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Elemental stoichiometry of a heterotrophic bacterial community in a freshwater lake: implications for growth- and resource-dependent variations

Wataru Makino[1,2,*], James B. Cotner[1]

[1]Department of Ecology, Evolution and Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, Minnesota 55108, USA

[2]*Present address:* Graduate School of Life Science, Tohoku University, Aramaki Aza Aoba, Sendai, Miyagi 980-8578, Japan

ABSTRACT: The growth rate hypothesis (GRH) suggests that growth and elemental stoichiometry of organisms are coupled through variation in nucleic acid composition, specifically variation in ribosomal RNA. We examined these interactions in a bacterial community (<1 µm water) from the moderately productive Lake Owasso (Minnesota, USA). A mixed bacterial community was grown in chemostats with different supply carbon:phosphorus ratios (93, 373 and 933 by atoms) and dilution (=growth) rates (0.25, 0.5 and 0.7 h^{-1}). We measured bacterial C, N, P, DNA and RNA content to determine resource- and growth-dependent variations in the elemental stoichiometry of bacterial biomass at the community level. In chemostats with high supply C:P ratios (≥373), biomass P increased proportionally to growth rate as a result of increased RNA content. High growth rates generated low biomass C:P and N:P ratios. At a low supply C:P (93:1), biomass C:P and N:P increased with increasing growth rate, even though RNA content increased, suggesting that non-nucleic acid P (presumably polyphosphate) was the dominant P-pool in this community and may have been more important in altering biomass stoichiometry at increasing growth rates. Therefore, the coupling of stoichiometry to growth predicted by the growth rate hypothesis was applicable only under P-limited conditions. These chemostat results revealed more complicated responses of bacterial C:N:P stoichiometry at a community level than in individual strains. The Lake Owasso bacterial community was more homeostatic in terms of biomass C:P and N:P stoichiometry than other bacterial communities described in the literature, and the degree of homeostasis for the Lake Owasso bacterial community was similar to that of single bacterial strains. However, few similar measurements have been made, and the degree of biomass C:N:P homeostasis may vary between systems and even within a system during different seasons.

KEY WORDS: Bacterial communities · Growth · Nucleic acids · Phosphorus · Stoichiometry

INTRODUCTION

Heterotrophic bacteria are one of the most nutritious components in the plankton, since they often have a lower biomass carbon:phosphorus ratio than phytoplankton (Bratbak 1985, Vadstein & Olsen 1989). One reason for high P content/low C:P ratios is related to growth rate; bacteria have larger amounts of P-rich RNA (9% P) (accounting for up to 25% of dry wt: see Neidhardt et al. 1990) than eukaryotes such as phytoplankton (RNA up to 5–6% dry wt: see Dortch et al. 1983). These differences in RNA content reflect different life-history strategies; organisms with higher growth rates require a large allocation to ribosomal RNA to satisfy higher rates of protein synthesis. This interpretation is called the 'growth rate hypothesis', GRH (Elser et al. 1996, 2000, Sterner & Elser 2002). The growth rate dependence of RNA and P contents is also reflected in intraspecific variations such as ontogenetic differences in P content, because growth rates and RNA content generally decline with increasing age of organisms (McKee & Knowles 1987).

The GRH is not relevant for organisms that can store P (e.g. vascular plants and vertebrates), since the amount

*Email: wmakino@mail.tains.tohoku.ac.jp

of stored P could be large enough to mask the effect of increased RNA on the total cellular P (Elser et al. 1996, 2000, 2003). Some heterotrophic bacteria are also known to store P, especially inorganic polyphosphate (e.g. Kornberg et al. 1999), suggesting that not only growth but surplus P could explain variations in biomass C:P ratios in bacteria also. In support of this, Tezuka (1990) demonstrated a positive relationship between the C:N:P ratios of organic substrates and that of bacterial community biomass collected from Lake Biwa, Japan. Therefore, both resource-supply ratios and the GRH have the potential to explain variation in bacterial stoichiometry in nature; however, we do not fully understand these interactions and/or the relative importance of these 2 factors.

To address some of these issues, we cultured *Escherichia coli* K-12 in chemostats at different growth rates (0.5 to 1.5 h^{-1}) and supply C:P ratios (9 to 933 by atoms; at.) and measured C, N, P and nucleic acid contents (Makino et al. 2003). RNA and P contents increased, and biomass C:P and N:P decreased with increasing growth rate at all supply C:P levels, indicating that *E. coli* conformed to the GRH. In contrast, P content did not vary strongly with supply C:P levels, but varied only as a function of growth (=RNA content). The growth-dependent variation in biomass C:P (between 65 and 55 at.) and N:P ratios (between 17 and 15) was much smaller than the variation in supply C:P and N:P (3 orders of magnitude), suggesting that *E. coli* is strongly homeostatic in terms of biomass C:N:P stoichiometry. On the basis of these data and data from the literature, Makino et al. (2003) suggested that probably the regulation of the elemental composition of individual bacterial strains is tightly bound to characteristic biomass C:P and N:P ratios, i.e. is stoichiometrically homeostatic. Furthermore, we suggested that shifts in the dominance of different strains in the environment are probably responsible for the large variation in bacterial biomass C:P seen in nature (e.g. variation shown by Tezuka 1990), as has been suggested for crustaceans (Gulati et al. 1991, Hessen et al. 1992). This hypothesis raises the following question: do bacterial communities respond differently than a single strain to changes in growth and in the elemental composition of supplied nutrients?

In the present study, we cultured lake water (<1 µm fraction) containing many bacterial strains (i.e. communities) in chemostats with different supply C:N:P and growth rates, and then measured the C, N, P, RNA and DNA content of the bacterial communities. One question addressed was whether the bacterial communities would conform to the GRH, i.e. in what way would whole-community bacterial P-pools vary with supply C:N:P and growth rates? Another question addressed was whether the bacterial communities were homeostatic in terms of C:N:P stoichiometry. A literature survey was also conducted to answer the second question.

MATERIALS AND METHODS

Bacteria were collected from Lake Owasso (Minnesota, USA), a moderately productive (5 µg chlorophyll l^{-1}), shallow (10 m maximum depth) lake (Biddanda et al. 2001, Cotner et al. 2001). In September and October 2001, lake water was collected and filtered though a 1 µm pore-size Nuclepore filter to separate bacteria from other organisms. Direct observations revealed that there was little contamination by flagellates and ciliates after this process. Of this <1 µm fraction water (referred to hereafter as the bacterial community), 20 ml was inoculated into an Erlenmeyer flask containing 200 ml defined culture media (Table 1). We used autoclaved, filtered (<0.2 µm) lake water for the culture media, and held organic C and N concentrations constant (Table 1). The concentration of potassium phosphate was manipulated to create an arbitrary gradient in the supply C:P level (93, 373 and 933 at.). The pH of the culture media after mixing all components was 7.3 to 7.4, and it changed little throughout the experiment.

The bacterial community was incubated for 24 to 36 h in the culture medium with a target supply of C:P (either 93, 373, or 933) prior to inoculation into a chemostat. The inoculum was distributed into three 33 ml chemostats, and the same culture media was provided at dilution rates of 0.25, 0.5 and 0.7 h^{-1}, corresponding to the specific growth rates of 4.2, 8.3 and 11.6 d^{-1}, respectively. The chemostats were continuously mixed with filtered, hydrated air, and maintained at 25°C in the dark.

We began to collect samples when the cell number of bacteria in the chemostat stabilized (at ca. 1 to 2 d). Outflowing culture media containing the bacterial community was collected, and filtered onto pre-combusted

Table 1. Composition of culture medium used in this study

Compound	Chemicals	Conc. (mM)
Buffer	Tris-HCl, pH 7.6	16
Chelate	EDTA	0.02
Macronutrients	KCl	0.50
	$MgSO_4$	0.53
	$CaCl_2$	0.10
Vitamins	p-aminobenzoic acid	5×10^{-3}
	p-dihydroxybenzoic acid	5×10^{-3}
	p-hydroxybenzoic acid	5×10^{-3}
	Panthothenate, hemicalcium salt	5×10^{-3}
	Thiamine-HCl	5×10^{-3}
C source	Glucose	1.984
N source	NH_4Cl	9.52
P source	KH_2PO_4 (C:P = 93:1, N:P = 72:1)	0.132
	KH_2PO_4 (C:P = 373:1, N:P = 288:1)	0.033
	KH_2PO_4 (C:P = 933:1, N:P = 721:1)	0.013

glass-fiber filters (Whatman GF/F) or polycarbonate filters (Osmotics, 0.2 µm pore size). The former were used for bacterial C and N content analyses while the latter were processed for bacterial P and nucleic acid analyses; 4 samples were made for each analysis, but the chemostats for a given supply ratio and dilution rate were not replicated. Thus, the interpretation of differences was based on the authors' assessment (i.e. not based on statistical significance) when comparing results among supply ratios and dilution rates.

C and N contents were determined using a CHN analyzer (Perkin-Elmer Model 2400) and P contents were measured by acid-persulfate digestion and subsequent soluble reactive phosphorus analysis (APHA 1992) using an Alpkem flow solution 3000 analyzer. The bacteria in the chemostats were relatively large (0.6 to 1.1 μm^3 average), and the vacuum pressure of the filtration process was kept low. Thus we assume that we collected the entire bacterial community on the GF/F filters as well as the polycarbonate filters, which enabled us to compare C, N, and P (and nucleic acids) data.

Nucleic acids were determined by extraction of cellular contents via sonication followed by staining with the fluorochrome RiboGreen (Molecular Probes), which reacts with both DNA and RNA (Jones et al. 1998, Gorokhova & Kyle 2002). Bacterial samples on the polycarbonate filter, negative control samples (containing all reagents but no bacteria), standard DNA (calf thymus) and RNA (baker's yeast) were processed with Tris-EDTA buffer containing *N*-laurosarcosyl. Bacterial RNA was distinguished from DNA by adding RNase in the extracts (for details see Makino et al. 2003). The relative amounts of RNA-P to total biomass P were calculated by assuming that P represents 8.6% of the mass of nucleic acids (see Sterner & Elser 2002).

Duplicate samples from the inoculum were also measured for C, N, and P content when the inoculum was transferred to the chemostat. These CNP data were added to the chemostat data to examine the degree of C:P and N:P homeostasis, which was evaluated according to Sterner & Elser (2002) by plotting log-transformed biomass elemental ratios against log-transformed supply elemental ratios. The slope of this regression line reflects the degree of homeostasis, with greater homeostasis reflected by a decreasing slope.

We also counted the number of bacteria in the chemostat and estimated their average cell volume by image-analysis. Samples were preserved with formalin (2% final concentration), stained with acridine orange, filtered onto 0.2 µm black polycarbonate filters, and examined by epifluorescence microscopy. At least 250 stained bacteria were counted and photographed with a SPOT digital camera, and the images analyzed with Image Pro Plus software. All cells were assumed to be ellipsoidal.

RESULTS

Bacterial biomass in the chemostats typically decreased with increasing dilution (= growth) rates (Fig. 1A), an exception being the intermediate growth rate in the lowest C:P treatment, where biomass reached the maximum value (ca. 90 µg C ml^{-1}). At the same growth rate, biomass was higher when supply C:P was lower. These trends were also reflected in cell numbers in the chemostats (Fig. 1B); however, cell numbers at the intermediate supply C:P of 373 were similar, but slightly higher than those at the highest supply C:P of 933. These observations are consistent with the idea that the 2 highest C:P treatments were P-limited.

Biomass C:N was nearly constant, 5.0 to 5.5 at. at all growth rates and supply C:P (Fig. 2). The responses of biomass C:P and N:P to growth rate varied with supply C:P. Under P-limited conditions (i.e. supply C:P ≥ 373), biomass C:P and N:P decreased with increasing growth rate. As shown in Table 2, cellular P content increased with increasing growth rate, while cellular C and N did not show clear growth-dependent changes (increasing when supply C:P was 933 but decreasing when supply C:P was 373). RNA dominated the cellular nucleic acid pool,

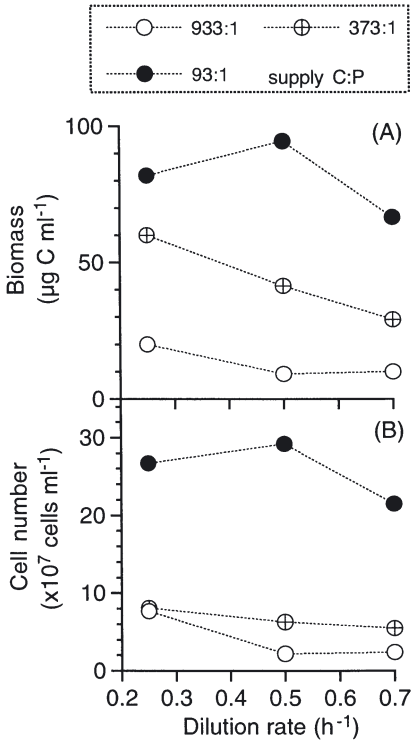

Fig. 1. Responses of (A) biomass and (B) cell numbers of Lake Owasso bacterial community grown in chemostats at different supply C:P levels to increasing dilution (= growth) rates

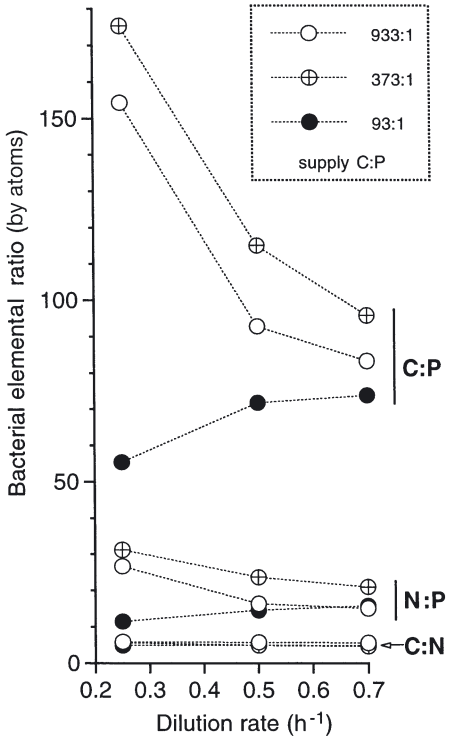

Fig. 2. Responses of biomass elemental ratios of Lake Owasso bacterial community grown in chemostats at different supply C:P levels to increasing dilution (=growth) rates

and cellular RNA content also tended to increase with increasing growth rate. We calculated the contribution of RNA-P to total cellular-P and found that it was always high (>70%), reaching 90% at the highest growth rate at the supply C:P of 933. The increased RNA content associated with increased growth raised the cellular relative P content, and consequently reduced the biomass C:P, as expected from the GRH (Fig. 3).

In contrast to the GRH, however, increased growth rates resulted in increased biomass C:P and N:P when supply C:P was 93:1 (Fig. 2). Although RNA dominated the cellular nucleic acid pool and cellular RNA content was highest at the highest growth rate, cellular P content decreased with increasing growth rate (Table 2). Cellular P contents at the supply C:P of 93 were similar to those at the other supply C:P ratios; however, cellular RNA contents were 35 to 50 fg lower than those in other supply C:P treatments. Thus, RNA-P contributed much less to the cellular P (only 40%) even at the highest growth rate of 0.7 h^{-1}, and represented only 25% when growth rates were ≤0.5 h^{-1}, while the contribution of non-nucleic acid P (NNA-P) was 50 to 70%, larger than that of RNA-P. Furthermore, cellular C and N contents were 310 to 325 and 74 to 77 fg lower, respectively, than at other supply C:P ratios. These lower C and N contents but

Table 2. Average (SD) cell volume, cellular C, N, P, RNA and DNA content, and composition of cellular P-pool for Lake Owasso bacterial community grown in chemostats at different dilution (=growth) rates and supply C:P ratios. NNA-P: P-sources that are neither RNA-P nor DNA-P; nd: no data

Supply C:P	Dilution rate (h^{-1})	Cell volume (μm^3)	Cellular content (fg)					Cellular P composition (% total cellular P)		
			C	N	P	RNA	DNA	RNA-P	DNA-P	NNA-P
93:1	0.25	0.89 (0.36)	307.1 (10.8)	74.0 (2.1)	14.3 (1.0)	41.7 (4.4)	8.4 (1.0)	25.2 (4.1)	5.0 (0.6)	69.8 (4.4)
	0.5	0.92 (0.50)	324.5 (51.3)	76.9 (13.1)	11.7 (1.1)	34.5 (1.4)	9.1 (1.4)	25.7 (3.1)	6.8 (1.3)	67.6 (4.2)
	0.7	0.96 (0.52)	310.5 (34.8)	77.2 (7.6)	11.1 (2.3)	53.2 (2.5)	9.6 (1.3)	43.1 (12.0)	7.5 (0.8)	49.3 (12.7)
373:1	0.25	0.70 (0.47)	675.3 (52.2)	140.3 (11.6)	9.9 (0.6)	89.1 (10.2)	10.6 (1.5)	77.6 (12.7)	9.2 (1.7)	13.2 (14.4)
	0.5	0.97 (0.74)	660.8 (28.0)	158.4 (7.8)	14.9 (1.1)	129.9 (7.7)	14.6 (1.4)	75.3 (3.3)	8.4 (0.7)	16.3 (4.0)
	0.7	1.12 (0.68)	528.8 (42.4)	135.0 (10.3)	14.5 (2.1)	115.3 (9.4)	11.7 (1.1)	69.0 (15.7)	7.1 (1.7)	23.9 (17.4)
933:1	0.25	0.61 (0.51)	278.0 (15.5)	56.0 (6.6)	4.7 (<0.1)	42.5 (9.0)	9.1 (0.9)	78.5 (16.7)	16.8 (1.6)	4.7 (15.3)
	0.5	0.59 (0.45)	425.2 (34.5)	87.3 (5.9)	11.8 (1.0)	nd –	nd –	nd –	nd –	nd –
	0.7	0.72 (0.54)	420.3 (12.6)	88.6 (1.1)	13.0 (0.3)	140.8 (17.1)	6.1 (1.0)	92.8 (10.9)	4.0 (0.7)	3.2 (11.3)

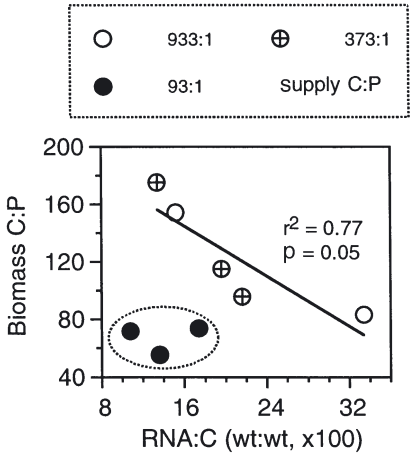

Fig. 3. Relationship between RNA content (RNA:C ratio) and biomass C:P of Lake Owasso bacterial community grown in the chemostats at different supply C:P levels. Data from lowest supply C:P of 93 (encircled) were excluded from regression analysis

comparable P content resulted in relatively low biomass C:P and N:P (Figs. 2 & 3).

We also found that cell volume at a given growth rate was highest at the lowest supply C:P of 93, indicating that it was significantly diluted in the cells. We did not measure the dry mass of the bacterial community, but can regard the volume-specific content as a biomass-specific characteristic and compare the values among treatments (Fig. 4). Volume-specific RNA contents tended to be lower, while volume-specific NNA-P content tended to be higher when supply C:P became lower. As a result of this complementary pattern, volume-specific P content was relatively stable. Coefficients of variation (CV, for all data) indicated that volume-specific P and DNA content were more conservative than volume-specific C and N content in the present study.

In the chemostat experiments, growth-dependent decreases in biomass C:P and N:P were observed when supply C:P was ≥373, but the magnitude of variation was <2-fold (e.g. 100 to 180 at the supply C:P of 373, Fig. 2). The 10-fold range in supply C:P and N:P produced only a 3-fold variation in biomass C:P (55 to 180) and N:P (10 to 30) at the lowest growth rate of 0.25 h^{-1}. We also measured C:P and N:P of bacteria in the inoculum, which was probably in the early stationary phase, and found up to 2-fold variations in biomass C:P (78, 72, and 169 for supply C:P levels of 93, 373 and 933, respectively) and N:P (17, 17, and 25 for supply C:P levels of 93, 373 and 933, respectively). We used these chemostat and inoculum data to determine the degree to which the bacterial communities were homeostatic in these incubations (Fig. 5). There was a positive relationship between supply and biomass C:P ratios; the slope of the regression line was 0.24 (Table 3) and was significantly less than 1 ($F_{1,10}$ = 68, p < 0.001). Biomass N:P also tended to increase with increasing supply N:P, but this relationship was not insignificant and therefore the slope was treated as zero.

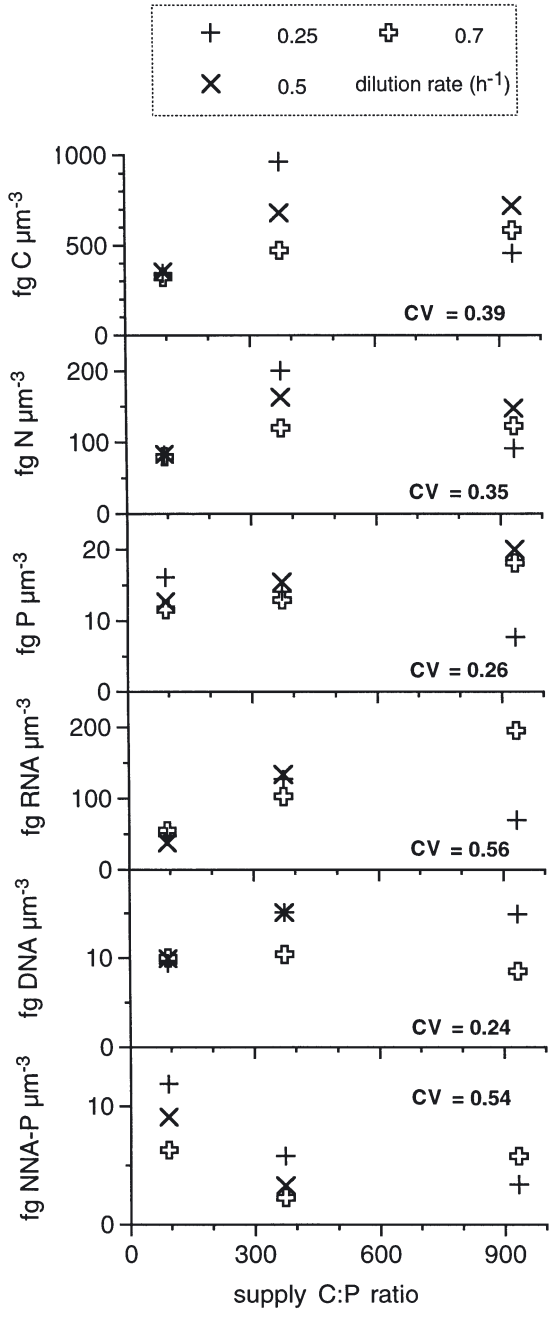

Fig. 4. Relationships between supply C:P and volume-specific carbon, nitrogen, phosphorus, RNA, DNA, and non-nucleic-acid P (NNA-P) in Lake Owasso bacterial community grown in chemostats. CV: coefficient of variation

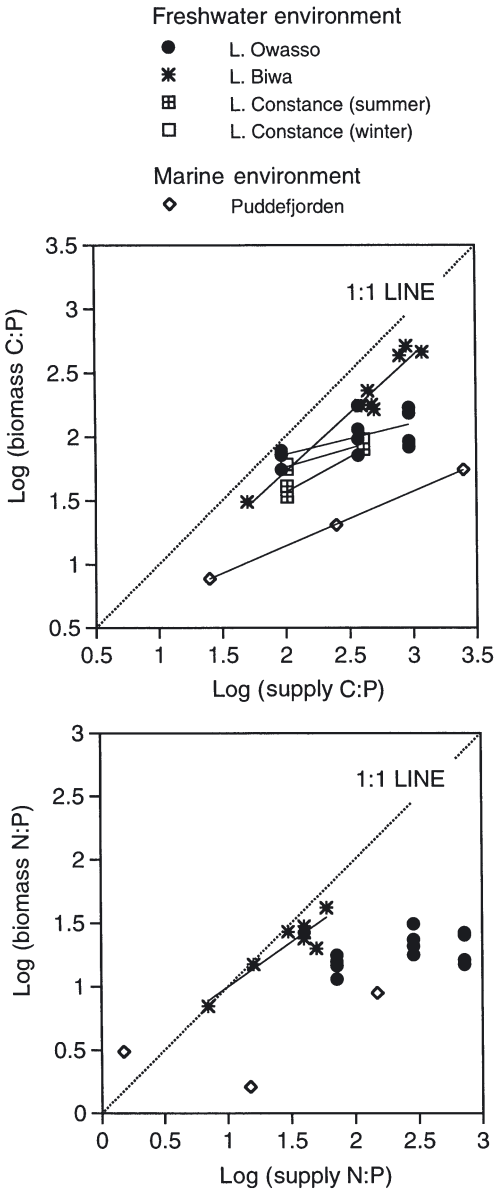

Fig. 5. Degree of homeostasis in terms of biomass C:P and N:P stoichiometry in bacterial communities from 4 different locations (Table 3)

DISCUSSION

Did the bacterial community conform to the GRH?

The GRH postulates that increased RNA content associated with increased growth raises the cellular/relative P content and consequently reduces biomass C:P and N:P (Elser et al. 1996, 2000, Sterner & Elser 2002). This hypothesis has been verified for various kinds of organisms, including the cultured heterotrophic bacterium *Escherichia coli* (Makino et al. 2003), but also for planktonic zooplankton and terrestrial insects (Elser et al. 2003). In the present study, we extended the GRH to freshwater heterotrophic bacterial communities. Unlike other studies, we conducted our experiments with a mixed inoculum from a natural system, which might have contained many strains that can accumulate P as polyphosphate. Polyphosphate accumulation in non-P-limited organisms complicates interactions between growth, RNA, and P content of organisms, and also complicates predictions of the GRH (Elser et al. 2003). Indeed, we found that the extent to which the cultures were limited by P-availability played an important role in allocation of P to RNA and impacted the mechanism of the GRH.

When P-limited (supply C:P of 373 and 933), the responses of cellular P and RNA contents of the Lake Owasso bacterial communities to changes in growth rate were exactly as predicted by the GRH, and were very similar to the response of a single strain (*Escherichia coli*) grown in chemostats (Makino et al. 2003), indicating that growth rates can profoundly affect biomass C:P and N:P stoichiometry in not only single bacterial strains, but also in mixed communities.

However, in contrast to the predictions of the GRH, we observed increased biomass C:P and N:P with increased growth when P-limitation was relaxed (i.e. at the supply C:P of 93:1). The contribution of RNA-P to total cellular P was lowest at this supply ratio, but that of NNA-P was highest. We consider that the NNA-P was probably inorganic polyphosphate that is known to accumulate in some strains whenever P is not limiting (e.g. Felzenberg et al. 1996). In support of this, the proportion of NNA-P increased at higher growth rates at the supply C:P of 373. The higher dilution rate in this chemostat represented a higher nutrient (P in this particular case) loading rate that could have alleviated the P-limitation of the bacteria, and resulting in an increased proportion of P being available for storage as polyphosphate, rather than RNA. Thus, at the lowest supply C:P of 93, we consider that NNA-P (presumably polyphosphate) comprised >60% of the cellular P pool, and that this large pool of excess biomass P masked the effect of increased RNA content at the highest growth rate. It is likely that this supply C:P level selected for strains that can store large amount of polyphosphate, and, in agreement with Elser et al. (2003), we suggest that bacterial communities in general conform to the GRH only when they are P-limited.

So far, we have discussed the role of P as the determinant of biomass C:N:P stoichiometry. In addition, the volume-specific nutrient-content analysis of the present study revealed that the volume-specific P content was relatively stable (as a result of the complementary behavior of RNA-P and NNA-P over the supply C:P range), while volume-specific C and N contents were

more variable over the gradient of supply C:N:P. One implication of this result is that Lake Owasso bacteria were very resilient to variations in P availability. This finding indicates that variations in specific C and N contents are sometimes as important as variation in specific P content (i.e. the GRH) in determining biomass C:N:P stoichiometry. The influence of carbon and nutrient supply regimes on cell volume and specific elemental content (e.g. Vrede et al. 2002) and the response of these parameters to variations in the nutrient supply may differ from species to species (e.g. Fagerbakke et al. 1996). Thus, the response of a bacterial community to growth and resource stoichiometry would be more complicated than that of a single strain.

Goldman et al. (1979) emphasized that the biomass C:P of marine phytoplankton decreased with increasing growth rate, reaching a Redfield ratio of 106:1 (Redfield 1958), and argued that phytoplankton in the open ocean are growing at maximum rate. In the present study, although the response of biomass C:P to growth varied with supply C:P, the biomass C:P converged around 80:1, similar to but lower than the Redfield ratio of 106:1 (Fig. 2). One implication of a decreased C:P ratio is that rapidly growing bacteria are generally more nutritious for their consumers than phytoplankton. In nature, bacteria often have lower biomass C:P than phytoplankton (Vadstein et al. 1988, Elser et al. 1995), and even achieve sub-Redfield values (Vadstein et al. 1988, Watanabe 1990, Chrzanowski et al. 1996, Fagerbakke et al. 1996), despite the fact that bacterial growth rates are on the order of days rather than hours, and are similar or even lower than algal growth rates (see Cotner & Biddanda 2002). Our experiments indicated that bacterial communities do not always conform to the GRH, especially if P is not limiting. Consequently, even when growing slowly, bacterial community can have sub-Redfield C:P and N:P ratios by allocating P to (presumably) polyphosphate rather than RNA and/or reducing C and N content also. Thus, as has been pointed out by Lee & Kemp (1994), the growth rates of a bacterial community in nature cannot be inferred from nutrient ratios and/or RNA content.

Bacterial community homeostasis in terms of C:N:P stoichiometry

The lack of homeostasis in terms of biomass C:N:P stoichiometry in bacterial communities can be clearly deduced from the study of Tezuka (1990), who cultured lake water collected from Lake Biwa (Japan) across a range of supply C:P and N:P levels of 50 to 1200 and 7 to 60, respectively, and found biomass C:P and N:P ranges of 31 to 464 and 7 to 41, respectively. The slopes of the log-log plot for these data (Fig. 5, Table 3) were not significantly different from 1 ($p > 0.05$, $F_{1,6} = 1.1$ and 5.7 for C:P and N:P, respectively), suggesting that the Lake Biwa bacterial community was completely non-homeostatic in terms of biomass C:N:P stoichiometry.

In the present study, Lake Owasso bacterial communities were cultured across a similar variation in supply C:P levels (93 to 933) and an even broader variation in supply N:P levels (72 to 721) than in Tezuka's (1990) study. This created a 3-fold variation in biomass C:P (55 to 175, minimum to maximum) and biomass N:P (11 to 31), a much smaller variation than the nearly 10-fold variation in the Lake Biwa bacterial community (Tezuka 1990). The slope of log(supply C:P) vs log(biomass C:P) plot for Lake Owasso communities (Fig. 5, Table 3) was statistically different from 1 (i.e. it was homeostatic) and similar to the slopes for single bacterial strains (e.g. 0.19 for *Pseudomonas fluorescens*;

Table 3. Summary of regression analyses of data in Fig. 5. Literature sources: (1) present study; (2) Tezuka (1990); (3) Jürgens & Güde (1990); (4) Bratbak (1985). SE: standard error

Locations	Culture conditions	Category	Regression analysis					
			N	R^2	F	p	Slope	SE of slope
Lake Owasso (USA) (1)	Batch culture and chemostat (0.25–0.7 h^{-1})	C:P	12	0.41	7.0	0.025	0.243	0.092
		N:P	12	0.26	3.6	0.088	0.152	0.081
Lake Biwa (Japan) (2)	Batch culture, stationary phase	C:P	8	0.95	107.0	<0.001	0.906	0.088
		N:P	8	0.85	33.6	0.001	0.710	0.122
Lake Constance (Germany) (3)	Summer observation, chemostat (1 d^{-1})	C:P	4	0.97	66.3	0.015	0.550	0.068
	Winter observation, chemostat (1 d^{-1})	C:P	4	0.89	16.2	0.056	0.292	0.073
Puddefjorden (Norway) (4)	Batch culture, log-phase. Blackish water	C:P	3	1	1.8×10^5	0.005	0.429	0.003
		N:P	3	0.38	0.6	0.575	0.231	0.294

Makino et al. 2003). The slope for the Lake Owasso bacterial community seems to be the lowest among bacterial communities reported in the literature (Table 3), though available data on this issue are still very limited. Furthermore, the Lake Owasso community appears strongly homeostatic in terms of biomass N:P stoichiometry, since the regression in Fig. 5 is insignificant. Overall, therefore, the response of the Lake Owasso bacterial community was more similar to that observed in single strains than the responses of other communities we have examined.

Given the variability in the C:P and N:P homeostasis in different bacterial communities, we suggest that there may be site-specific variations and even perhaps seasonal variations within the same site in the degree of biomass C:N:P homeostasis (Fig. 5, Table 3). We speculate that differences in the diversity of bacterial communities could have an important influence on this variation, although we did not examine the community structure in our experiments. Nonetheless, it seems reasonable that if there is little diversity in a bacterial community, there may be a decreased potential for that community to respond to changes in supply C:nutrient. Under such conditions, a community would probably be more homeostatic, whereas a more diverse community would probably have greater potential to respond to variations in supply C:nutrient.

Because of its effects on nutrient recycling ratios, the degree of homeostasis of a bacterial community has important biogeochemical feedbacks to the entire aquatic community. If a bacterial community is, for example, not homeostatic and the elemental ratios of its community routinely track supply elemental ratios, the bacterial community would regenerate nutrients at about the same ratio as they are supplied (Elser & Urabe 1999, Sterner & Elser 2002). Combining these ideas with recent advances in techniques for phylogenetic identification of *in situ* bacterial communities could provide invaluable information on biogeochemical cycling and the efficiency of energy flow within food webs mediated by natural bacterial communities. A comparison of marine and freshwater systems from this standpoint would also be useful since our (limited) data indicate that marine bacteria may have different homeostasis regulatory systems in terms of biomass C:P, as their C:P levels seem to be lower at given supply C:P levels than those of freshwater bacteria (Fig. 5).

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