Modulation of microbial predator–prey dynamics by phosphorus availability: Growth patterns and survival strategies of bacterial phylogenetic clades

Michaela M. Salcher[1], Julia Hofer[2], Karel Horňák[3], Jan Jezbera[3], Bettina Sonntag[2], Jaroslav Vrba[3], Karel Šimek[3], Thomas Posch[1]

[1]Department of Limnology, Institute of Plant Biology, University of Zurich, Kilchberg, Switzerland; [2]Institute of Ecology, University of Innsbruck, Innsbruck, Austria; and [3]Institute of Hydrobiology, Biology Centre ASCR and Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic

Correspondence: Thomas Posch, Department of Limnology, Institute of Plant Biology, University of Zurich, Seestrasse 187, CH-8802 Kilchberg, Switzerland. Tel: +41 44 716 1224; fax: +41 44 716 1225; e-mail: posch@limnol.unizh.ch

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Abstract

We simultaneously studied the impact of top-down (protistan grazing) and bottom-up (phosphorus availability) factors on the numbers and biomasses of bacteria from various phylogenetic lineages, and on their growth and activity parameters in the oligo-mesotrophic Piburger See, Austria. Enhanced grazing resulted in decreased proportions of bacteria with high nucleic acid content (high-NA bacteria) and lower detection rates by FISH. There was a change in the composition of the bacterial assemblage, whereby *Betaproteobacteria* were heavily grazed while *Alphaproteobacteria* and *Cytophaga–Flavobacterium–Bacteroides* were less affected by predators. Changes in bacterial assemblage composition were also apparent in the treatments enriched with phosphorus, and even more pronounced in the incubations in dialysis tubes (allowing relatively free nutrient exchange). Here, *Betaproteobacteria* became dominant and appeared to act as successful opportunistic competitors for nutrients. In contrast, *Actinobacteria* did not respond to surplus phosphorus by population growth, and, moreover, maintained their small size, which resulted in a very low biomass contribution. In addition, significant relationships between high-NA bacteria and several bacterial phylogenetic clades were found, indicating an enhanced activity status. By combining several single-cell methods, new insight is gained into the competitive abilities of freshwater bacteria from a variety of phylogenetic lineages under contrasting sets of bottom-up and top-down constraints.

Introduction

Grazing by bacterivorous protists and nutrient availability are known to be the major limiting factors faced by bacterial assemblages in freshwater habitats. The interplay of these factors has been the subject of a multitude of experimental studies during the recent decades (for reviews, see Sanders *et al.*, 1989; Jürgens & Güde, 1994). Laboratory studies (e.g. batch cultures or chemostat experiments) and field experiments have provided new insight into the general rules of the interaction between different taxonomic groups of bacteria and their protistan predators (Horňák *et al.*, 2005; Jezbera *et al.*, 2005; Pernthaler, 2005; Salcher *et al.*, 2005; Šimek *et al.*, 2005).

To elucidate bottom-up effects on bacterial phylogenetic lineages, several manipulation studies have been carried out by enrichment with a variety of nutrients (e.g. Pérez & Sommaruga, 2006) or by transplanting bacterial assemblages from nutrient-poor to nutrient-rich habitats (Gasol *et al.*, 2002; Šimek *et al.*, 2006) or vice versa (Gasol *et al.*, 2002; Horňák *et al.*, 2005).

Datasets from lakes with different trophic status point to a relationship between the response of bacterial assemblages to bottom-up and top-down manipulations and the ambient nutrient levels. Oligotrophic systems are thought to be more bottom-up-controlled, while top-down pressure plays just a minor role (Sanders *et al.*, 1992). The opposite seems to apply for eutrophic lakes, where grazing by different trophic levels is assumed to be the major limiting factor for bacterial development. However, the effects of predation on bacterial assemblages can be buffered by high nutrient availability, and consequently by rapid growth of the

prominent bacterial lineages (Šimek *et al.*, 2003, 2005). Hence, opportunistic bacteria with high growth rates can establish high standing stocks even though their numbers are heavily reduced by grazers. Moreover, bacteria could also be stimulated as a result of nutrient recycling by grazers (Pernthaler *et al.*, 1997; Jezbera *et al.*, 2006).

It is known, however, that certain bacteria can resist high grazing pressure. Associated strategies include morphological adaptations that allow the bacteria to escape the optimal prey-size spectrum of the predators, i.e. shifts towards smaller or larger cell sizes or the formation of inedible aggregates or microcolonies (for reviews see Hahn & Höfle, 2001; Jürgens & Matz, 2002; Pernthaler, 2005). Bacteria with high phenotypic plasticity can be found in a wide range of phylogenetic clades (e.g. within *Cytophaga–Flavobacterium–Bacteroides* or several subgroups of *Proteobacteria*), but the underlying mechanisms (e.g. triggering of filament–formation) are still controversial (Hahn *et al.*, 1999; Corno & Jürgens, 2006).

A further aspect of protistan grazing is its pronounced impact on the activity status of bacterial assemblages. Several studies have shown that protistan predators selectively feed on active bacteria (Del Giorgo & Gasol, 1995; Posch *et al.*, 1999; Šimek *et al.*, 2003; Hornák *et al.*, 2005). Therefore, high grazing pressure may often lead to a decrease in bacterial bulk activity. However, grazing can also have a beneficial effect on bacterial activity owing to nutrient recycling (Jürgens & Sala, 2000; Sherr & Sherr, 2002).

The activity status of bacteria can be followed indirectly by the ratio of bacteria with high to those with low nucleic acid content (high NA vs. low NA), as revealed by means of flow cytometry (Gasol *et al.*, 1999), or by the amount of ribosomes that can be determined with FISH techniques (EUB detection rate, Oda *et al.*, 2000).

We present here a size-fractionation and nutrient-enrichment experiment carried out in the oligo-mesotrophic Piburger See, Austria. We intended to deepen our knowledge about the underlying factors shaping the dynamics of bacterial clades present in the lake by simultaneous bottom-up and top-down manipulations. Our aims were to elucidate: (i) which bacterial groups provided the opportunistic competitors for the uptake of surplus phosphorus (P); (ii) which clades were able to form grazing-resistant morphologies under different nutrient regimes; (iii) the impact of grazing and nutrients on the activity status of bacteria; and (iv) the synergistic effects of top-down and bottom-up factors.

Materials and methods

Study site and set-up of the experiment

The experiment was carried out at the oligo-mesotrophic Piburger See, Austria (for details, see Tolotti & Thies, 2002). Lake water from 0.5 m depth was filtered by means of two stainless steel filtration devices. Two size fractions of lakewater microbial assemblages were produced by sequential filtration of raw water through filters (Osmonics) with pore sizes of 5 μm or 0.8 μm. Duplicate set-ups (2 L each) of these fractions were incubated in (i) bottles (2-L Schott glass bottles, termed $< 5\,\mu m$ Bottle or $< 0.8\,\mu m$ Bottle), (ii) bottles enriched with *c.* three-fold *in situ* phosphorus (P) concentration ($15\,\mu g\,P\,L^{-1}$; termed $< 5\,\mu m$ Bottle+P or $< 0.8\,\mu m$ Bottle+P), or (iii) prerinsed dialysis tubes (Spectra-Por, 12 000–14 000 molecular weight cut-off termed $< 5\,\mu m$ Dialysis or $< 0.8\,\mu m$ Dialysis). All variants were incubated in the lake for four days and subsamples of 400 mL were taken daily.

Abundance of microorganisms, flagellate grazing rates

Twenty-millilitre subsamples were fixed with formaldehyde (3% final concentration), and organisms were stained with DAPI (Porter & Feig, 1980), filtered on black polycarbonate filters (0.22-μm pore size, Osmonics), and further processed as described in Posch *et al.* (1999).

We used fluorescently labelled bacteria (FLB) to determine flagellate grazing rates (Šimek *et al.*, 1999). Briefly, we added FLB (*c.* 18% of actual bacterial concentration) to 50-mL unfixed subsamples of each $< 5\,\mu m$ treatment for 10–20 min. After fixation with the alkaline Lugol's solution – formaldehyde – thiosulfate decolorization technique (Sherr *et al.*, 1987), 10–30 mL were stained with DAPI and filtered onto black polycarbonate filters (1-μm pore size, Osmonics). At least 50 flagellates were counted and inspected for FLB ingestion by means of epifluorescence microscopy (Zeiss Axioplan). Average FLB uptake rates were multiplied by flagellate abundance to estimate total grazing rates (TGR) of each treatment.

Flow cytometry

Duplicate 10-mL samples were fixed with paraformaldehyde (1% final concentration, pH 7.4) and stained with the nucleic acid stain SYTO 13 (Molecular probes, Eugene, OR) at a final concentration of 2.5 μM. In addition, Triton-X 100 (0.1% final concentration) and fluorescent microspheres (1 μm TransFluoSpheres 488/560, Molecular Probes) as a counting and internal fluorescence reference were added. Flow-cytometric analyses were performed on a MoFlo (DakoCytomation, Glostrup, Denmark) equipped with a water-cooled argon ion 4 W Innova 90 C1 laser (Coherent, Santa Clara, CA) tuned to 488 nm with an output power of 200 mW. The orthogonal side scatter (SSC) was measured at 488/10 nm, the green fluorescence of SYTO 13 at 530/40 nm, and the yellow signals from the microspheres at 570/40 nm. Detectors were R-1477

photomultiplier tubes (Hamamatsu, Hamamatsu City, Japan) at 540, 470, and 570 V for SSC, SYTO 13, and yellow signals, respectively. Measurements were triggered on logarithmically amplified SYTO 13 or yellow signals using a logical OR on a custom multiple trigger board (DakoCytomation). The heterotrophic bacterial community was discriminated by manual gates from background caused by electronic noise, microspheres, and protists. Likewise, gates were drawn to distinguish between bacteria with high and low NA content. These discriminations were clearly visible, and identical gates were applied to all samples.

FISH and catalyzed reporter deposition (CARD-FISH)

Subsamples of 10–15 mL were fixed with buffered paraformaldehyde (2% final concentration, pH 7.4), filtered onto white polycarbonate filters (Millipore, Type GTTP, 0.2-μm pore size, 47-mm diameter), and stored at $-20\,^{\circ}C$ until further processing. CARD-FISH was carried out as previously described by Sekar *et al.* (2003) using the following horseradish peroxidase-labelled probes: EUB I-III for all *Bacteria* (Daims *et al.*, 1999); the probes ALF968 (Neef, 1997) and BET42a (Manz *et al.*, 1992) for the *Alpha* and *Beta* subgroups of *Proteobacteria*, respectively; probe CF319 (*Cytophaga-Flavobacterium-Bacteroides*, Manz *et al.*, 1996); the actinobacterial probe HGC69a (Roller *et al.*, 1994); and probe R-BT065 (Šimek *et al.*, 2001), which detects a subcluster within *Betaproteobacteria*. Signal-amplification was carried out with fluorescein isothiocyanate (FITC)-labelled tyramides. For counting and determination of biomasses, a Zeiss Axiophot epifluorescence microscope was used. Cell dimensions of all FISH-positive FITC-labelled bacterial cells within the studied bacterial phylogenetic lineages were measured by image analysis from images at blue excitation (488-nm wavelength). As the dimensions of CARD-FISH-stained bacteria overestimate the cell size in comparison with DAPI-stained cells, we show only the percentage of biomass contribution of each group to the total biomass of cells hybridized with probe EUB.

Growth rates of the various bacterial clades in the <0.8-μm variants were calculated from the abundances at two consecutive sampling dates, assuming exponential growth.

Results

Bacterial abundance

Bacterial counts acquired by direct counting with epifluorescence microscopy were highly significantly correlated with values gained by flow cytometry ($r^2 = 0.93$, $P < 0.001$). Bacterial abundance passed through significant changes during the experiment (Table 1). While abundances increased significantly in all <0.8-μm treatments and in the <5-μm dialysis variant, the opposite was true for the <5-μm bottle treatments. More detailed data of bacterial abundances, biomasses and stoichiometry can be found in Posch *et al.* (submitted).

Table 1. Flagellate abundances (10^6 cells L^{-1}) and total grazing rates (10^7 bacteria L^{-1} h^{-1}) of the <5-μm variants at times 0 and 96 h Bacterial abundances (10^9 cells L^{-1}) and proportions of the various bacterial clades (EUB in % of DAPI; ALF, BET, ACT, CFB, and R-BT in % of EUB and % of EUB biomass) of all variants at times 0 and 96 h. Each value is a mean of two replicates

	<0.8-μm variants				<5-μm variants			
	96 h				96h			
	0 h	Bottle	Bottle+P	Dialysis	0 h	Bottle	Bottle+P	Dialysis
HNF abundance (10^6 cells L^{-1})†	–	–	–	–	1.41	11.54***	11.44***	6.30***
Total grazing rate (10^7 bacteria L^{-1} h^{-1})†	–	–	–	–	2.64	21.30*	35.52***	12.29**
Bacterial abundance (10^9 cells L^{-1})†	3.23	5.17**	7.10***	9.72***	3.47	3.45	3.76	10.34**
EUB (% of DAPI)	70.3	81.0	86.3*	84.5*	74.4	53.9**	41.0***	78.0
ALF (% of EUB)	1.8	2.2	5.1	11.9***	1.9	8.7***	12.0***	15.1*
BET (% of EUB)	10.3	23.4***	36.9***	57.2***	10.6	16.8	21.0	59.4**
ACT (% of EUB)	33.0	20.3	30.0	23.7	31.8	37.8	42.4	16.9*
CFB (% of EUB)	1.7	2.1	4.9***	6.4**	2.0	8.3*	12.4***	7.3**
R-BT065 (% of EUB)	4.8	10.7	10.1	15.5	4.8	10.3	27.0	28.2
Sum of general probes (without R-BT065)	46.8	48.0	76.9	99.2	46.3	71.6	87.8	98.7
ALF (% of EUB biomass)	6.4	7.0	4.6	11.7	10.1	15.0	21.6*	31.2*
BET (% of EUB biomass)	27.4	42.0	51.0*	84.6	41.0	17.2*	44.4	70.0
ACT (% of EUB biomass)	12.7	7.5	4.1	4.0	14.6	8.3	7.2	2.9*
CFB (% of EUB biomass)	7.7	5.7	4.1**	4.8	9.6	19.0	15.0*	10.1

Asterisks indicate significant differences between 0 and 96 h (one-way ANOVA with Turkey's HSD posthoc test:
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).
†Data from Posch et al. (submitted).

Abundance of heterotrophic nanoflagellates, and total grazing rates

Decoupling of flagellates from higher trophic levels resulted in accelerated growth rates and significant increases of heterotrophic nanoflagellates (HNF) and their bacterivory in all three $<$ 5-μm variants (Table 1, Fig. 1a). The highest increase was found in $<$ 5 μm Bottle+P, followed by $<$ 5 μm Bottle, while in dialysis tubes HNF abundance and total grazing rates showed just a slight rise after four days. Overall, bacterial abundances were negatively correlated with total grazing rates ($r^2 = 0.70$, $P < 0.001$).

Changes in bacterial activity parameters

In the grazing-free ($<$ 0.8-μm) fractions, both EUB detection rates and proportions of high-NA bacteria increased, but to different extents (Fig. 1b–g). While EUB-positive cells increased slightly in all treatments, there was a significant shift from initially dominant low-NA bacteria to high-NA bacteria within bottles+P and dialysis tubes. In the bottle treatments without P enrichment, high- and low-NA bacteria remained in stable proportions throughout the experiment.

In the $<$ 5-μm bottle treatments, EUB detection rates decreased significantly and were negatively correlated with total grazing rates ($r^2 = 0.58$, $P < 0.001$). Within dialysis tubes, where the lowest grazing rates were observed, EUB detection rates increased slightly, while in the lake, detection remained constant (68.4 ± 4.9%). In the presence of grazers, the fraction of high-NA bacteria reacted differently depending on the nutrient manipulation. It either stayed stable (bottles), went back to approximately initial values after a slight increase (bottle+P), or increased until the end of the experiment (dialysis tubes).

Changes in bacterial assemblage composition

We detected 38–99% (mean: 61%) of EUB-positive bacteria with the four general probes ALF968, BET42a, CF319a, and HGC69a. In lake-water samples, all bacterial groups showed stable percentages throughout the investigation period.

In the absence of grazers, *Betaproteobacteria* (BET) were the most prominent group, with two-fold higher percentages at the last date than initally (Table 1, Fig. 2), and, therein, the R-BT065 subcluster of BET showed nearly the same increase ($\times$ 2.2). There was a clear effect of the P-addition on members of *Cytophaga—Flavobacterium–Bacteroides* (CFB: $\times$ 2.9), *Alphaproteobacteria* (ALF: $\times$ 2.8), and BET ($\times$ 3.6), which increased, whereas *Actinobacteria* (ACT) maintained constant values. This effect of surplus nutrients was even more pronounced in the dialysis tubes, where ALF and BET showed almost six-fold higher proportions at the end of the experiment, CFB increased by four-fold, while ACT decreased ($\times$ 0.7).

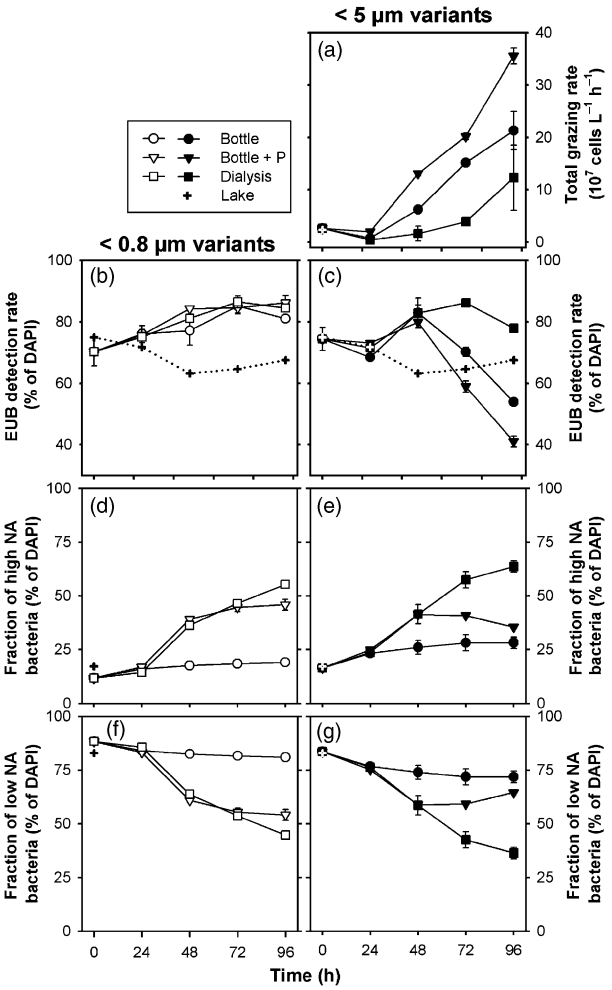

Fig. 1. (a): Total grazing rates (10^7 cells L^{-1} h^{-1}); (b, c): EUB detection rates (% of DAPI); (d, e): proportions of high-NA bacteria (% of DAPI); (f, g): proportions of low-NA bacteria (% of DAPI). (a, c, e, g): Data from $<$ 5-μm variants (with HNF); (b, d, f): data from $<$ 0.8-μm variants (without HNF). Values are the means of two replicate set-ups, and whiskers show the SE of these values.

In the $<$ 5-μm treatments (Table 1, Fig. 2), the percentages of ALF and CFB increased more than four-fold, while BET and ACT remained stable. The addition of P also stimulated growth of ALF ($\times$ 6.5) and CFB ($\times$ 6.2) in these variants, but not of BET nor of ACT. In the dialysis-tube treatments, BET dominated the assemblages, although ALF showed the highest rise ($\times$ 8.1). CFB did not respond to high nutrient concentrations, while ACT declined to 50% of their original abundance.

Contribution of bacterial phylogenetic lineages to total bacterial biomass

While ACT accounted for about 29% of total bacterial abundance, on average they contributed only 7.7% to

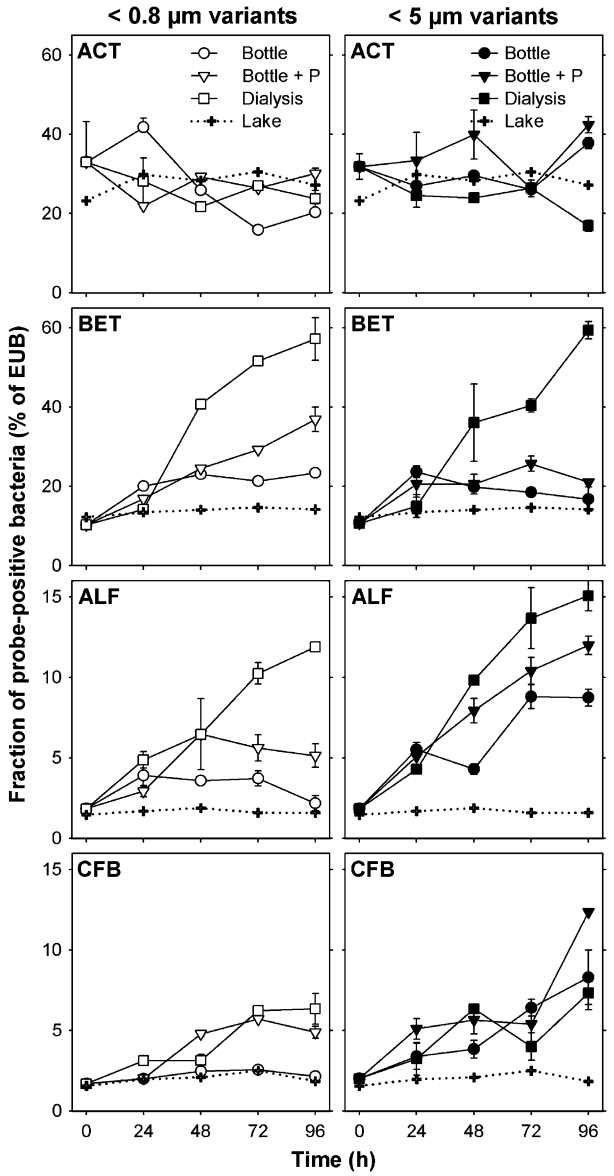

Fig. 2. Proportions of the bacterial clades *Actinobacteria* (ACT), *Betaproteobacteria* (BET), *Alphaproteobacteria* (ALF), and *Cytophaga–Flavobacterium–Bacteriodes* (CFB) as a percentage of EUB-positive cells. Values are the means of two replicate set-ups, and whiskers show the SE of these values.

bacterial biomass (Table 1). These bacteria were always very small and of uniform vibrioid morphology. The opposite was found for nearly all other bacterial groups: their contribution to EUB biomass was disproportionately higher than their contribution to total abundance. This was most apparent for ALF and CFB in the < 5-µm variants (more than two-fold higher values for biomasses), whereas in the < 0.8-µm variants such phenotypic adaptations were found only for BET (nearly two-fold higher values for biomasses). ALF comprised filamentous strains in all < 5-µm variants and in < 0.8-µm Dialysis, but not in the < 0.8-µm bottle treatments, whereas CFB formed filaments only in the dialysis-tube treatments, regardless of grazing pressure.

Growth rates of the bacterial groups

We found highly contrasting patterns of growth rates of the bacterial clades in the < 0.8-µm fractions (Fig. 3). While net growth rates in the lake ranged around 0, uncoupling of the food web by filtration resulted in significantly higher values.

EUB-positive and DAPI-positive bacteria featured similar values, whereas high-NA bacteria always had higher, and low-NA bacteria had much lower growth rates. Moreover, group-specific growth rates were higher compared with total bacteria and eubacteria. ALF, BET and CFB showed almost the same growth patterns, while growth of ACT was delayed for about 1 day compared with the other groups.

In the bottle treatments, growth rates declined very quickly, although it was mainly ALF and BET that benefitted from the absence of predators during the first 2 days. At the end of the experiment, only ACT showed positive growth rates, even though these bacteria had exhibited negative values the days before, while growth of ALF and CFB ceased.

This 'bottle effect' was partially buffered in the bottles+P, where very high growth rates during the first 2 days were followed by a sharp decline. Here, high-NA bacteria showed their highest growth rates between days 2 and 3 (corresponding to a net doubling time of 13 h), which were quite similar to the growth rates of ALF, BET and CFB (corresponding to net doubling times of 13, 17, and 12 h, respectively).

In the dialysis tubes, all bacterial groups profited from continuous nutrient supply. BET grew fastest (corresponding to a doubling time of 11 h) and showed a high similarity to growth-rate patterns of high-NA bacteria. Again, ACT exhibited a delay of one day in their growth rate responses.

Relationships between high-NA bacteria and bacterial phylogenetic lineages determined by CARD-FISH

Abundances of high-NA bacteria correlated with most of the bacterial phylogenetic lineages (Fig. 4), while low-NA bacteria showed only a weak positive correlation with EUB abundances ($r^2 = 0.62$, data not shown). There were highly significant correlations with EUB, ALF, BET and CFB (Fig. 4). However, the highest correlation was found by comparing the sum of the latter 3 groups (ALF, BET, CFB) with high-NA bacteria ($r^2 = 0.95$). Interestingly, the slope of the regression line corresponded almost exactly to the 1 : 1 line. ACT was the only bacterial group that showed no significant positive correlation with either high- or low-NA bacteria.

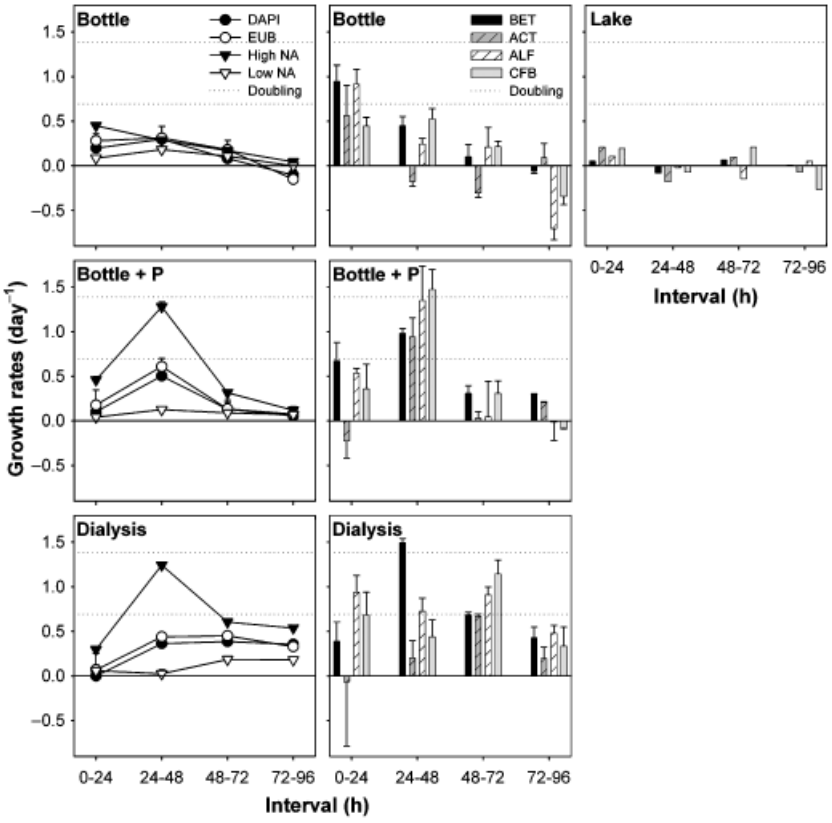

Fig. 3. Growth rates (d^{-1}) of the bacterial clades in the < 0.8-μm variants and the lake. Abbreviations as in Figs 1 and 2. Lines show the growth rates for one and two duplications per day.

Discussion

Activity status determined by flow cytometry and CARD-FISH

Very often, scatter plots of aquatic prokaryotes labelled with DNA stains gained by flow cytometry show two distinct subgroups: the high-NA fraction is assumed to be the proportion of 'active' bacteria (Gasol *et al.*, 1999), while bacteria with a low nucleic acid content seem to be more or less inactive. This assumption was confirmed by several cell sorting studies, where high-NA bacteria had higher amino acid uptake and growth rates (Servais *et al.*, 1999; Lebaron *et al.*, 2002; Longnecker *et al.*, 2006; Zubkov *et al.*, 2006). Moreover, a transplant and grazing experiment in a eutrophic reservoir (Gasol *et al.*, 2002) revealed a significant correlation between the fraction of high-NA bacteria and EUB detection rates, as was also found in our experiment (Fig. 4). In the above-mentioned study, the abundance of EUB-positive bacteria vs. high-NA bacteria fitted almost the 1 : 1 line, while this was not the case in our study. This could be explained by the different methodologies used, because Gasol and *et al.* (2002) applied FISH with fluorescently monolabelled probes (Alfreider *et al.*, 1996; Glöckner *et al.*, 1996) to determine bacterial assemblage composition. Owing to the higher detection rates of CARD-FISH, the intercept of the regression line between high-NA and EUB in our study was higher than 1 (Fig. 4). The best relationship was found between high-NA bacteria and the sum of bacteria hybridized with probes ALF, BET and CFB ($r^2 = 0.95$), with a regression line that fitted nearly exactly the 1 : 1 line. The exclusion of ACT from the regression could make sense, if their small cell size and morphology is interpreted as inactivity and a low nucleic acid content. However, the opposite effect was found by Warnecke *et al.* (2005), who detected high DNA *de novo* synthesis rates of ACT in various mountain lakes. Moreover, MAR-FISH (microautoradiography combined with FISH) investigation of ACT in a high mountain lake revealed a high leucine uptake activity (Pérez & Sommaruga, 2006). Similarly, Nielsen *et al.* (2006) found higher thymidine uptake activity among ACT than among all *Bacteria* in a drinking-water reservoir. In our study, abundances of ACT were also not correlated with low-NA bacteria (data not shown), and ACT showed positive growth rates (Fig. 3), which can be regarded as additional proof that they were not inactive or dormant in the lake.

Do different bacteria from large phylogenetic lineages form coherent functional guilds?

Until recently, aquatic bacteria were seen as a 'black box', and their ecological role was studied regardless of their

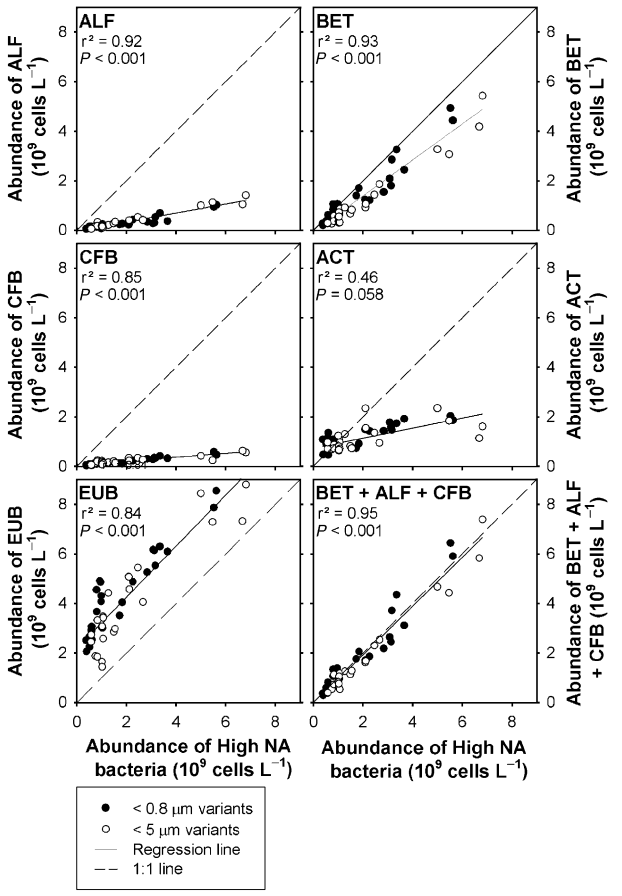

Fig. 4. Correlations between high-NA bacteria (10^9 cells L^{-1}) and the various bacterial clades (10^9 cells L^{-1}). Abbreviations as in Fig. 2.

phylogenetic affiliation. In this context, Pedrós-Alió (1989) suggested the introduction of 'functional guilds'; that is, even if the taxonomic background of certain bacterial species is not known, they could still be grouped together in terms of their physiology and ecological function. Moreover, one could even claim that such functional guilds could not only be classified physiologically, but also morphologically or even phylogenetically. To date, little is known about the physiological features of abundant freshwater bacterial clades, because most of them have not yet been cultivated and are known only from 16S (or 23S) rRNA gene sequence data. Moreover, it is problematic that FISH probes for large phylogenetic groups often cover physiologically and ecologically very diverse microorganisms (e.g. CFB). More specific probes are rarely used because they detect only a small fraction of total bacterial abundances.

Our results support the assumption that BET follow 'opportunistic' growth strategies; that is, they are favoured under limited grazer control and good nutrient availability, but are heavily grazed when environmental conditions change towards the dominance of top-down control. This phylogenetic group plays a very important role in freshwater ecosystems owing to its high abundance and biomass. However, in several lakes ACT are the most abundant group (Warnecke *et al.*, 2005), although, in terms of biomass, BET can still account for the largest part in bacterioplankton assemblages (Table 1). Several strains of the prominent *Polynucleobacter necessarius* cluster (also called beta II cluster) have recently been isolated, with very small cell sizes and distinct morphologies (Hahn, 2003). This group and another very abundant group of the beta I cluster (belonging to the *Rhodoferax* sp. BAL47 cluster, detectable with probe R-BT065, Šimek *et al.*, 2001) were able to overgrow other bacterial groups within a few days and become dominant in the absence of predators (Šimek *et al.*, 2001; Burkert *et al.*, 2003; Pérez & Sommaruga, 2006). This was also found in our experiment, in which BET accounted for 60% of all bacteria within the dialysis tubes, but to only 17% in the bottles experiencing the most intense HNF bacterivory (Fig. 2). Moreover, bacteria belonging to the narrower phylogenetic linage of the R-BT065 cluster accounted for up to 50% of all BET on the last day (Table 1), while in the lake they remained stable at around 22% (data not shown).

There was a clear difference between the < 0.8-μm bottle+P and the dialysis treatments, in which bacteria were able to incorporate nutrients from surrounding water (Fig. 2), and therefore it seems that BET were not exclusively limited by P. In a dissolved organic matter (DOM)-enrichment experiment conducted in a high mountain lake, BET and especially the R-BT065 subcluster showed high abundances and activities when enriched with algal-derived DOM (Pérez & Sommaruga, 2006), whereas in an enrichment with soil-derived DOM, ACT were more efficient. On the one hand, BET (and particularly R-BT065) show very high activity and growth rates (Jürgens *et al.*, 1999; Šimek *et al.*, 2001, 2003, 2005; Gasol *et al.*, 2002; Hahn, 2003), even under high grazing pressure. On the other hand, these bacteria are preferably ingested by flagellates, as revealed with FISH-analysis of HNF food vacuoles (Jezbera *et al.*, 2005, 2006; Salcher *et al.*, 2005). In conclusion, this opportunistic growth strategy with fast physiological shift-ups could represent a certain protection against grazing by compensating for the losses with high growth rates.

ACT are very abundant in limnetic ecosystems (Glöckner *et al.*, 2000; Sekar *et al.*, 2003; Allgaier & Grossart, 2006) and can show a certain resistance to UV radiation (Warnecke *et al.*, 2004; 2005). However, it is only recently that our knowledge of their dynamics has begun to expand, with the application of the more sensitive CARD-FISH protocol (Sekar *et al.*, 2003). ACT are known to be less grazing-vulnerable because of their small size, which could be below the uptake limits of certain protists (Pernthaler *et al.*, 2001),

as recently confirmed by Jezbera *et al.* (2005, 2006) in a series of *in situ* experiments. Speculations about their inedibility include their cell-wall structure and even toxicity. Hahn *et al.* (2003) demonstrated the complete protection of an isolated strain of ACT against predation by *Ochromonas* sp. strain DS. In our experiment, ACT showed no clear trend in their abundance (Fig. 2), which is in contrast to prior experiments (Pernthaler *et al.*, 2001; Šimek *et al.*, 2001, 2005; Horňák *et al.*, 2005). However, one effect was visible: ACT were not very efficient competitors in taking up nutrients; that is, they were often outcompeted by other, rapidly growing bacterial clades such as BET. In the dialysis variants (highest nutrient availability), we found that ACT decreased, pointing to an antagonistic relationship between these two clades. The same phenomenon was described for an enrichment experiment in an acidic bog lake (Burkert *et al.*, 2003), where members of the beta II cluster outcompeted ACT within a few days. In our study, ACT growth rates were lower than those for all other groups (Fig. 3). Besides, they were of uniform, small size, and, although they formed the bulk of hybridized cells (up to 42% of EUB), their contribution to bacterial biomass was small (between 3% and 15% of EUB biomass, Table 1).

In contrast, ALF and CFB showed different patterns. In the < 5-μm fraction, these bacteria increased both in abundance and in biomass (Table 1). The increase in biomass of these bacteria was about two-fold higher than that in abundance; that is, they increased not only numerically, but also in size at the same time. This can be seen as a grazing-protection mechanism and is further evidence of their high phenotypic plasticity (Jürgens *et al.*, 1999; Kirchman, 2002). In the predator-free variants, ALF showed no or just a slight increase in cell length, and CFB became even smaller (data not shown). CFB can display very high growth rates when heavily grazed by protists (Jürgens *et al.*, 1999), but slowed down when grazing pressure is eliminated experimentally (Fuchs *et al.*, 2000; Šimek *et al.*, 2003). Moreover, this clade contains strains that are able to form filaments (Hahn *et al.*, 1999; Hahn & Höfle, 2001; Pernthaler *et al.*, 2004; Salcher *et al.*, 2005) or aggregates (Šimek *et al.*, 2001) under high grazing pressure.

The same strategy seems to hold true for ALF. Several studies have shown that these bacteria are enriched when protistan grazing is high (Pernthaler *et al.*, 1997; Jürgens *et al.*, 1999; Langenheder & Jürgens, 2001; Salcher *et al.*, 2005), partly because they form filaments and aggregates together with other bacterial phyla (Langenheder & Jürgens, 2001; Salcher *et al.*, 2005). Although they are present in small proportions in lakes (2% of EUB in Piburger See) they can eventually increase if top-down pressure occurs (15% in < 5-μm dialysis, Fig. 2).

Synergistic effects of bottom-up and top-down factors

The assemblage composition in our enrichment experiment does not reflect the usual composition of freshwater bacterioplankton. Specifically, it seems peculiar that grazing-resistant ALF and CFB do not dominate natural assemblages. However, HNF grazing pressure is always linked to several other factors and cannot be seen as the only influence on bacteria. Grazing by zooplankton, substrate availability, intraspecific and interspecific competition, and host-specific virus-induced mortality are of major importance for shaping bacterial assemblages.

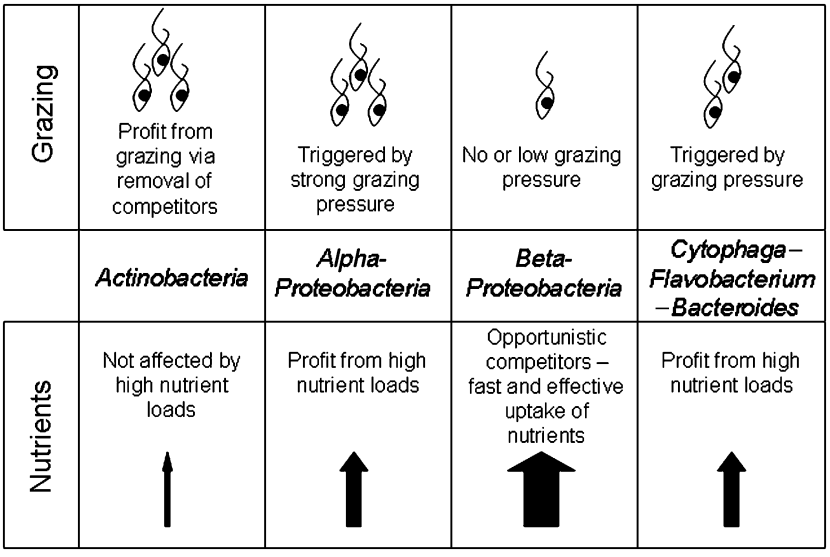

Fig. 5. Schematic depiction of the synergistic effects of bottom-up and top-down factors inducing the dominance of bacterial clades in Piburger See.

However, some experimentally observed patterns can also be found in freshwater habitats. At certain time points in the seasonal cycle (late spring bloom), bacteria allow themselves the 'luxury' of filament formation (Jürgens & Güde, 1994; Jürgens & Stolpe, 1995; Pernthaler *et al.*, 1996, 2004) but this is induced not only because of high grazing pressure but also as a result of high nutrient availability. In such situations nutrients might provide the necessary preconditions for filamentation, whereas grazing acts as a trigger. Mainly bacteria of the CFB cluster (Hahn *et al.*, 1999; Pernthaler *et al.*, 2004), but also some ALF and BET (Jürgens *et al.*, 1999; Langenheder & Jürgens, 2001) dominate these filamentous morphotypes that are less vulnerable to HNF predation. Later on, during the clear-water phase with peaking mesozooplankton, this morphology disappears because filaments are filtered out by Daphnids (Jürgens & Stolpe, 1995; Degans *et al.*, 2002; Vrba *et al.*, 2003; Pernthaler *et al.*, 2004).

Moreover, grazing by protists may not exclusively have a negative impact on bacterioplankton (Fig. 5). Some strains (e.g. ALF, CFB) might benefit from nutrients released by predators, while others (e.g. ACT) might benefit from changed competition owing to the removal of competitive but grazing-vulnerable bacteria (e.g. BET). Moreover, strains from ALF and CFB with high phenotypic plasticity could be enriched because of their inedible size (filaments, aggregates). Jürgens & Matz (2002) showed a schematic depiction of possible regulatory mechanisms for the development of grazing-resistant morphologies that includes some of the above-mentioned factors. However, in that review, bacteria were still seen as a black box; that is, their phylogenetic background was not considered. Therefore, we want to highlight that, with the application of single-cell techniques, some bacterial groups, even if they are categorized into large phylogenetic entities (i.e. ALF, BET, CFB, and ACT), are now successfully identified as being supported or repressed by at least some of the above-mentioned mechanisms (Fig. 5).

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