

# Water level and fish-mediated cascading effects on the microbial community in eutrophic warm shallow lakes: a mesocosm experiment

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**Abstract** Information on the effects of water level changes on microbial planktonic communities in lakes is limited but vital for understanding ecosystem dynamics in Mediterranean lakes subjected to major intra- and inter-annual variations in water level. We performed an in situ mesocosm experiment in an eutrophic Turkish lake at two different depths crossed with presence/absence of fish in order to explore the effects of water level variations and the role of top-

down regulation at contrasting depths. Strong effects of fish were found on zooplankton, weakening through the food chain to ciliates, HNF and bacterioplankton, whereas the effect of water level variations was overall modest. Presence of fish resulted in lower biomass of zooplankton and higher biomasses of phytoplankton, ciliates and total plankton. The cascading effects of fish were strongest in the shallow mesocosms as evidenced by a lower zooplankton contribution to total plankton biomass and lower zooplankton:ciliate and HNF:bacteria biomass ratios. Our results suggest that

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a lowering of the water level in warm shallow lakes will enhance the contribution of bacteria, HNF and ciliates to the plankton biomass, likely due to increased density of submerged macrophytes (less phytoplankton); this effect will, however, be less pronounced in the presence of fish.

**Keywords** Bacteria · Ciliates · Heterotrophic nanoflagellates · Macrophytes · Phytoplankton

## Introduction

Water level fluctuations (WLFs) are of critical importance in shallow lakes (Coops et al., 2003; Leira & Cantonati, 2008). WLFs, often triggered by natural variations in net precipitation, are thought to have a decisive effect on the ecology, functioning and management of shallow lakes located in semi-arid regions via their effects on nutrient concentrations (Jeppesen et al., 2009, 2011; Özen et al., 2010), macrophyte growth (Gafny et al., 1992; Coops et al., 2003; Havens et al., 2004; Beklioglu et al., 2006; Özkan et al., 2010; Bucak et al., 2012), phytoplankton growth (Naselli-Flores, 2003) as well as on invertebrate and fish community structure and abundance (Leira & Cantonati, 2008; Özkan et al., 2010; Bucak et al., 2012). Due to the present global climate change, WLFs may become just as significant as nutrients for the change in the functioning of shallow lakes in the future, especially in the Mediterranean region (Coops et al., 2003; Beklioglu et al., 2007; Bucak et al., 2012).

How WLFs affect the microbial community structure is relatively unexplored, and experimental evidence is limited of direct and indirect effects through changes in nutrient availability, macrophyte coverage and zooplankton grazing on the structure of the microbial community (Tzaras et al., 1999; Farjalla et al., 2006). The structure of the microbial community changes with lake trophic state (Carrick et al., 1991; Nixdorf & Arndt, 1993; Gaedke & Straile, 1994), and in eutrophic lakes the transfer of carbon from the primary producers through the microbial community to the higher trophic levels decreases (Weisse, 1991; Muylaert et al., 2002; Auer et al., 2004; Saad et al., 2013). The impact of macrophytes on the microbial community is two-sided. Macrophytes may promote bacterial growth and biomass by providing dissolved organic carbon (DOC) to

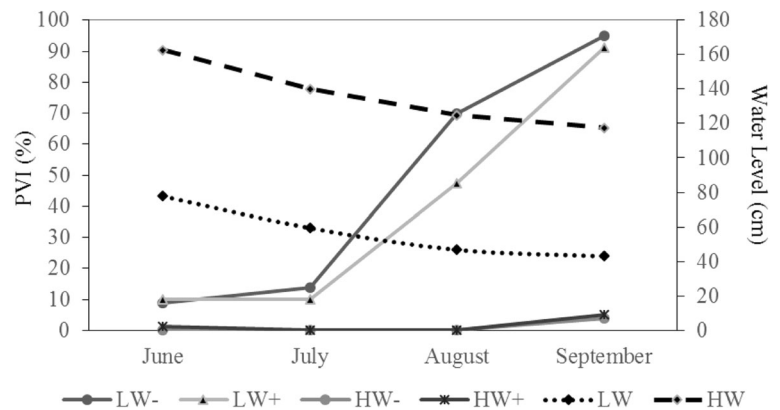
bacteria in the water column (Wetzel & Søndergaard, 1998; Wilcock et al., 1999; Stanley et al., 2003), but they may also negatively affect bacteria production and phytoplankton via allelopathy (Stanley et al., 2003). Moreover, by acting as a refuge for large-bodied zooplankton, macrophytes may negatively affect the abundance of bacteria, heterotrophic nanoflagellates (HNF) and ciliates through enhanced competition with and predation by the zooplankton (Jürgens & Jeppesen, 2000; Zingel & Nöges, 2008).

Several studies have elucidated the top-down effects of zooplankton on the biomass and community structure of the microbial community (Jürgens et al., 1994; Jürgens & Jeppesen, 2000; Wickham, 1998; Zöllner et al., 2003, 2009), and results show that the impacts of fish-mediated trophic cascades on microbial loop processes can be strong (Riemann, 1985; Pace & Funke, 1991; Pace & Cole, 1994; Fonte et al., 2011; Nishimura et al., 2011). Some studies have revealed that the presence of planktivorous fish changes the biomass and composition of the zooplankton community (Riemann, 1985; Christoffersen et al., 1993), which, in turn, influences the biomass of bacteria (Riemann, 1985; Markosova & Jezek, 1993) via the altered grazing pressure on bacteria by zooplankton. Fish, may, however, also affect the microbial community via bottom-up control, nutrient excretion and regeneration, inducing an increase in nutrient concentrations and phytoplankton biomass (Vanni & Layne, 1997; Vanni, 2002; Roozen et al., 2007; Nishimura et al., 2011).

Although there are numerous studies on the effect of fish predation on the structure of phytoplankton and microbial communities (e.g., Christoffersen et al., 1993; Nishimura et al., 2011) and a few examples of water level change effects on the structure of the microbial community (Tzaras et al., 1999; Farjalla et al., 2006), the combined effect of water level change and fish on aquatic food webs as a whole, including zooplankton, phytoplankton, ciliate, HNF and bacteria, has, to the best of our knowledge, never been assessed before.

Bucak et al. (2012) studied the influence of water level (0.8–1 and 1.6–1.7 m at the beginning of the experiment) and fish on macrophyte growth and trophic interactions in a mesocosm set-up in Lake Eymir, located in central Anatolia, Turkey. During the experiment, the water depth decreased by  $0.41 \pm 0.06$  m in all mesocosms (Fig. 1) and macrophytes, expressed as the proportion of plant volume

**Fig. 1** Monthly percent plant volume inhabited (PVI %) in low water level fishless (LW-), low water level with fish (LW+), high water level fishless (HW-) and high water level with fish (HW+) mesocosms and changes in mean water level in low (LW) and high (HW) water level mesocosms throughout the experiment (data from Bucak et al., 2012)



inhabited, became, most abundant in the shallow fishless mesocosms, while macrophyte growth was limited at the higher water depth (Fig. 1). They further reported higher chlorophyll *a* (chl-*a*) in the low water level-fish (LW+) mesocosms than in the other mesocosms. Moreover, they found a shift in the zooplankton community from large-bodied zooplankton to small-bodied zooplankton during the experiment, and the biomass of cladocerans was higher in the fishless mesocosms than in those with fish. The present study was undertaken in the same experimental set-up, from June to September 2009, and focused on ciliates, HNF and bacteria, hereafter called the microbial community, in the water column.

We hypothesised that (i) water level would indirectly influence the biomass of bacteria and phytoplankton by affecting the growth of submerged macrophytes; (ii) presence of fish would alter the top-down control of the microbial community through reduced zooplankton grazing; and (iii) interactions between water level and fish treatments would be important, with fish effects being stronger at higher water level due to lower abundance of macrophytes.

## Materials and methods

### Study site and experimental design

The study was conducted in Lake Eymir, which has a surface area of 125 ha and a mean depth of 3.1 m. This lake was chosen as it has demonstrated high sensitivity to water level changes (Beklioğlu & Tan, 2008; Özen et al., 2010). During the study period, the lake was eutrophic with total phosphorus (TP) and chl-

*a* concentrations varying between 162–253 and 30–82  $\mu\text{g l}^{-1}$ , respectively. Detailed information about the history of the lake can be found in several publications (Beklioğlu et al., 2003; Tan & Beklioğlu, 2006; Beklioğlu & Tan, 2008; Özen et al., 2010).

The experiment was carried out in 16 cylindrical-shaped mesocosms with a diameter of 1.2 m combining two contrasting depths (low water level, 0.8 m—LW and high water level, 1.6 m—HW, respectively); 8 mesocosms were placed at each water depth of which half had fish (+), while the other half was fishless (-). The effect of WLFs was simulated by placing mesocosms at different water depths (0.8 and 1.6 m) in the lake. The mesocosms were kept open to the sediment and to the atmosphere. Before the mesocosms were set-up, macrophytes were removed by scuba divers. After set-up, the mesocosms were left for a week to recover from disturbance. Zooplankton collected from the lake with a 50- $\mu\text{m}$  plankton net were inoculated in all mesocosms as were ten shoots of *Potamogeton pectinatus*. To imitate the natural fish density of the lake, 12 small fish (<10 cm), six *Tinca tinca* and six *Alburnus* spp., were stocked to half of the mesocosms at each depth.

TP and total nitrogen in the mesocosms ranged between 77–441 and 749–2,200  $\mu\text{g l}^{-1}$ , respectively. Bucak et al. (2012) reported that the LW mesocosms had higher TN concentrations than the HW mesocosms and that both reduced water level and presence of fish had a significant positive effect on TP concentrations.

### Sampling procedures and analyses

Phytoplankton biomass was estimated from the chl-*a* in the water using a carbon to chl-*a* ratio of 30 (Reynolds,

1984). The carbon (C) content of zooplankton was calculated using a conversion factor of  $0.48 \mu\text{g C per } \mu\text{g dry weight}$  (Andersen & Hessen, 1991).

The microbial community was sampled monthly between June and September 2009. From each mesocosm, a 4 l composite water sample integrating the whole water column was taken using a tube sampler without disturbing the bottom or the macrophytes. The water samples were used for enumeration of the microbial communities, including bacteria, HNF and ciliates. From the bulk water sample, 50 ml subsamples for bacteria and HNF and a 100 ml subsample for ciliate analyses were taken. Samples for enumeration of bacteria and HNF were fixed immediately after collection by adding glutaraldehyde (Sigma, Taufkirchen, Germany) to a final concentration of 2% (v/v). Ciliates were fixed with acidic Lugol (4% Lugol's iodine (v/v)).

#### Enumeration of the microbial community

Subsamples for bacteria and HNF analyses were stained for 10 min with 4',6-diamidino-2-phenylindole (DAPI, Sigma, Taufkirchen, Germany) at a final concentration of  $10 \mu\text{g DAPI ml}^{-1}$  (Porter & Feig, 1980). A Whatman GF/C glass microfiber filter with a pore size of  $1.2 \mu\text{m}$  as a pad was used to obtain a uniform distribution of cells under low pressure ( $<0.2 \text{ bar}$ ). Within 2 h following sampling, we filtered the subsamples to count bacteria (2 ml) and HNF (15 ml) onto  $0.2$  and  $0.8 \mu\text{m}$  pore size black Nuclepore filters, respectively. The filters were stored at  $-20^\circ\text{C}$  until enumeration. The abundances of bacteria and HNF were determined by direct counting of cells using epifluorescence microscopy (Leica, DM 6000B, Wetzlar, Germany) at  $1,500\times$  magnification. At least 400 bacteria cells from different fields were counted for each sample with a UV filter (420 nm). All HNF specimens found within  $1.6 \text{ mm}^2$  of each filter (randomly selected) were counted. The microscope was equipped with a UV (420 nm) and a blue (515 nm) filter to distinguish heterotrophs from mixo- and autotrophs for HNF counting. Conversion to carbon biomass was made using a factor of  $0.22 \text{ pg C } \mu\text{m}^{-3}$  for bacteria and HNF (Bratbak & Dundas, 1984; Borsheim & Bratback, 1987).

Counting of ciliates was performed in sedimentation chambers following Utermöhl (1958) in an inverted microscope with  $630\times$  magnification (Leica DMI 4000B, Wetzlar, Germany). At least 200 ciliate cells or

the entire chamber were counted and identified to genus or species level according to Foissner & Berger (1996) and Foissner et al. (1999). The biovolume of ciliates was calculated from measurements of length and width dimensions approximated to an appropriate geometric shape. Conversion to carbon biomass was done using the factor  $0.14 \text{ pg C } \mu\text{m}^{-3}$  (Putt & Stoecker, 1989).

#### Statistical analyses

To test for treatment effects (water level and fish) with time (months), we applied repeated measures ANOVA (RM-ANOVA) using the SAS 9.2 software (SAS Institute Inc, Cary, NC, USA). Data were log-transformed before analysis to reduce skewness and to approximate to normal distribution.

## Results

### Microbial community

#### *Bacteria biomass*

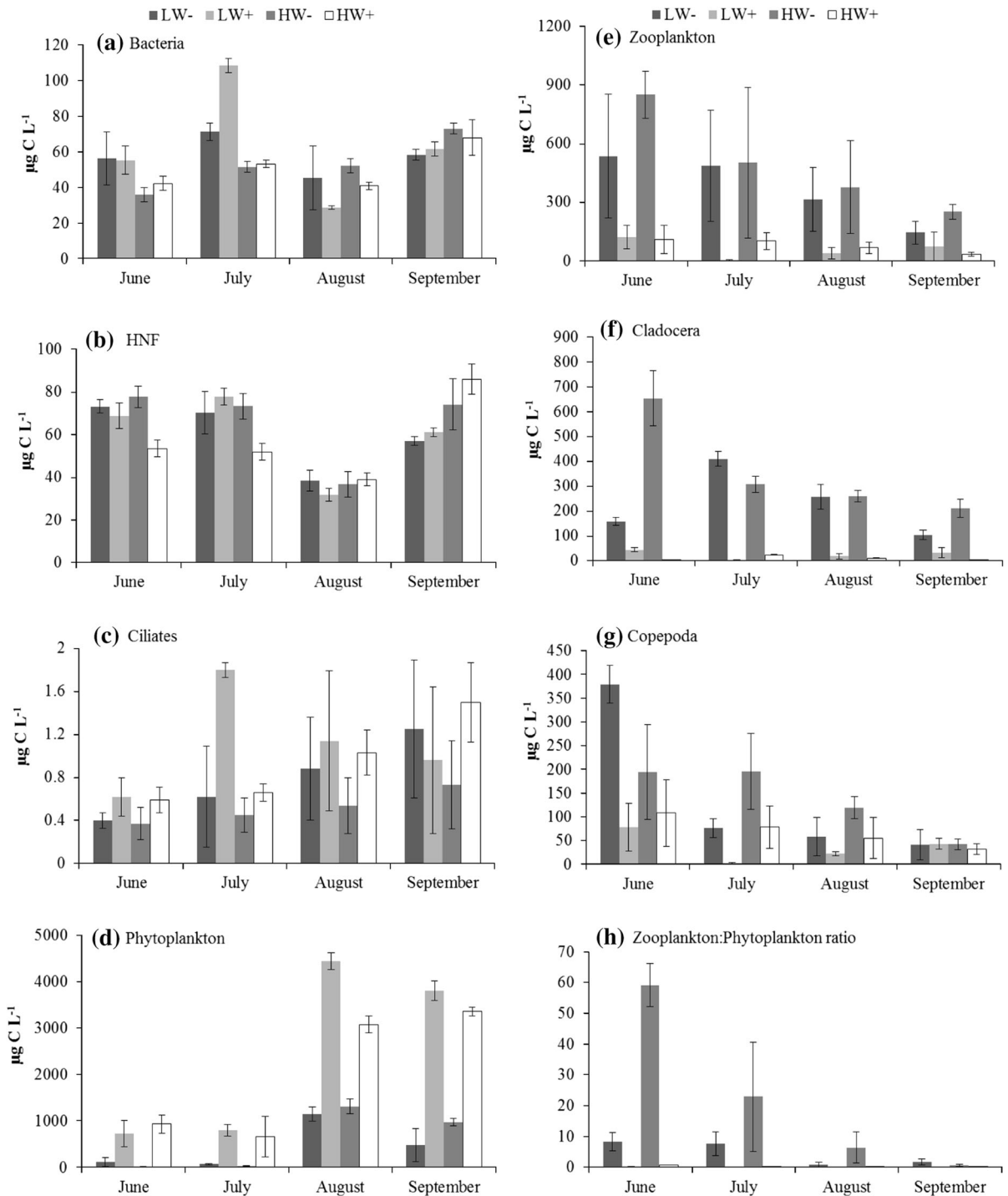
Bacterial abundance ranged between 0.5 and  $1.9 \times 10^6 \text{ cells ml}^{-1}$  and the biomass between 28 and  $109 \mu\text{g C l}^{-1}$ . A significant negative effect of water level on bacterial biomass was observed (Fig. 2a; Table 1).

#### *Heterotrophic nanoflagellates (HNF)*

HNF abundance ranged between 0.7 and  $1.7 \times 10^4 \text{ cells ml}^{-1}$  and the biomass between 39 and  $86 \mu\text{g C l}^{-1}$ . An interactive negative water level-fish effect on HNF biomass was recorded (Fig. 2b; Table 1). In August, there was a noticeable decrease in the HNF biomass in all mesocosms and it increased again in September (Fig. 2b). The HNF:bacteria biomass ratio was lower in the LW+ mesocosms and significantly higher in the LW- and HW- mesocosms (Fig. 3; Table 1).

#### *Ciliates*

Ciliate abundance ranged between 0.2 and 2 individuals  $\text{ml}^{-1}$  and the biomass between 0.4 and  $1.8 \mu\text{g C l}^{-1}$  (Fig. 2c). Oligotrichida dominated in



**Fig. 2** Monthly biomasses ( $\pm 1$ SD) of (a) bacteria, (b) HNF, (c) ciliates, (d) phytoplankton, (e) total zooplankton, (f) Cladocera, (g) Copepoda and (h) the zooplankton:phytoplankton ratio

in low Water level fishless (LW-), low Water level with fish (LW+), high Water level fishless (HW-) and high Water level with fish (HW+) mesocosms

**Table 1** Summary of the univariate repeated measures of two-way ANOVA testing the effect of water level and fish on the biomass of microbes and other plankton

	Water level (WL)	Fish (F)	WL × F
Bacteria	*** ↓	NS	NS
HNF	NS	**	** ↓
Ciliate	NS	** ↑	NS
T. microbial community	**	NS	** ↓
% T. microbial community	NS	** ↓	NS
Phytoplankton	* ↑	*** ↑	NS
% Phytoplankton	NS	*** ↑	NS
Zooplankton	NS	** ↓	NS
% Zooplankton	NS	*** ↓	NS
Cladocera	NS	** ↓	NS
Copepoda	NS	* ↓	NS
The whole community	NS	*** ↑	NS
HNF:bacteria	** ↑	* ↓	NS
Ciliate:bacteria	NS	* ↑	NS
Ciliate:HNF	NS	* ↑	NS
Copepoda:bacteria	NS	* ↓	NS
Copepoda:HNF	NS	* ↓	NS
Copepoda:ciliate	NS	* ↓	NS
Copepoda:phytoplankton	*	**	* ↓
Cladocera:bacteria	NS	* ↓	NS
Cladocera:HNF	NS	** ↓	NS
Cladocera:ciliate	NS	* ↓	NS
Cladocera:phytoplankton	NS	* ↓	NS
Zooplankton:bacteria	NS	* ↓	NS
Zooplankton:HNF	NS	** ↓	NS
Zooplankton:ciliate	NS	* ↓	NS
Zooplankton:phytoplankton	NS	* ↓	NS
Bacteria:phytoplankton	***	***	*** ↓
HNF:phytoplankton	***	***	*** ↓
Ciliate:phytoplankton	*	***	* ↓

Arrows show the direction of the treatment effect on the organisms and ratios

NS not significant

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

most samples, including the genera *Strobilidium* and *Strombidium*. RM-ANOVA showed no significant effect of water level on ciliate biomass, whereas a positive effect of fish on ciliate biomass was observed (Fig. 2c; Table 1). Fish also had a significant positive effect on the ciliate:bacteria and ciliate:HNF biomass ratios (Fig. 3; Table 1).

### Interaction between phytoplankton, zooplankton and the microbial community

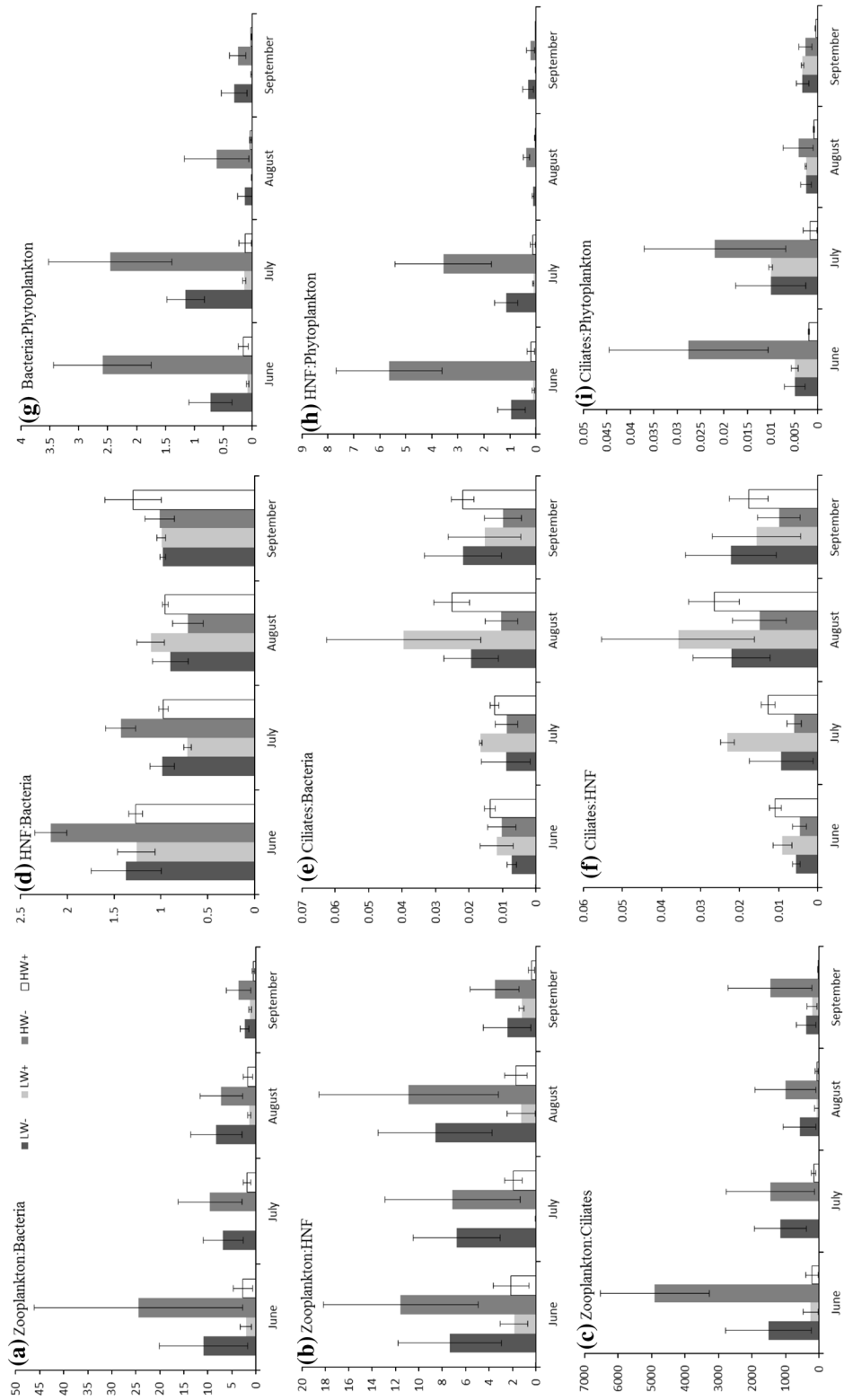
Phytoplankton biomass was estimated from chl-*a* concentrations and ranged between 16 and 4,440  $\mu\text{g C l}^{-1}$  (Fig. 2d). We found a significant negative effect of water level–fish interactions on the bacteria:phytoplankton, HNF:phytoplankton and ciliate:phytoplankton biomass ratios (Fig. 3; Table 1).

Total zooplankton biomass ranged between 4 and 850  $\mu\text{g C l}^{-1}$  (Fig. 2e). We found no significant effect of water level on zooplankton biomass, whereas a negative fish effect occurred (Table 1). We recorded a significant negative effect of fish on the zooplankton:phytoplankton, zooplankton:bacteria, zooplankton:HNF and zooplankton:ciliate biomass ratios (Fig. 3; Table 1). The zooplankton:phytoplankton biomass ratio was high in the fishless mesocosms and low in the mesocosms with fish (Fig. 2h).

Cladoceran biomass ranged between 2 and 654  $\mu\text{g C l}^{-1}$  in the monthly samples (Fig. 2f). A negative fish effect was recorded on cladoceran biomass (Table 1). We found a negative effect of fish on the Cladocera:phytoplankton, Cladocera:bacteria, Cladocera:HNF and Cladocera:ciliate biomass ratios (Fig. 3; Table 1). Copepoda biomass ranged between 2 and 379  $\mu\text{g C l}^{-1}$  in the monthly samples (Fig. 2f). A negative effect of fish on copepod biomass was traced (Table 1). The Copepoda:bacteria, Copepoda:HNF and Copepoda:ciliate biomass ratios were also significantly and negatively affected by fish (Fig. 3; Table 1). The interaction of water level and fish had a negative impact on the Copepoda:phytoplankton biomass ratio (Fig. 3; Table 1).

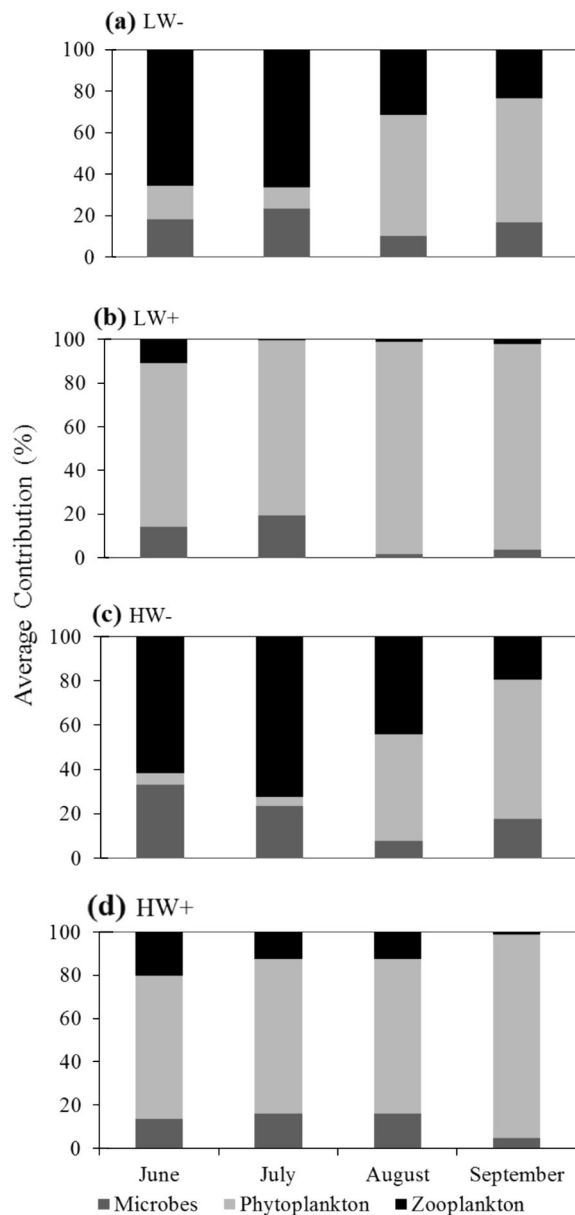
### Contribution of zooplankton, phytoplankton and microbes to plankton biomass

The contributions of zooplankton and the microbial communities to total plankton biomass were significantly higher in the fishless mesocosms (Fig. 4). We found no effect of water level or water level–fish interactions on the relative contribution of the three plankton groups to total plankton biomass; a negative effect of fish was recorded on the microbial and zooplankton community contribution, while a positive fish effect was observed for phytoplankton (Table 1).



**Fig. 3** Monthly ratios ( $\pm$ 1SD) of **(a)** zooplankton:bacteria, **(b)** zooplankton:HNF, **(c)** zooplankton:ciliates, **(d)** HNF:bacteria, **(e)** ciliates:bacteria, **(f)** ciliates:HNF, **(g)** bacteria:phytoplankton, **(h)** HNF:phytoplankton and **(i)** Ciliates:phytoplankton in low water level fishless (LW-), low water level with fish (LW+), high water level fishless (HW-) and high water level with fish (HW+) mesocosms





**Fig. 4** Average contribution (%) of zooplankton, phytoplankton and microbes (the sum of HNF, ciliates and bacterioplankton) to total plankton biomass in low water level fishless (LW-), low water level with fish (LW+), high water fishless (HW-) and high water level with fish (HW+) mesocosms

## Discussion

We found a strong effect of fish on zooplankton, which abated gradually towards the lower trophic levels at both high and low water levels, while the effect of water level difference was comparatively weak: the

contribution of the microbial community to total plankton biomass was higher in the low water mesocosms at the expense of phytoplankton, likely reflecting the observed higher density of submerged macrophytes, the depth effect being lower in the presence of fish.

Mesocosms with fish had a higher biomass of ciliates and slightly lower biomass of HNF. The higher biomass of ciliates likely reflects reduced predation and competition from zooplankton as a result of fish predation on the zooplankton. This is supported by the much lower zooplankton biomass and the lower biomass ratios of total zooplankton, cladocerans and copepods to ciliates in the mesocosms with fish than in the fishless mesocosms. Higher ciliate biomass and thus presumably higher ciliate predation on HNF may explain the lower HNF biomass (higher ciliate:HNF biomass ratios) in the mesocosms with fish, as has also been observed in other studies though often with stronger effect on HNF (and bacteria) (e.g., Pace & Funke, 1991; Müller-Solger et al., 1997; Wickham, 1998; Yamazaki et al., 2010; Nishimura et al., 2011), also in mesocosms open to the sediment (Jürgens & Jeppesen, 2000; Jeppesen et al., 2002).

However, despite lower HNF:bacteria, lower Cladocera:bacteria biomass ratios and lower biomasses of HNF and Cladocera, we did not find any difference in bacterial biomass between the mesocosms with and without fish. Apparently, the bacteria did not take advantage of the reduced predation on them as also evidenced by the lower bacteria:phytoplankton biomass ratio. Moreover, a higher ciliate:bacteria ratio in the fishless mesocosms did not result in a lower bacterial biomass than in the mesocosms with fish, perhaps reflecting that lower zooplankton grazing on phytoplankton (a lower zooplankton:phytoplankton biomass ratio) may lead to lower overall DOC production (decreasing algal carbon assimilation by zooplankton, decreasing defecation, sloppy feeding of zooplankton), implying lower availability of organic carbon for bacteria growth per unit of algal biomass (Gasol & Duarte, 2000). Accordingly, the presence of fish also negatively affected the contribution of the total microbial community to plankton biomass with increasing phytoplankton biomass.

A water level increase, both in the presence and absence of fish, enhanced the phytoplankton biomass and lowered the bacteria biomass and led to increased



HNF: bacteria ratios. This higher phytoplankton biomass likely reflects the concurrent lower macrophyte coverage (Bucak et al., 2012). Fish–water level interactions were demonstrated by lower biomass ratios of bacteria, HNF and ciliates against phytoplankton at high water level in the presence of fish, mainly reflecting a higher phytoplankton biomass. Moreover, during the experiment a declining water level resulted in higher PVI% of submerged macrophytes in the shallow mesocosms (Fig. 1, Bucak et al., 2012) and, concurrently, the biomass of phytoplankton decreased and the bacteria biomass increased, not least when fish were absent (Fig. 3). Nõges et al. (2010) found that increasing mean depth in shallow Lake Vortsjärv, Estonia, had a negative effect on the phytoplankton biomass in summer and autumn and attributed this to light limitation during periods with high water level. In contrast, we found positive effects of an increasing water level on phytoplankton biomass, attributable to an increase in submerged macrophyte PVI% in shallow water. However, DOC derived from macrophytes is probably of lower quality than DOC derived from phytoplankton (Zhang et al., 2013), higher bacterial biomass at higher submerged macrophyte PVI% may reflect enhanced bacteria growth stimulated by organic matter released from the plant-periphyton community (Wetzel & Søndergaard, 1998; Wilcock et al., 1999; Stanley et al., 2003), but also lower grazing by HNF (lower HNF:bacteria biomass ratio) may have contributed. Higher availability of nutrients in the shallow mesocosms may be another contributory factor as bacterial biomass generally increases with lake trophity (Biddanda et al., 2001; Cotner & Biddanda, 2002; Muylaert et al., 2002; Auer et al., 2004).

In summary, strong effects of fish were found on the zooplankton. These weakened through the food chain to ciliates, HNF and with no effect on bacterioplankton, whereas the direct effect of water level variations was overall weak. Indirect effects of changes in water level via macrophyte cover (higher macrophyte growth in low water mesocosms) and zooplankton (higher cascading effect of fish in low water mesocosms) were, however, observed for phytoplankton, ciliates and HNF. We found higher bacteria and lower phytoplankton biomasses in the shallow mesocosms, coinciding with higher submerged macrophyte density. The lowest biomass ratios of bacteria, HNF and ciliates against phytoplankton and the lowest total microbial and HNF

biomasses were recorded in the deep mesocosms with fish. Our study is correlative and measurements of processes such as grazing losses and bacterial production would have provided a more solid picture of the dynamics and regulating factors of the microbial community (top-down or bottom-up processes) in these systems. Our results suggest, however, that a lowering of the water level in warm shallow lakes will enhance the importance of the microbial community at the expense of phytoplankton, likely reflecting higher density of submerged macrophytes; the effect will be less pronounced in the presence of fish.

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