Biomass reallocation within freshwater bacterioplankton induced by manipulating phosphorus availability and grazing

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ABSTRACT: Grazing by bacterivorous protists and the availability of nutrients strongly affect the taxonomic composition of freshwater bacterioplankton. However, so far, studies have focussed mainly on changes in abundance. Therefore, we studied biomass reallocation within different phylogenetic lineages of bacteria under varying regimes of protistan grazing and nutrient supply in the oligo-mesotrophic Piburger See (Austria). Size fractionation of lake water was used to create setups with and without bacterivores. These treatments were incubated in bottles (with and without added P) and in dialysis tubes (allowing for free nutrient exchange) for 96 h. The release of bacteria from grazing resulted in a small increase in total abundance, but more pronounced changes of production and biomass. Addition of P to bottles seemed to indicate P limitation of bacterial growth. However, this was contradicted by the results of dialysis tube incubations. In these treatments, highest bacterial biomass and production were observed as well as a substantial increase in particulate P in the bacterial size fraction. While *Betaproteobacteria* abundance and biomass increased greatly in P-surplus bottles (4× and 12×, respectively), biomass increased even more in the dialysis tubes (28×). After 48 h incubation, virtually all imported P in predator-free dialysis tubes could be attributed to newly produced betaproteobacterial biomass. These bacteria were significantly reduced by the presence of predators in the bottle incubations only, probably due to the delayed growth of bacterivores in the dialysis tubes. By contrast, although *Actinobacteria* numerically dominated in the lake, as well as in the grazing-exposed bottle treatments, their contribution to total biomass was always low. Our results illustrate that the quantification of the biomass of specific lineages may allow for a better assessment of C and P fluxes within microbial food webs. Moreover, nutrient addition during bottle incubations might result in misleading conclusions about growth limitation.

KEY WORDS: *Actinobacteria* · Bacterial biomass · Bacteria–flagellate interactions · Bacterial lineages · *Betaproteobacteria* · Fluorescence *in situ* hybridization · Phosphorus enrichment

INTRODUCTION

Analysis of the bacterial assemblage composition in various freshwater systems initially suggested a numerical dominance of *Betaproteobacteria* (Alfreider et al. 1996, see also Table 1 in Bouvier & del Giorgio 2003). However, subsequent methodological improve-ments, namely the combination of catalyzed reporter deposition with fluorescence *in situ* hybridization (CARD-FISH), have changed this view by highlighting the important role of Gram-positive *Actinobacteria* within freshwater bacterioplankton (Sekar et al. 2003, Wu et al. 2006). Several studies have confirmed the numerical dominance of these 2 phylogenetic lineages

in freshwater lakes (Table 6 in Klammer et al. 2002, Bouvier & del Giorgio 2003, Warnecke et al. 2005). However, most investigations have primarily focused on abundance, whereas our knowledge about the biomass allocation within freshwater phylogenetic lineages is limited (Yokokawa et al. 2004). A detailed look at the contribution of different bacteria to total biomass might substantially change our view on the importance of these groups, leading us to the understanding that less-abundant bacteria with larger cell sizes (e.g. filamentous cells) might contribute substantially to the microbial C pool (Schauer & Hahn 2005).

The proportion of bacteria from different phylogenetic groups is regulated by top-down (grazing) and bottom-up (nutrient availability) controls (Horňák et al. 2005). While our knowledge about the role of nutrients for the success of distinct lineages is still restricted to single observations (Pérez & Sommaruga 2006, Šimek et al. 2006), the structuring impact of bacterivores on freshwater assemblages has been elucidated in detail (see Table 3 in Salcher et al. 2005). *Betaproteobacteria* are often selectively eliminated by grazing protists (Jezbera et al. 2006), whereas *Actinobacteria* maintain or even increase their relative proportions at high grazing pressure. This holds true for both field experiments and laboratory-based experimental assemblages, and it has partially been explained as a consequence of size-selective protistan feeding (Pernthaler et al. 2001, Hahn et al. 2003). Since bacterivorous protists selectively consume larger bacterial morphotypes, they will affect not only cell numbers, but also (and to a greater extent) the biomass ratios of different freshwater lineages. Moreover, grazing is not only a key factor in directly shaping bacterial assemblages, but also an intrinsic step in the remineralization of nutrients (Rothhaupt 1997). As a consequence, grazing might also modify the competition within the bacterioplankton assemblage in cases of strong nutrient limitation (Salcher et al. 2007).

We analyzed the biomass reallocation between freshwater bacterial lineages in the context of P availability and grazing pressure. Raw water of the oligomesotrophic Piburger See (Austria) was size fractionated to generate variants consisting of bacteria only or of bacteria and their major consumers, heterotrophic nanoflagellates (HNF). We chose 2 approaches to manipulate P availability: (1) the incubation of samples in non-penetrable bottles with and without additional P under *in situ* conditions; and (2) the *in situ* exposure of filtrates in dialysis tubes, allowing for the relatively free exchange of nutrients. In these setups we investigated the effects of P availability and grazing on the biomass of 4 prominent phylogenetic lineages in the context of bacterial production and P partitioning between bacteria and HNF (see also Salcher et al. 2007).

MATERIALS AND METHODS

Study site. Piburger See (Austria) is a small pre-alpine lake which was strongly affected by anthropogenic-induced eutrophication during the 1960s and 1970s. For the restoration of the lake, in 1970 a tube was permanently installed at the 24 m depth to continuously remove oxygen-depleted and P-rich hypolimnetic water. Although the oxygen saturation in deeper water subsequently improved, it took nearly 30 yr to decrease P concentrations (Tolotti & Thies 2002). Current annual averages of the epi-, meta-, and hypolimnetic water strata are around 6, 7 and 9 $\mu g \, P \, l^{-1}$, respectively. At the time of sampling (September 2003) the major limnological variables (at 0.5 m depth) were as follows: temperature = 18.5°C, oxygen = 8.8 mg l^{-1}, chlorophyll *a* (chl *a*) = 3.8 $\mu g \, l^{-1}$, dissolved organic carbon (DOC) = 2.4 mg l^{-1}.

Experimental setup. Lake water (50 l) from 0.5 m depth was transported in 25 l bottles to the laboratory. We produced 2 size fractions by sequential filtration of the raw water samples. Size fractionations were conducted with 2 autoclaved stainless steel filtration devices (140 mm diameter). Gravity filtration over 5 µm pore-size polycarbonate filters (Osmonics) generated a filtrate consisting of bacteria and their major predators, i.e. mostly HNF. Half of the 5 µm filtered sample was subsequently filtered through 0.8 µm pore-size polycarbonate filters using a peristaltic pump in order to remove the bacterivores. The different filtrates were incubated *in situ* (at 0.5 m depth) for 4 d in setups of the following design: 2 l Schott glass bottles served as controls (referred to as <0.8 or <5 µm Bottle) for treatments (bottles) enriched with 15 µg l^{-1} of P (K_2HPO_4), assigned as <0.8 or <5 µm Bottle & P. In a third variant, 2 l of each size fraction were incubated in pre-rinsed dialysis tubes (assigned as <0.8 or <5 µm Dialysis; Spectra-Por, 12 000–14 000 molecular weight cut off). Each variant was set up in duplicate, i.e. in sum 8 bottles and 4 dialysis bags were incubated in the lake. Subsamples of 400 ml were taken each morning (08:00 h) for the analyses described below.

Abundance and biomass of the total bacterial assemblage. Twenty ml of the subsamples were fixed with 0.2 µm prefiltered formaldehyde (2% final concentration). Organisms were stained with DAPI (5 to 10 µg ml^{-1} final concentration, Porter & Feig 1980), filtered on black polycarbonate filters (0.22 µm pore-size, Osmonics) and further processed as described in Posch et al. (1997). We used the image analysis software LUCIA (Laboratory Imaging Prague) for the size determination of organisms. At least 500 bacteria per sample were measured and bacterial mean cell volumes (MCV_{BAC} in μm^3) were converted to cellular C contents (CC_{BAC} in fg C $bacterium^{-1}$) applying the formula

$CC_{BAC} = 218 \times MCV_{BAC}^{0.86}$ (Loferer-Krößbacher et al. 1998). Since this equation was developed using bacterioplankton data from Piburger See, a high precision of this volume-to-C conversion factor can be presumed (see also Posch et al. 2001). Bacterial biomass (in µg C l^{-1}) was calculated by multiplying CC_{BAC} by bacterial abundance (in bacteria l^{-1}).

HNF numbers and feeding rates. HNF grazing was estimated using fluorescently labeled bacterioplankton (FLB) following the protocol of Šimek et al. (1999). HNF cell-specific FLB uptake rates were multiplied with HNF abundance to estimate total grazing rates (TGR) of each treatment, used for the calculation of bacterial net production rates (see below). All methodological details on the quantification of HNF are described in Salcher et al. (2007).

Bacterial production. Triplicate samples of 5 to 10 ml of raw water were incubated with [methyl-^{3}H]thymidine (5 nM final concentration, 83 Ci mmol^{-1} specific activity, Amersham) for 1.5 h at *in situ* temperatures. For each treatment 2 formaldehyde-fixed control samples were incubated under the same conditions. After fixation with formaldehyde (2% final concentration) samples were filtered onto white polycarbonate filters (0.2 µm pore-size, 25 mm diameter, Osmonics). Bacterial cells were disrupted and macromolecules extracted with 5 ml of 5% ice cold tricloroacetic acid (TCA) for 5 min. Filters were further processed as previously described (Bell 1993). Disintegrations per min (dpm) were measured with a Beckman Scintillation counter (Beckman LS 6000 IC) after addition of 5 ml Ready Safe (Beckman) scintillation cocktail. We calculated empirical conversion factors for each of the 3 experimental setups (Bottle, Bottle & P, Dialysis — all variants in duplicate) based on the increase of bacterial cell numbers in the <0.8 µm variants and corresponding uptake rates of thymidine. For the calculation of conversion factors, we followed the modified derivative method of Ducklow et al. (1992). Net production rates of the <5 µm variants were determined as the gross productions (measured via radiotracer uptake) minus total grazing rates.

Total and size-fractionated P. Total P (TP) in the <0.8 µm variants was determined spectrophotometrically using the molybdate method after digestion with H_2SO_4 and H_2O_2 (Schmid & Ambühl 1965) from 100 ml subsamples. To achieve a rough separation of the P content of HNF and bacteria in the <5 µm variants, subsamples (100 ml) were filtered at low vacuum (13.3 kPa) onto 1.2 µm pore-size polycarbonate membrane filters (47 mm in diameter, Millipore). TP was estimated for the filters (organisms >1.2 µm corresponding to the 'HNF' fraction) and the filtrates (organisms <1.2 µm corresponding to the 'bacterial' fraction). Therefore, in the <1.2 µm fraction, P was

determined as the sum of particulate P and dissolved P (DP). However, values of DP in the raw water were always <1 µg l^{-1} (after filtration through 0.2 µm pore-size filters). In addition, we determined the TP of the unfiltered sample in the <5 µm variants to control the efficiency of the described size differentiation. On 2 sampling dates we took 2 ml of subsamples from the filtrates to account for the potential loss of bacterial cells during filtration (following the staining procedure as described above). At each time point we also analysed samples from the lake to follow the *in situ* TP concentration.

CARD-FISH and biomass of phylogenetic lineages. We applied CARD-FISH (Sekar et al. 2003) with oligonucleotide probes EUB I-III (Daims et al. 1999), ALF968 (Neef 1997), BET42a, CF319a and HGC69a (Amann et al. 1995) to quantify the abundance and biomasses of *Bacteria* (hereafter referred to as EUB), *Alpha-* and *Betaproteobacteria* (ALF and BET, respectively), the *Cytophaga–Flavobacterium–Bacteroides* group (CFB) and *Actinobacteria* (ACT). More details of the hybridization technique are given in Salcher et al. (2007).

Cell dimensions of hybridized bacteria were measured directly at blue excitation (488 nm) with the image analysis system described above. Since CARD-FISH stains cellular proteins, which results in apparently larger cell dimensions than size measurements from DNA staining (DAPI), we applied the following volume-to-C conversion for hybridized cells: $CC_{HYB} = 120 \times MCV_{HYB}^{0.72}$, where MCV_{HYB} is the mean cell volume of hybridized bacteria (µm^3) and CC_{HYB} is the cellular C content of hybridized bacteria (fg C bacterium^{-1}). This conversion factor has been previously used to compensate for the larger dimensions of cells stained with Acridine Orange (AO) (Posch et al. 2001). The biomass of bacteria from different phylogenetic lineages (in µg C l^{-1}) was calculated by multiplying CC_{HYB} by their specific abundance (in bacteria l^{-1}).

RESULTS

Bacterial and HNF abundance and biomass

Initial values of bacterial abundance and biomass in the <0.8 µm variants were 3.2×10^6 cells ml^{-1} and 39 µg C l^{-1}, respectively. The release from top-down control caused an increase in bacterial numbers and biomasses in all variants, but to a different extent (Figs. 1 & 2, Table 1). The addition of P resulted in a 2.2- and 3.6-fold rise in numbers and biomasses, respectively. The highest values were found in samples incubated in dialysis tubes. Here, bacterial abundance increased 3.0-fold, and the mean bacterial cell volumes also
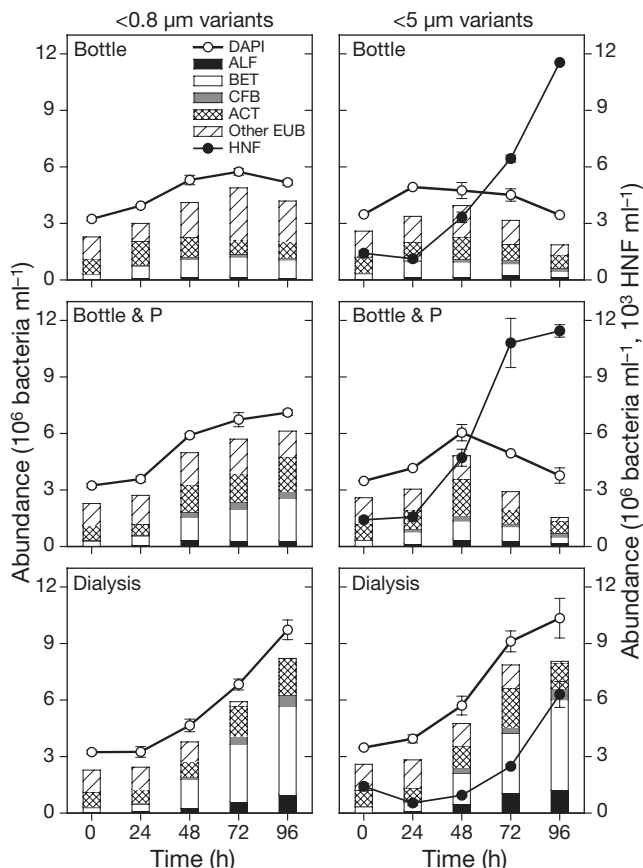
Fig. 1. Development of bacterial (DAPI, ○) and heterotrophic nanoflagellate (HNF) numbers (●) in all experimental variants. Bars show abundances of 4 phylogenetic lineages. Values are means of duplicate setups and whiskers represent the deviation from the arithmetic mean. ALF = *Alphaproteobacteria*, BET = *Betaproteobacteria*, CFB = *Cytophaga–Flavobacterium–Bacteroides* group, ACT = *Actinobacteria*, Other EUB = difference between the sum of specific groups and the abundance of *Bacteria*

increased, which resulted in an 8.9× higher total biomass than at the beginning of the experiment (Fig. 2, Table 1).

As opposed to the <0.8 µm variants, bacterial biomass was negatively affected in all <5 µm variants incubated in bottles where protistan predators were present (Fig. 2). Bacterial abundance and biomass were 3.5×10^6 cells ml^{-1} and 49 µg C l^{-1}, respectively, at the beginning of the experiment. Initial HNF abundance was 1.4×10^6 cells l^{-1}. The addition of P to these variants led to a weak increase in bacterial biomass, but also to a faster increase in HNF numbers (Fig. 1). The highest and relatively stable increase in bacterial biomass (6.0-fold) was again observed in the dialysis tubes (Table 1). In these treatments, the development of protistan grazers was delayed in comparison to the bottle variants (Fig. 1).

Abundance and biomass of bacterial phylogenetic lineages

In the <0.8 µm variants, increases in total numbers could be mainly attributed to BET and ACT. The abundance of ACT increased by a factor of 2.4 after P addition and by a factor of 2.5 after 96 h in the dialysis tubes, but remained stable in unamended bottles. In contrast, numbers of BET were 10 and 20 times higher in these setups, but less than 5 times higher in bottles without P (Fig. 1, Table 1). The specific biomass of BET increased 12 and 28 times after P addition and in the dialysis variant, respectively (Fig. 2, Table 1). The abundance of ALF and CFB also increased in all variants, but their relative contribution to total hybridized cell abundance remained low. However, ALF and CFB reached higher biomass than ACT in the dialysis tubes.

Increasing grazing rates in all <5 µm bottle variants resulted in stagnating or even declining numbers of BET and ACT, whereas CFB and ALF became more numerous (Fig. 1, Table 1). These patterns were also reflected in the development of group-specific bio-
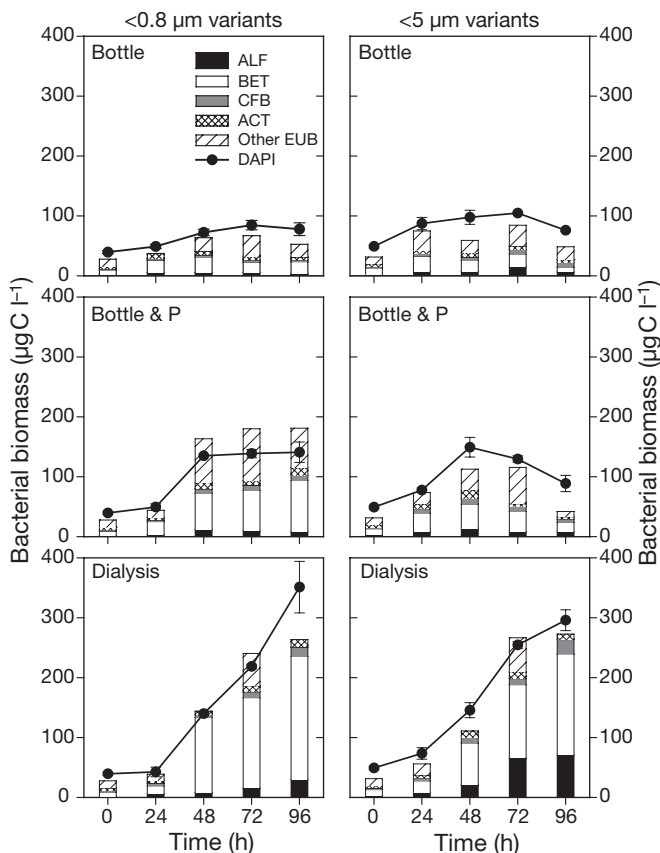

Fig. 2. Development of total bacterial biomass (●) and biomasses of 4 phylogenetic lineages (bars). Values are means of duplicate setups and whiskers represent deviations from the arithmetic mean. For abbreviations see Fig. 1

masses. Since grazing rates in the <5 µm dialysis tubes increased more slowly than in bottles, BET dominated the assemblage in terms of abundance and biomass at the end of the experiment, followed by ALF and CFB (Fig. 2, Table 1).

We compared the BET-to-ACT ratio in terms of bacterial abundance (Fig. 3A,B) and biomass (Fig. 3C,D). ACT was 3 times more abundant than BET at the beginning of the experiment, but BET dominated by a factor of 2 in terms of biomass. The physical separation of bacteria from predators in the <0.8 µm variants shifted numerical dominance from ACT to BET (Fig. 3A). This shift in abundance ratios did not seem to be influenced by P addition, but was very pronounced in the dialysis tubes. Biomass ratios changed even more drastically in the <0.8 µm variants, reflecting a positive effect of P addition and dialysis tube incubations on BET biomass (Fig. 3C). This was due to a pronounced increase in CC_{HYB} in these 2 treatments (Fig. 3E), whereas ACT maintained low C content (Fig. 3G). High grazing rates in the <5 µm bottle variants maintained abundance ratios (Fig. 3B) of the 2 groups as found in the lake and even the biomass ratio (Fig. 3D) remained constant in the unamended bottle. In contrast, biomass ratios were also shifted in favour of BET after P addition and in the <5 µm dialysis tubes.

Table 1. Relative increases (values >1) or decreases (bold values <1) of bacterial parameters (abundance and biomass) after 96 h. Size-fractionated samples (<0.8 and <5 µm variants) were either incubated in bottles (Bottle), in bottles enriched with 15 µg P l^{-1} (Bottle & P) or in dialysis tubes (Dialysis). DAPI = total bacterial parameters determined via DAPI-staining; the following parameters were determined from CARD-FISH preparations: EUB = *Bacteria*, ALF = *Alphaproteobacteria*, BET = *Betaproteobacteria*, CFB = *Cytophaga-Flavobacterium-Bacteroides,* and ACT = *Actinobacteria*

	DAPI	EUB	ALF	BET	CFB	ACT
Bacterial numbers						
<0.8 µm variants						
Bottle	1.6	1.8	2.0	4.2	2.0	1.1
Bottle & P	2.2	2.7	6.8	9.6	6.4	2.4
Dialysis	3.0	3.6	21.2	19.8	11.3	2.5
<5 µm variants						
Bottle	1.0	**0.7**	3.2	1.1	3.0	**0.8**
Bottle & P	1.1	**0.6**	3.7	1.2	3.9	**0.8**
Dialysis	3.0	3.1	24.2	17.5	11.2	1.7
Bacterial biomass						
<0.8 µm variants						
Bottle	2.0	1.9	1.9	2.8	1.3	1.0
Bottle & P	3.6	6.5	4.9	11.6	3.6	3.0
Dialysis	8.9	9.5	18.2	27.9	6.3	2.9
<5 µm variants						
Bottle	1.5	1.6	2.6	**0.8**	3.6	**0.9**
Bottle & P	1.8	1.3	3.1	1.6	2.5	**0.7**
Dialysis	6.0	8.1	28.2	16.3	11.2	1.8

Grazing led to a decrease in the cellular C of BET only in the dialysis treatment (Fig. 3F), whereas the cellular C of ACT increased at the beginning (Fig. 3H).

Bacterial production

If other potential sources of mortality (viral lysis) are neglected, gross production rates in the <0.8 µm variants can be set equivalent to net production rates. In all bottle variants, decreasing production rates after 48 h possibly reflected substrate or nutrient depletion (Fig. 4). Enrichment with P resulted in slightly higher production rates, as also mirrored by elevated bacterial numbers (see Fig. 1). Bacterial production rates showed the most pronounced increase in the <0.8 µm dialysis tubes. Grazing pressure resulted in negative net production already after 48 h (Fig. 4) in the <5 µm bottle variants, but only at the last sampling time point (96 h) in the <5 µm dialysis tubes.

P partitioning

TP concentrations in the <0.8 µm bottle variants were very stable, indicating only small losses due to wall growth of biofilm (Fig. 5). Concomitant with the steep increase in bacterial biomass, we detected a 3-fold increase in P bound in bacteria in the <0.8 µm dialysis variant. In contrast, the TP concentrations of the surrounding lake water remained stable at 6.2 ± 0.4 µg l^{-1} during 4 d (Fig. 5).

The applied separation method of P in all <5 µm variants was very effective, as reflected by linear regression of directly measured TP versus the sum of the 2 P fractions (regression slope = 0.94, r^2 = 0.96, n = 30). Only a negligible overestimation of the real TP concentrations was observed when summing up the 2 size fractions (<10%). Direct counts of bacteria in the <1.2 µm size fractions revealed that on average >95% of bacterial cells of the raw samples passed the filter. In the <5 µm bottle variants, the 2 size fractions accounted for equal amounts of P (Fig. 5), which moreover represented roughly the *in situ* TP concentrations (as determined at time point 0 h). After the enrichment with 15 µg P l^{-1}, approximately half of TP was still bound in the >1.2 µm fraction at 96 h, but 3-fold higher values were reached than in the bottle variants without P enrichment. Interestingly, the accumulation of P by the microbial food web led to an almost equally high increase (2.6×) in particulate P in the <5 µm dialysis tubes. However, less P was found in the largest size fraction than in P-enriched bottles, probably reflecting the slower development of HNF (Fig. 1).
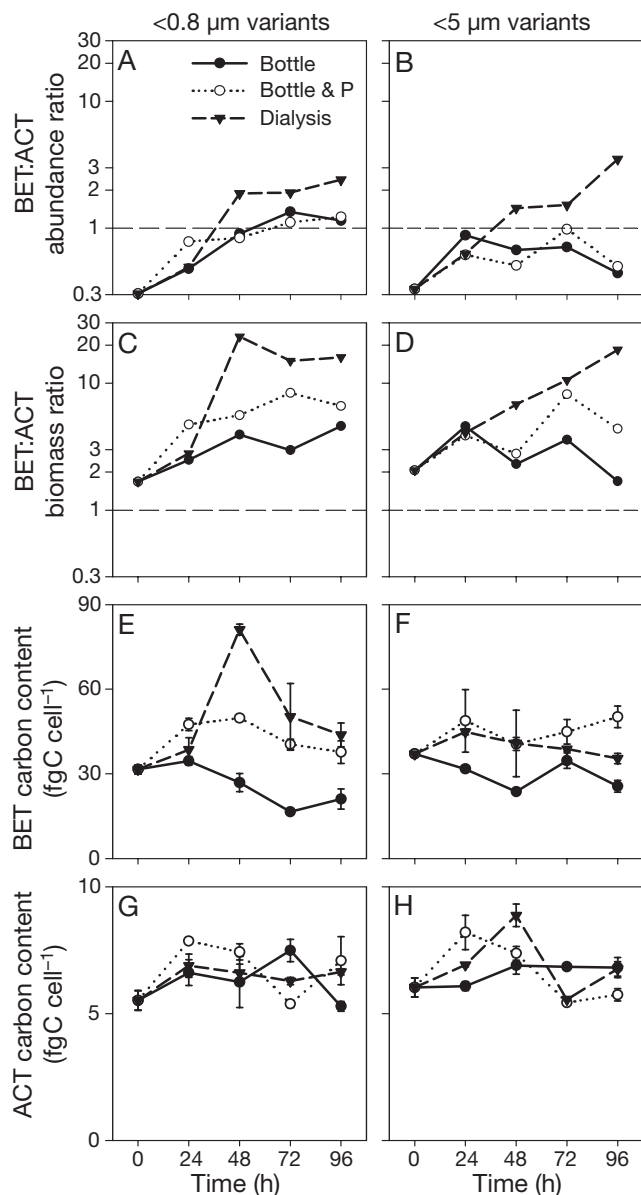
Fig. 3. Ratio between *Beta-* (BET) and *Actinobacteria* (ACT) with respect to their (A,B) abundance and (C,D) biomass over time. Note the logarithmic scale. Cellular C contents of (E,F) BET and (G,H) ACT (note different scale) in all treatments

Fig. 4. Bacterial production rates determined via [3H]thymidine uptake rates, which were converted to cell numbers with empirical conversion factors (see text for further explanations). Net production rates for the <5 µm variants were calculated by subtracting total grazing rates from the gross production rates. All values are means of 2 replicate setups; whiskers represent deviations from the arithmetic mean. Intervals at the *x*-axis symbolize periods during which the presented production values are valid

DISCUSSION

Bacterial production potential versus standing stock in Piburger See

In many freshwater lakes, numbers and biomass of heterotrophic bacteria seasonally vary only within a narrow range. However, the determination of bacterial standing stock could be regarded as a 'snapshot,' which does not inform us about the balance of growth and loss processes,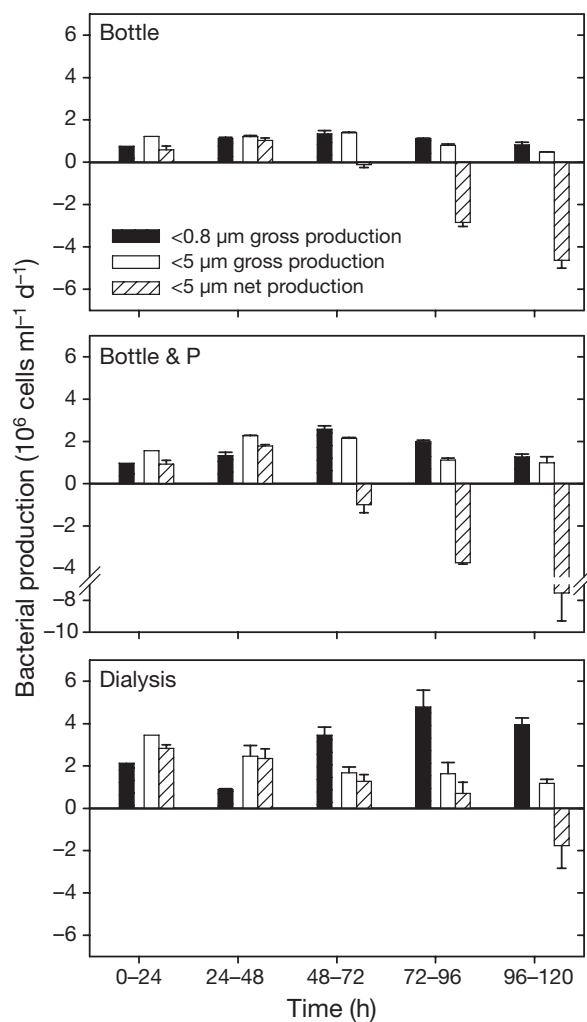 and does not take into account substantially higher fluctuations of bacterial productivity. In Piburger See, total bacterial abundance and biomass at the surface layer vary only slightly throughout the year. Sommaruga & Psenner (1995) reported seasonal ranges of bacterial numbers between 1.2×10^6 and 4.7×10^6 cells ml^{-1}. In contrast, leucine uptake rates varied from 0.039 to 1.22 nmol l^{-1} h^{-1}. Even at sampling frequencies of 2 to 3 d, Pernthaler et al. (1996) only found moderate changes in abundance (1.4×10^6 to 3.5×10^6 cells ml^{-1}), whereas thymidine uptake rates varied more than 10-fold (3.7 to 40.2 pmol l^{-1} h^{-1}).

Numerous approaches have been developed to investigate bacterial growth and loss processes in more
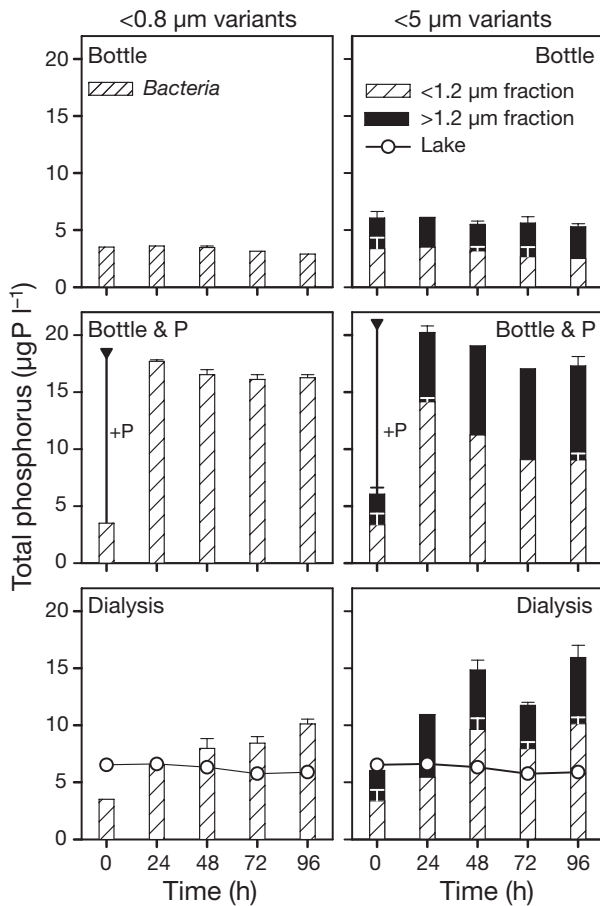
Fig. 5. Total P (TP) concentrations of the bacterial (white bars) and HNF fraction (black bars). For the <5 µm variants, these 2 fractions were separated via filtration over a 1.2 µm pore-size membrane filter. For the Bottle & P variants, the added amount of P (15 µg P l⁻¹) is symbolized by a triangle and a line. TP values of the untreated lake water during the investigation period are shown in the Dialysis panels (O). Values are means of 2 replicate setups; whiskers represent deviations from the arithmetic mean

highest increase of abundances and biomasses at reduced grazing pressure, while ACT only moderately responded to experimental manipulations.

P enrichment and bacteria as a sink or link of P

As outlined by Vadstein et al. (1993), bacteria can act either as 'sink' or 'link' for different elements in aquatic food webs. Thus, bacteria can contribute in very different ways to the total pool and flux of C and P in ecosystems.

If the development of bacterial parameters in our experiments were regarded only from the unamended bottle variants, the potential production of the microbial assemblage would be significantly underestimated. Addition of P revealed much higher production potential (Fig. 4). Thus, if only bottle incubations with and without P addition were compared, one could conclude that bacterial growth was P limited. However, the incubation of filtrates in dialysis tubes indicated a much more complex situation. A release from grazing pressure illustrated a high growth potential of the total bacterial assemblage and of distinct phylogenetic lineages, if free access to nutrients at ambient concentrations was allowed for (Fig. 2). Therefore, we might need to interpret our addition of P to bottles as a study about the effects of allochthonous (surplus) nutrient addition, rather than as a nutrient limitation experiment.

On the one hand, bacteria showed a high potential to incorporate P after the addition of surplus P in 'bottle systems' (Fig. 5). On the other hand, the bacterial assemblage in the dialysis tubes was capable of accumulating TP at nearly 3-fold higher than ambient levels when released from grazing. These observations indicate that bacteria in Piburger See can act as an effective sink of P at very different concentrations of this nutrient. Few studies have reported the total amount of particulate P within the 'bacterial size fraction' in freshwater lakes. Most of these investigations pointed at a high bacterial contribution (from 30 up to more than 50%) to the pool of TP, typically with high seasonal variability (Rigler 1956, Vadstein et al. 1988, Jürgens & Güde 1990, Rothhaupt & Güde 1992, Vadstein et al. 1993, Elser et al. 1995). In Piburger See, approximately 100% of the TP was found in the size fractions <5 µm, and 50% in the <0.8 µm size fraction during the investigation period (Fig. 5). We conclude that both the bacterial and (presumably) the HNF fraction may act as powerful sinks for P if decoupled from higher trophic levels.

It has been suggested that growth rates of BET may be related to P concentrations (Šimek et al. 2006). Here we provide more direct evidence for a stimulation of

detail. In the present study, we chose size fractionations of raw water samples to separate bacteria from larger planktonic compartments (Šimek et al. 1999) in combination with nutrient enrichment experiments. Our experimental manipulations resulted in rapid changes of total bacterial abundances that were at least in the range of annual variability. The decoupling of bacteria from larger planktonic components revealed even higher dynamics of P incorporation, production rates and especially of biomasses, reflecting the complexity of processes that lead to the realized levels of bacterial standing stock. These observed changes of bulk parameters could moreover be attributed to the dynamics of distinct phylogenetic bacterial lineages, some of which exhibited disproportional growth or loss compared to the total assemblage (see also Salcher et al. 2007). Specifically, BET showed the

these bacteria by surplus P (Figs. 1 & 2). Moreover BET seemed most effective in collecting P from lake water during incubation in dialysis tubes. This is reflected in the marked increase of P in the bacterial size fraction and the concomitant dominance of bacterial biomass by BET in the <0.8 µm dialysis tubes after 48 h (Figs. 2 & 5). Consequently, our experiments helped to clarify which members within the assemblage profited most strongly from P addition (or were most successful in P acquisition at low ambient levels). By contrast, abundances and biomasses of the total assemblage (all DAPI-stained cells) seemed to be much less affected by grazing or nutrients (Table 1). Thus, one might underestimate the effect of limitation and predation on distinct phylogenetic lineages by studying bacterial bulk parameters only.

Abundances versus biomasses of phylogenetic lineages

Recently, a number of studies have used FISH for analyzing freshwater bacterioplankton. Yet, the majority of these investigations have focused on the quantification of cell numbers but not on biomass. However, a major advantage of the FISH approach is that it simultaneously provides information about both bacterial morphotype and identity, and consequently, about the biomass contributions of different lineages. At present, the quantification of the biomass of hybridized cells is still technically challenging. FISH with fluorescently monolabeled oligonucleotide probes may result in very variable fluorescence intensities of hybridized cells (compared to DNA staining by DAPI), thus complicating the measurements of cell dimensions via standard image analysis procedures. CARD-FISH preparations are characterized by low bleaching and bright signal intensities, but the apparent cell dimensions are typically larger than of DAPI-stained cells. Discrepancies between sizes of cells stained with different fluorochromes are well known, e.g. when using the nucleic acid stains DAPI and AO (Sieracki & Viles 1992). For the correct quantification of biomass (in µg C l^{-1}), such differences can be compensated for by the use of adequate, stain-specific volume-to-C conversion factors (Posch et al. 2001). Unfortunately, to date, there is no rigorous comparison of cell sizes determined by CARD-FISH and other fluorochromes. Therefore, we applied a volume-to-C conversion factor, which was originally described for AO-stained cells, to calculate biomasses of hybridized cells. Considering the staining properties of AO (it interacts with DNA and RNA), this seems to be a good approximation. Moreover, the biomasses of all hybridized cells (stained by probe EUB I–III) seemed to match the biomasses determined from

DAPI-stained preparations reasonably well at time points when the vast majority of DAPI-stained cells could be hybridized (see our Fig. 2 and Salcher et al. 2007). In any case, the biomass ratios (Fig. 3) and contributions of different lineages to total biomass of hybridized cells (Fig. 6) are unaffected by conversion factors.

The determination of the abundances and biomasses of distinct phylogenetic lineages revealed complementary aspects of the studied microbial assemblage (Fig. 6). Although ACT numerically dominated the assemblage *in situ*, their biomass was only half of BET. This discrepancy was even more pronounced in all
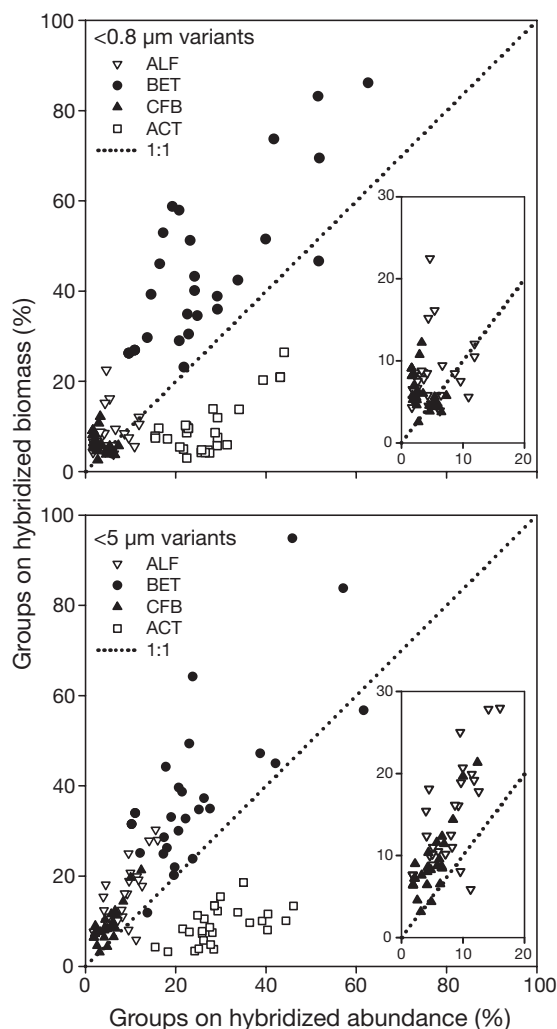

Fig. 6. Abundance and biomass contributions of 4 phylogenetic lineages to total hybridized *Bacteria* (determined via hybridization with probe EUB I–III). All data from the bottle variants (with and without P addition) and the dialysis variants were pooled for the <0.8 µm and the <5 µm setup, respectively. Note the 2 inserts highlighting development of *Alphaproteobacteria* (ALF) and *Cytophaga–Flavobacterium–Bacteroides* (CFB). BET = *Betaproteobacteria*, ACT = *Actinobacteria*

variants where HNF were removed via filtration (Fig. 3A,C). This may point to the importance of size-selective protistan grazing in affecting biomass ratios of different phylogenetic groups. This aspect is also likely to influence the competition between bacterial lineages (Salcher et al. 2007).

At present, it is still unclear if abundance or biomass represents a more appropriate link to the activity patterns of distinct phylogenetic lineages (Yokokawa et al. 2004). While there is evidence for a correlation between relative abundance and the percentage of [3H]leucine active cells for distinct lineages in freshwater systems (Zhang et al. 2006), this relationship may change dramatically due to substrate availability and quality (Pérez & Sommaruga 2006). Therefore, it might be necessary to synoptically study the contribution of different bacterial lineages to the activity, abundance and biomass dynamics of the total microbial assemblage in the context of top-down control and nutrient limitation. The methodological tools for such investigations have become available, namely quantification of lineage-specific biomasses via image analysis procedures, analysis of food vacuole contents of bacterivores (Jezbera et al. 2006), and specific uptake preferences via microautoradiography (Zhang et al. 2006). Thus, the combination of these approaches might substantially refine our view on C and P fluxes within microbial food webs.

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