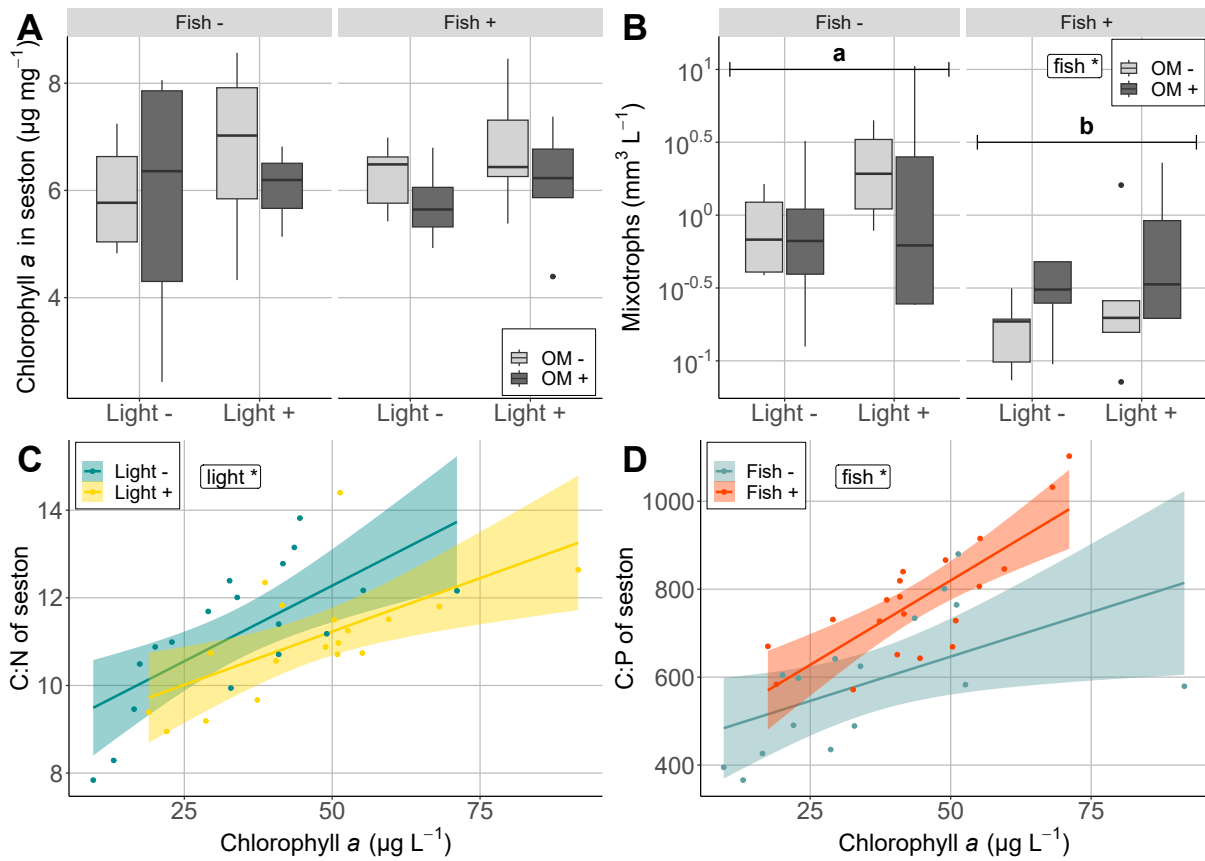


Figure 4: Physiological response of phytoplankton. **A)** Ratio chlorophyll *a* concentration to seston concentration ($\mu\text{g mg}^{-1}$). **B)** Concentration of mixotrophic algae (from the taxa identification) ($\mu\text{m}^3 \text{mL}^{-1}$). **C)** C:N ratio of seston and **D)** C:P ratio of seston depending on chlorophyll *a* concentration. Significant effects of treatments and interactions are notified in each panel.

a classical cascading effect of top-predators on primary producers, mediated by a reduction of grazing pressure by zooplankton (see the meta-analysis of Hulot et al. (2014)). This reduction of phytoplankton grazing has not always been associated to a decrease in zooplankton biomass, Cyprinidae or Leuciscidae fish tending to shift zooplankton composition by suppressing large Cladocerans, which are the most efficient grazers (Bertolo et al., 1999a; Danger et al., 2008). In our study, zooplankton biomass was not affected by fish presence (Fig. 2B), nor was the relative abundance of large zooplanktonic organisms. Instead, zooplankton biomass remained 3-5 times lower in all treatments (close to $100 \mu\text{g}_{\text{DW}} \text{L}^{-1}$) compared to similar mesocosm experiments in temperate systems (Bertolo et al., 1999b; Danger et al., 2012). Furthermore, large zooplankton organisms, such as Daphnidae and Calanoida, were absent in our mesocosms, which were dominated by small herbivorous and detritivorous species (Chydoridae and small rotifers such as Lecane) and omnivorous Cyclopidae as in Garnier et al. (2023). Clearly, the very low zooplankton biomass, the absence of efficient grazers, and the very high level of chlorophyll *a* in the enclosures strongly suggest that primary producers were not strongly top-down controlled by herbivores in our experiment. This has also been reported in some previous studies (Leibold, 1989; Pace et al., 1998; Faithfull et al., 2011; Garnier et al., 2023).

An alternative, and more probable, explanation for the observed positive effect of fish on phytoplankton is associated with nutrient excretion by fish. Considering the rapid and important growth of primary producers in the first weeks of the experiment (Fig. S1-3A in the supporting information), phytoplankton probably became rapidly nutrient-limited (Fig. S1-1 in the supporting information). This nutrient demand was likely partly satisfied by consumer-driven recycling, and in particular by fish excretion as zooplankton represented less than 2% of fish biomass in our experiment. Indeed, consumer-driven recycling has been proposed to play a key role in determining nutrient availability for phytoplankton (Vanni and Layne, 1997; Vanni et al., 1997; Vanni, 2002). This explanation is also supported by the higher abundance of mixotrophic phytoplankton in the absence of fish (Fig. 3D and Fig. 4B), which was probably able to overcome nutrient limitation by consuming heterotrophic prokaryotes or organic matter.

The significant interaction between the fish and light treatments is also in agreement with effects asso-

ciated with nutrient excretion by fish (Fig. 5). Phytoplankton is known to have highly plastic C:nutrient ratios depending on nutrient availability and daylight exposure (Sterner et al., 1997, 2002; Dickman et al., 2006; Hall et al., 2007; Mette et al., 2011). Indeed, when light exposure is reduced, phytoplankton cells compensate the lack of energy by an increased demand of nitrogen or phosphorus to produce more proteins, RNA (Rhee and Gotham, 1981; Healey, 1985) or chlorophyll *a* (Felip, 2000), which are involved in photosynthetic structures and could explain the positive effect of nutrient excretion by fish on chlorophyll *a* in our experiment. However, our results did not support this compensation hypothesis since the chlorophyll:seston ratio did not respond to our treatments (Fig. 4A) and the C:N ratio of the seston was even higher when light was depleted (Fig. 4C). In addition, the C:P ratio of the seston was significantly higher in enclosures with fish (Fig. 4D), which contradicts the hypothesis of a higher nutrient content in phytoplankton and is probably due to the immobilisation of P in fish biomass (Rock et al., 2016). Instead, we observed a similar increase in the C:N and C:P ratios when phytoplankton biomass increased, while a limitation by N or P would have led to a constant C:N or C:P as in (Danger et al., 2007b). This change in phytoplankton stoichiometry can neither be explained by a shift in the species composition since one species of Zygnematophyceae, *Cosmarium laeve*, drove the chlorophyll *a* response to treatments. Therefore, we conclude that fish had a positive bottom-up effect on phytoplankton, which was probably mediated by the limitation by nutrients other than N and P.

Top-down effects of fish on brown food web: importance of the quality of settling organic matter?

Fish had a positive bottom-up effect on the brown food web represented by an increase in the density of heterotrophic protists and microbial eukaryotes (Fig. 2D and E), through the higher accumulation of DOC in the pelagic compartment (Fig. 2G) and sediments in the benthic compartment (Fig. 2F). This observation is related to previous experiments in which the presence of Leuciscidae increased the production of labile organic matter, in particular exopolysaccharides such as transparent exopolymer particles (TEP) that aggregated with particulate matter and were transported downward (Danger et al., 2012; Harrault et al., 2012). However, this increased excretion would be due to fish directly because of the absence of difference in phytoplankton abundance when light was not depleted. This was paralleled with a significant fish effect on the catabolic activity of the free microbial communities collected in the sediment traps (Fig. 3F). The utilisation patterns of the 31 substrates of the EcoPlates™ Biolog suggest that the relative oxidation of amines, amino acids, carbohydrates, phenolic acids and carboxylic acids was lower in the sediment of fish enclosures. The relative metabolic potential of the microbial community on a particular category of substrate has been interpreted as dependent upon its availability (Leflaive et al., 2008) or upon its relative concentration within the bulk of dissolved organic matter Pommier et al. (2014). Our results on the benthic microbial communities are in total agreement with those of Pommier et al. (2014), who observed that the addition of highly bioavailable organic matter to aquatic microbial communities favoured more specialised communities and reduced their metabolic potential. Interestingly, this specialisation trend was not observed within pelagic microbial communities. This might be due to the fact that a large part of fresh organic matter settled, probably associated to TEP, at the bottom of enclosures as shown by the positive effect of fish on sediment accumulation. All things being equal, pelagic microbial communities should sustain on less abundant and more diverse resources, and thus remain more generalist than benthic microbial communities.

Effects of light and organic matter addition: weak cascades between the green and the brown food web?

Light had clear bottom-up effects on the green food web, in particular on total chlorophyll *a* and green algae, with clear bottom-up repercussions on fish growth rate in accordance with Rock et al. (2016) who had a similar experimental set-up with light reduction performed by shade mesh (Table 3). Our results also showed that an increase in light exposure resulted in an increase in DOC concentration, which is produced during active photosynthesis by phytoplankton. First, this increase in DOC concentration might be simply an effect of phytoplankton biomass (more primary producers implies more exudation). Indeed, the significant interaction between light filtering and fish presence on DOC concentration could be related to weaker light effects on phytoplankton concentration in enclosures with fish. Although primary production is considered as an important autochthonous source of DOC for heterotrophic microbial communities in aquatic ecosystems (*e.g.* Baines and Pace, 1991 and Morana et al., 2014), light filtering did not significantly affect the abundance of heterotrophic microbial communities. It only marginally affected microbial metabolic activities despite its negative effect on DOC concentration. Similarly, the addition of organic matter appeared to have only limited effects on heterotrophic prokaryote community functioning. The relative metabolic potential of the microbial community was only marginally affected

by DOC addition, in contrast with the results of Pommier et al. (2014).

Concerning the cascading effects from brown to green food webs, DOC addition neither affected the green algae concentration nor the algal taxa composition (Fig. 3A-D). This absence of effect is in contradiction with previous experiments showing reduced phytoplankton biomass in response to addition of labile DOC because of the immobilisation of nutrients by heterotrophic prokaryotes (Joint et al., 2002; Degerman et al., 2018). Faithfull et al. (2011) did not find such effects because the phytoplankton community was dominated by mixotrophic taxa in their experiment, which was not the case in our experiment. Rather, the contrast between our results and those of Joint et al. (2002) and Degerman et al. (2018) might be partly explained by the strong differences in experiment duration and levels of organic carbon added in the two studies. We added $5.4 \text{ mg}_C \text{ L}^{-1}$ over a period of 15 weeks while Joint et al. (2002) added between 4.5 and $9 \text{ mg}_C \text{ L}^{-1}$ over a period of 6 days only (Table 3). Although our organic carbon addition was lower than in previous experiments, it was representative of carbon transfers from terrestrial ecosystems to lakes reported by Gounand et al. (2018) (between 1.2 and 465 (median 6.4) $\text{g C m}^{-2} \text{ year}^{-1}$, $46.8 \text{ g C m}^{-2} \text{ year}^{-1}$ in our experiment). In addition, our organic matter addition relied on a mixture of three molecules but the low degradation capacity of two of them (Fig. S1-14A and B in the supporting information) might have made this treatment less efficient than pure glucose addition as done in previous studies, although it is more realistic than a massive input of glucose. However, this must be considered carefully because phytoplankton is osmo-mixotrophic for glucose (G erard Lacroix’s personal observations) and glucose carbon enrichment may not target heterotrophic prokaryotes and the brown food web. Joint et al. (2002) and Degerman et al. (2018) also found an increase in prokaryote production following DOC addition.

Table 3: Summary of experimental treatments in previous studies testing the effects of light reduction, fish presence, organic carbon addition and nutrient addition in aquatic mesocosms.

Study	Light	Fish	OM ($\text{mg}_C \text{ L}^{-1}$)	Nutrient	Days
Our study	10-50%	rudds	5.4 (mixture)	-	105
Joint et al., 2002	-	-	4.5-9	NP	6
Faithfull et al., 2011	-	perch	4.2-21 (glucose)	-	45
Mette et al., 2011	4-40%	gizzard shad	-	NP	56
Hitchcock et al., 2016	-	-	1.5-16 (leachate)	-	22
Rock et al., 2016	35-90%	bluegill sunfish	-	NP	42
Degerman et al., 2018	-	stickle-back	120.5 (glucose)	NP	63
Garnier et al., 2023	~50%	young perch	-	NP	21

Conclusion and perspectives

Our results showed some bottom-up effects of light and organic matter addition on green and brown food webs, but the cascading effects from bottom-up effects in one web to the other seemed to be weak. Fish presence had a strong effect on both green and brown food webs: it changed phytoplankton abundance in the green web, increased the abundance of heterotrophic prokaryotes, altered their metabolic activity and increased the abundance of their protist predators in the brown web, and changed zooplankton community composition. Our results suggest that fish effects were mostly mediated by their impact on detritus decomposition and nutrient recycling in this experiment, which would reduce the sequestration of nutrients in poorly degradable sediments and increase their availability for phytoplankton. This contrasts with other studies in which phytoplankton was strongly controlled by large Cladocerans in absence of fish. This led to our most important observation, which concerns the interaction between fish and light treatments where light depletion only reduced phytoplankton abundance in the absence of fish. To our knowledge, such an interaction has not been reported in previous experiments (Mette et al., 2011; Rock et al., 2016; Garnier et al., 2023). Thus, our results highlight the importance of the interactions between bottom-up and top-down effects for ecosystem functioning mediated by the physiological response of organisms. We advocate for the development of mathematical models in line with those of Zou et al. (2016) and Qu evreux et al. (2021) including the functional traits of organisms to establish detailed theoretical predictions of the functioning of aquatic ecosystem (Dutkiewicz et al., 2021; Wickman et al., 2024). In addition, future studies should pay attention to two major features of aquatic ecosystems highlighted by our results: First, the bottom-up effect of fish should be better analysed by identifying in detail their effect on nutrient cycling and phytoplankton stoichiometry. Second, the composition of the

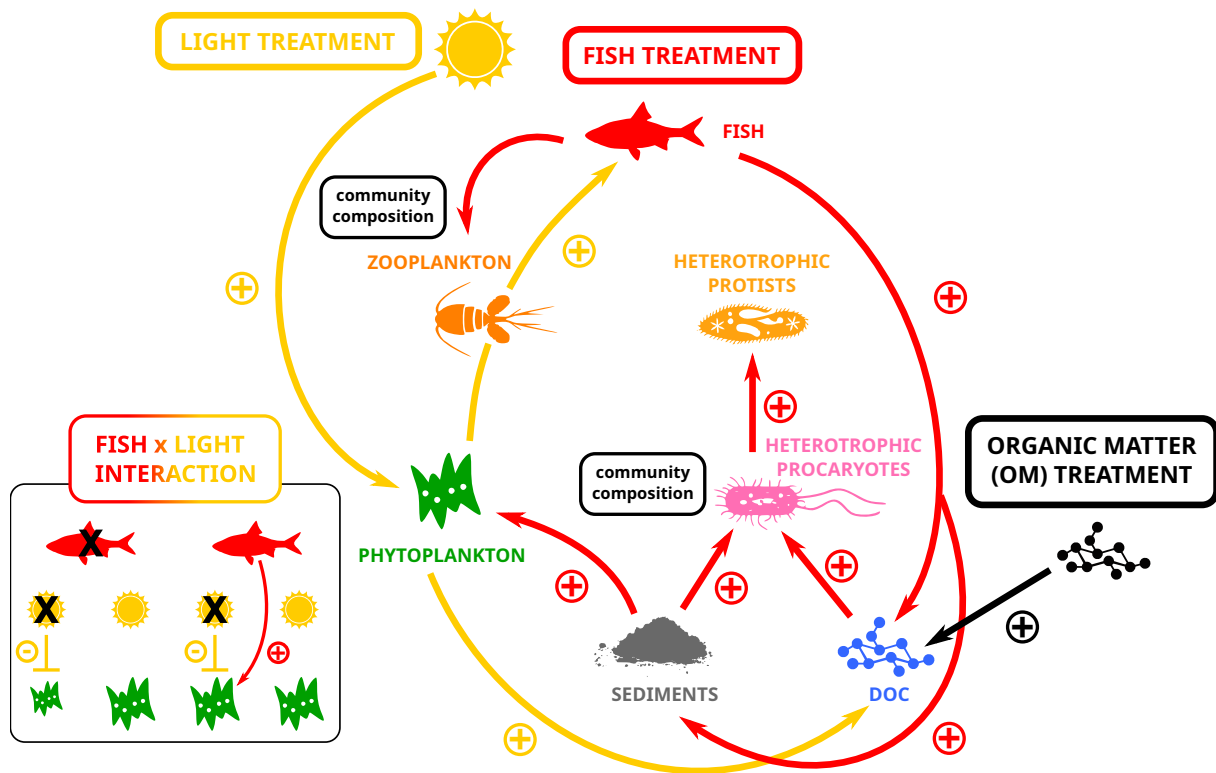


Figure 5: Summary of the effects of the three treatments on the different compartments of the ecosystem present in our mesocosms. The light treatment represents the effect of full daylight exposure (Light+ in the previous figures). The bottom-left box represents the fish×light interaction in which the positive bottom-up effect of fish compensates for the negative effect of light depletion on phytoplankton, whose abundance is represented by the size of the silhouettes.

initial zooplankton community and the presence or absence of large Cladocerans, in particular, should be documented since it seems to condition the top-down effect of fish.

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Author contribution

Conceptualisation: PQ, KZ, SB, ÉT, GL. Developing methods: PQ, KZ, SB, ÉT, ERN, LJ, ÉE, GL. Conducting the research: PQ, KZ, ÉT, SA, JM, SF, GL. Data analysis: PQ, SB, ÉT, ÉE, GL. Data interpretation: PQ, KZ, SB, ÉT, ERN, LJ, ÉE, GL. Preparation of figures and tables: PQ. Writing: PQ, KZ, SB, ÉT, ERN, LJ, ÉE, SF, GL.

Data availability

The data and R code used to perform the statistical analyses and plot figures are available on GitHub at the following link: https://github.com/PierreQuevreur/stat_mesocosm_green_brown_CEREEP.

Ethical statement

All the procedures were conducted in accordance with appropriate European (Directive, 2010/63/EU) and French national guidelines, permits, and regulations regarding animal care and experimental use (Agreement n° B77-431-1).

Conflict of interest

We have no conflict of interest to disclose.

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S1 Supplementary results

S1-1 Water physico-chemistry

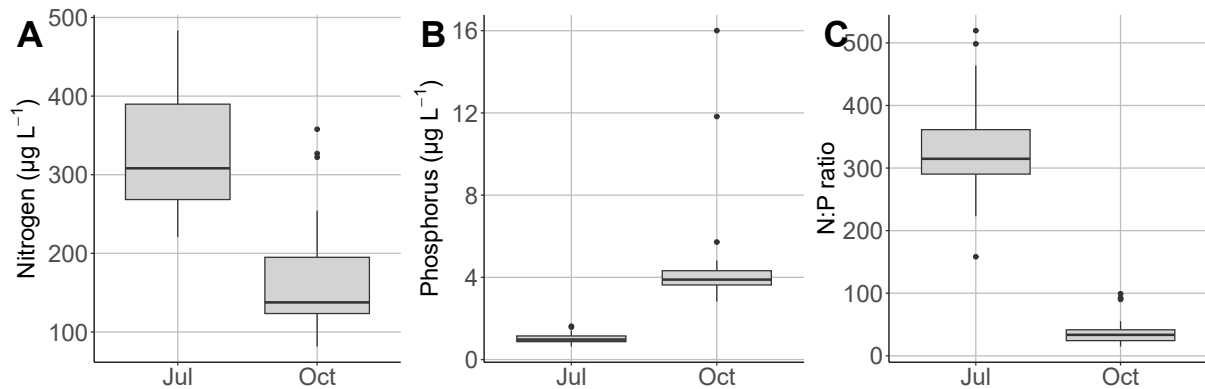


Figure S1-1: Mineral nutrient concentration. Total **A**) nitrogen and **B**) phosphorus concentrations $\mu\text{g L}^{-1}$. **C**) N:P ratio in mesocosms (mass ratio). The average N:P is equal to 330 at the beginning of the experiment and equal to 40 at the end of the experiment.

The water column was stratified for all parameters (but the difference between 50 cm and 150 cm is on average equal to 0.5°C for temperature, Fig. S1-2A). Oxygen concentration (Fig. S1-2B) and pH (Fig. S1-2C) were positively affected by fish but not by light, while turbidity responded to our treatments similarly to chlorophyll *a* (Fig. S1-2D).

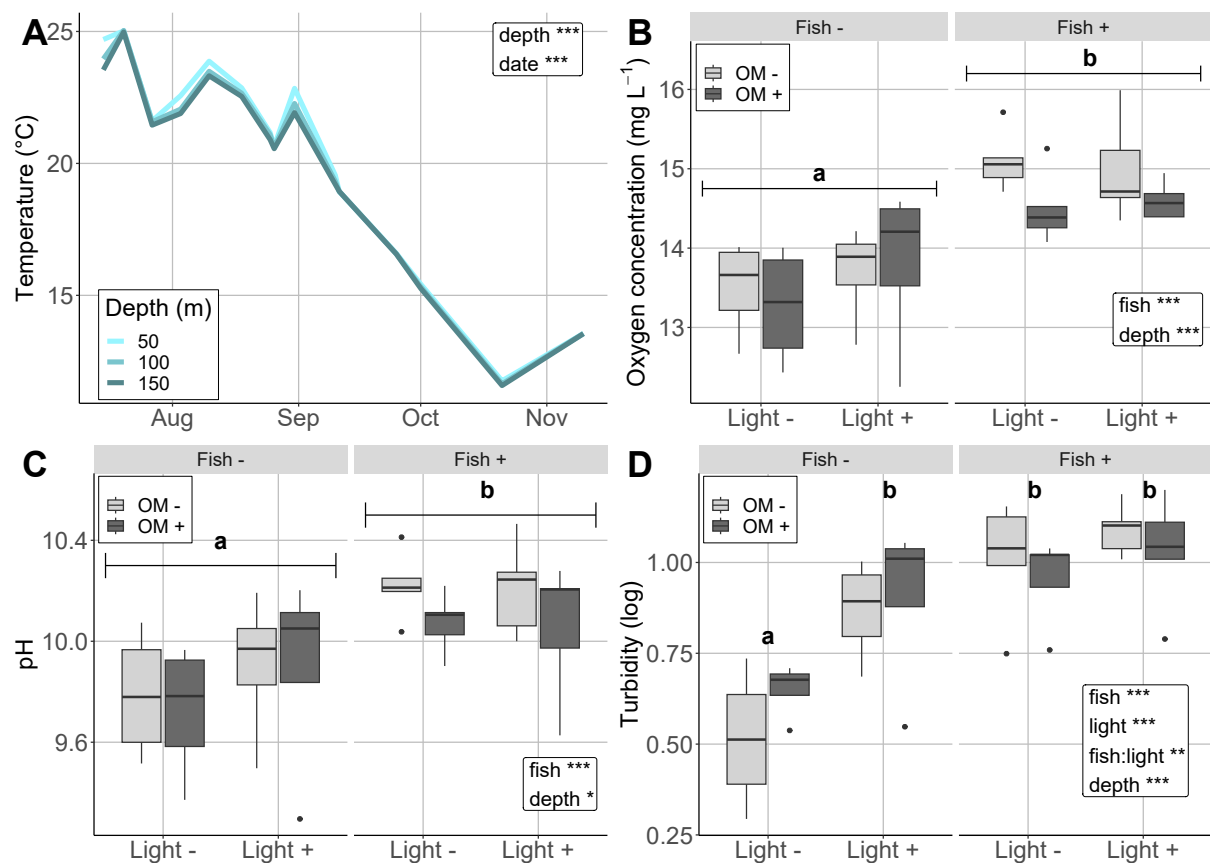


Figure S1-2: Physico-chemistry of the water measured the multiparameter probe and aggregated from July to November. **A**) Temperature ($^\circ\text{C}$) at three depths. **B**) Oxygen concentration (mg L^{-1}). **C**) pH. **D**) Turbidity (log scale).

Table S1-4: Significance (p-values) of treatments on water physico-chemistry measured the multiparameter probe.

Variable		Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM	Depth
Chlorophyll	p-value	<.001	<.001	0.759	0.012	0.392	0.701	0.834	<.001
	χ^2	30.83	21.26	0.094	6.382	0.733	0.147	0.044	421.65
	Df	1	1	1	1	1	1	1	2
Temperature	p-value	0.956	0.056	0.682	0.574	0.23	0.202	0.554	0.003
	χ^2	0.003	3.654	0.168	0.316	1.443	1.627	0.35	11.89
	Df	1	1	1	1	1	1	1	2
Oxygen	p-value	<.001	0.693	0.052	0.205	0.144	0.813	0.595	<.001
	χ^2	34.01	0.156	3.773	1.606	2.13	0.056	0.283	35.44
	Df	1	1	1	1	1	1	1	2
pH	p-value	<.001	0.445	0.174	0.277	0.435	0.896	0.85	0.035
	χ^2	17.5	0.584	1.847	1.18	0.61	0.017	0.036	6.71
	Df	1	1	1	1	1	1	1	2
Turbidity	p-value	<.001	<.001	0.91	0.008	0.149	0.941	0.859	<.001
	χ^2	43.6	15.19	0.013	6.966	2.086	0.006	0.031	45.27
	Df	1	1	1	1	1	1	1	2

S1-2 Phytoplankton

Table S1-5: Significance of treatments on the response of phytoplankton (p-values).

Variable		Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM
Seston	p-value	0.012	0.027	0.493	0.026	0.101	0.977	0.974
	F	7.218	5.491	0.483	5.523	2.88	0.001	0.001
	Df	1	1	1	1	1	1	1
C:N seston	p-value	0.15	0.796	0.461	0.094	0.451	0.525	0.794
	F	2.196	0.068	0.56	3.002	0.584	0.415	0.069
	Df	1	1	1	1	1	1	1
C:P seston	p-value	0.001	0.401	0.802	0.179	0.222	0.773	0.902
	F	14.41	0.728	0.064	1.899	1.559	0.085	0.016
	Df	1	1	1	1	1	1	1
Cyanobacteria (BBE)	p-value	<.001	0.143	0.922	0.278	0.671	0.219	0.645
	χ^2	21.13	2.15	0.01	1.177	0.181	1.514	0.212
	Df	1	1	1	1	1	1	1
Diatoms (BBE)	p-value	0.02	0.035	0.517	0.874	0.073	0.249	0.453
	χ^2	5.437	4.469	0.421	0.025	3.209	1.329	0.562
	Df	1	1	1	1	1	1	1
Pico-phytoplankton	p-value	0.441	0.907	0.386	0.695	0.64	0.558	0.824
	χ^2	0.594	0.014	0.751	0.154	0.219	0.343	0.049
	Df	1	1	1	1	1	1	1
Nano-phytoplankton	p-value	<.001	<.001	0.745	0.001	0.068	0.634	0.594
	χ^2	12.93	24.45	0.106	11.22	3.327	0.227	0.284
	Df	1	1	1	1	1	1	1

Table S1-6: Significance of treatments on the response of phytoplankton taxa (p-values).

Variable		Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM
Chlorophyceae	p-value	0.574	0.562	0.172	0.157	0.906	0.573	0.297
	F	0.324	0.344	1.961	2.112	0.014	0.326	1.128
	Df	1	1	1	1	1	1	1
Cyanobacteria	p-value	0.315	0.156	0.998	0.09	0.624	0.54	0.48
	F	1.047	2.123	0	3.092	0.246	0.384	0.513
	Df	1	1	1	1	1	1	1
Dinophyceae	p-value	<.001	0.065	0.769	0.439	0.186	0.896	0.348
	F	18.2	3.676	0.088	0.617	1.84	0.017	0.912
	Df	1	1	1	1	1	1	1
Trebouxiophyceae	p-value	0.08	0.272	0.696	0.031	0.092	0.886	0.233
	F	3.293	1.258	0.156	5.161	3.048	0.021	1.488
	Df	1	1	1	1	1	1	1
Zygnematophyceae	p-value	0.022	0.022	0.91	0.012	0.444	0.796	0.968
	F	5.856	5.895	0.013	7.275	0.603	0.068	0.002
	Df	1	1	1	1	1	1	1
Shannon index	p-value	0.026	0.239	0.936	0.258	0.406	0.928	0.978
	F	5.492	1.445	0.007	1.335	0.712	0.008	0.001
	Df	1	1	1	1	1	1	1

Chlorophyll *a* strongly varied during the experiment (Fig. S1-3A), its concentration being maximum at the beginning because of the initial nutrient enrichment (Fig. S1-1). Chlorophyll *a* concentration measured by the multiparameter probe (Fig. S1-3B) had the same response to our treatments as the chlorophyll *a* concentration measured by the BBE probe (see Fig. 2A in the main text) with positive effects of light and fish and a fish×light interaction (Table S1-4). It was also stratified with a larger concentration at greater depth (Fig. S1-3C). However, fish presence did not decrease chlorophyll *a* concentration stratification, thus fish did not affect phytoplankton through water mixing and resuspension.

Seston concentration displays a response to treatments similar to the response of chlorophyll *a* (Fig. S1-4A and Table S1-5), thus confirming the robustness of our measure of the phytoplankton compartment. Our treatments did not affect the C:N of the seston (Fig. S1-4B) if we do not account for the dilution effect of phytoplankton abundance (as done in Fig. 4C in the main text). Fish presence has a positive effect on the C:P ratio (Fig. S1-4C), interpreted as a P sink effect of fish (Rock et al., 2016).

Pico-phytoplankton abundance (Fig. S1-5A) did not respond to treatments but nano-phytoplankton was significantly affected by the interaction between fish and light treatments (Fig. S1-5B and Table S1-5): light had a positive effect only in enclosures without fish and fish had an effect only in enclosures where daylight was filtered, which is similar to the response of chlorophyll *a* (see Fig. 2A in the main text and Fig. S1-3B). Given the contrast between taxa cell abundance (Fig. S1-7A) and taxa biovolume (Fig. 3A in the main text), we can identify the pico-phytoplankton as Chlorophyceae (Fig. 3B) and the nano-phytoplankton as Zygnematophyceae (Fig. 3C) and Dinophyceae (Fig. 3D).

The different taxa forming the phytoplankton community inhabiting our mesocosms responded more or less to treatments (Fig. S1-7A and Table S1-6) and we present the response of the most abundant taxa in the following. Chlorophyceae (Fig. S1-7B), cyanobacteria (Fig. S1-7C) and trebouxiophyceae (Fig. S1-7E) did not respond to treatments. Dinophyceae (Fig. S1-7D) were more abundant in the absence of fish, while zygnematophyceae (Fig. S1-7F) responded positively to fish and light treatments, and their interaction, as for chlorophyll *a* (see Fig. 2A in the main text). The Shannon index was higher in the absence of fish (Fig. S1-7G), thus indicating a negative effect of fish on phytoplankton taxonomic diversity.

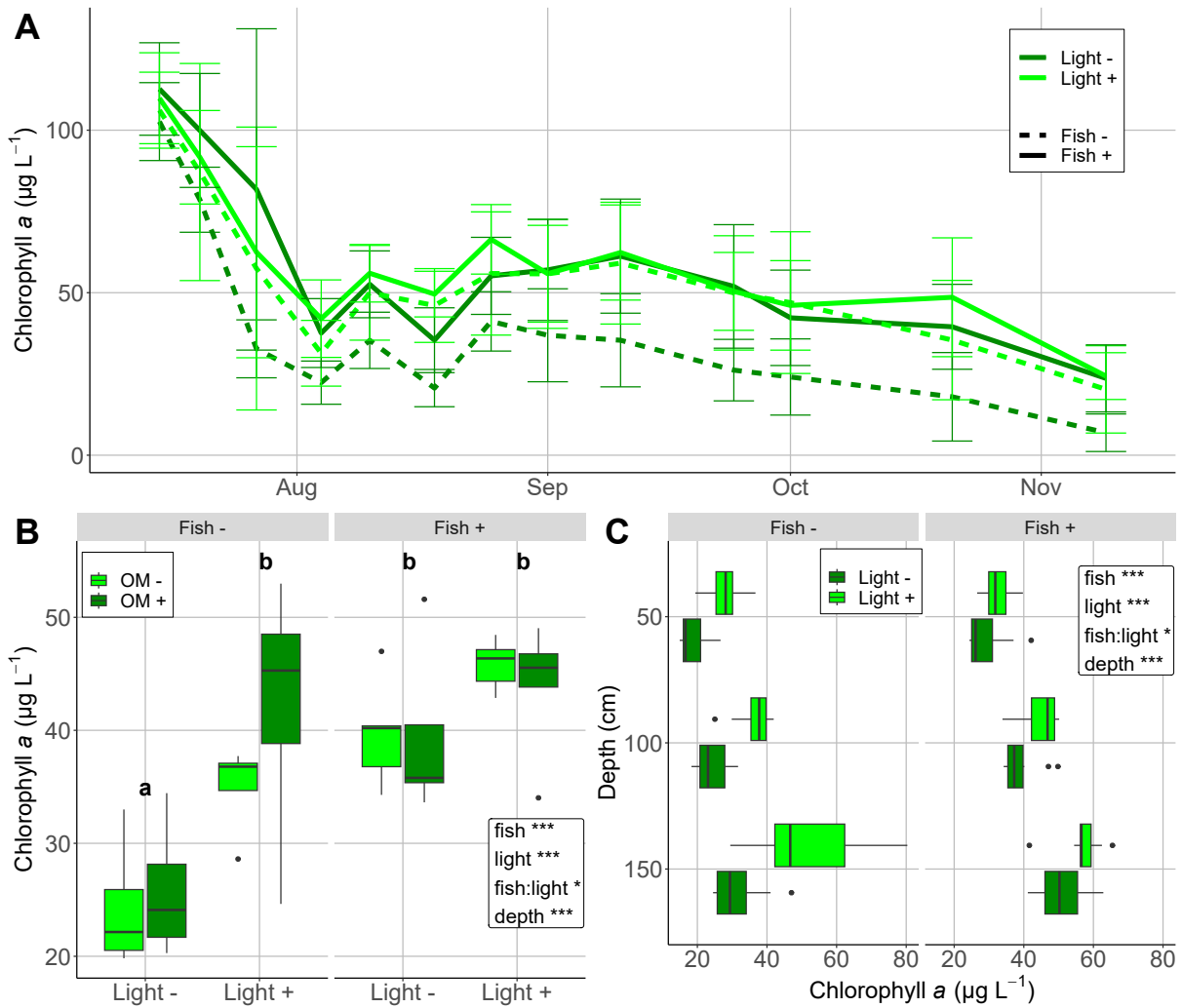


Figure S1-3: Detailed response of chlorophyll *a* concentration ($\mu\text{g L}^{-1}$). **A**) Temporal dynamics of chlorophyll *a* (BBE probe data). **B**) Average chlorophyll concentration (multiparameter probe data). **C**) Chlorophyll *a* concentration at three depths (multiparameter probe data).

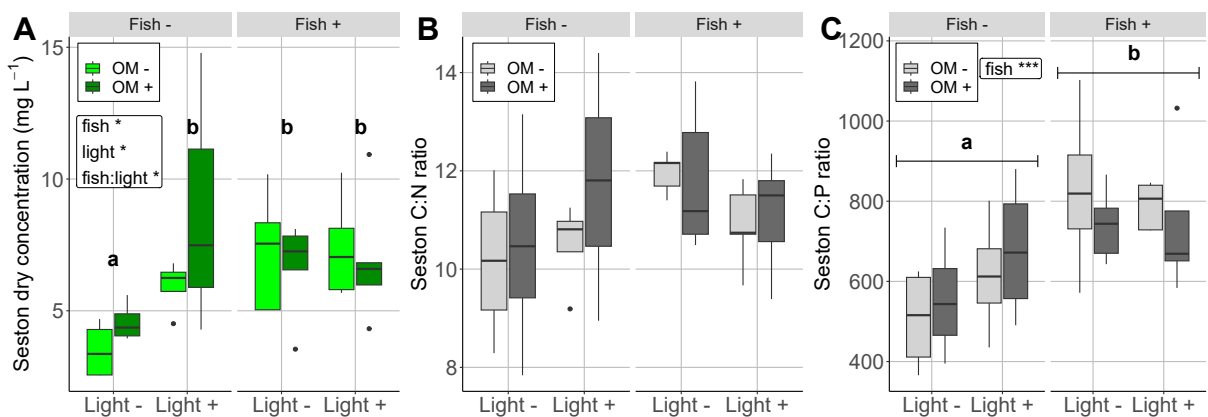


Figure S1-4: response of the seston. **A**) Seston dry mass concentration (mg L^{-1}). **B**) C:N ratio of the seston. **C**) C:P ratio of the seston.

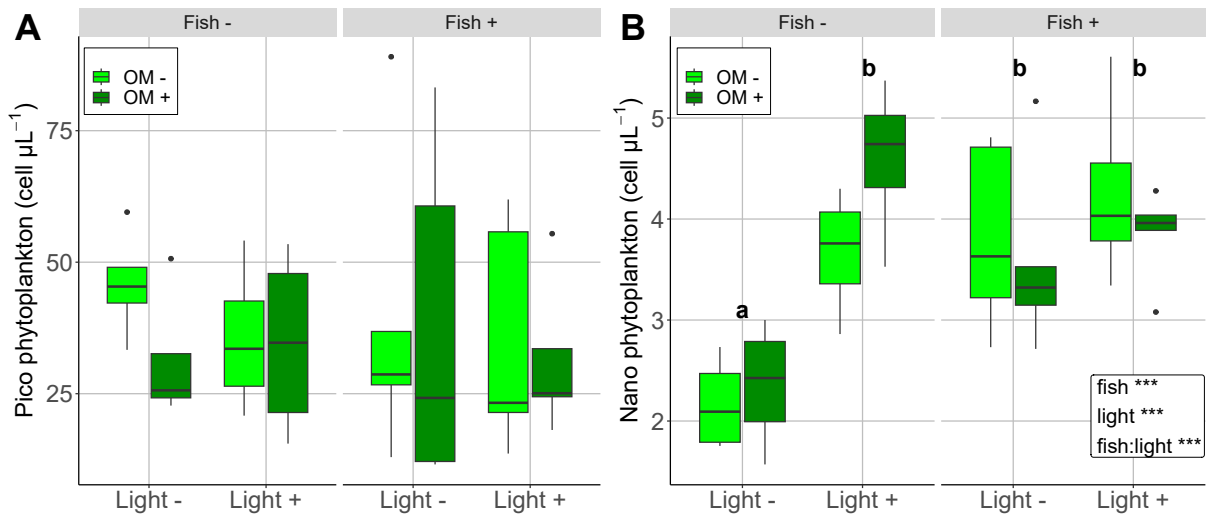


Figure S1-5: Response of phytoplankton concentration measured by FACS ($\text{cell } \mu\text{L}^{-1}$). **A**) Nano-phytoplankton. **B**) Pico-phytoplankton.

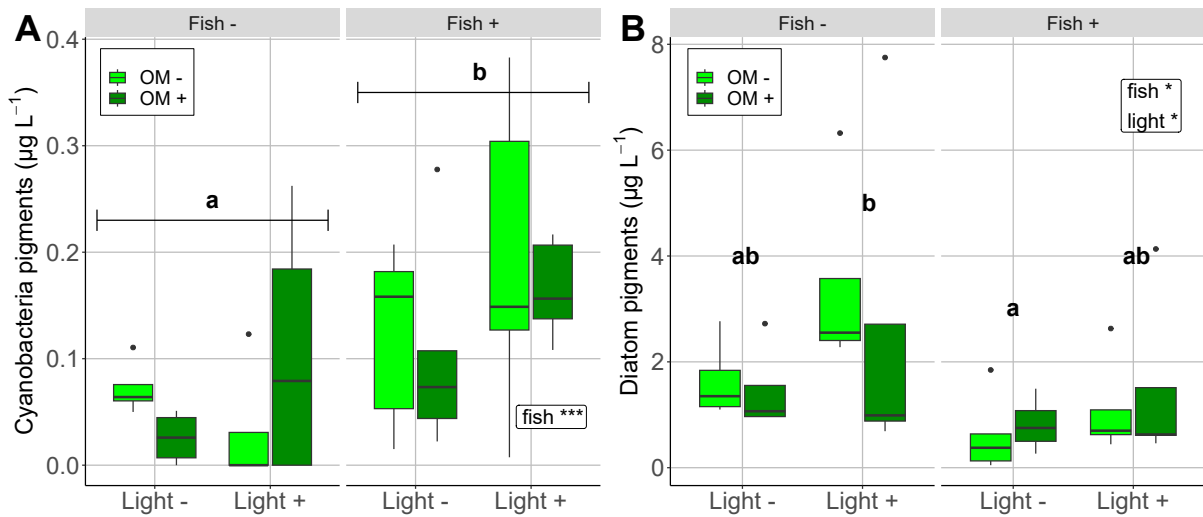


Figure S1-6: Response of **A**) cyanobacteria and **B**) diatoms measured by the concentration of their pigments ($\mu\text{g L}^{-1}$) by the BBE probe.

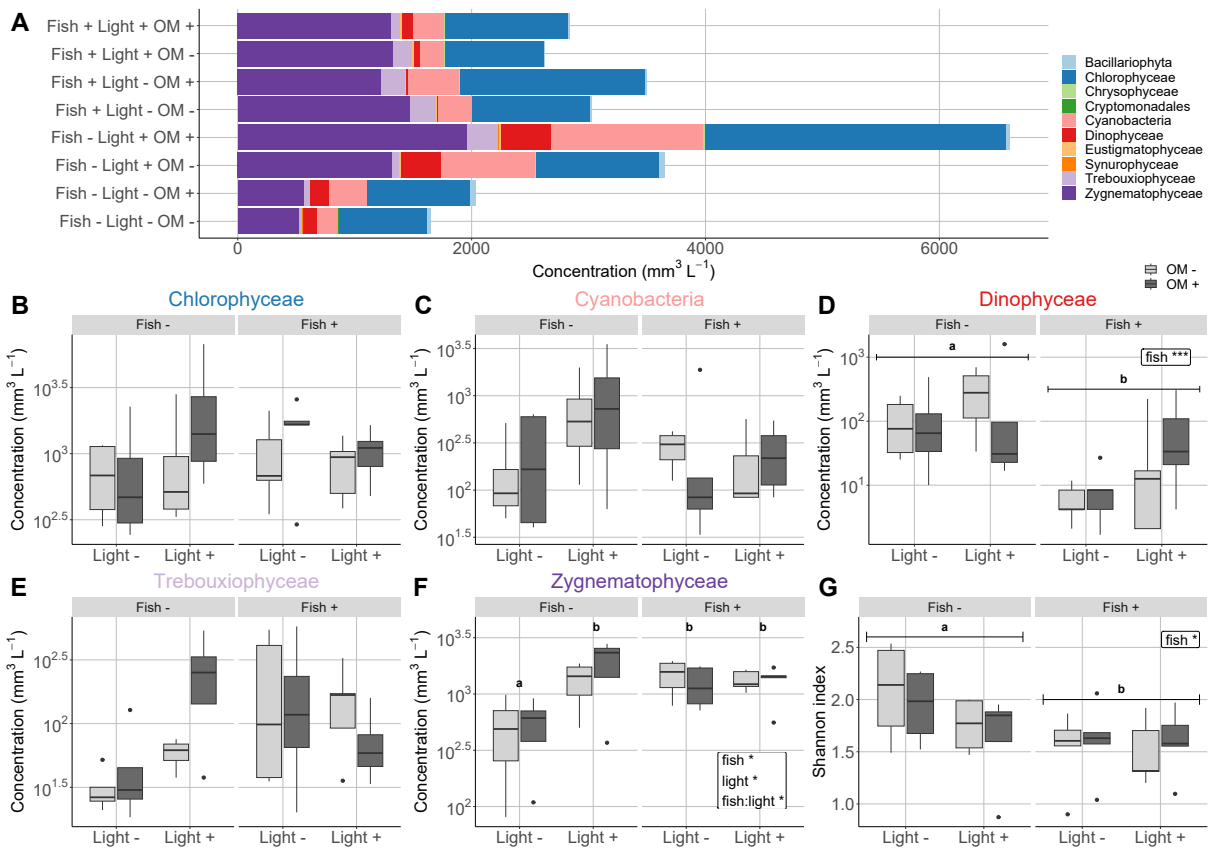


Figure S1-7: Response of the main phytoplankton taxa (cell mL^{-1}). **A**) Average abundance of the main taxa. **B**) Chlorophyceae. **C**) Cyanobacteria. **D**) Dinophyceae. **E**) Trebouxiophyceae. **F**) Zygnetatophyceae. **G**) Shannon index.

S1-3 Zooplankton

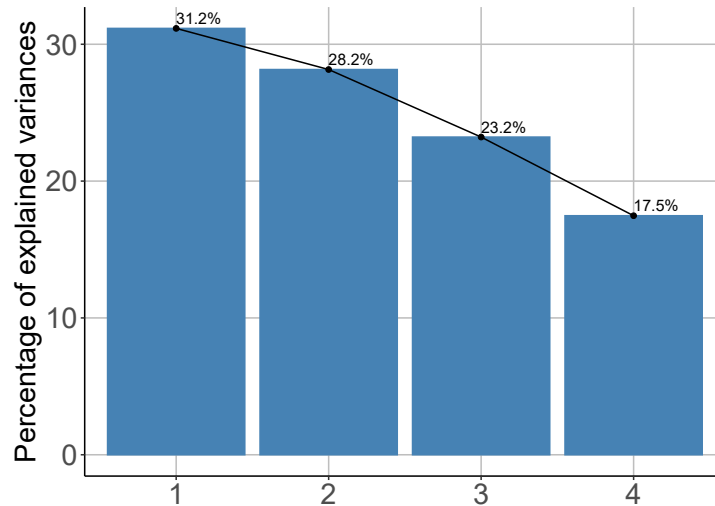


Figure S1-8: Percentage of variance explained by each eigenvector of the PCA of the zooplankton composition.

Table S1-7: Contributions of zooplankton taxa (%) to the main eigenvectors.

	Dim 1	Dim 2	Dim 3	Dim 4
Small cladocerans	41	12.4	10.3	36.3
Copepods	5.5	49.3	26.3	18.9
Chaoborus	36.3	17.3	12.3	34.1
Rotifers	17.2	21	51.1	10.7

In the following, we present zooplankton counting at the genus level. We aggregated the two dates or treated them as independent data sets to test the robustness of the effects of our treatments over time. When dates were aggregated, sampling date was included in the model.

Copepods were positively affected by light when Cyclopidae and nauplii were aggregated over the two sampling dates ($+0.55 \pm 0.27$ (Log individuals per 24L), Table S1-8). However, we only see a positive effect of fish in August sampling (Fig. S1-9A and Fig. S1-10A and B) for aggregated and non aggregated data and light effect is not significant. Thus, fish had a positive effect on Copepoda at the beginning of the experiment but this effect vanished later, while the positive effect of fish on Chydoridae (the only Cladocera in our experiments, except in one mesocosm) held at the two sampling dates ($+2.26 \pm 0.7$ (Log individuals per 24L) Fig. S1-9B and Fig. S1-10C). Chaoborus (large Diptera larvae) were only present in enclosures without fish (Fig. S1-9C).

Rotifers were not affected by our treatments when the two sampling dates were aggregated (Fig. S1-9D). However, a significant effect of the addition of organic matter was observed in September. This effect was observed in September for Bdelloid (Fig. S1-11C), Polyarthra (negative effect, Fig. S1-12A) and Brachionus (positive effect, Fig. S1-12C). Polyarthra were negatively affected by organic matter addition while full light exposure had a positive effect. Thus Bdelloid and Brachionus can be associated to the brown food web while Polyarthra would be assimilated to the green food web. Fish had a negative effect on Bdelloid (Fig. S1-11C) and Polyarthra (Fig. S1-12A) and a positive effect on Keratella (Fig. S1-12B) in August. In September, the effect of fish on Bdelloid (Fig. S1-11C) and Keratella (Fig. S1-12B) was still visible. Anuraeopsis (Fig. S1-12D) and Lecane (Fig. S1-11A) were negatively affected by fish. Thus, except for Keratella, fish had a negative effect on many rotifer species.

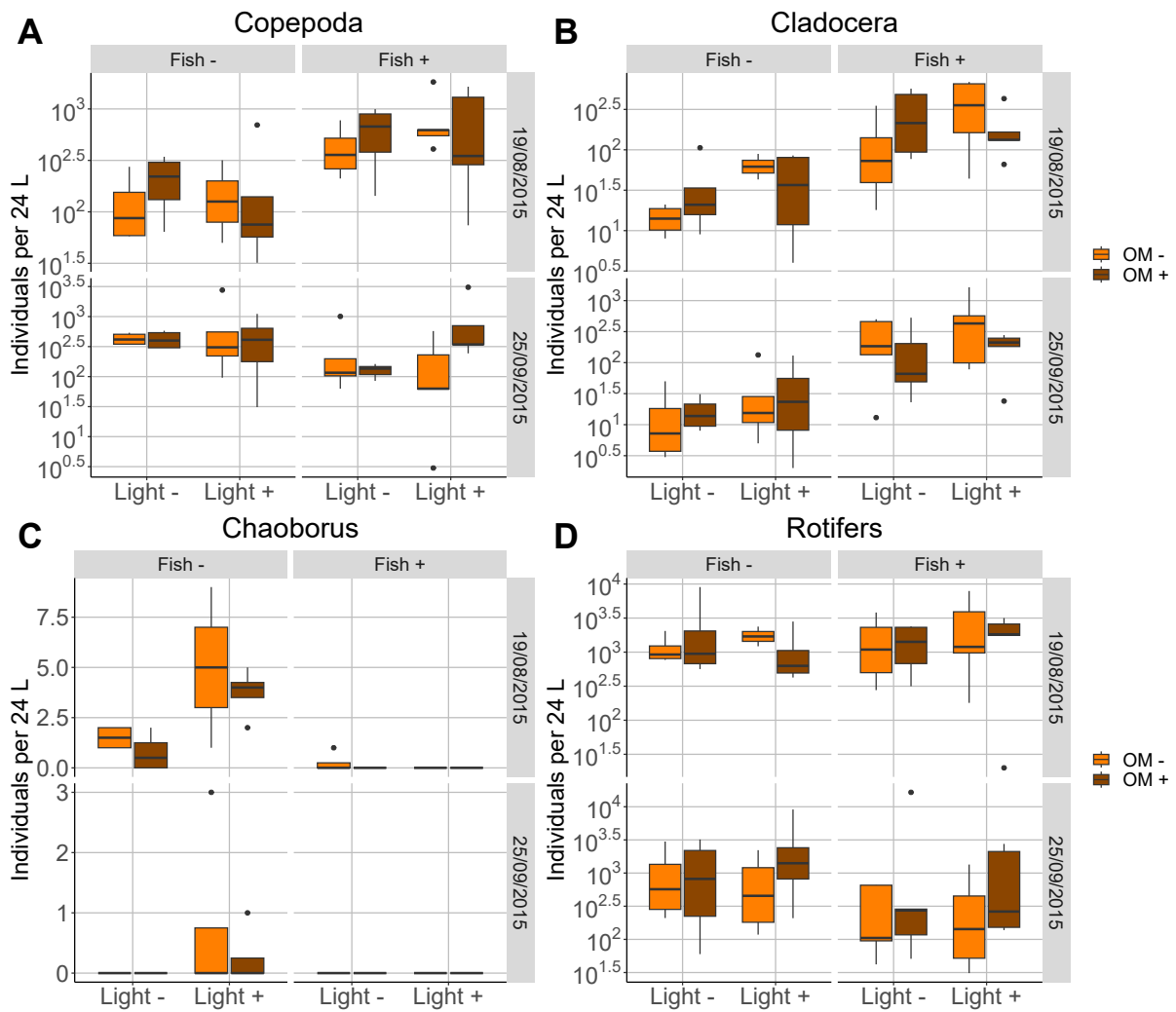


Figure S1-9: Response of zooplankton from three main aggregated groups. **A**) Cladocerae (Chydoridae and Bosminiae), **B**) Copepods (adults and nauplii), **C**) Chaoborus (large Diptera larvae) and **D**) rotifers (Lecanes, Lepadella, Bdelloid, Polyarthra, Brachionus, Hexarthra and Anuraepsis), Keratella were not included because of their strong variance.

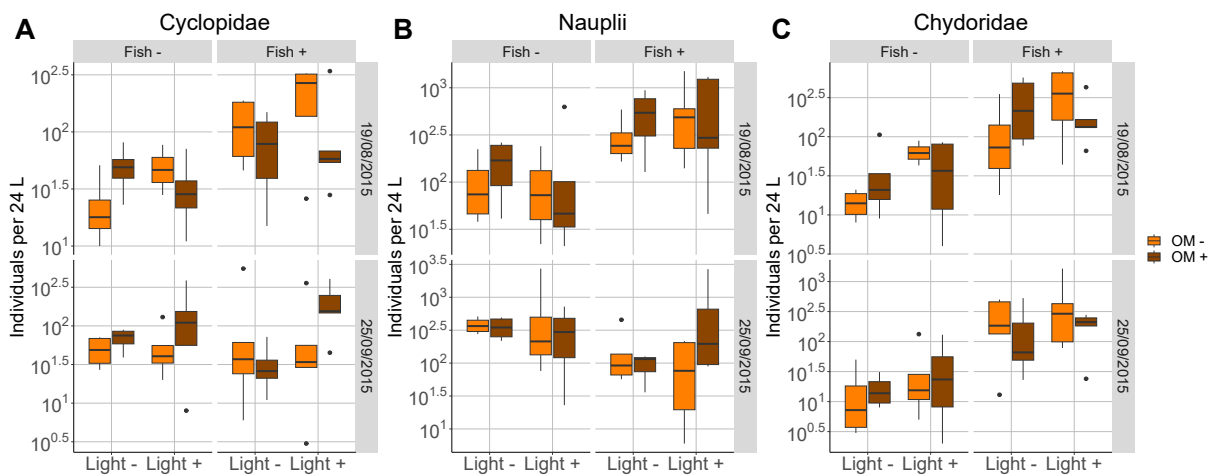


Figure S1-10: Zooplankton numeration of large zooplankton from 24 L samples **A**) Cyclopidea (large Copepoda) **B**) Nauplii (Copepoda juveniles) and **C**) Chydoridae (small Cladocera).

Table S1-8: Significance (p-values) of treatments on zooplankton taxa abundance. Each subpart corresponds respectively to data at the genus level aggregated for dates, data at the genus level analysed independently for the two dates and aggregated genus analysed independently for the two dates.

	Taxa	Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM
global	Copepoda	0.34	0.037	0.68	0.81	0.28	0.95	0.28
	Cladocera	10^{-8}	0.072	0.078	0.52	0.39	0.039	0.80
	Chaoborus	-	-	-	-	-	-	-
	Rotifer	0.98	0.85	0.18	0.83	0.87	0.39	0.51
19/08	Copepods	0.0009	0.26	0.67	0.81	0.80	0.46	0.92
	Chladocerans	10^{-5}	0.36	0.76	0.56	0.77	0.022	0.93
	Chaoborus	-	-	-	-	-	-	-
	Rotifers	0.81	0.89	0.97	0.36	0.51	0.37	0.50
25/09	Copepoda	0.41	0.11	0.7	0.72	0.22	0.50	0.093
	Chladocera	10^{-5}	0.11	0.05	0.70	0.48	0.41	0.78
	Chaoborus	-	-	-	-	-	-	-
	Rotifers	0.74	0.70	0.046	0.24	0.44	0.89	0.38
global	Cyclopidae	0.014	0.053	0.79	0.69	0.13	0.25	0.51
	Nauplii	0.60	0.052	0.61	0.75	0.11	0.84	0.32
	Chydoridae	10^{-8}	0.11	0.11	0.51	0.42	0.055	0.83
	Lecane	0.033	0.73	0.20	0.75	0.79	0.64	0.77
	Lepadella	0.28	0.62	0.84	0.22	0.25	0.030	0.0051
	Bdelloide	0.0083	0.40	0.011	0.59	0.55	0.12	0.38
	Polyarthra	0.0055	0.50	0.80	0.79	0.76	0.023	0.25
	Keratella	10^{-8}	0.19	0.015	0.98	0.91	0.36	0.90
	Brachionus	0.47	0.076	0.0038	0.045	0.38	0.60	0.10
	Chaoborus	-	-	-	-	-	-	-
19/08	Cyclopidae	0.0012	0.16	0.13	0.69	0.36	0.43	0.58
	Nauplii	0.0021	0.34	0.41	0.86	0.93	0.52	0.82
	Chydoridae	10^{-5}	0.36	0.76	0.56	0.77	0.022	0.93
	Chaoborus	-	-	-	-	-	-	-
	Lecane	0.054	0.15	0.33	0.97	0.81	0.67	0.93
	Lepadella	0.29	0.91	0.93	0.36	0.43	0.066	0.034
	Bdelloide	0.033	0.72	0.85	0.79	0.55	0.45	0.062
	Polyarthra	0.017	0.087	0.23	0.82	0.76	0.32	0.23
	Keratella	0.0005	0.68	0.16	0.99	0.43	0.061	0.12
	Brachionus	-	-	-	-	-	-	-
25/09	Cyclopidae	0.40	0.20	0.56	0.74	0.35	0.070	0.41
	Nauplii	0.28	0.12	0.77	0.57	0.094	0.78	0.098
	Chydoridae	10^{-5}	0.18	0.085	0.69	0.53	0.50	0.81
	Bosmine	-	-	-	-	-	-	-
	Lecane	0.0001	0.30	0.81	0.26	0.64	0.17	0.42
	Lepadella	0.66	0.055	0.51	0.81	0.30	0.70	0.11
	Bdelloide	0.044	0.42	0.029	0.66	0.64	0.26	0.52
	Polyarthra	0.12	0.0050	0.0046	0.89	0.66	0.59	0.95
	Keratella	10^{-5}	0.36	0.087	0.99	0.93	0.53	0.93
	Brachionus	0.61	0.21	0.042	0.17	0.55	0.72	0.26
Anuraeopsis	0.037	0.65	0.66	0.10	0.25	0.19	0.85	
Chaoborus	-	-	-	-	-	-	-	

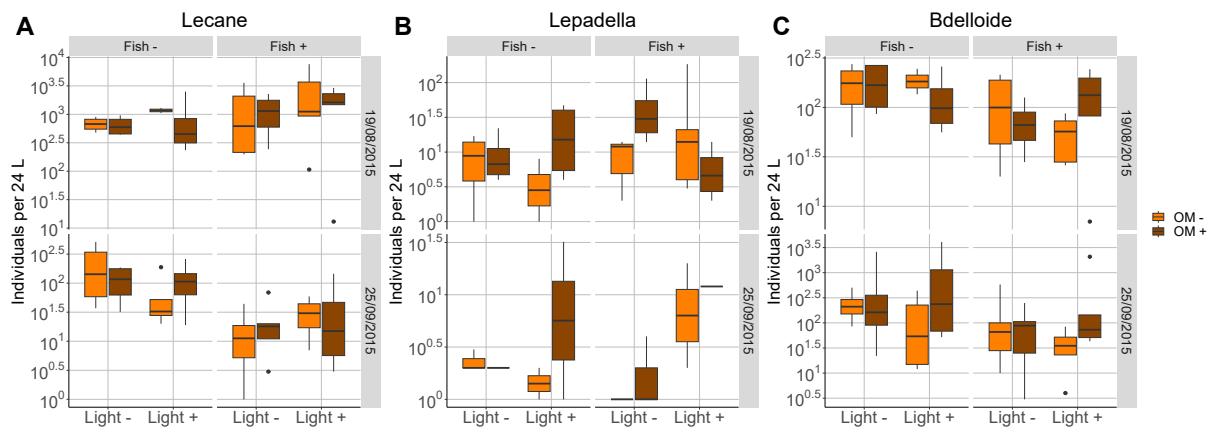


Figure S1-11: Zooplankton numeration of small rotifers from 24 L samples **A)** Lecane **B)** Lepadella and **C)** Bdelloid.

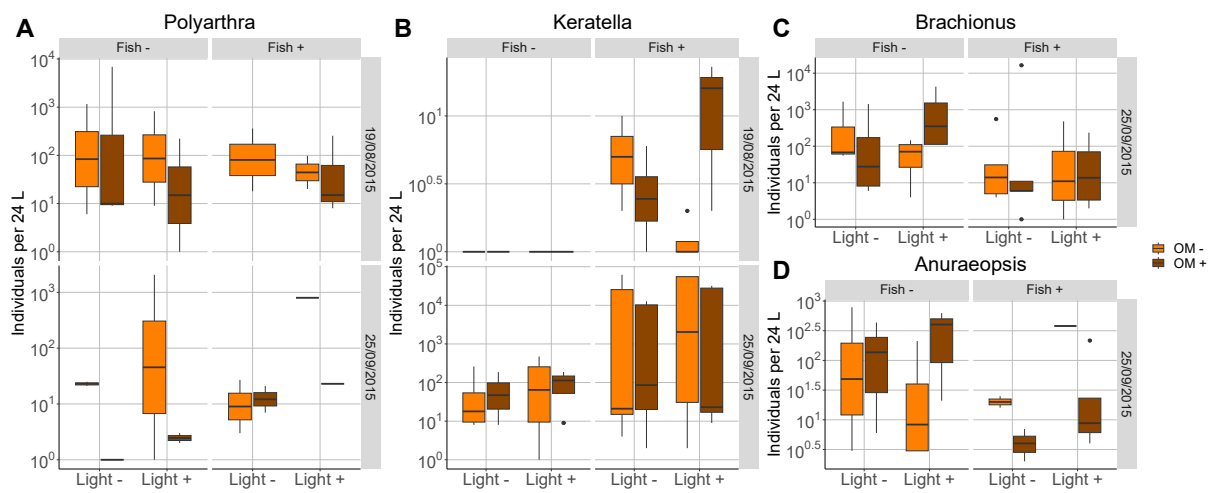


Figure S1-12: Zooplankton numeration of large rotifers from 24 L samples **A)** Polyarthra, **B)** Keratella, **C)** Brachionus and **D)** Anuraeopsis.

S1-4 Functional diversity of heterotrophic prokaryotes, EcoPlates™

Table S1-9: Significance (p-values) of treatments on the degradation capacity by heterotrophic prokaryotes of substrate families present in the EcoPlates™ Biolog.

Variable		Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM	
pelagic	Amine	p-value	0.623	0.342	0.545	0.801	0.299	0.078	0.847
		F	0.242	0.91	0.368	0.064	1.086	3.161	0.037
		Df	1	1	1	1	1	1	1
	Amino acids	p-value	0.158	0.134	0.041	0.8	0.184	0.896	0.733
		F	2.005	2.259	4.211	0.064	1.771	0.017	0.117
		Df	1	1	1	1	1	1	1
	Carbohydrates	p-value	0.666	0.794	0.208	0.32	0.426	0.178	0.324
		F	0.187	0.068	1.591	0.992	0.635	1.819	0.976
		Df	1	1	1	1	1	1	1
	Carboxylic acids	p-value	0.5	0.012	0.184	0.308	0.805	0.951	0.98
		F	0.456	6.385	1.767	1.041	0.061	0.004	0.001
		Df	1	1	1	1	1	1	1
	Phenolic acids	p-value	0.75	0.394	0.788	0.862	0.997	0.511	0.821
		F	0.102	0.731	0.072	0.03	0	0.434	0.051
		Df	1	1	1	1	1	1	1
	Polymer	p-value	0.58	0.174	0.17	0.558	0.248	0.213	0.169
		F	0.307	1.855	1.89	0.345	1.342	1.557	1.905
		Df	1	1	1	1	1	1	1
benthic	Amine	p-value	<.001	0.344	0.833	0.437	0.05	0.304	0.408
		F	57.12	0.9	0.044	0.608	3.907	1.066	0.69
		Df	1	1	1	1	1	1	1
	Amino acids	p-value	<.001	0.367	0.089	0.303	0.102	0.539	0.581
		F	76.61	0.815	2.903	1.064	2.688	0.378	0.305
		Df	1	1	1	1	1	1	1
	Carbohydrates	p-value	<.001	0.008	0.41	0.156	0.015	0.977	0.243
		F	13.94	7.096	0.679	2.014	5.988	0.001	1.363
		Df	1	1	1	1	1	1	1
	Carboxylic acids	p-value	<.001	0.564	0.011	0.552	<.001	0.627	0.206
		F	75.24	0.333	6.495	0.355	16.56	0.236	1.602
		Df	1	1	1	1	1	1	1
	Phenolic acids	p-value	<.001	0.125	0.176	0.706	0.073	0.579	0.207
		F	41.06	2.379	1.853	0.143	3.274	0.31	1.605
		Df	1	1	1	1	1	1	1
	Polymer	p-value	0.401	0.205	0.2	0.526	0.053	0.631	0.011
		F	0.707	1.613	1.653	0.403	3.774	0.231	6.624
		Df	1	1	1	1	1	1	1

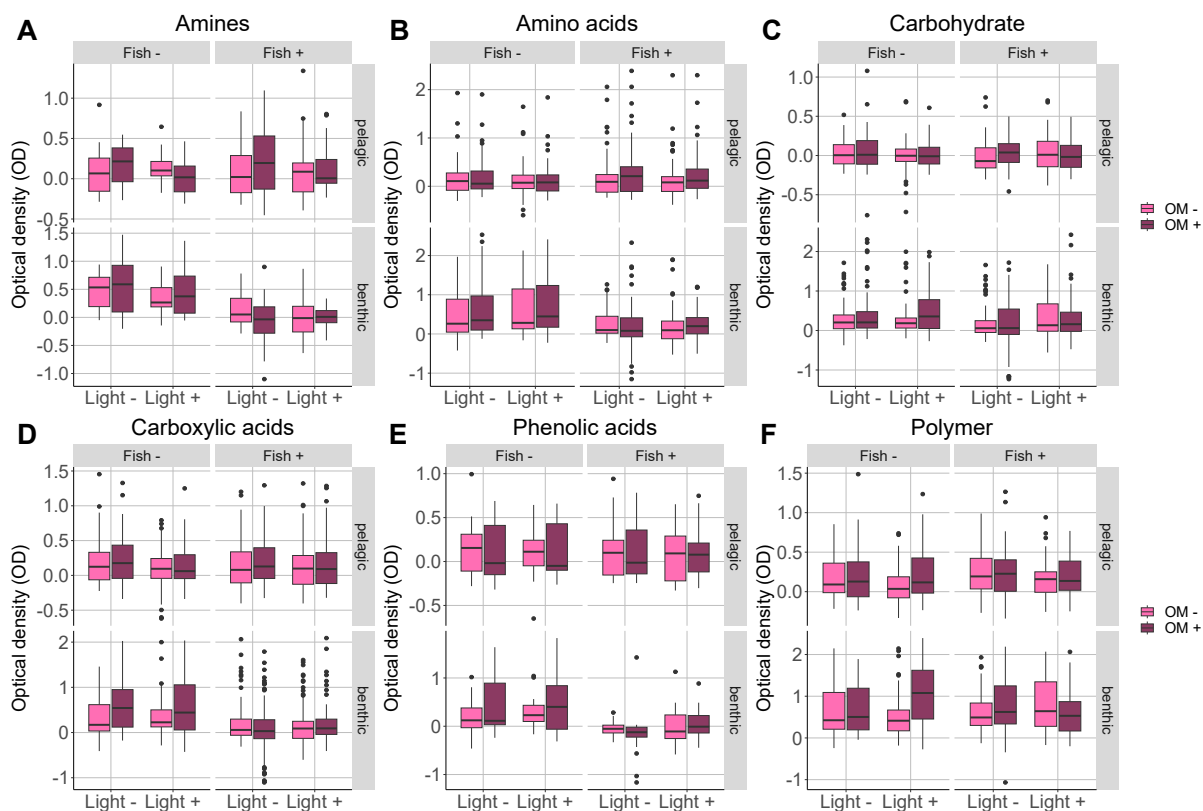


Figure S1-13: Optical density (OD) after 7 days for the different families of substrates after incubation of heterotrophic prokaryote samples from the pelagic or benthic compartments.

Table S1-10: Significance (p-values) of treatments on the degradation capacity of α -cyclodextrine, D-cellobiose (two of the molecules used in the organic carbon addition treatment (OM)) and L-asparagine by heterotrophic prokaryotes.

Variable		Fish (F)	Light (L)	OM	F:L	F:OM	L:OM	F:L:OM	
pelagic	α -cyclodextrine	p-value	0.055	0.323	0.924	0.007	0.886	0.654	0.125
		F	3.807	0.992	0.009	7.714	0.021	0.203	2.419
		Df	1	1	1	1	1	1	1
	D-cellobiose	p-value	0.633	0.789	0.764	0.031	0.278	0.373	0.137
		F	0.23	0.072	0.091	4.859	1.198	0.806	2.273
		Df	1	1	1	1	1	1	1
	L-asparagine	p-value	0.189	0.234	0.126	<.001	0.846	0.79	0.95
		F	1.759	1.447	2.404	32.48	0.038	0.071	0.004
		Df	1	1	1	1	1	1	1
benthic	α -cyclodextrine	p-value	0.294	0.777	0.959	0.475	0.444	0.492	0.096
		F	1.122	0.081	0.003	0.517	0.594	0.477	2.852
		Df	1	1	1	1	1	1	1
	D-cellobiose	p-value	0.477	0.251	0.751	0.243	0.236	0.009	0.303
		F	0.512	1.341	0.102	1.388	1.428	7.36	1.077
		Df	1	1	1	1	1	1	1
	L-asparagine	p-value	<.001	0.477	0.084	0.762	0.19	0.883	0.864
		F	27.37	0.511	3.083	0.093	1.751	0.022	0.03
		Df	1	1	1	1	1	1	1

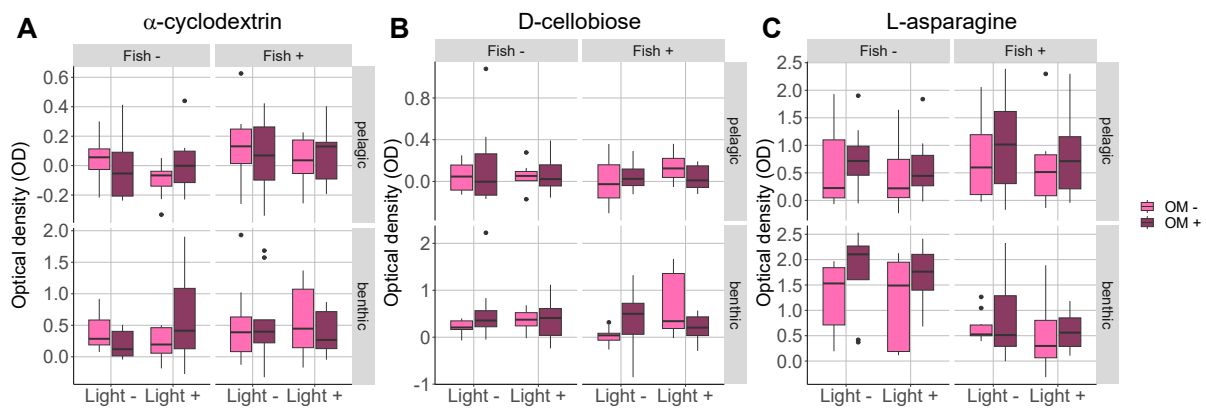


Figure S1-14: Optical density after 7 days for: **A)** α -cyclodextrin, **B)** D-cellobiose and **C)** L-asparagine.

As presented in Fig. 3C, fish had a strong negative effect on the degradation capacity of the substrates of the EcoPlates™ (Fig. S1-13). In addition, heterotrophic prokaryotes had a poor degradation capacity of the two recalcitrant molecules of our organic matter addition: α -cyclodextrin (Fig. S1-14A) and cellobiose (Fig. S1-14B), compared to asparagine (Fig. S1-14C) for instance. The degradability capacity for both molecules was not significantly affected by our organic matter addition treatment (Table S1-10, which might explain the weak general effects of our organic matter addition compared to previous studies (Faithfull et al., 2011; Degerman et al., 2018)).

References

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- Rock, A. M., Hall, M. R., Vanni, M. J., & González, M. J. (2016). Carnivore identity mediates the effects of light and nutrients on aquatic food-chain efficiency. *Freshwater Biology*, 61(9), 1492–1508. <https://doi.org/10.1111/fwb.12790>

S2 Supplementary methods

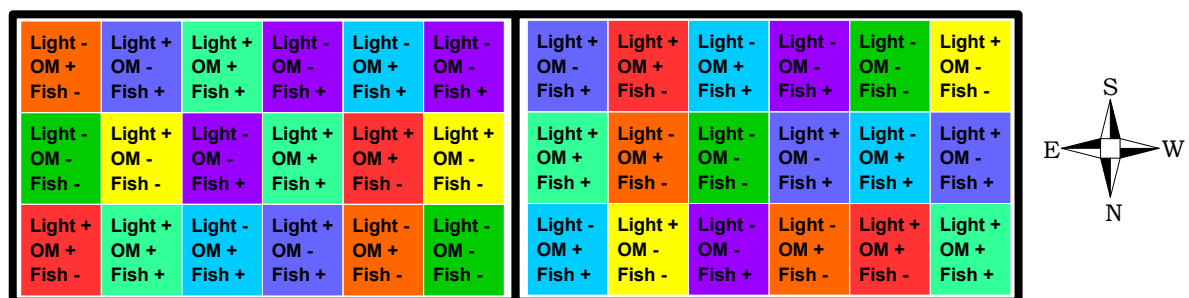


Figure S2-1: Experimental design consisting of three crossed treatments: light filtration (Light+ = no light filtration), soluble organic carbon addition (OM+ = carbon addition) and fish addition (Fish+ = fish addition). Each category of treatment combination is represented by a different colour and has four replicates, except for combinations with Fish+ that have five replicates.

Table S2-11: Taxonomy and mixotrophic ability of identified phytoplankton species.

Species	Class	Division	Mixotrophic
Chlamydomonas	Chlorophyceae	Chlorophyta	no
Crucigeniella	Trebouxiophyceae	Chlorophyta	no
Dictyosphaerium	Trebouxiophyceae	Chlorophyta	no
Dictyosphaerium subsolitarium	Trebouxiophyceae	Chlorophyta	no
Kirchneriella	Chlorophyceae	Chlorophyta	no
cf. Neochloris	Chlorophyceae	Chlorophyta	no
Oocystis	Trebouxiophyceae	Chlorophyta	no
Oocystis cf. marssonii	Trebouxiophyceae	Chlorophyta	no
Oocystis cf. parva	Trebouxiophyceae	Chlorophyta	no
Pediastrum boryanum	Chlorophyceae	Chlorophyta	no
Pediastrum tetras	Chlorophyceae	Chlorophyta	no
Scenedesmus (2 cells)	Chlorophyceae	Chlorophyta	yes
Scenedesmus ecornis var. concavus	Chlorophyceae	Chlorophyta	yes
Scenedesmus gr. Abundantes/ Spinosi	Chlorophyceae	Chlorophyta	yes
Scenedesmus gr. Acutodesmus	Chlorophyceae	Chlorophyta	yes
Tetraedron minimum	Chlorophyceae	Chlorophyta	no
Tetraedron minimum "Kivu form"	Chlorophyceae	Chlorophyta	no
Tetraedron triangulare	Chlorophyceae	Chlorophyta	no
Cosmarium inconspicuum	Zygnematophyceae	Streptophyta	no
Cosmarium laeve	Zygnematophyceae	Streptophyta	no
Mallomonas	Synurophyceae	Stramenopiles	no
Ochromonas	Chrysophyceae	Stramenopiles	yes
Synura	Synurophyceae	Stramenopiles	no
Undetermined Chrysophyceae	Chrysophyceae	Stramenopiles	unknown
Chrysophyceae kyst	Chrysophyceae	Stramenopiles	unknown
Trachydiscus sexangulatus	Eustigmatophyceae	Stramenopiles	no
Achnanthidium	Bacillariophyta	Stramenopiles	no
Fragilaria	Bacillariophyta	Stramenopiles	no
Cryptomonas	Cryptomonadales	Cryptophyta	yes
Plagioselmis nannoplanctica	Cryptomonadales	Cryptophyta	yes
Gymnodinium	Dinophyceae	Alveolata	yes
Peridiniopsis cf. penadiforme	Dinophyceae	Alveolata	yes
Peridinien	Dinophyceae	Alveolata	yes
Aphanocapsa	Cyanobacteria	Terrabacteria	no
Aphanothece	Cyanobacteria	Terrabacteria	no
Chroococcus	Cyanobacteria	Terrabacteria	no
Cyanogranis ferruginea	Cyanobacteria	Terrabacteria	no
Cyanogranis libera	Cyanobacteria	Terrabacteria	no
Merismopedia tenuissima	Cyanobacteria	Terrabacteria	no
Pseudanabaena	Cyanobacteria	Terrabacteria	no