Effects of phosphorus loading on interactions of algae and bacteria: reinvestigation of the 'phytoplankton–bacteria paradox' in a continuous cultivation system

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ABSTRACT: The effect of different phosphorus loads (L_P) on the phosphorus (P) and carbon (C) content (biomass) of algae and bacteria was assessed in continuous culture. We tested if a mixed freshwater microbial assemblage co-cultured with a phytoflagellate (*Cryptomonas phaseolus*) would comply with the 'phytoplankton–bacteria paradox' (sensu Bratbak & Thingstad 1985). This hypothesis states that the ratio of bacterial to algal abundance changes to the benefit of bacteria with decreasing L_P. However, the phenomenon was originally investigated by simultaneously altering L_P and microbial growth rates, and it is unclear to which extent it can be assigned to either parameter. Therefore, we set up 3 chemostat systems in triplicate at equal dilution rates, but with daily L_P of 21, 41 or 62 µg l^{-1} d^{-1} (corresponding to 50, 100 and 150 µg P l^{-1}). Higher L_P led to a 5-fold increase in total algal abundance and biomass but to less than a doubling of these parameters in the bacterial assemblage. Total biomass ratios of bacteria to algae changed from 0.18 to 0.06 with increasing L_P, while the bacteria–algae total phosphorus ratios decreased from 0.80 to 0.17. The cellular C:P ratio of algae remained similar at all P concentrations, whereas the molar C:P ratios of bacterial cells significantly increased at higher L_P (from 44 to 73). An enrichment experiment with the 50 µg P l^{-1} treatment demonstrated that bacteria at the lowest L_P were co-limited by P and C, and that increased P stimulated mainly the algal fraction. The phytoplankton–bacteria paradox at the level of a mixed microbial assemblage is thus characterised by the following aspects: (1) bacteria profit from their high affinity to P and are better competitors at lower L_P; (2) although algae compete with bacteria for P, P-limited algae release extracellular C that stimulates growth of their bacterial competitors; (3) when bacteria depend on algae as their sole source of organic C, this provides a feedback mechanism by which algae limit the abundance of their competitors at higher L_P; (4) large oscillations in the bacteria–algae ratios at the lowest L_P point to a greater instability of this interaction with stronger P competition. However, bacteria were not able to outcompete *C. phaseolus*, as algae were their only C source.

KEY WORDS: Algae–bacteria interaction · Chemostat · Continuous cultivation system · *Cryptomonas phaseolus* · Eutrophication · Phosphorus · Phytoplankton–bacteria paradox

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INTRODUCTION

Traditionally, pelagic heterotrophic bacteria have almost exclusively been regarded as 'mineralisers' of nutrients bound in detrital organic matter, thus supporting algal production with recycled dissolved phosphorus and other inorganic nutrients. However, planktonic algae and bacteria exhibit more complex ecological interactions that cannot easily be described by simple mineralization-based models (Fogg 1971, Currie 1990, Grossart 1999, Gurung et al. 1999). If bacterial production is limited by the supply of key

nutrients, such as nitrogen or phosphorus, the relationship between bacteria and algae is not considered mutualistic, but competitive (Currie & Kalff 1984b, Elser et al. 1995, Vadstein 2000).

The competition for P between bacteria and algae cannot easily be studied *in situ*, i.e. in a planktonic food web of high complexity. Continuous cultivation systems have the advantage that algae and bacteria experience fewer environmental fluctuations and can be cultivated at known specific growth rates. In addition, the systems can easily be replicated. Seminal chemostat experiments by Bratbak & Thingstad (1985) showed that a marine bacterial isolate acquired P more efficiently than a diatom when the phosphorus loads (L_P) was decreased by reduction of the dilution rate. Since extracellular organic carbon (EOC) release by algae was the only carbon source for the bacteria, a paradoxical situation developed: although bacteria became more competitive in sequestering P at low P concentrations (or lower growth rate), algae still supported bacterial carbon demand by EOC release. Thus, the algae supported the growth of their own competitors, and the bacterial versus algal P ratio increased with stronger P limitation. The authors termed their observations the 'phytoplankton–bacteria paradox'. However, since the P limitation in the chemostat system was induced indirectly by a change of the dilution rate, the possibility cannot be excluded that this phenomenon was related to physiological adaptations of the algae or bacteria to decreased growth rates.

Rapidly growing bacterial cells are characterised by lower C:P ratios than slowly growing cells (Chrzanowski et al. 1996, Makino et al. 2003). The C:P of algae may decrease from ~1000 to 100 with increasing growth rates (Gächter & Bloesch 1985). Both bacteria and algae have the ability to store P and N surplus in excess of their actual demand, which is reflected by the high variability of the intracellular nutrient content in natural populations (Vrede et al. 2002). Thus, the nutrient flux within the microbial food web and the competition for nutrients are also influenced by growth rate-related stoichiometric constraints.

To assess the effect of different L_P on bacteria-algae interactions, we performed an experiment with the phytoflagellate *Cryptomonas phaseolus* and a mixed bacterial assemblage in continuous culture. The following questions and hypotheses were addressed: (1) Is the phytoplankton–bacteria paradox (Bratbak & Thingstad 1985) confirmed for a mixed bacterial assemblage if the dilution rate (growth rate) is kept constant, and only the P loadings are varied? (2) If so, to which extent is this paradoxical situation reflected in different community parameters, specifically in the abundance, biomass (i.e. total organic C) and P content of the algal and bacterial assemblage? (3) Which single-cell characteristics of bacteria and algae (elemental content, stoichiometry, cell dimensions) change with different P loads? (4) Are bacteria increasingly C-limited at lower P concentrations, in parallel with increasing bacterial to algal biomass ratio?

MATERIALS AND METHODS

Experimental design. Experiments were performed in 9 chemostat vessels (see Posch et al. 1999 for a schematic drawing) with a dilution rate of 0.41 d^{-1}, corresponding to approximately 20 analysed generations of bacteria and algae over the investigation period (37 d). The phytoflagellate *Cryptomonas phaseolus* was grown on an inorganic medium (Guillard & Lorenzen 1972) in these continuous-flow systems together with an accompanying mixed bacterial assemblage (Pernthaler et al. 2001) fuelled by organic substrates released by the algae. *C. phaseolus* seems to be a true photoautotrophic organism, as we never observed any uptake of bacteria by the phytoflagellate in former experiments (Šimek et al. 1997, Posch et al. 2001). Phosphorus concentrations were adjusted with K_2HPO_4 to obtain P levels of 50 µg P l^{-1} (P50), 100 µg P l^{-1} (P100) and 150 µg P l^{-1} (P150) in triplicate flow-systems under controlled laboratory conditions. The daily phosphorus loads (L_P) were 21 µg P l^{-1} d^{-1} (0.6 µM d^{-1}) in P50, 41 µg P l^{-1}d^{-1} (1.3 µM d^{-1}) in P100, and 62 µg P l^{-1} d^{-1} (1.9 µM d^{-1}) in P150. Nitrogen was supplied as NO_3^- at non-limiting concentrations and the N:P weight ratios were 280:1, 140:1, and 93:1 for P50, P100, and P150, respectively.

We used 3 glass bottles of 10 l each to store the inorganic media, and these had to be refilled every 8 d. The culture vessels with a volume capacity of 780 ml were aerated from the bottom and had a lateral outflow. Silicon tubes with an inner diameter of 2 to 5 mm were utilised for the connections. A peristaltic pump (Digital Drive MCP with cartridge pump heads; Ismatec) and pumping tubes with inner diameters of 2.06 mm (Ismatec) were used for the transport of the media. All parts were rinsed with distilled water and sterilised before the system was assembled in a walk-in chamber at 16°C. All cultures were kept under constant light intensity (~120 µE m^{-2} s^{-1}) with a 16:8 h light:dark cycle. The vessels were filled with media and inoculated with 20 ml each of a pure stock culture of *Cryptomonas phaseolus* and its accompanying bacteria. Pumping was stopped for 4 d to establish the experimental communities. Subsequently, the dilution rate was set at 0.41 d^{-1} and the first samples were taken (Day 0 of the experiment).

Sampling and sample preparation. Subsamples of 120 ml were collected from each vessel into sterile

250 ml Schott-bottles every second day until Day 10. Afterwards, samples were taken at 5 to 9 d intervals. Occasionally, the inner walls of the vessels were scrubbed manually with a bar magnet to reduce algal and bacterial wall-growth. All subsamples for counting and sizing of bacteria and algae were prefixed with alkaline Lugol´s solution (0.5% final conc.) immediately followed by formaldehyde (2% final conc.) fixation, and bleached with some drops of sodium thiosulphate (3% stock solution).

Heterotrophic bacteria and algae. Bacterial and algal cells were counted after DAPI staining with a Zeiss Axioplan or a Zeiss Axiophot 2 (for algal counts) epifluorescence microscope following the protocol of Porter & Feig (1980). For a more detailed description of the methods see Šimek et al. (1997) and Posch et al. (2001). Mean cell volumes of organisms were determined by image-analysis (Lucia D, Laboratory Imaging: see www.lim.cz). At least 300 algae and 500 bacteria per sample were measured. Bacterial cell carbon content and total bacterial biomass were calculated according to Loferer-Krößbacher et al. (1998): $CC = 218 \cdot V^{0.86}$, $BM = CC \cdot N$, where CC is bacterial cell carbon (fg C $cell^{-1}$), V is bacterial cell volume (μm^3), BM is bacterial biomass (μg C l^{-1}), and N represents bacterial abundance (cells l^{-1}). The carbon content of *Cryptomonas phaseolus* cells was estimated using the conversion factor of Menden-Deuer & Lessard (2000): $CC = 216 \cdot V^{0.939}$. It was verified during a separate pilot study by direct measurements of algal carbon content in treatments enriched with 50 and 150 μg P l^{-1}. For that purpose, subsamples (3 to 5 ml) of the algal cultures were filtered at low vacuum (100 mm Hg) onto pre-combusted glass-fibre filters GF8 (13 mm in diameter, Schleicher and Schüll) which were frozen (–20°C) until analysis (Bertoni 1978). The organic elemental analyses were done by the company ThermoFinnigan with a Flash EA 1112 NC analyzer, and atropine (1 to 1.5 mg) was used as a standard. No correction for bacterial contamination was applied, because only <15% of the bacteria were retained by the GF8 filters used for the concentration of algae.

Phosphorus content of bacteria and algae. In order to separately measure the P content of algae and bacteria, subsamples (25 ml) were filtered at low vacuum (100 mm Hg) onto white 3 μm pore-sized polycarbonate membrane filters (47 mm in diameter; Millipore). For measurements of total dissolved phosphorus (TDP), we filtered subsamples (25 ml) through 0.2 μm polycarbonate membrane filters. Phosphorus concentrations of the whole sample (TP) and of the different size fractions, i.e. >3 μm (algae), >0.2 and <3 μm (bacteria), and <0.2 μm (TDP) were determined by spectrophotometry using the molybdate method after digestion with H_2SO_4 and H_2O_2 (Schmid & Ambühl

1965). The percentage of bacteria retained on the 3 μm filters was checked by epifluorescence microscopy (up to 15% of total bacterial number were retained). The number of retained bacteria was multiplied by the mean bacterial P content and this value was used to correct the determinations of total algal and bacterial P content (resulting in a 5 to 15% correction of measured values).

Nutrient enrichment experiment. We took a 120 ml subsample of the P50 chemostat vessels and prepared 4 treatments (30 ml each) that were kept as batch-cultures for 1 wk: 1 treatment served as a control, whereas the other 3 were enriched with carbon (+C), phosphorus (+P) or carbon plus phosphorus (+CP). Surplus phosphorus was added as K_2HPO_4 to give a final concentration of 150 μg P l^{-1}, corresponding to the P150 chemostat vessels. Carbon was added as glucose to a molar C:P ratio of 100:1, resulting in a final concentration of 14.5 mg glucose l^{-1}. Abundance and biomass of organisms were determined daily during 1 wk.

Statistical analyses. We used a 2-way ANOVA (factors: P concentration and time) for repeated measurements, with post-hoc comparisons using Bonferroni-corrected *t*-tests. All statistical analyses were performed using SigmaStat (Version 2.03, SPSS). To control the overall experimental Type I error, we corrected the significance level according to the Bonferroni method as $\{\alpha/[n(n-1)/2]\}$, where α is the probability level and n is the number of observations.

RESULTS

The continuous cultivation systems were sampled 10 times within 37 d. Since P concentrations were regulated via the concentration of the media and not by changed dilution rates, growth rates were fairly constant over the investigation period (Table 1). Total dissolved phosphorus was generally below the detection limit (<1 μg P l^{-1}; data not shown), i.e. all P was incorporated by the algae and bacteria.

Abundance and biomass

EOC released by algae growing on the mineral medium was the sole substrate source for the accompanying bacterial assemblage. Neither trace amounts of vitamins nor an organic buffer (115 mg l^{-1} of TES buffer, Sigma) allowed bacterial growth in batch experiments. We observed 2 phases of population development. After inoculation of the stock culture the system was rather instable between Days 0 and 10. After Day 10 (approximately 5 generations), however, all cultures reached a steady state. Average values of bac-

Table 1. Algae *Cryptomonas phaseolus* and their associated mixed bacterial assemblage. Abundance, biomass, total phosphorus content and growth rate at 3 phosphorus concentrations: 50 µg P l^{-1} (P50), 100 µg P l^{-1} (P100), 150 µg P l^{-1} (P150). Values are means (n = 10) of 3 parallel systems over whole investigation period of 37 d. Minimum and maximum data given in parentheses. B: bacteria; A: algae. Values of P100 and P150 were compared with values of P50 to quantify increase in bacterial and algal parameters

Parameter	P50		P100		P150	
Abundance						
Bacteria (10^6 cells ml^{-1})	6.84	(4.27–9.94)	10.30	(7.23–14.44)	13.20	(8.23–18.12)
Algae (10^4 cells ml^{-1})	2.36	(1.39–3.58)	7.96	(7.14–8.62)	11.75	(10.51–13.99)
B:A ratio	290		129		112	
B increase vs. P50	–		1.5		1.9	
A increase vs. P50	–		3.4		5.0	
Biomass						
Bacteria (µg C l^{-1})	258	(180–451)	391	(266–643)	480	(339–738)
Algae (µg C l^{-1})	1471	(839–2229)	4544	(3262–5972)	7407	(5468–8871)
B:A ratio	0.18		0.09		0.06	
B increase vs. P50	–		1.5		1.9	
A increase vs. P50	–		3.1		5.0	
Phosphorus content						
Bacteria (µg P l^{-1})	16.5	(12.0–22.7)	16.9	(12.3–22.9)	18.4	(12.5–23.7)
Algae (µg P l^{-1})	19.5	(10.5–27.8)	63.7	(55.7–70.1)	108.0	(91.4–123.4)
B:A ratio	0.80		0.31		0.17	
B increase vs. P50	–		1.0		1.1	
A increase vs. P50	–		3.3		5.6	
Growth rate						
Bacteria (d^{-1})	0.46	(0.38–0.49)	0.43	(0.34–0.46)	0.43	(0.36–0.52)
Algae (d^{-1})	0.39	(0.27–0.51)	0.40	(0.27–0.46)	0.40	(0.37–0.45)
B:A ratio	1.18		1.08		1.08	

terial and algal abundance and biomass in the 3 different treatments are compiled in Table 1. In all set ups, bacterial cells increased during the first 10 d and stabilised thereafter. Algal cell numbers remained more or less stable throughout the experiment – with the exception of the P50 treatment, in which algal abundance decreased during the first 8 d from 3.5 to 1.8 × 10^4 cells ml^{-1}. The increase in P concentrations of the medium from 50 to 150 µg l^{-1} caused a doubling of bacterial cell numbers, but a 5-fold increase in algal abundance (Fig. 1, Table 1). Algal cell numbers significantly correlated with the P supply rates (r^2 = 0.93, n = 30, p < 0.001), while bacteria did not respond as strong on P supply (r^2 = 0.42, n = 30, p < 0.05), i.e. bacterial growth did not keep pace with the increasing algal abundance. Algal and bacterial biomass showed the same trend as the corresponding abundance (Fig. 2), and biomass ratios of bacteria versus algae were 2 (P100) to 3 (P50) times higher than at P150, i.e. 0.18 (P50), 0.09 (P100), and 0.06 (P150) (n = 30, p < 0.05, Table 1).

Algal and bacterial phosphorus

The L_P of the medium had a significant effect on the total P content of algae (n = 30, p < 0.001), which increased more than 5-fold from P50 to P150. In contrast, the total P content of bacteria remained almost constant

due to a drop in the per-cell P content (Fig. 3, Tables 1 & 2). Thus, in P50, 46% of the TP was found in bacteria, whereas in P100 and P150 the bacterial contribution of TP decreased to 21 and 15%, respectively. Therefore, the bacteria can be regarded as an important P-pool in
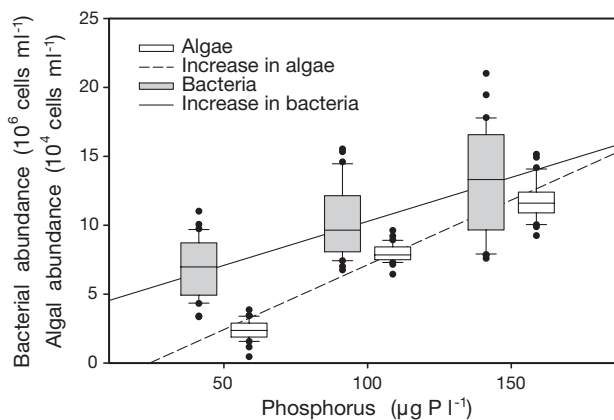

Fig. 1. Algae *Cryptomonas phaseolus* and mixed bacterial assemblage. Box-and-whisker plots of abundance over whole investigation period of 37 d at 3 P concentrations (50, 100, 150 µg P l^{-1}). Each box is based on n = 30 (3 triplicates sampled 10 times each). Bars show 25th, 50th and 75th percentiles of all data and whiskers 10th and 90th percentiles; data points are outliers. Trend lines show steep increase in algal and slower increase in bacterial abundance with increasing P load
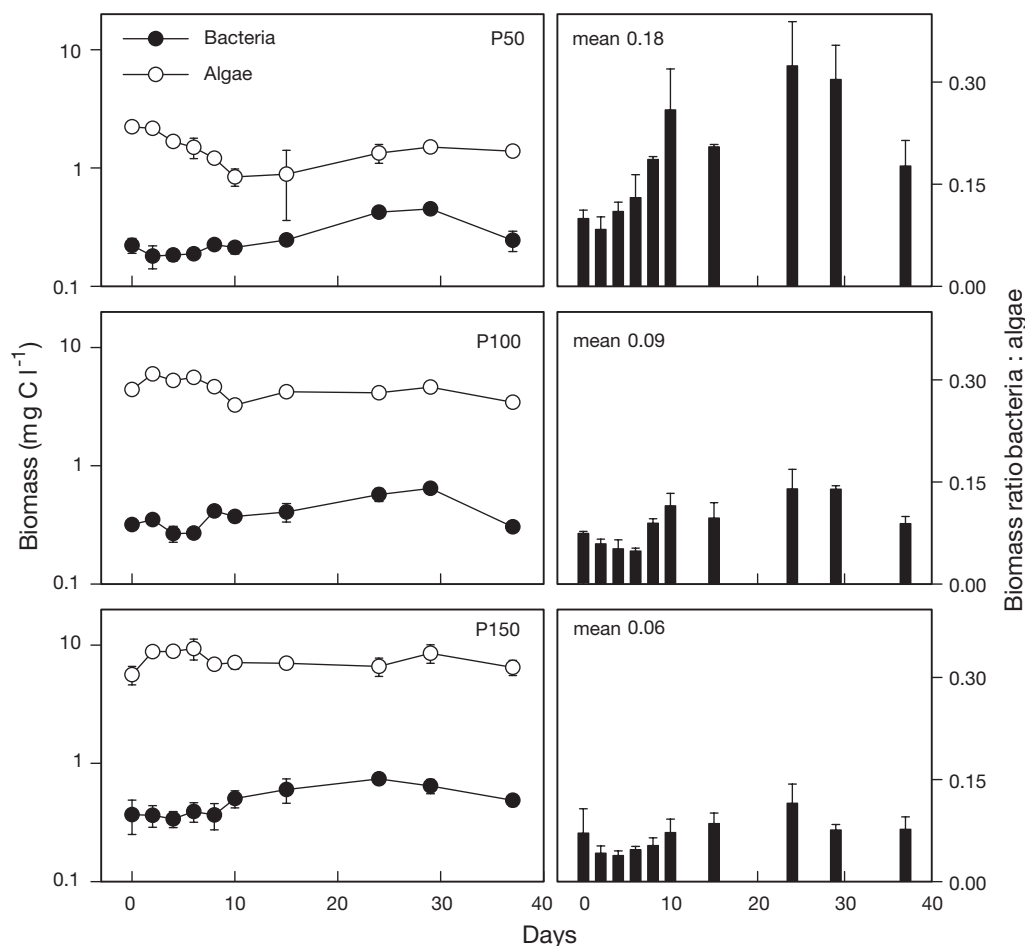
Fig. 2. Algae *Cryptomonas phaseolus* and mixed bacterial assemblage. Biomass over whole investigation period of 37 d at 3 P concentrations (P50, P100, P150: 50, 100, 150 µg P l^{-1}, respectively). Values are means (±1 SD) of 3 parallel chemostats

the treatment with the lowest P supply, but their relative contribution to TP diminished at higher L_P.

Cell-specific carbon and phosphorus content

We found no distinct changes in the mean cell volume over the investigation period of either bacteria or algae (Table 2). The development of abundance and biomass showed similar trends. Phosphorus enrichment in the medium significantly affected the cellular P content of bacteria but not of algae. The mean P content of the bacterial cells was 2.4, 1.7, and 1.5 fg P cell^{-1} in the P50, P100, and P150 treatments, respectively, and showed a significantly higher value in P50 than in the other treatments (n = 30, p < 0.05, Table 2). Therefore, the C:P ratio of bacterial cells in P50 (C:P = 44) was significantly lower than in P150 (C:P = 73; n = 30, p < 0.05). In contrast to bacteria, the C:P ratio of algae varied only within a narrow range (179 to 193), with

slightly higher values in P50 (n = 30, not significant, Table 2). Microscopic observations of bacterial cells revealed the likely presence of polyphosphate granules in P50 during the first 20 d, as indicated by very bright yellow grains inside the DAPI-stained cells.

Nutrient enrichment experiment

The transfer of organisms from the continuous cultivation to the batch-system caused a slight increase in bacterial abundance and biomass in the control (Fig. 4). Therefore, the bacterial to algal biomass ratio slowly increased until Day 6 (average = 0.23, n = 7). The enrichment with glucose (+C) caused mainly an increase in bacterial cell volume (data not shown) and total biomass, with no increase in abundance (Fig. 4). The bacterial to algal biomass ratio strongly increased (average = 0.45, n = 7). Enrichment with phosphorus (+P) stimulated both bacterial and algal growth, while bacterial

Table 2. Algae *Cryptomonas phaseolus* and their associated mixed bacterial assemblage. Cell volume and cell-specific element content at 3 phosphorus concentrations: 50 µg P l^{-1} (P50), 100 µg P l^{-1} (P100), 150 µg P l^{-1} (P150). Further details in Table 1

Parameter	P50		P100		P150	
Cell volume						
Bacteria (µm^3)	0.132	(0.088–0.180)	0.131	(0.102–0.164)	0.127	(0.092–0.159)
Algae (µm^3)	418	(356–476)	395	(286–508)	434	(332–522)
Cellular carbon						
Bacteria (fg C cell^{-1})	38	(27–50)	38	(31–46)	37	(28–44)
Algae (fg C cell^{-1})	62386	(53715–70652)	57234	(40049–75098)	63098	(48744–76981)
Cellular phosphorus						
Bacteria (fg P cell^{-1})	2.4	(1.8–3.1)	1.7	(1.2–2.5)	1.5	(0.9–2.7)
Algae (fg P cell^{-1})	888	(629–1085)	805	(703–880)	833	(925–1053)
C:P atomic ratio						
Bacteria	44	(25–70)	64	(47–89)	73	(45–99)
Algae	193	(135-267)	183	(131-227)	179	(154-201)

cell volumes decreased. Bacterial growth rate increased especially during the first 2 d; afterwards, algal growth rates were higher (Fig. 4). The bacterial to algal biomass ratios were the lowest of all treatments, with an average value of 0.21 (n = 7). The addition of carbon

and phosphorus (+CP) resulted in the highest increase in bacterial growth rate and total bacterial biomass; thus, bacteria attained the same biomass as algae on Days 3 and 4. Thereafter, the ratio changed again to the benefit of the algae, with a value of 0.16 on Day 6.
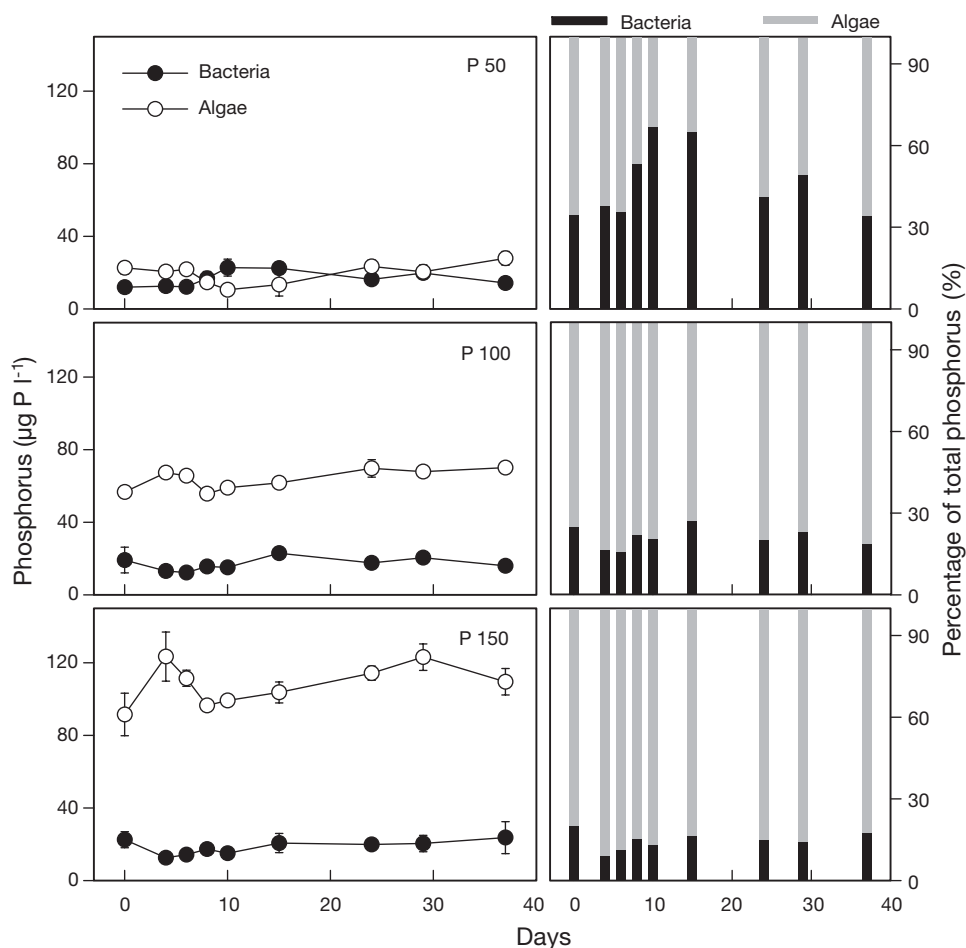

Fig. 3. Algae *Cryptomonas phaseolus* and mixed bacterial assemblage. Development of total phosphorus over whole investigation period of 37 d at 3 P concentrations (P50, P100, P150: 50, 100, 150 µg P l^{-1}, respectively)
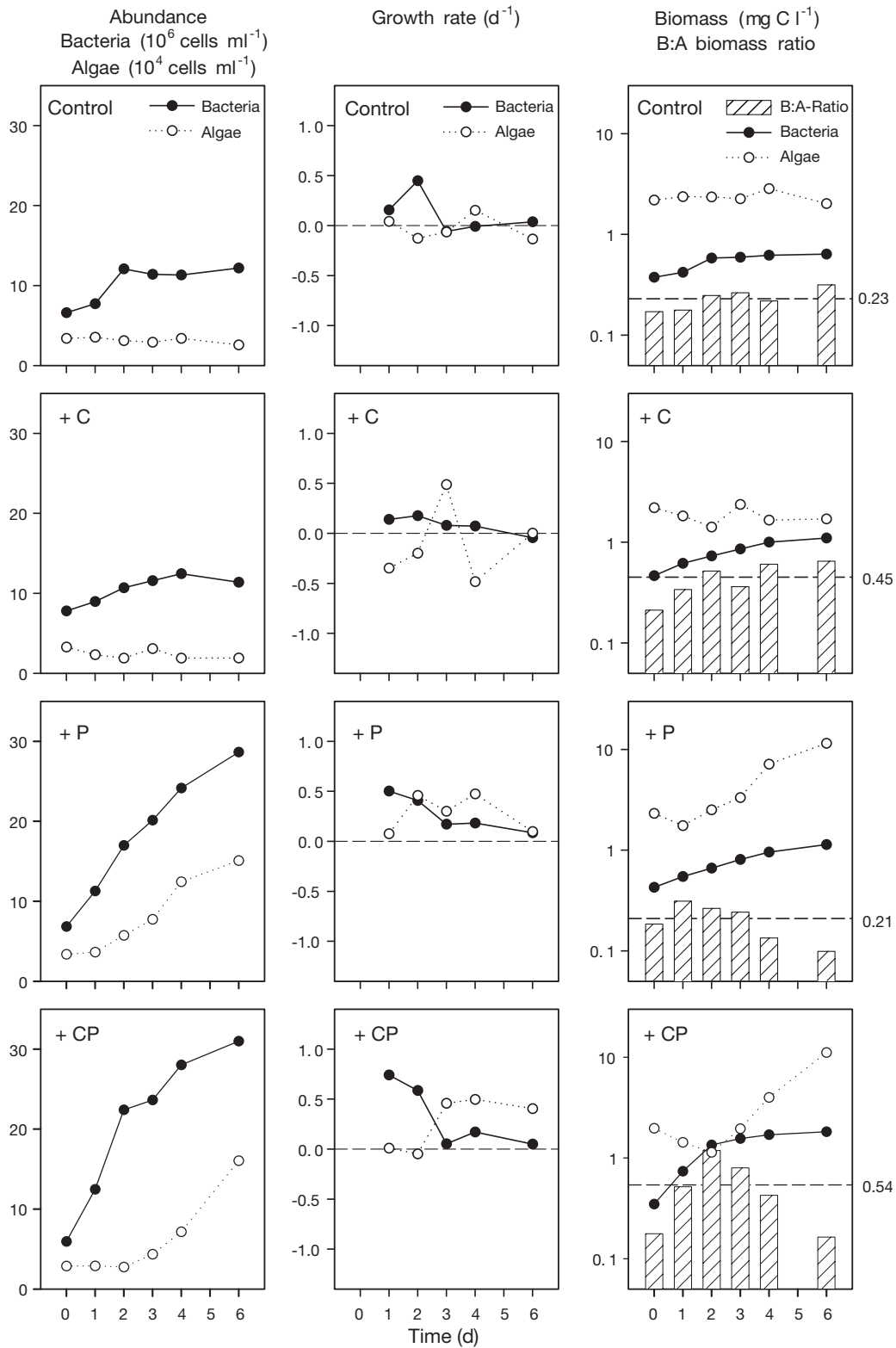
Fig. 4. Algae *Cryptomonas phaseolus* and mixed bacterial assemblage. Nutrient enrichment of samples originally grown in chemostats at 50 μg P l^{-1}. Samples were kept as batch-cultures for 1 wk. Algal and bacterial abundance, growth rate, biomass and biomass ratio are shown for 4 different treatments. Control: no nutrient enrichment; +C: enriched with glucose (final conc. 14.5 mg glucose l^{-1}); +P: enriched with K_2HPO_4 (final conc. 150 μg P l^{-1}); +CP: glucose and K_2HPO_4 (final conc. 150 μg P l^{-1}, resulting in molar C:P ratio of 100:1). Note logarithmic scale of right-hand graphs

DISCUSSION

General remarks on experimental set-up

In recent years, we have used continuous cultivation for modelling resource-limitation, competition, and predation among planktonic microorganisms (Pernthaler et al. 2001, Posch et al. 2001 and references therein). In several previous studies of algae-bacteria interactions in continuous cultivation, the P concentrations were defined by varying the dilution rates of the systems (e.g. Currie & Kalff 1984a, Bratbak & Thingstad 1985). As growth rates of organisms cultivated in chemostats are regulated by the dilution rate, such an approach causes a decrease in both L_P and growth rates. This 'side-effect' on growth rates might substantially influence the interaction between bacteria and algae. For example, lower dilution rates may lead to higher biomass (Makino et al. 2003) and even to higher growth yields of bacteria (Vadstein & Olsen 1989). Highly increased affinities to P and faster P uptake of bacteria has been observed as a result of decreased dilution rates (Vadstein & Olsen 1989), suggesting that the 'phytoplankton–bacteria paradox' might be a consequence of growth rates rather than of P concentrations. Therefore, we chose a different approach by keeping the dilution rate constant. Instead, we varied the P concentrations in the respective cultivation media. Our continuous-flow systems were designed to mimic a gradient of inorganic P supply corresponding to the observed decrease in P load in many temperate lakes after decades of eutrophication in the second half of the 20th century. Although growth rate-related artifacts can be excluded by such an approach, this set-up ignores the possibility that increased P loads might potentially stimulate higher growth rates (Vadstein 2000, Makino et al. 2003).

Phytoplankton–bacteria paradox

In our experimental communities we confirmed that the phytoplankton–bacteria paradox (sensu Bratbak & Thingstad 1985) is also valid for constant growth rates, and applicable to interactions of a freshwater microbial community. In addition, we performed a quantitative assessment of this phenomenon with respect to bacterial and algal parameters. Bacterial to algal abundance and biomass ratios closely followed the pattern of P loads (Fig. 2). At 3-fold higher L_P, the bacteria to algae ratio was 3 times lower (Table 1). Interestingly, a similar trend has been observed in field studies. The bacteria to algae biomass ratio is usually higher in more oligotrophic waters, and the bacterial biomass increases more slowly than that of the phytoplankton

along trophic gradients (Currie 1990, Simon et al. 1992, Gasol & Duarte 2000, Biddanda et al. 2001). Vrba et al. (2003) suggested a similar pattern, i.e. a decreasing proportion of bacteria in the entire plankton biomass with an increasing trophic status of lakes, where higher trophic levels were absent due to acidification. This might indicate that a basic feature of natural bacterial and algal assemblages such as biomass ratios at different P concentrations can be modelled adequately in a cultivation system without bacterial and algal predators. In addition, our own observations showed that the phytoplankton–bacteria paradox was still valid for the tested microbial community, even in the presence of hetero- or mixotrophic bacterivores (Posch et al. unpubl.).

In our experiments, the partitioning of P between bacteria and algae at different L_P also reflected the changes in the relative proportions of abundance and biomass. The bacterial to algal P ratio was 4-fold higher in P50 than in P150. In other words, in P50, 46% of the TP consisted of bacterially bound P, whereas in P100 and P150 the percentages of TP bound to bacteria were 21 and 15%, respectively. Similar values were reported by Olsen et al. (2002) for a chemostat experiment with the algae *Tetraselmis* sp. and a mixed bacterial assemblage. Although bacteria to *Tetraselmis* biomass ratios were low (0.18 to 0.12 with increasing L_P), bacteria bound nearly the same amount of P as algae. Such high percentages are also known from natural systems. Elser et al. (1995) determined the relative contribution of bacterial P to epilimnetic particulate P, and obtained values of 25 to 34% for 3 lakes with different trophic status. Jürgens & Güde (1990) found that on average ~62% of the particulate P was incorporated into the bacterial size fraction in Lake Constance (Germany/Switzerland) during summer. In our experiment, the extremely high fraction of P bound in the bacterial fraction at the lowest L_P might, in part, also reflect the lack of P transfer to primary producers by bacterivorous flagellates (Rothhaupt 1997).

Cell characteristics

Cell morphologies and mean cell volumes of both bacteria and algae varied within a very narrow range, and no relationship between these parameters and P supply could be established. Algal carbon content calculated from cell volumes was in good agreement with the results of direct carbon analyses (Table 3). The bacterial volume to carbon conversion factor (Loferer-Krößbacher et al. 1998) has been verified for both cultures and bacterioplankton from lakes of different trophic status. Therefore, this factor provides a good tool for the estimation of cellular carbon content for a

Table 3. *Cryptomonas phaseolus*. Direct measurements of total carbon biomass (μg C l^{-1}) in cultures enriched with 50 and 150 μg P l^{-1}. Carbon biomass measured with a carbon analyser (means of triplicate measurements ± SD) and calculated according to volume:carbon conversion factor of Menden-Deuer & Lessard (2000): CC = 216 · V$^{0.939}$, BM = CC · N, where CC = cellular carbon (fg C cell^{-1}), BM = biomass (μg C l^{-1}), N = algal abundance (cells l^{-1}), and V = algal cell volume (μm^3); 400 cells measured per triplicate. All data are from pilot study conducted prior to main experiment

| Day | P50 | | | P150 | | |
	C analyzer	C calculated	Percentage	C analyzer	C calculated	Percentage
0	2718 ±414	2682 ±519	99	8481 ±1853	10045 ±749	118
29	2148 ±103	1943 ±21	90	9806 ±653	9479 ±371	97
40	2140 ±465	1787 ±123	84	9319 ±559	9888 ±304	106

comparison of C:P ratios. We found that the carbon content per algal or bacterial cell as inferred from these allometric factors was similar in all treatments (Table 2).

The molar C:P ratio of *Cryptomonas phaseolus* was ca. 3 times larger than that of bacteria (approx. 185), independent of the amount of P supply. In contrast, the mean P content of the bacteria significantly varied with decreasing L$_P$, from 1.4 to 2.5 fg P cell^{-1}, i.e. it was approximately 40% higher in P50 than in P100 or P150. Little information is available about the changes in the cellular P content of bacteria in the context of P availability (Table 4). Nucleic acids usually represent the largest P pool of microbial cells. Since cellular DNA and ribosome content is typically related to growth rate (Cooper 1991, Kemp et al. 1993), the growth rate affects the cellular P content. However, in our study, we kept the growth rate constant at 0.41 d^{-1}, and thus, we can exclude the possibility that the observed change in the bacterial cellular P content was a growth rate-related effect. In some bacteria, P can also be stored as polyphosphate during growth limitation (Weltin et al. 1996), and microscopic observations of bacterial cells in the P50 treatment did indeed reveal the presence of such polyphosphate granules (data not shown). The bacterial molar C:P ratio fluctuated

Table 4. Summary of published bacterial phosphorus contents and molar C:P ratios. E: estuarine; F: freshwater; M: marine; DAPI: sizing of DAPI-stained bacteria; Size-TEM: sizing of bacteria by transmission electron microscopy; XRMA: X-ray microanalysis. Values are means; in most cases observed ranges are also listed in parentheses

P per cell (fg cell^{-1})	C:P	Cell volume (μm^3)	Habitat	Bacteria	Method	Source
0.23 (0.01–7.5)	81 (59–143)	0.048 (0.002–2)	M	Native bacteria	Size-TEM, XRMA	Gundersen et al. (2002)
0.8 (0.5–1.4)	54 (39–66)	0.253 (0.11–0.41)	E, F, M	Native bacteria, cultures	Size-TEM, XRMA	Fagerbakke et al. (1996)
1.3 3.9	56–61 34–41	0.11–0.12 0.17–0.23	F	Cultures, C-limitation	Molybdate method	Jürgens & Güde (1990)
3.0 3.5	81–96 80	0.21–0.26 0.20–0.23	F	Cultures, P-limitation	Molybdate method	Jürgens & Güde (1990)
1.5 (0.7–2.6)	48 (39–70)	0.35 (0.15–0.60)	M	Native bacteria, enriched with amino acids and P	Size-TEM, XRMA	Tuomi et al. (1995)
1.5 (0.9–2.7)	73 (45–99)	0.127 (0.09–0.16)	F	Culture together with algae, high P	DAPI, molybdate method	This study
1.7 (1.2–2.5)	64 (47–89)	0.131 (0.10–0.16)	F	Medium P	DAPI, molybdate method	This study
2.4 (1.8–3.1)	44 (25–70)	0.132 (0.09–0.18)	F	Low P	DAPI, molybdate method	This study
2.3 (2.2–2.3)	178 (176–180)	2.38 (1.70–3.31)	M	Isolates, P-limitation	Size-TEM, XRMA	Vrede et al. (2002)
2.8 (1.4–4.4)	42 (25–70)	0.45 (0.28–0.91)	M	Isolates, C-limitation	Size-TEM, XRMA	Vrede et al. (2002)
5.7 (4.7–8.3)	49 (29–56)	1.40 (0.85–1.99)	M	Isolates, N-limitation	Size-TEM, XRMA	Vrede et al. (2002)
12 (8.5–16)	35 (26–50)	1.78 (0.81–3.00)	M	Isolates, Exponential growth	Size-TEM, XRMA	Vrede et al. (2002)

between 25 and 100 between treatments and increased with increasing L_P (Table 2). Bacterial cells grown under P limitation typically have C:P ratios in the range of 100:1 and higher (Jürgens & Güde 1990, Vrede et al. 2002, Makino et al. 2003). The C:P ratio for cells grown under C or mineral limitation other than P falls below 50:1 (Jürgens & Güde 1990, Vrede et al. 2002). The observed bacterial C:P ratios thus provided indirect evidence that bacteria in all treatments were C-limited. Therefore, we performed enrichment experiments to test this hypothesis (Fig. 4).

Mechanistic explanation of phytoplankton–bacteria paradox

Our enrichment experiments (Fig. 4) provide a first insight into why the interactions between bacteria and algae change with increasing L_P. The exclusive addition of C to the microbial assemblages from the P50 treatments primarily resulted in an increase in bacterial cell volume and total biomass. In contrast, enrichment with P led to a steep increase in the bacterial growth rate at the beginning of the experiment, followed by increased growth dynamics of the algae. This is in agreement with previous observations, and it probably reflects the different uptake kinetics of these organisms (Rothhaupt & Güde 1992). Bacteria usually show higher specific uptake rates and affinities to P than algae, and therefore are frequently superior competitors of algae at lower P concentrations in aquatic systems (Rhee 1972, Currie & Kalff 1984a, Bratbak & Thingstad 1985, Gurung et al. 1999). In contrast, the competition for P can shift towards the benefit of algae at increased amounts of available P (Rothhaupt & Güde 1992). In our enrichment study, algal biomass increased much faster than bacterial biomass after the addition of surplus P.

However, bacteria from the P50 treatments seemed to be simultaneously co-limited by both C and P, as the addition of both elements resulted in significantly higher initial growth rates and higher total biomass (Fig. 4). Moreover, the bacterial to algal biomass ratio more than doubled compared to the addition of P only (0.54 and 0.21, respectively, Fig. 4). This suggests that the bacteria in our systems profited from their higher P uptake capacities at lower L_P, but that bacteria were also C-limited in all 3 treatments (P50, P100, P150).

Variability in bacteria-algae relationships

The observed simultaneous C and P limitation of the bacterial assemblage raises the interesting question as to whether such a double feedback between bacterial and algal growth could result in increased instability of the community as a whole. Already, in a pilot study of the present experiment run at 50 µg P l^{-1}, we noted that the high ratio of bacterial to algal biomass (and P) did not seem to be stable during longer periods (Table 1, Figs. 2 & 3). We found that the ratio of bacteria to algae strongly shifted to the benefit of bacteria for certain periods (days to weeks). During these phases of imbalance, the bacteria incorporated up to 80% of available P and algal abundance and biomass decreased rapidly. These imbalance situations can partly be compensated by grazing activities of bacterivorous protists, thus decreasing the strength of bacteria as competitors for algae (Salcher et al. 2005, this issue). However, the mixed bacterial community could not displace or outcompete the algae, as *Cryptomonas phaseolus* was still the only available carbon source for the bacteria. As a consequence, the ratios changed again to the benefit of algae, but showed more instability, i.e. periodical oscillations in the bacteria-algae ratio (Salcher et al. 2005). To our knowledge, such an imbalance situation has not been described previously, and we intend to follow-up these observations in future studies on bacteria-algae interactions. Continuous cultivation studies might provide an adaequate tool for exploring the long-term stability of bacteria-algae relationships at different P loads.

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