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Response of heterotrophic bacteria, autotrophic picoplankton and heterotrophic nanoflagellates to re-oligotrophication

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We investigated the response of the microbial components of the pelagic food web to re-oligotrophication of large, deep Lake Constance where total phosphorus concentrations during mixing decreased from a maximum of 2.81 μmol L ^{1} in 1979 via 1.87 μmol L ^{1} in 1987 to 0.26 μmol L ^{1} in 2007. Measurements of heterotrophic bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF) in 2006 and 2007 were compared to values from 1987 to 1997. We hypothesized that the biomass and seasonal variability of all groups will decrease under more oligotrophic conditions due to reduced resource availability, particularly for APP and HNF but less for the competitively stronger bacteria. Average bacterial biomass between spring and autumn was unrelated to phosphorus, whereas the ratio of bacterial biomass to chlorophyll a concentration increased with decreasing trophy due to declining chlorophyll concentrations. In contrast, a unimodal relationship was found between APP and phosphorus with low biomass at low and high phosphorus concentrations and maximum biomass in between. Average HNF biomass decreased strongly by a factor of 10 30 with decreasing trophy, and chlorophyll-specific HNF biomass was unimodally related to phosphorus. The relative seasonal biomass variability did not change for any group during re-oligotrophication. To conclude, HNF responded much more strongly and bacteria less so than chlorophyll concentrations to oligotrophication, whereas APP exhibited a more complex pattern.

INTRODUCTION

Natural or anthropogenically induced disturbances provide valuable information on the complex functional relationships and feedback mechanisms in natural food webs. For example, many lakes worldwide underwent an eutrophication process during the second half of the last century. Mainly the increased phosphorus inputs led to enhanced levels of phytoplankton biomass, chlor ophyll concentration and pelagic primary production influencing also the microbial food web and higher trophic levels such as zooplankton and fish (e.g. Jeppesen *et al.*, 2000). Furthermore, the utility of lakes, e.g. for drinking water supply or for recreation, deterio rated. Therefore, intensive restoration efforts were undertaken for many lakes to reduce the external phos phorus load (Sas, 1989), to remove the phosphorus from the water by chemical precipitation (Deppe *et al.*, 1999; Mehner *et al.*, 2008) and/or to reduce algal biomass (e.g. by food web manipulation; e.g. Benndorf, 1990; Søndergaard *et al.*, 2008). As a consequence, in lake phosphorus concentrations decreased in numerous lakes (re oligotrophication) with concomitant changes in the biomass of phytoplankton and higher trophic levels (Jeppesen *et al.*, 2005). Today, re oligotrophication is

required by law for many eutrophic lakes in Europe (Water Framework Directive of the European Union). However, our understanding of re oligotrophication effects on pelagic food webs is almost entirely restricted to the classical food chain, whereas hardly any studies document the response of the microbial food web.

One well documented example of the response of the pelagic food web to eutrophication and re oligotro phication is that of the deep, large Lake Constance located between Germany, Austria and Switzerland. The plankton community is largely autochthonous, and studies of the planktonic food web including the microbial part and of its regulating factors started very early on resulting in long term data sets. The eutrophi cation which began early in the 20th century, reached concentrations of total phosphorus at winter circulation of 0.29 μM in 1959 and peaked around 1979 with 2.81 μM (87 μg P L ^{1}; Güde *et al.*, 1998). As a conse quence of sewage treatment and phosphorus substi tution in detergents (Güde *et al.*, 1998), the concentration decreased continuously to 0.71 μM in 1996. Due to the pronounced change in total phos phorus, this lake provides good opportunities to analyze the response of a pelagic food web to re oligotrophication (Bäuerle and Gaedke, 1998). The Special Collaborative Program "Cycling of matter in Lake Constance" enabled measurements of all plankton groups including bacteria (Simon *et al.*, 1998), auto trophic picoplankton (APP; Weisse, 1988; Gaedke and Weisse, 1998) and heterotrophic nanoflagellates (HNF; Weisse and Müller, 1998). According to expectations, total phytoplankton biovolume decreased from 30 to 15 cm^{3} m ^{2}, primary production from 1.5 to 1 g C m ^{2} day ^{1} and Secchi depth increased from 4 to 6 m during the period 1979 1996 (Gaedke, 1998b; Häse *et al.*, 1998; Anneville *et al.*, 2005). In contrast, bacterial, APP, ciliate and crustacean biomass showed no clear trend during this period (Gaedke, 1998b; Simon *et al.*, 1998; Gaedke and Weisse, 1998; Straile and Geller, 1998; Gaedke and Wickham, 2004). Interestingly, the biomass of mixotrophic algae such as *Dinobryon* spp. increased (Kamjunke *et al.*, 2007) which was attributed to the uptake of phosphorus by bacterivory and to the increasing light availability due to the decreasing biomass of other phytoplankton resulting in a release from self shading.

During the subsequent 10 years (1997 2007), the in lake total phosphorus concentration declined further to 0.26 μM (8 μg P L ^{1}) strongly enhanced phosphorus depletion motivating follow up investigations. The present study focuses on the response of the microbial components of the pelagic food web, particularly on the heterogeneous groups of heterotrophic bacteria, APP and heterotrophic nanoflagellates. Biomass of bacteria, APP and HNF were measured during 2006 and 2007, compared with data from 1987 to 1997, and are dis cussed in relation to phosphorus and chlorophyll *a* con centration. In general, we expect decreasing biomass of these groups due to reduced resource availability, par ticularly for APP and HNF but less for bacteria which are stronger competitors for phosphorus (Currie and Kalff, 1984) and often top down controlled (Simon *et al.*, 1998). Furthermore, the seasonal variability of organisms is expected to decrease under more oligo trophic conditions when resources for pronounced bloom formation are lacking as it was described for the classical food web (the PEG model; Sommer *et al.*, 1986).

METHOD

Study site and sample collection

Lake Constance (47°40′N, 9°20′E) is a large and deep prealpine lake with a surface area of 472 km^{2} and a mean depth of 101 m. Sampling took place at the deepest site (147 m) of the north western basin (Überlinger See). Water samples from the euphotic layer were taken weekly in most cases and every 2 to 4 weeks during winter. The water from a 2 m long tube sampler was pooled for the depth intervals 0 8 m and 8 20 m prior to counting. For all analyses, we used a weighted average of the biomass of the water strata 0 8 m and 8 20 m. Biomass of bacteria, APP and HNF was deter mined for 1987 1997, 1987 1996 and 1987 1992, respectively, and for 2006 2007.

Plankton biomass

Plankton samples were preserved with formalin (1%, final concentration). Counting was done within a few months after sampling. Organisms were stained with DAPI (4′6 diamidino 2 phenylindole; 1 mg L ^{1}, final concentration) according to Porter and Feig (Porter and Feig, 1980). Bacteria and APP were collected on 0.2 μm Nuclepore filters (Whatman), whereas HNF samples were filtered onto 0.8 μm Nuclepore filters. Cells were counted by epifluorescence microscopy (Zeiss, magnifi cation ×1000, UV excitation for DAPI and green exci tation for chlorophyll *a*). The sizes of bacteria, APP and HNF were measured using an eyepiece micrometer esti mating 50, 50 and 35 cells per filter, respectively. Cell volumes were estimated according to simple geometric shapes. Consistent with previous studies, the carbon content of bacteria was calculated following Simon and

Azam (Simon and Azam, 1989). For APP and HNF, a carbon content of 19% and 22% of fresh weight was assumed, respectively (Gaedke, 1992). Chlorophyll *a* concentration was measured spectrophotometrically after extraction with hot ethanol and corrected for phaeopigments after acidification (Häse *et al.*, 1998).

Calculations

Biomass averages were calculated for the growing season between spring and autumn to account for the fact that direct responses to re oligotrophication are not to be expected during winter when light limitation, deep mixing and high phosphorus concentrations prevail. The growing season lasted from early spring (starting with the first algal spring bloom in March or April) until the end of autumn (mid of November; modified after Gaedke, 1998a). In contrast to plankton biomass, for total phosphorus we used the concen tration after late winter mixing as a measure of the available resource at the beginning of the growing season. As a measure of relative seasonal biomass varia bility, we calculated the coefficient of variance of biomass measured between spring and autumn of each year.

RESULTS

Chlorophyll *a* concentration showed the highest peaks in spring and decreased during the period 1987 1997 (Fig. 1). Concentrations were lower in 2006 and 2007. Bacterial biomass was high during spring and summer and lower during the clear water phase and in winter during 1987 1997 (Fig. 2). The peaks ranged between 40 and 90 μg C L ^{1}. In 2006, the bacterial biomass was relatively low except for a peak in early August. In contrast, it was higher in 2007 when it peaked in early May and reached a maximum of 154 μg C L ^{1} in late August. Bacteria were relatively small in 2006: the size class <10 fg C cell ^{1} contributed considerably to the total biomass, and large filaments were not observed (Table I). Conversely, small bacteria were rare in 2007, but large filaments contributed significantly and were responsible for the bulk of the biomass maximum in late August. The APP biomass reached summer maxima ranging between 10 and 45 μg C L ^{1} and low winter values in the period 1987 1996 (Fig. 2). In 2006 and 2007, APP biomass was as low as during 1995 1996 with summer peaks of 10 15 μg C L ^{1}. The size of APP was not considered specifically since no change was expected as it is defined as comprising only cells <2 μm. The HNF biomass showed maxima between 12 and 23 μg C L ^{1} in the period 1987 1992 and was much lower in 2006 and 2007 (Fig. 2). Peaks of only 0.5 1.5 μg C L ^{1} were observed in August 2006 and in April and July 2007. Most of the HNF were small in 2006 and the biomass was almost equally distributed over the size classes, whereas HNF size was larger and small cells contributed less to total biomass in 2007 (Table I).

The seasonal dynamics of biomass differed between years which were different also in water temperature: the average temperature (December to March) in the water layer 0 20 m was 4.62 ± 1.03°C (mean $\pm$ SD) in winter 2005/2006 and 6.19 ± 1.30°C in winter 2006/ 2007 (Straile *et al.*, unpublished results). Spring peaks of bacteria and HNF occurred after the mild winter in 2007 but not after the cold winter in 2006, whereas summer peaks were observed independent of winter temperature for bacteria and HNF (Fig. 3). In 2006, biomass of all groups showed lower values compared with the other years during most of the time. In 2007, bacterial biomass fell mostly within the range of former years, APP biomass was lower in spring but within the average range in summer and HNF biomass was con sistently lower than during 1987 1992. The seasonal variability of biomass between spring and autumn (CV, mean $\pm$ SD) was lowest for bacteria (1987 1997: 0.38 ± 0.12, 2006: 0.48, 2007: 0.54), highest for APP (1987 1996: 0.81 ± 0.22, 2006: 0.84, 2007: 0.59) and intermediate for HNF (1987 1992: 0.71 ± 0.11, 2006: 1.01, 2007: 0.68). There was no long term trend in variability: we did not find significant relationships between the coefficients of variation and the total phos phorus concentration for any group of organisms (neither for the period 1987 1997 nor for 1987 2007). This also holds when including winter values (data not shown).

Chlorophyll specific biomass of bacteria showed the highest peaks in 1988 and 2007 (Fig. 4). Specific APP biomass was maximal in 1993, whereas it was low in 2006 and 2007. Values of specific HNF biomass were much lower in 2006 and 2007 than during the period 1987 1992. The mean seasonal biomass of bacteria and HNF were unrelated to total phosphorus concen tration for the periods 1987 1997 and 1987 1992, respectively ($P > 0.8$, linear regressions). If the data of 2006 and 2007 were included, the regression for bac teria remained non significant (Fig. 5). As chlorophyll *a* concentrations declined, there was a negative relation ship between the ratio of bacterial biomass to chloro phyll *a* concentration and total phosphorus (y $0.107x + 9.16$, P 0.007, r^2 0.50; Fig. 6). Mean APP biomass exhibited a unimodal relationship versus total phosphorus for the period 1987 1996 with

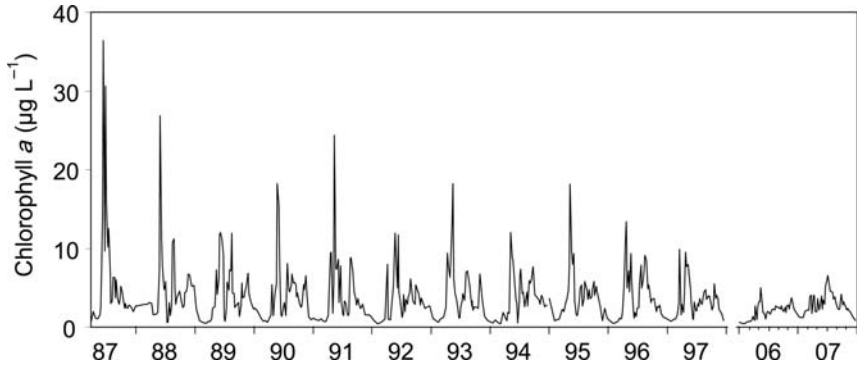

Fig. 1. Dynamics of chlorophyll *a* concentration in Lake Constance between 1987 and 1997 and in the years 2006 and 2007.

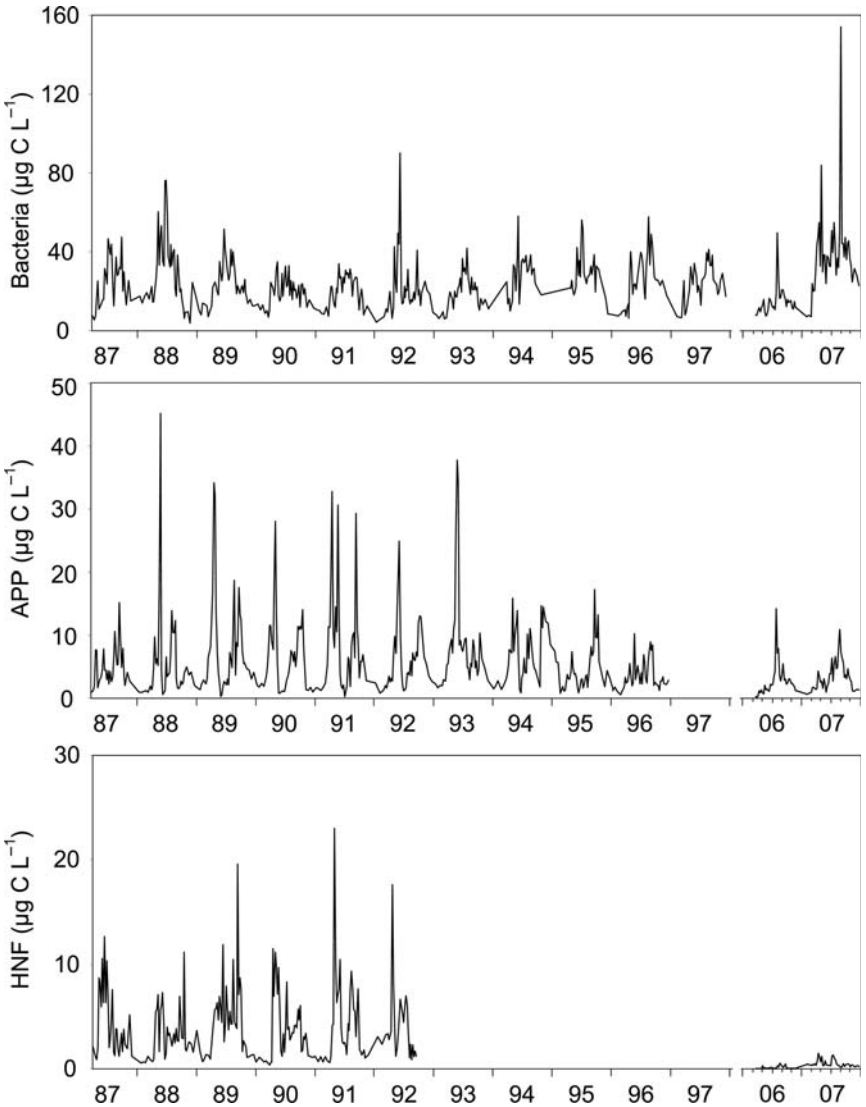

Fig. 2. Dynamics of carbon biomass of bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF) in Lake Constance between 1987 and 1997 and in the years 2006 and 2007. Values of the period 1987–1997 based on abundance data of Simon *et al.* (Simon *et al.*, 1998), Weisse (Weisse, 1988), Gaedke and Weisse (Gaedke and Weisse, 1998) and Weisse and Müller (Weisse and Müller, 1998).

Table I: Contribution (%) of different size classes of bacteria and heterotrophic nanoflagellates (HNF) to their total biomass in 2006 and 2007 (between spring and autumn) in Lake Constance

	Cell biomass	2006	2007
Bacteria	<10 fg C	35	6
	10 100 fg C	65	73
	>100 fg C	0	21
HNF	<1 pg C	40	3
	1 10 pg C	27	69
	>10 pg C	33	28

low biomass at low and high phosphorus concentrations and maximum average biomass at intermediate phos phorus values (P 0.045, r^2 0.59) and the additional measurements of 2006 and 2007 confirmed the unimo dal relationship (y $0.0054x^2 + 0.398x + 0.268$, P 0.008, r^2 0.66; Fig. 5). There was neither a significant linear nor a unimodal relationship (P 0.14) between the ratio of APP biomass to chlorophyll *a* concentration and total phosphorus (Fig. 6). Compared to the mean seasonal HNF biomass during the period 1987 1992, their biomass decreased by a factor of about 30 (2006) and 10 (2007) with declining phosphorus concentrations (Fig. 5). Chlorophyll specific biomass of HNF increased with decreasing phosphorus concentration during 1987 1992 but was lower in 2006 and 2007 resulting in a unimodal relationship (y $0.001x^2 + 0.0717x$ 0.394, P 0.003, r^2 0.90; Fig. 6).

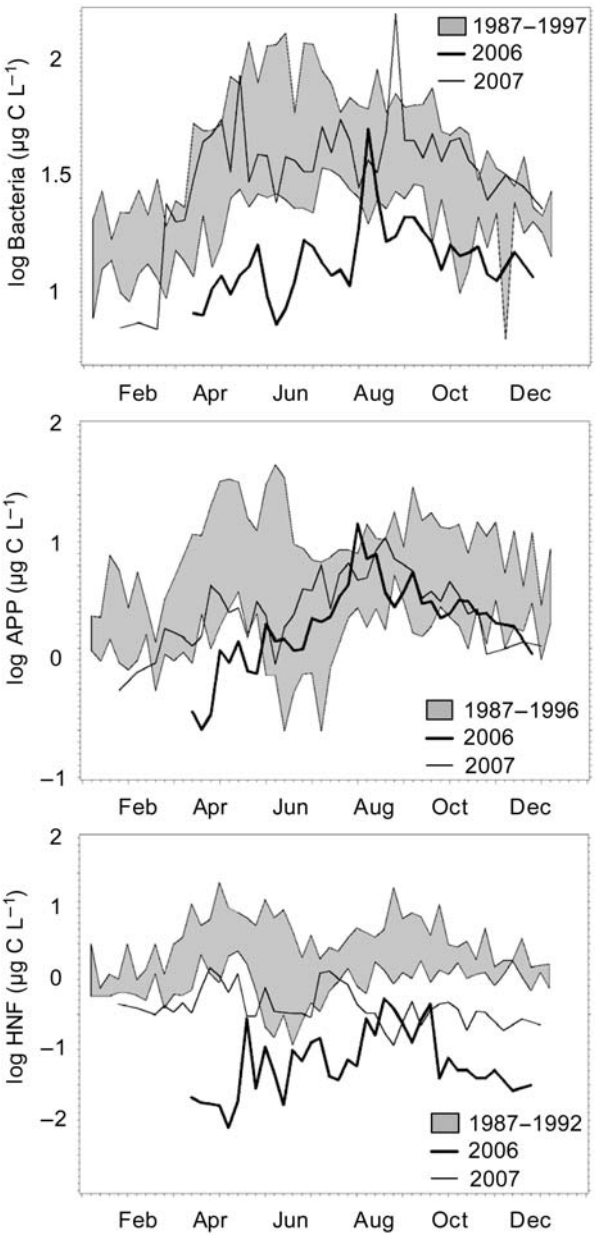

Fig. 3. Dynamics of logarithmically transformed biomass values of bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF) in 2006 and 2007. The grey areas indicate the biomass range of the period 1987–1997.

DISCUSSION

The biomass of purely phototrophic phytoplankton decreased during the re oligotrophication from 1979 to 1997 in Lake Constance which was attributed to the depletion of soluble reactive phosphorus below 0.08 μM in the upper water layer during summer (Gaedke, 1998a). Very high molar carbon to phosphorus ratios of phytoplankton (180 700:1) indicated a strong phos phorus depletion of the algae in 1995 (Hochstädter, 2000). In accordance with the hypothesis of decreasing APP biomass, the biomass of APP was lower in 2006 and 2007 than in most of the preceding years. However, we found no linear but a unimodal relation ship between APP biomass and phosphorus concen tration (Fig. 5). In contrast, chlorophyll specific APP biomass was not dependent on phosphorus and rather constant (Fig. 6). This contrasts with other studies which found an increased proportion of APP in total phytoplankton biomass with declining trophy (Bell and Kalff, 2001) or a unimodal pattern in mesocosm exper iments with low proportions at high trophy and at very low nutrient concentrations (Agawin *et al.*, 2000). The lack of a significant relationship in Lake Constance was probably due to the limited number of data points (n 12, P 0.14).

In contrast to the PEG model predicting declining seasonal variability with decreasing phosphorus

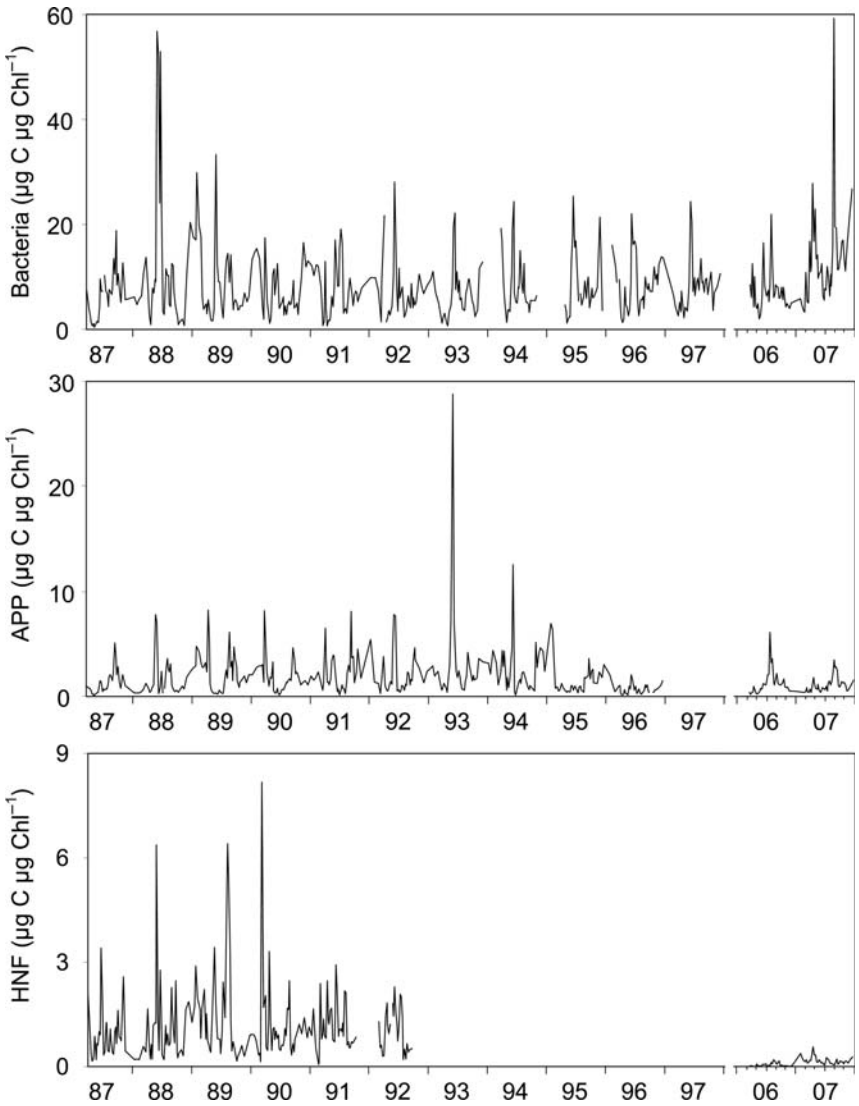

Fig. 4. Dynamics of chlorophyll-specific carbon biomass of bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF) in Lake Constance between 1987 and 1997 and in the years 2006 and 2007.

concentration for the classical food web (Sommer *et al.*, 1986), the seasonal variability of bacteria, APP and HNF did not decrease under more oligotrophic con ditions. A potential response to re oligotrophication may have partially passed unnoticed because we con sidered bacteria, APP and HNF as homogeneous func tional groups. As already observed for larger phytoplankton (Gaedke, 1998a; Anneville *et al.*, 2005), a change in taxonomic composition is also probable for the components of the microbial food web during re oligotrophication and is likely to precede a change in total biomass (Gaedke, 1998b). Regarding APP for example, the proportion of certain *Synechococcus* geno types varied seasonally in Lake Constance (Becker *et al.*, 2007). However, these investigations (required particularly for heterotrophic bacteria) need molecular methods which were not yet available at the beginning of the investigations in 1987.

In contrast to APP, bacterial biomass did not decrease with declining phosphorus concentrations, and the highest seasonal average on record was observed in 2007 (Fig. 5). Since total phytoplankton biomass decreased during re oligotrophication, the ratio of bac terial to phytoplankton biomass should have increased. This was confirmed by the negative relationship between the ratio of bacterial biomass to chlorophyll *a* and total phosphorus (Fig. 6) which is consistent with increasing ratios of bacterial production to primary pro duction with decreasing trophy of lakes found in pre vious studies (Jeppesen *et al.*, 1992; Pace and Cole,

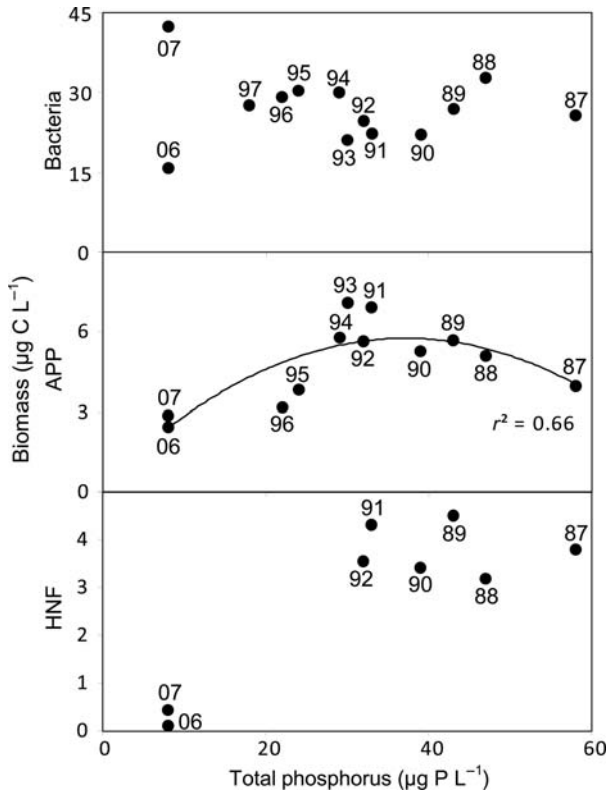

Fig. 5. Biomass averages between spring and autumn of bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF) as a function of total phosphorus concentration.

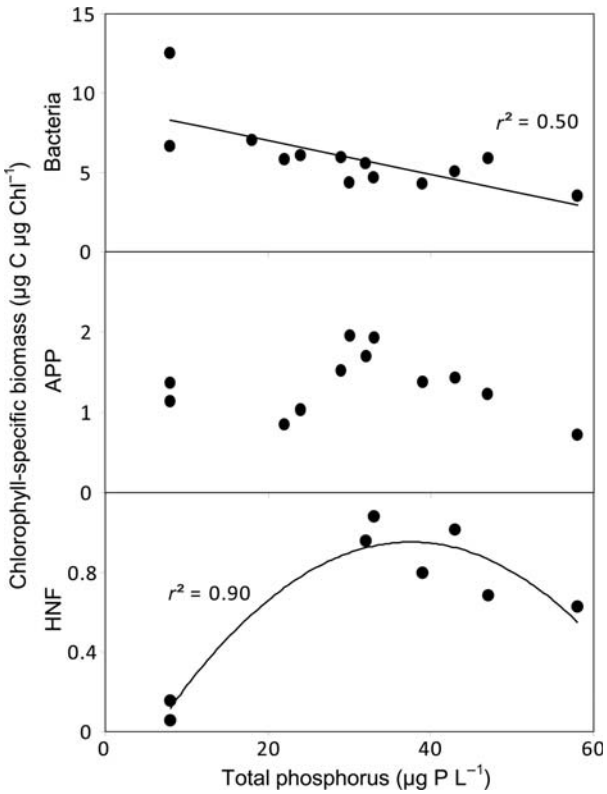

Fig. 6. Chlorophyll-specific biomass averages between spring and autumn of bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF) as a function of total phosphorus concentration.

1994). Bacteria are regarded as superior competitors for phosphorus (Currie and Kalff, 1984), and presumably they were not as phosphorus limited as phytoplankton. The bacterial cell sizes were larger in the year with higher HNF biomass (2007) than with low HNF biomass (2006; Table I) which is in agreement with expectations predicting the formation of large, grazing resistant bacteria in the presence of high flagel late abundance (Jürgens, 1994). The seasonal dynamics of bacterial biomass seemed to be influenced by temp erature and showed a delayed development after cold winters compared with years after mild winters (Fig. 3).

The biomass of HNF declined most pronouncedly of all groups considered with decreasing phosphorus con centrations. The low biomass in 2006 was partly due to the small cell size, whereas cells were larger in 2007. Low biomass of heterotrophic flagellates (1 4 µg C L ^{1}) was also found in several unproductive Swedish lakes (Bergström *et al.*, 2003). In principle, there are three potential reasons for the strongly decreased HNF biomass: (i) a decline in prey availability, (ii) a stronger competition for bacteria with increasing biomass of mixotrophs (Kamjunke *et al.*, 2007) and (iii) an increase in predation pressure. The biomass of bacteria and APP as main food sources for HNF was not considerably lower in 2006 and 2007 than during the period 1987 1992 which makes simple food limitation less likely. The average summer biomass of the most important mixo troph, *Dinobryon* spp., amounted to 4.6 and 2.7 µg C L ^{1} in 2006 and 2007, respectively, which was not higher than in the period 1987 1992 (3.2 63 µg C L ^{1}; Kamjunke *et al.*, 2007) indicating no substantial change in the competition. The abundance of potential preda tors, i.e. daphnids, rotifers and ciliates, was lower during the growing seasons of 2006 and 2007 com pared with the period 1987 1992 (Straile *et al.*, unpub lished results), and, in contrast to 1992 (Weisse, 1997), there were no minima of HNF during the clear water phases in 2006 and 2007 (Fig. 3). Therefore, also an enhanced top down control of HNF in 2006 and 2007 appears very unlikely. One might argue that a change in methods of HNF counting may have caused the low values. However, the biomass values of the two other groups, bacteria and APP, agreed quite well with the biomasses of former years. Possibly, the HNF compo sition shifted from a dominance of large algivorous

flagellates such as *Katablepharis* (in agreement with higher phytoplankton biomass available) towards smaller bacterivorous taxa such as *Spumella* sp. (Weisse, 1997). Further investigations are necessary to clarify the reasons for the decline in HNF.

Overall, bacterial biomass did not change during re oligotrophication, whereas chlorophyll specific biomass of bacteria increased, APP biomass and phos phorus concentration showed a unimodal pattern and HNF biomass strongly declined with decreasing trophy resulting in a unimodal relationship between chlorophyll specific HNF biomass and phosphorus. This shows that the different components of the microbial food web responded in a complex and group specific way to alterations in nutrient and chlorophyll concen tration. In conclusion, HNF responded much stronger and bacteria less than chlorophyll concentrations to re oligotrophication, whereas APP exhibited a more complex pattern. In the future, a further reduction in phosphorus concentration will decelerate since the recent level is relatively low already. Instead, the influ ence of climate change on aquatic food webs will increase. Further research is necessary to investigate the effects of warming, changed stratification and cloudiness, and increased carbon loads from the terrestrial environment.

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