# Changes in nutrient concentration and water level affect the microbial loop: a multi-seasonal mesocosm experiment

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**Abstract**

Eutrophication and lake depth are of key importance in structuring lake ecosystems. To elucidate the effect of contrasting nutrient concentrations and water levels on the microbial community, we manipulated water depth and nutrients in a mesocosm experiment in north temperate Estonia and followed the microbial community dynamics during a 6-month period. We used two nutrient levels crossed with two water depths, each represented by four replicates. We found treatment effects on the microbial food web structure, with nutrients having a positive and water depth a negative effect on bacterial biomass, heterotrophic nanoflagellates (HNF) and metazooplankton biomass. Nutrients and water depth had both positive impacts on phytoplankton biomass. Bacterivorous ciliates had lowest biomass in shallow and nutrient rich mesocosms, whilst predaceous ciliates had highest biomass here, influencing trophic interactions in the microbial loop. Overall, increased nutrient concentrations and decreased water level resulted in an enhanced bacterial biomass and a decrease in their main grazers. These differences appeared to reflect distinctive regulation mechanisms inside the protozoan community and in the trophic interactions in the microbial loop community.

Keywords: eutrophication, protozoa, bacteria, heterotrophic nanoflagellates, top-down and bottom-up control

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**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Priit Zingel and Helen Agasild. The first draft of the manuscript was written by Priit Zingel and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Introduction

Shallow lakes are thought to be more vulnerable to climate change than deep lakes (Kundzewicz et al. 2008; Jeppesen et al. 2009, 2011; Kernan et al. 2010). High temperature and precipitation resulting from climate change in northern temperate region may further enhance eutrophication in shallow lakes (Jeppesen et al. 2014, 2015; Moss et al. 2012). However, in southern Mediterranean lakes reduced precipitation and stronger evaporation induced hydrological deficient (e.g. reduced water level) contributes to eutrophication via prolonged water residence time and increased internal loading (Jeppesen et al. 2009; Özen et al. 2010; Coppens et al. 2016). Climate change may thus enhance eutrophication with changes in community structure and dynamics in shallow lake ecosystems.

How water level changes affect the microbial community is relatively unexplored. There is only limited experimental evidence of direct and indirect effects of changes in water level, nutrient availability, macrophyte coverage and zooplankton grazing on the structure of the microbial community (Tzaras et al. 1999; Jezbera et al. 2003; Farjalla et al. 2006; Christoffersen et al. 2006; Özen et al. 2013, 2014; Zingel et al. 2018; Šimek et al. 2019). Global warming may affect microbial communities indirectly through warming-induced eutrophication (Jeppesen et al. 2009, 2010) as these communities are highly sensitive to the changes in nutrient status and the top-down effect by consumers (Carrick et al. 1991; Nixdorf and Arndt, 1993; Gaedke and Straile, 1994; Mathes and Arndt, 1994). Water level fluctuations may become just as significant as nutrients for changes in the functioning of microbial communities in the context of global change (Özen et al., 2014; Porcel et al., 2019). As the microbial loop represents an important compartment in the food webs of shallow lakes (Zingel and Nõges, 2010), it is crucial to understand how changes in water level and nutrients affect microbial communities.

To elucidate the effect of contrasting nutrient concentrations and water levels on the microbial community, we manipulated both in mesocosm experiment undertaken in Estonia, and followed the microbial community dynamics during a 6-month period, from June to November 2011.

We hypothesised that: i) bacterioplankton abundance at different nutrient concentrations and water levels is controlled by different protozoan groups due to different impacts of top-down grazing

which leads to dissimilarities in the microbial loop functioning; ii) We expected to have more bacteria biomass and abundance in SH mesocosms as water level indirectly influences the biomass of bacteria by affecting nutrient concentrations and the growth of submerged macrophytes.

## Material and methods

We conducted an *in situ* mesocosm experiments in Lake Võrtsjärv (58° N 26° E), Estonia. Our study was part of a comprehensive study of the impact of water level fluctuations under high and low nutrient conditions along a north-south gradient across continental Europe (Landkildehus et al., 2014). The experiment lasted from May to November 2011 and the mesocosms were sampled monthly. We let the mesocosms to settle during the first month and for the current study we used samples from June to November (6 monthly sampling occasions). The mesocosms and the experimental set-up are described in detail by Landkildehus et al. (2014). Briefly, 16 cylindrical (diameter 1.2 m) fiberglass mesocosms were installed and filled with water sieved through a mesh size of 500 µm. Eight mesocosms were shallow (S) with a depth of 1 m and the other 8 were deep (D) with a depth of 2 m. To test the effect of nutrient loading, we created two levels of total phosphorus (TP) concentrations (25 µgP $L^{-1}$ low (L) and 200 µgP $L^{-1}$ high (H)). The final set of mesocosms consisted of two treatments, each with four replicates (shallow with low (SL) and high (SH) nutrient loading and deep with low (DL) and high (DH) nutrient loading). All mesocosms were dosed on a monthly basis with nutrients with an N:P ratio by weight of 20:1. All mesocosms contained a layer of sediment (thickness 10 cm). The sediment contained 90% (by volume) washed sand (grain size < 1 mm) and 10% lake sediment. Large particles (e.g. plant fragments, mussels, stones, debris, etc.) were removed by sieving through a 10 mm mesh. Before the sediment was added, it was equilibrated to the two experimental TP treatment levels (25 and 200 µg TP $L^{-1}$) (Landkildehus et al. 2014). All mesocosms contained macrophytes (*Myriophyllum spicatum*) and a mixture of phyto- and zooplankton species assemblages collected from five different lakes (see Landkildehus et al. 2014). Six planktivorous fish (three-spined sticklebacks *Gasterosteus aculeatus*) were added to each enclosure. On each sampling date and for

each mesocosm, a Percent Plant Volume Inhabited (PVI%) was estimated based on visual coverage percentage and measured mean macrophyte height (see Fig. 1 for mean TP, total nitrogen (TN) and PVI values). The mean water temperature in the mesocosms was ca 17 ˚C and the mean air temperatures was 15 ˚C (Landkildehus et al. 2014).

The microbial food web community was sampled monthly between June and November 2011. From the bulk water sample, 50 mL subsamples for bacteria and HNF analyses and a 100 mL subsample for ciliate analysis were taken. Samples for enumeration of bacteria and HNF were fixed immediately after collection by adding glutaraldehyde to a final concentration of 2% (v/v) and stained for 10 min with 4'6-diamidino-2-phenylindole (DAPI) at a final concentration of 10 µg DAPI $mL^{-1}$ (Porter and Feig, 1980). Within 2 h following sampling, we filtered the subsamples to count bacteria (2 mL) and HNF (15 mL) onto 0.2- and 0.8-µm pore size black Nuclepore filters, respectively. A Whatman GF/C glass microfiber filter with a pore size of 1.2 µm was used as a pad to obtain a uniform distribution of cells under low pressure (< 0.2 bar). Filters were stored at -20 $^0$C until enumeration. The abundances of bacteria and HNF were determined by direct counting of cells using epifluorescence microscopy (Nikon Eclipse Ti) at 1000X magnification. At least 400 bacteria cells from different fields were counted for each sample with a UV filter (420 nm). All specimens of HNF found within 1.6 $mm^2$ of each filter were counted. The microscope was equipped with a pale yellow UV (420 nm) and a blue (515 nm) filter to distinguish heterotrophs from mixo- and autotrophs at HNF counting. Conversion to carbon biomass was made using a factor of 0.22 pg C $\mu m^{-3}$ for bacteria and HNF (Bratback and Dundas, 1984; Borsheim and Bratback, 1987). For calculations all results from different months were averaged and the six-month seasonal mean was used for analysis.

A crucial links in the microbial loop is HNF. Therefore, it is important to understand the main mode of regulation of HNF abundance. For this purpose, we used Gasol's (1994) theoretical model to plot corresponding abundances of HNF and bacteria. According to Gasol's theory, data points located below the Mean Realized Abundance (MRA) line suggest top-down control on HNF. Points above the MRA line imply low top-down control on HNF. Points that are close to the Maximal Attainable Abundance (MAA) line point to strong bottom-up control on HNF.

Ciliates were fixed with acidic Lugol (4% Lugol's iodine (v/v)) and counted in sedimentation chambers under inverted microscopes at 600X magnification (Nikon Eclipse Ti) following Utermöhl (1958). At least 200 ciliate cells or the entire chamber were counted and identified to genus or species level according to Foissner and Berger (1996) and Foissner et al. (1999). Ciliate biovolumes were calculated from measurements of length and width dimensions of animals with approximations to an appropriate geometric shape. For conversion to carbon biomass, the factor 0.19 pg C $\mu m^{-3}$ was used (Putt and Stoecker, 1989). Besides using the monthly samples, we also analysed the six-month seasonal means.

Ciliates were divided into five functional groups using data gathered during several former feeding experiments (e.g. Kisand and Zingel, 2000; Agasild et al. 2007; Zingel et al. 2007; Zingel and Nõges, 2008). In these experiments, we used either fluorescently labelled bacteria or fluorescently labelled microparticles of different sizes to estimate ciliate feeding types. Additionally, we used published data on ciliate ecology (Foissner et al. 1991, 1992, 1994, 1995) and live observations during the experiments for estimating proper feeding types. As functional groups, we distinguished between bacterivores (picovores), herbivores (nanovores), bacteri-herbivores (pico-nanovores), predators (consumers of ciliates and small metazooplankters) and omnivores. We are fully aware that this division is somewhat arbitrary, but the most common ciliate species found in our experiment could quite reasonably be divided in these feeding groups.

Metazooplankton samples were collected monthly (from June to November). A 5 L subsample was filtered through a 20-µm mesh and preserved in 4% Lugol's solution. In the laboratory, 25% of the original sample volume was carefully subsampled and all subsamples were pooled into a single sample representing the entire experimental season. Further metazooplankton analysis and biomass calculation are described in detail by Tavşanoğlu et al. (2017). For carbon biomass, a conversion factor of 0.48 mg C per mg dry weight was used (Andersen and Hessen1991).

Phytoplankton samples were collected monthly (from June to November). Phytoplankton cells were enumerated and measured with an inverted microscope (Ceti Versus) at 100X or 400X

magnification. Samples were counted until at least 400 counting units (filaments, cells, colonies) had been processed, which gives a counting error of ±10% for the total biomass. Phytoplankton biomass in carbon units was calculated using a biovolume conversion factor of 0.22 mg C $mm^{-3}$ (Reynolds, 1984).

The results are expressed as the mean ± standard deviation of the quadruplicate parallel measurements. Significance of the impacts of environmental factors on microbial community was analysed with R version 4.1.1 (2021-08-10) using two-way repeated measures ANOVA with nutrient dosing and depth as fixed factors. Data were log-transformed before analysis to reduce skewness and to approximate to normal distribution. One-way ANOVA and Tukey's test were used for multiple mean comparisons.

**Results**

The seasonal mean bacterial abundances were 4.88 ± 1.08 × $10^6$ cells $ml^{-1}$ and the mean biomass was 173 ± 38 µg C $l^{-1}$. Bacterial abundance and biomass were significantly affected by depth and nutrients separately and by their interactions (Table 1; RM-ANOVA). Nutrients had a positive and water depth had a negative effects on bacterial abundance and biomass (Fig. 2). The highest seasonal mean bacterial abundances and biomasses were found in the SH treatment (6.59 ± 0.3 × $10^6$ cells $ml^{-1}$; 233 ± 11 µg C $l^{-1}$).

The seasonal mean HNF abundances were 2998 ± 406 (SD) cells $ml^{-1}$ and the mean biomass was 20.8 ± 3.9 (SD) µg C $l^{-1}$. HNF abundance was significantly affected by neither depth nor nutrients separately nor by their interactions but only nutrients had significant effect on HNF seasonal mean biomass (Table 1; RM-ANOVA, $p<0.05$). The highest seasonal mean HNF biomass was found in the SH treatment (24.5 ± 2.3 (SD) µg C $l^{-1}$). The highest seasonal mean HNF abundances were found in the SL treatment (3206 ± 588 (SD) cells $ml^{-1}$) and lowest in SH treatment (2664 ± 256 (SD) cells $ml^{-1}$). The mean biomass of individual HNF cells was significantly affected by depth and nutrients separately

and by their interactions (Table 2; RM-ANOVA). The seasonal mean biomass of individual HNF cells was highest in the SH treatment (biomass of $10^6$ cells 42.1 ± 1.6 (SD) µg WW; Fig 3).

We plotted abundances of HNF and bacteria according to Gasol (1994). We found that most data points were above the MRA line, indicating that HNF control by predation was relatively weak. Only exception were the SH mesocosms where a slightly higher predation pressure was visible (Fig. 4).

The seasonal mean total ciliate abundance was 126 ± 41 (SD) cells $ml^{-1}$ and the seasonal mean total biomass 1379 ± 195 (SD) µg C $l^{-1}$. Water depth and the combination of water depth and nutrients had significant effect on total ciliate abundances (Table 1; RM-ANOVA, $p<0.05$). The highest seasonal mean ciliate abundances were found in the DH treatment (170 ± 21 (SD) cells $ml^{-1}$) and biomass in the SH treatment (1466 ± 55 (SD) µg C $l^{-1}$) (Fig. 5). The mean biomass of individual ciliate cells was significantly affected by depth and nutrients separately and by their interactions (Table 2; RM-ANOVA). The seasonal mean biomass of individual ciliates was highest in the SH treatment (biomass of $10^6$ cells 158.4 ± 92.4 (SD) µg WW; Fig. 3).

The most abundant ciliate group was small-sized bacterivores (mean abundance 74 ± 36 (SD) cells $ml^{-1}$; mean biomass 157 ± 80 (SD) µg C $l^{-1}$), whilst in terms of ciliate biomass the larger predaceous species dominated (mean abundance 4.4 ± 2 (SD) cells $ml^{-1}$; mean biomass 608 ± 290 (SD) µg C $l^{-1}$). The highest seasonal mean bacterivorous ciliates abundance and biomass was found in the DH treatment (113 ± 14 (SD) cells $ml^{-1}$; 239 ± 32 (SD) µg C $l^{-1}$) and lowest in SH treatment (40 ± 2 (SD) cells $ml^{-1}$; 78 ± 5 (SD) µg C $l^{-1}$) (Fig. 5). Bacterivorous ciliate biomasses differed significantly between treatments (one-way ANOVA, $p<0.05$). The highest seasonal mean predaceous ciliate abundances and biomasses were found in the SH treatment (6.3 ± 0.3 (SD) cells $ml^{-1}$; 868 ± 67 (SD) µg C $l^{-1}$) and lowest in DH treatment (2.5 ± 0.4 (SD) cells $ml^{-1}$; 340 ± 47 (SD) µg C $l^{-1}$) (Fig. 5). Predaceous ciliate biomasses were significantly different between treatments (one-way ANOVA, $p<0.05$). The effects of depth and nutrients separately and by their interactions on the abundance and

biomass of ciliate feeding groups based on the covariance test of significance (RM-ANOVA) are shown in Table 3.

The seasonal mean metazooplankton biomass was 74 µg C $l^{-1}$, highest biomass was found in the SH treatment (126 ± 82 µg C $l^{-1}$) and lowest in DL treatment (30 ± 9 µg C $l^{-1}$) (Fig. 2). Metazooplankton biomasses differed significantly between treatments (one-way ANOVA, $p<0.05$). Mean abundances and biomasses and respective standard deviations of metazooplankton groups (copepods, cladocerans and rotifers) are shown in Table 4. Seasonal mean phytoplankton biomass was 951µg C $l^{-1}$, highest biomass was found in the DH treatment (1622 ± 377 µg C $l^{-1}$) and lowest in the DL treatment (821 ± 211 µg C $l^{-1}$) (Fig. 2). Phytoplankton biomasses differed significantly between treatments (one-way ANOVA, $p<0.05$).

The ratio between biomasses of ciliates and bacteria was lowest in SH treatment (median 6.2) and highest in DL treatment (median 11.2) (Fig. 6; one-way ANOVA, $p<0.05$). The respective ratio between biomasses of ciliates and metazooplankton was lowest in DH treatment (median 11.1) and highest in SL treatment (median 52.5) (Fig. 6; one-way ANOVA, $p<0.05$). The ratio between biomasses of bacteria and metazooplankton was lowest in DH treatment (median 1.4) and highest in SL treatment (median 5.4) (Fig. 6; one-way ANOVA, $p=0.10$).

## Discussion

Our study showed treatment effects on microbial food web structure. Nutrients had a positive and water depth a negative effect on bacterial biomass, HNF and metazooplankton biomass. Both nutrients and water depth had both positive impacts on phytoplankton biomass. Bacterivorous ciliates had lowest biomass and predaceous ciliates had the highest biomass in the SH treatment. Ratios between ciliate and bacterial biomasses were lowest in the SH and DH treatments, indicating that in these mesocosms bacteria were under the lowest grazing pressure. Ratios between ciliate and metazooplankton biomasses were in the same time also lowest in the SH and DH treatments, indicating that in these mesocosms ciliates were under the highest grazing pressure. The same trend

was observed in the HNF:metazooplankton biomass ratios. Therefore we assume that metazooplankton had indirect positive effect on bacteria biomasses. This is also reflected in the corresponding bacterial and metazooplankton biomass ratios (Fig. 6).

Bacterivorous ciliates were the dominant consumers of bacteria in all treatments (based on the fact that their mean biomass was 29 times greater than HNF mean biomass), and predaceous ciliates likely controlled the abundances of bacterivorous ciliates, which corresponds to the situation in shallow eutrophic water bodies (Šimek et al. 2019). We hypothesized that bacterioplankton abundance at different nutrient concentrations and water levels was controlled by different protozoan groups (ciliates or HNF) due to different impacts of top-down grazing which leads to dissimilarities in the microbial loop functioning, but this hypothesis was not supported. However, when we plotted abundances of HNF and bacteria according to Gasol (1994), the results indicated that HNF were controlled by top-down rather than by bottom-up mechanisms in all our mesocosms. The predator control was, however, weak, with the exception of the SH mesocosms where a slightly higher predation pressure was visible (Fig. 4). Almost all data points from the low nutrient mesocosms remained above the MRA line, suggesting that HNF experienced weaker top-down control under less eutrophic conditions. This is in accordance with Gasol and Vaque (1993), who showed that HNF control by predation was most important in eutrophic systems but not in nutrient poor ones. The same trend was demonstrated by Sanders et al. (1992) using a modelling approach. It is commonly agreed that in more eutrophic conditions diversity and biomass of organisms capable of preying on HNF usually increases (e.g. Riemann and Christoffersen, 1993) and thus also their predation pressure. Cell size of both HNF and ciliates were largest in the SH treatment (Fig. 3). The cell volume of protists is plastic and can respond rapidly to changes in environmental conditions and population abundances (Forster et al., 2013). The larger size in SH reflects the dominance of large predaceous ciliates and metazooplankton potentially leading to suppression of small sized bacterivorous species.

The water level likely had an indirect effect on the biomass of bacteria and phytoplankton by affecting the relative amount of submerged macrophytes in the mesocosms. Higher PVI% of

macrophytes may lead to a higher release of organic matter from the plant-periphyton community (Stanley et al. 2003), improving the conditions for bacterial growth. On the other hand, macrophytes can suppress the development of algae through shading, competition for nutrients and allelopathy (Mulderij, 2007). In another mesocosm experiment undertaken in Turkey, Özen et al. (2014) showed that declining water level led to an increase in PVI% of submerged macrophytes and a concurrent decrease in the biomass of phytoplankton and an increase in bacterioplankton biomass. Bucak et al. (2012) also reported that the lesser amount of macrophytes had a positive effect on phytoplankton biomass in similar type of experiments. These results concur with our findings of a higher phytoplankton biomass in the deep mesocosms and higher bacterioplankton biomass in the shallow mesocosms.

Several experiments have shown that crustaceans can control ciliates (Adrian and Schneider-Olt, 1999; Ventelä et al. 2002; Zöllner et al. 2003). This is substantiated by the experiment of Agasild et al. (2013) where removal of crustaceans led to a higher number of large predacious ciliates (known to feed actively on small-sized ciliates), demonstrating that selective grazing by crustaceans on large-sized ciliates can significantly alter ciliate community structure. In our study this trend was not generally visible as both metazooplankters and predaceous ciliates showed highest biomasses in the SH treatment. In this treatment the metazooplankton community consisted of species that do not select large sized predaceous ciliates as prey but rather consume small sized bacterivorous ciliates (Ventelä et al. 2002; Agasild et al. 2012) (Table 4). In the DH treatment, however, where metazooplankters showed second highest biomasses, the biomass of predaceous ciliates was lowest and biomass of bacterivorous ciliates highest.

In our experiment the number of fish added to each mesocosm was the same, leading to a relatively higher abundance of fish per volume in shallow mesocosms, as commonly found in lakes (Jeppesen et al. 2007). A former study carried out in shallow eutrophic ponds in Estonia (Karus et al. 2014) showed that the feeding of planktivorous fish had a remarkable indirect shaping effect on the microbial food web. Depending on the pressure of planktivorous fish, the main bacterial grazers can

be either HNF or small bacterivorous ciliates. The results of the same study also revealed that in the absence of planktivorous fish, the number of bacteria decreased due to the cascading grazing effects of zooplankton (Karus et al. 2014). We may, therefore, assume that also in the current mesocosm experiment the effect of fish predation cascaded down the food web and affected different trophic levels, as evidenced in other mesocosm studies by Özen et al. (2013). The higher fish density in the shallow mesocosms may, therefore, have contributed to the more significant effects on the microbial community in these mesocosms. However, as we lack data on fish feeding patterns in the mesocosms, we cannot elucidate the fish effects in more detail.

It is evident that nutrient manipulations and water level influenced trophic interactions in the microbial loop in our mesocosm experiment. Increased nutrient concentrations and decreased water level resulted in an enhanced bacterial biomass and a decrease in their main grazers (bacterivorous ciliates). Our results reveal that the interactions between water depth and nutrients significantly affected the microbial communities and that the trophic cascade between metazooplankters, predaceous ciliates, bacterivores and bacteria, was most notable in the shallow mesocosms with high nutrient concentration.

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**Table 1.** Significance of the impacts of environmental factors on microbial community indices based on two-way repeated measures ANOVA including the whole study period and all mesocosms and with nutrient dosing and depth as fixed factors. Significant p-values ($p<0.05$) are given in bold. To significant p-values a generalized Eta-Squared measure of effect size (ges) is given, followed by the direction of effect ((+) or (-)).

| | Abundance | | | Biomass | | |
|---|---|---|---|---|---|---|
| | Bacteria | HNF | Total ciliates | Bacteria | HNF | Total ciliates |
| Depth | **<0.0001** ges=0.90; (-) | 0.521 | **0.009** ges=0.44; (+) | **<0.0001** ges=0.90; (-) | 0.094 | 0.927 |
| Nutrient | **<0.0001** ges=0.95; (+) | 0.230 | 0.430 | **<0.0001** ges=0.95; (+) | **0.035** ges=0.32; (+) | 0.431 |
| Depth*Nutrient | **<0.0001** ges=0.83; (+) | 0.677 | **0.0001** ges=0.72; (+) | **<0.0001** ges=0.83; (+) | 0.177 | 0.197 |

**Table 2.** Significance of the impacts of environmental factors (p-values) on the individual biomass (wet weight) of protozooplankters (= size) based on two-way repeated measures ANOVA including the whole study period and all mesocosms and with nutrient dosing and depth as fixed factors. After p-values a generalized Eta-Squared measure of effect size (ges) is given, followed by the direction of effect ((+) or (-)).

| | Size | |
|---|---|---|
| | HNF | Total ciliates |
| Depth | **<0.0001** ges=0.90; (-) | **0.002** ges=0.57; (-) |
| Nutrient | **<0.0001** ges=0.95; (+) | **0.023** ges=0.36; (+) |
| Depth*Nutrient | **<0.0001** ges=0.84; (+) | **<0.0001** ges=0.80; (-) |

**Table 3.** Significance of the impacts of environmental factors on the abundance and biomass of ciliate feeding groups based on two-way repeated measures ANOVA including the whole study period and all mesocosms and with nutrient dosing and depth as fixed factors. Significant p-values ($p<0.05$) are given in bold. To significant p-values a generalized Eta-Squared measure of effect size (ges) is given, followed by the direction of effect ((+) or (-)).

| | Abundance of ciliate feeding groups | | | | |
|---|---|---|---|---|---|
| | Bacterivores | Bacteri-herbivores | Herbivores | Omnivores | Predators |
| Depth | **0.032** ges=0.33; (+) | **0.002** ges=0.57; (+) | 0.741 | **0.014** ges=0.41; (+) | 0.200 |
| Nutrient | 0.695 | **0.028** ges=0.34; (+) | 0.501 | 0.993 | 0.355 |
| Depth*Nutrient | **0.0002** ges=0.69; (+) | **<0.0001** ges=0.81; (+) | 0.238 | 0.493 | **0.001** ges=0.60; (-) |
| | Biomass of ciliate feeding groups | | | | |
| | Bacterivores | Bacteri-herbivores | Herbivores | Omnivores | Predators |
| Depth | **0.032** ges=0.33; (+) | **0.008** ges=0.46; (+) | 0.456 | **0.002** ges=0.58; (+) | 0.131 |
| Nutrient | 0.544 | 0.057 | 0.494 | 0.388 | 0.369 |

| | | | | | |
|---|---|---|---|---|---|
| Depth*Nutrient | **0.0002** ges=0.70; (+) | **<0.0001** ges=0.76; (+) | 0.226 | 0.873 | **0.001** ges=0.59; (-) |

**Table 4.** Mean abundances and biomasses and respective standard deviations (SD) of metazooplankton groups of copepods, cladocerans and rotifers in shallow and low-nutrient (SL), shallow and high-nutrient (SH), deep-low (DL) and deep-high (DH) mesocosms. In each treatment the taxa dominating among biomasses of metazooplankton groups are indicated.

| | Copepods | | | | Cladocerans | | | | Rotifers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Abundance | | Biomass | | Abundance | | Biomass | | Abundance | | Biomass | |
| Treatment | ind $l^{-1}$ | ±SD | µg C $l^{-1}$ | ±SD | ind $l^{-1}$ | ±SD | µg C $l^{-1}$ | ±SD | ind $l^{-1}$ | ±SD | µg C $l^{-1}$ | ±SD |
| DH | 123.0 | 29.7 | 35.5 | 9.5 | 236.3 | 126.2 | 60.6 | 32.9 | 1231.3 | 550.5 | 9.5 | 8.7 |
| Biomass dominants | Cyclopoid copepodites, *Mesocyclops leuckarti* | | | | *Bosmina longirostris, Chydorus sphaericus* | | | | *Keratella* spp., *Euchlanis dilatata* | | | |
| DL | 76.5 | 32.9 | 14.9 | 7.4 | 99.8 | 40.4 | 13.4 | 6.6 | 320.8 | 141.3 | 2.1 | 1.5 |
| Biomass dominants | Nauplii, Cyclopoid copepodites | | | | *Bosmina longirostris* | | | | *Polyarthra* spp., *Keratella* spp. | | | |
| SH | 88.2 | 26.7 | 20.3 | 5.8 | 388.8 | 323.4 | 90.0 | 76.1 | 4009.7 | 2473.1 | 15.8 | 8.3 |
| Biomass dominants | Nauplii, Cyclopoid copepodites | | | | *Bosmina longirostris* | | | | *Euchlanis dilatata* , *Anuraeopsis fissa* , *Keratella* spp. | | | |
| SL | 67.0 | 41.7 | 14.5 | 9.1 | 62.7 | 46.5 | 12.7 | 11.4 | 1364.6 | 1354.7 | 7.0 | 8.3 |
| Biomass dominants | Nauplii, Cyclopoid copepodites | | | | *Bosmina longirostris* | | | | *Keratella* spp., *Polyarthra* spp. | | | |

## Figure captions

**Fig. 1** Means and standard deviations (SD) of total phosphorus (TP) and total nitrogen (TN) in May–November and plant volume inhabited (PVI, %) in July–November in the mesocosm experiment conducted in 2011.

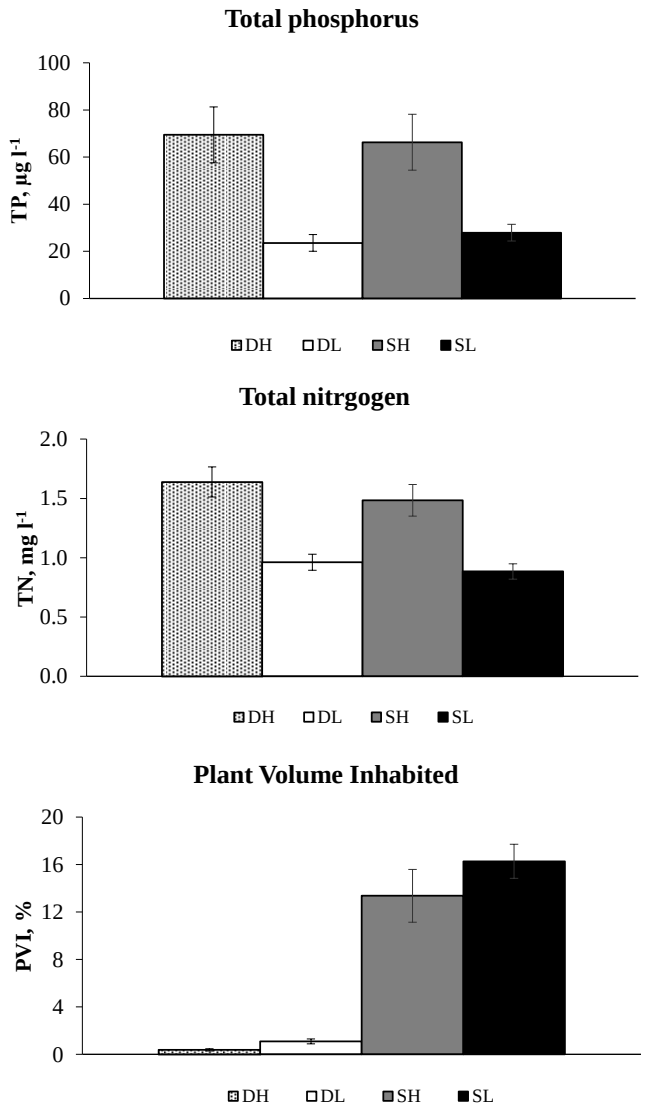
**Fig. 2** Biomass of bacteria, HNF, metazooplankton and phytoplanktont and respective standard deviations (SD) in shallow and low-nutrient (SL), shallow and high-nutrient (SH), deep-low (DL) and deep-high (DH) mesocosms. Notice the different scales.

**Fig. 3** Biomass (mg WW) of $10^6$ individuals of HNF and ciliates in shallow and low-nutrient (SL), shallow and high-nutrient (SH), deep-low (DL) and deep-high (DH) mesocosms. For HNF the difference in means (Tukey test) between SL:SH, SL:DH, SH:DL, SH:DH and DL:DH are statistically significant ($p<0.05$). For ciliates the difference in means (Tukey test) between SL:SH and SH:DH are statistically significant ($p<0.05$).

**Fig. 4** Bacterial and HNF abundance in mesocosms plotted following the Gasol's model (1994). MAA is the maximum attainable abundance line, and MRA is the mean realized abundance line.

**Fig. 5** Biomass of total ciliates, bacterivorous ciliates and predaceous ciliates and respective standard deviations (SD) in shallow and low-nutrient (SL), shallow and high-nutrient (SH), deep-low (DL) and deep-high (DH) mesocosms. Notice the different scales.

**Fig. 6** The ratio between ciliate and bacterioplankton biomass, ciliate and metazooplankton biomass, bacterioplankton and metazooplankton biomass and HNF and metazooplankton biomass and respective standard deviations (SD) in shallow and low-nutrient (SL), shallow and high-nutrient (SH), deep-low (DL) and deep-high (DH) mesocosms. Notice the different scales.

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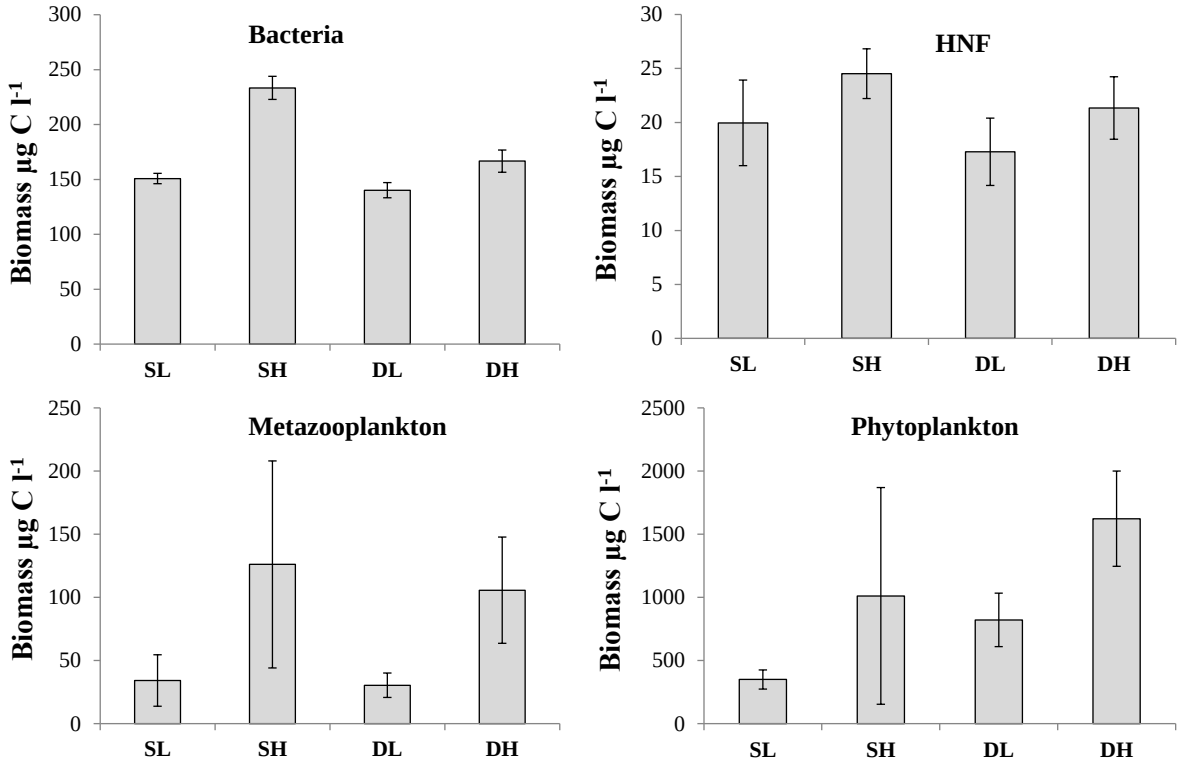


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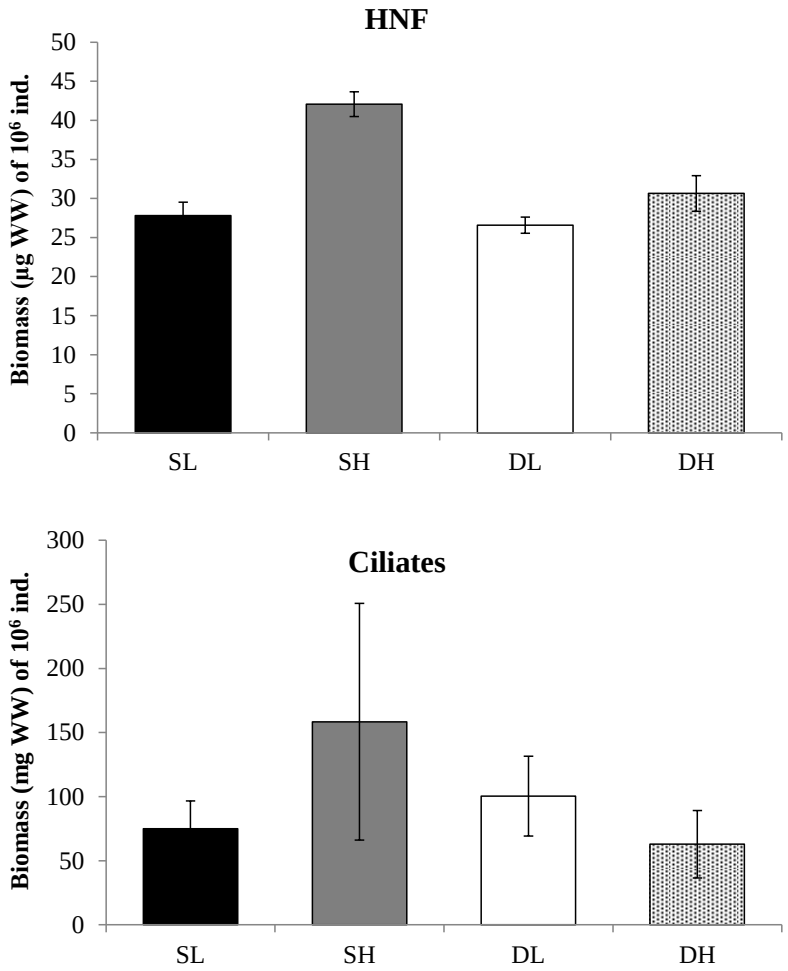


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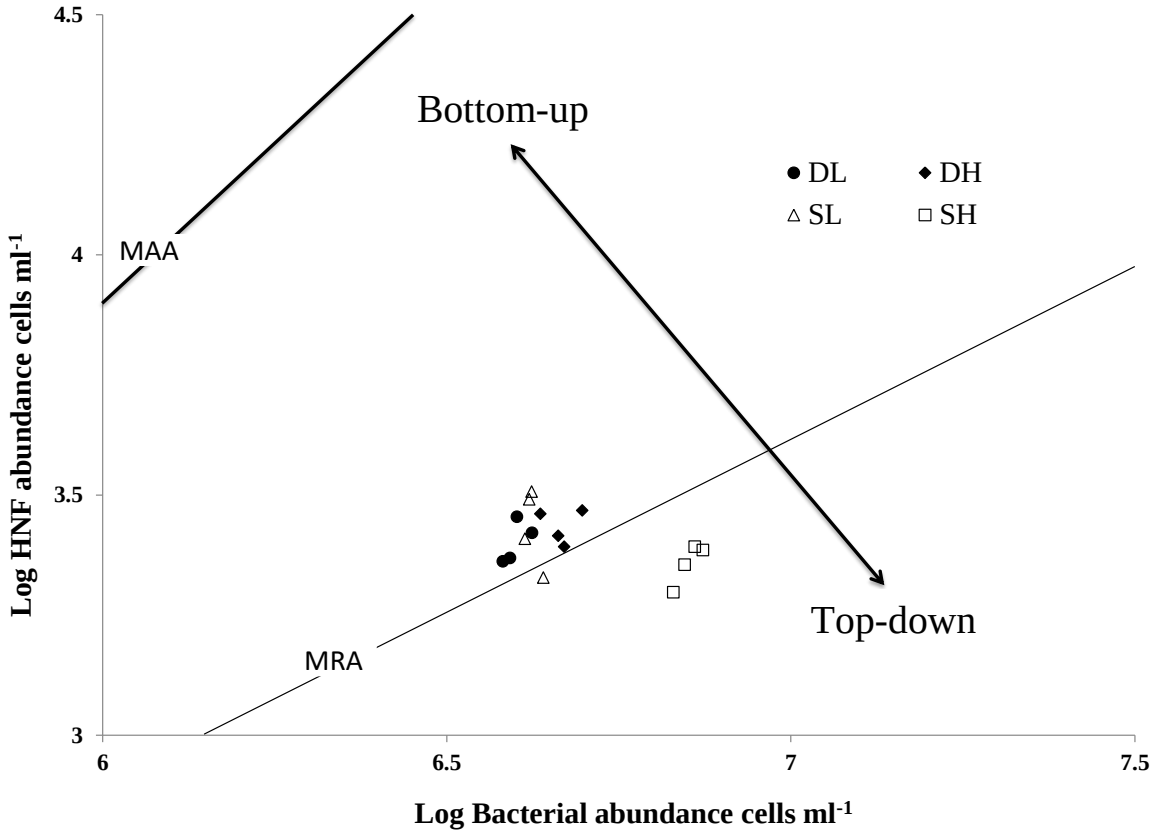


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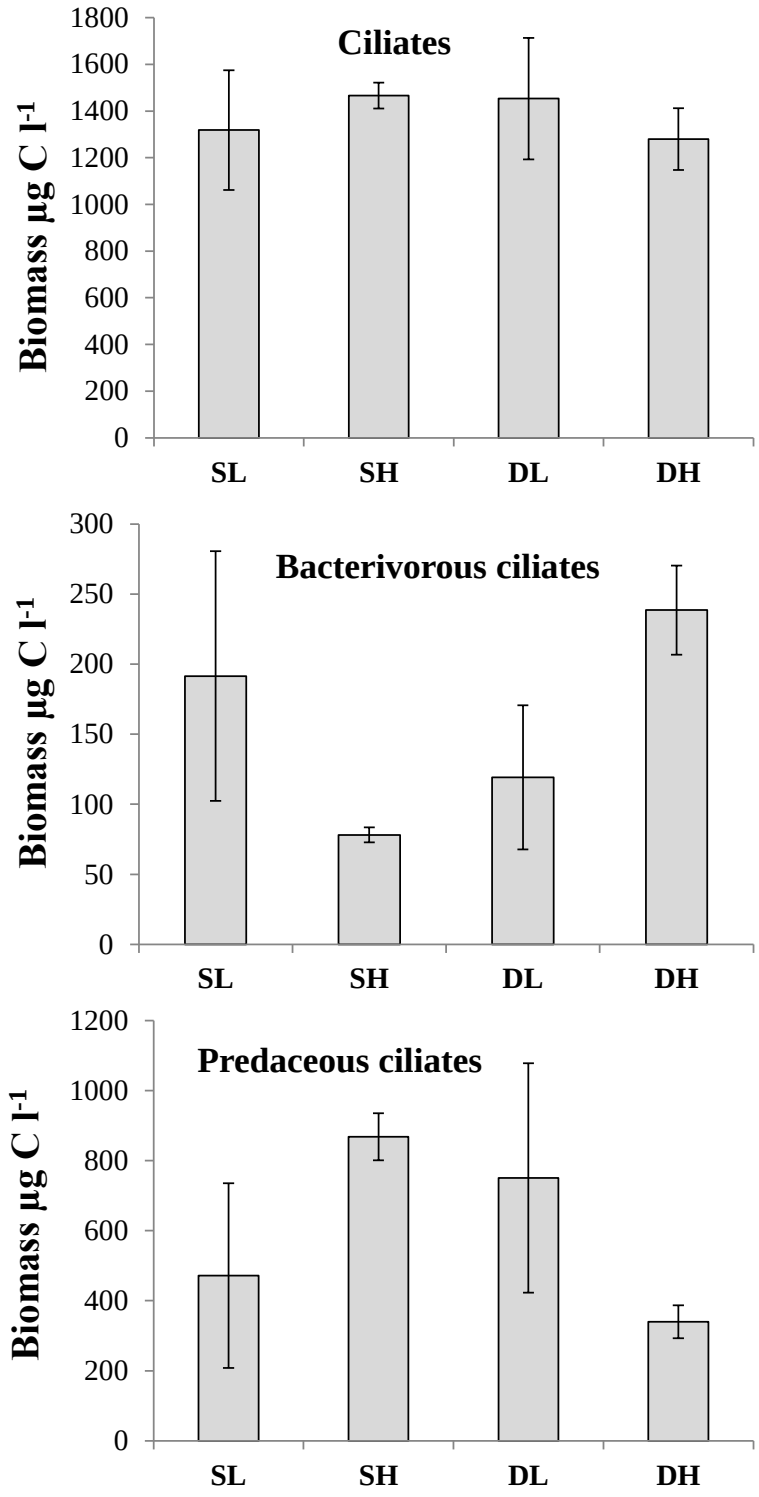


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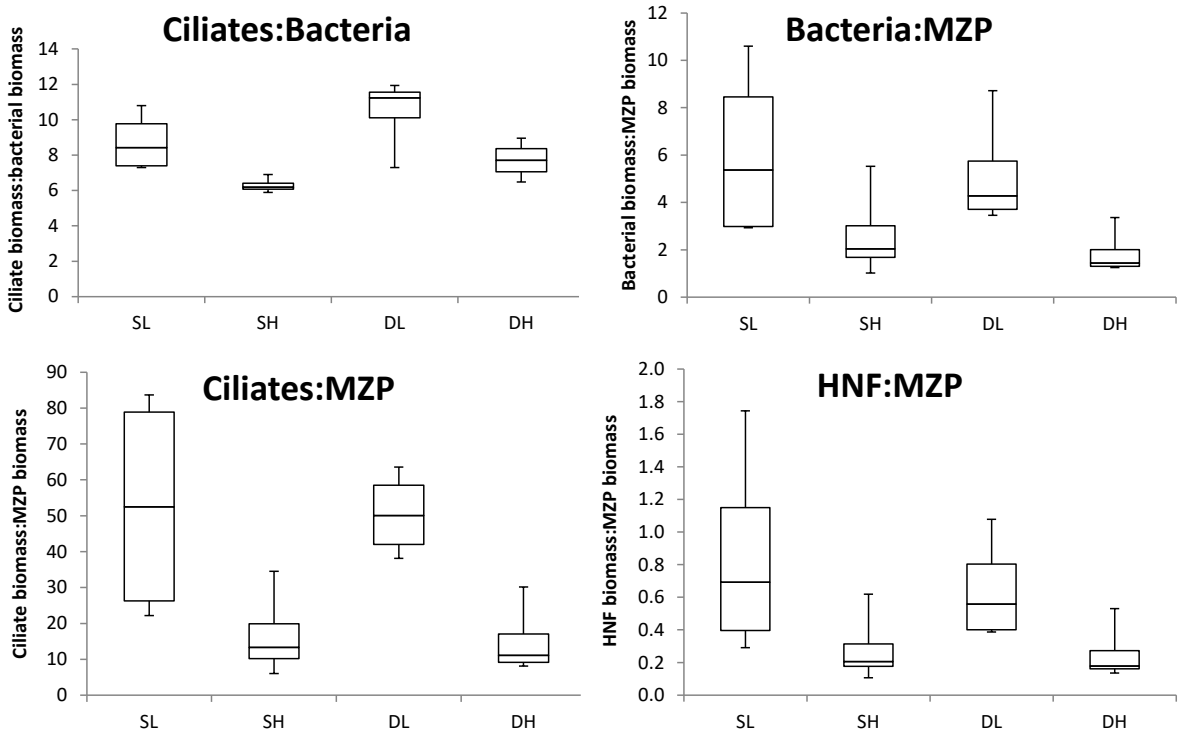


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