

Element ratios and growth dynamics of bacteria in an oligotrophic Canadian shield lake

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ABSTRACT: Basic bacterial physiology predicts that the element composition of cells changes as a function of generation time. Rapidly growing cells are characterized by low C:P and N:P ratios compared to ratios of slowly growing cells. Working from this premise, we (1) determined the element composition of newly synthesized bacteria collected from an oligotrophic Canadian shield lake and (2) compared the changes in element composition of bacteria to their temporal changes in abundance. The element composition of new biomass was determined from the change in bacterial element composition following nutrient enrichment and incubation with nutrients derived from seston concentrated from a eutrophic lake. Bacterial C:P and C:N ratios generally decreased during incubation but N:P ratios changed only slightly. When averaged, new bacterial biomass had a C:P ratio (atomic) of 44.4, N:P of 8.5, and C:N of 5.5 (C:N:P 44:9:1). The element composition of the *in situ* bacterial assemblage of an oligotrophic lake was compared to the temporal dynamics of the assemblage. Cell N:P and C:P ratios were low when cell abundance was increasing compared to the ratios obtained following a rapid decline in cell abundance. The data indicate that bacteria may alter their cellular element composition, and that the growth dynamics of bacterial assemblages may be reflected by the element ratios of their biomass.

KEY WORDS: Carbon · Nitrogen · Phosphorus · Element ratios · Nutrients · Bacteria · Growth

INTRODUCTION

Several studies describing growth limitation of the bacterioplankton by mineral nutrients have added additional layers of complexity to an already complex view of planktonic nutrient dynamics (Heinänen 1991, Wang et al. 1992, Chrzanowski et al. 1995). These studies reveal, in contrast to the catholic view that bacteria are C limited, that bacteria may often be limited by a single element other than C, and at times suffer co-limitation by C, N and P. Also stemming from these studies is the conclusion that there must be common situations (nutrient conditions) where the relationship between the bacterio- and phytoplankton is more competitive than commensal.

Nutrient limitation studies focus attention on the fact that bacteria, like other members of the plankton, have specific requirements for mineral nutrients. While the bacterial demand for nutrients is clearly recognized, less appreciated is the relationship between nutrient (element) content of cells and growth (Reiners 1986, Martinussen & Thingstad 1987, Thingstad 1987, Eccleston-Parry & Leadbeater 1995). There are very few data available on the element content of bacteria and even less on the relationship between element content and growth rate. Perhaps the most extensive data characterizing the element content of cells is that of Porter (1946, summarized in Reiners 1986), while the relationship between element content and growth rate may be derived from the data presented by Bremer & Dennis (1987).

In exponentially growing cultures, generation time is reflected in the nutrient demand and the element con-

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tent of cells. As generation time decreases (i.e. growth rate increases), cell volume increases as does, to a lesser extent, the concentration of DNA (Herbert 1976, Bremer & Dennis 1987). The concentration of RNA also increases with decreasing generation time, but to a disproportionately greater extent than other cellular constituents. Thus, during rapid growth the per capita demand for C, N, and P necessary for biosynthetic and energetic processes is greater than that required to maintain slower growth rates. Despite increases in the per capita demand for elements, bacteria appear to hold the cellular ratio of C:N constant over a wide range of generation time (Schweitzer & Simon 1985, and references within), so an increase in cell C must be accompanied by a corresponding (and apparently proportional) increase in cell N. However, as generation time decreases, cellular P concentrations increase disproportionately due to the accumulation of macromolecules rich in P (as RNA) compared to structural molecules. Consequently, cellular N:P and C:P ratios should be expected to fall as cells progress from long to short generation times. The trajectory in cellular N:P as a function of generation time is shown in Fig. 1 for a cell type capable of extremely rapid growth (*Escherichia coli*) and for a mixed assemblage of freshwater bacteria.

From this basic physiology stems the idea that cellular N:P or C:P ratios may reflect the growth dynamics of natural assemblages of aquatic bacteria. In this paper we summarize 2 experiments relating cellular element ratios to growth dynamics of entrained and *in situ* bacterial assemblages. In previous work (Chrzanowski et al. 1995, Sterner et al. 1995) we demonstrated that the growth rate of bacteria in an oligotrophic Canadian shield lake (L240) was constrained

by nutrient availability and that bacteria in these waters depended upon nutrients regenerated by consumers (macro- as well as microzooplankton). Consequently, in one series of experiments, we developed a 'natural enrichment' from a potentially nutrient-rich seston and used it to release bacteria in L240 water from reliance upon regenerated nutrients and to stimulate their growth. We subsequently compared element ratios of cells before enrichment to after enrichment and determined the C:N:P ratio of newly synthesized bacterial biomass. In a second experiment, we compared the changes in the N:P ratio of bacteria in an oligotrophic lake (L240) to temporal changes in abundance.

MATERIALS AND METHODS

Sampling sites. Eutrophic Lake 227 (L227, $z_{\max} = 10$ m, $z_{\text{mean}} = 4.4$ m) and oligotrophic Lake 240 (L240, $z_{\max} = 13.1$ m, $z_{\text{mean}} = 6.0$ m) lie within the Experimental Lakes Area, Ontario, Canada (93° 45' N, 49° 40' W). L227 has been receiving artificial nutrient enrichment for over 20 yr and currently receives annual P additions at 0.56 g P m⁻²; no N is currently being added. Total dissolved P (TDP) in L227 averaged 0.124 μM during the summer of this study. Nitrogen fixing bacteria dominate the phytoplankton by mid-summer. L240 is highly oligotrophic with dissolved reactive P concentrations typically near the analytical detection limit and TDP annually averages less than 0.04 μM.

Sampling. Water was collected from the deepest sections of each lake for enumeration of bacteria (L240, weekly) and for nutrient enrichment experiments (L240 and L227, 8 occasions between June and mid-August; see Table 1). Water was collected with an 8 l VanDorn bottle from 3 evenly spaced depths in the epilimnion (1 m below surface, ~1 m above the thermocline, and midway between), screened through 80 μm Nitex, and equal volumes from each depth pooled to create a composite sample ranging between 10 and 60 l. The procedure was repeated to produce independent triplicate samples.

Development of nutrient concentrate. A nutrient enrichment for L240 bacteria was created from L227 samples by concentrating the seston into approximately 200 ml using hollow-fiber concentration apparatus (0.2 μm cut-off, Microgon Corp.). The concentrate was frozen overnight, thawed, and sonified twice (50 ml aliquot held

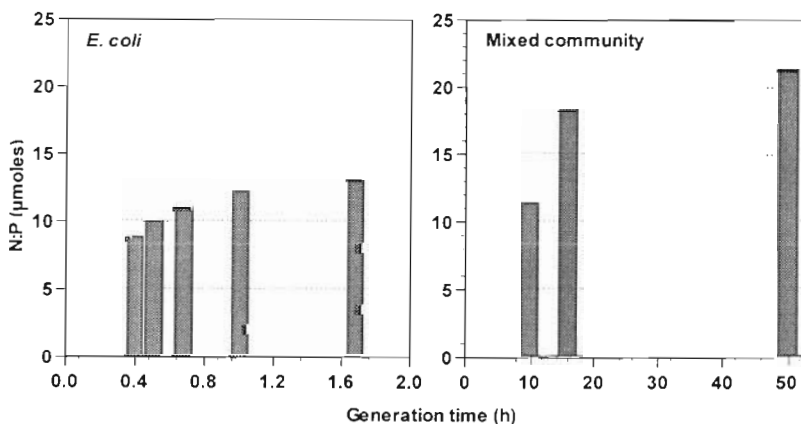


Fig. 1. Ratios of N:P in cells as a function of generation time. Data for *Escherichia coli* were generated from Bremer & Dennis (1987). Data for the mixed assemblage of freshwater bacteria were generated from Vadstein & Olsen (1989)

in ice; 80 W, 10 min intervals). Debris and unbroken cells were removed by filtering (2×) through glass-fiber filters (Whatman GF/F) and then through 0.2 µm polysulfone filters (Gelman). TDP concentration was determined immediately prior to using the concentrate in enrichment experiments (see below).

Enrichment experiments. Water collected from L240 was gently filtered through 1.0 µm porosity polycarbonate filters (Poretics) and 1 l dispensed into each of 6 acid-washed polycarbonate bottles. Flagellates were not detected in the filtrates (microscopic observation). Each bottle received a volume of L227 nutrient concentrate to raise the ambient total P by 1 µmol. Initial conditions were established immediately following the addition of nutrient concentrate; water was removed from each of 3 bottles for particulate C, N (duplicate), and P (triplicate), and for TDP analyses (triplicate). The remaining 3 bottles were incubated in the dark for 24 h at 22°C, after which final particulate C and N, and TDP were determined.

Analyses. Particulates were collected on pre-combusted glass-fiber filters (Whatman GF/F), dried, and C and N determined using a CHN analyzer (Perkin-Elmer). Particulate P, TDP, and total dissolved nitrogen (TDN) (both TDP and TDN from GF/F filtrates) were determined according to the methods of Strickland & Parsons (1972). Net incorporation of C and N into biomass was calculated (in µmol) as (final element content – initial element content) and net incorporation of P was calculated as [initial particulate P + (initial TDP – final TDP)]. Dissolved organic carbon (DOC, GF/F filtrates) was oxidized with persulfate (Strickland & Parsons 1972) and the resultant CO₂ measured with an infrared analyzer and subsequently converted to mol C. Bacterial cell abundance in unfiltered L240 water (triplicate samples) was determined by epifluorescence direct counts using DAPI (Porter & Feig 1980) as the fluorochrome.

RESULTS

Enrichment experiments

TDP concentrations in bottles receiving nutrients derived from L227 were near 1 µM (Table 1). At this concentration, TDP was approximately 20 times the average ambient TDP level (Sterner et al. 1995). TDN concentrations were approximately double ambient TDN following nutrient enrichment. DOC was abun-

Table 1. Element composition of oligotrophic L240 water following enrichment with nutrient concentrate developed from eutrophic L227 (DOC, TDN, and TDP = dissolved organic carbon, total dissolved nitrogen, and total dissolved phosphorus, respectively). Each estimate is the mean from 3 experimental bottles

Date	Element concentration (µM ± SE)			Element ratios		
	DOC	TDN	TDP	C:P	N:P	C:N
13 June	894 ± 81	36.0 ± 0.4	0.96 ± 0.02	931	38	25
20 June	780 ± 35	37.3 ± 0.2	0.96 ± 0.05	813	39	21
28 June	906 ± 49	48.8 ± 0.5	0.95 ± 0.21	954	51	19
5 July	968 ± 20	57.7 ± 1.5	0.89 ± 0.01	1088	65	17
14 July	901 ± 32		0.90 ± 0.02	1001		
19 July	949 ± 34	54.8 ± 0.2	1.03 ± 0.07	921	53	17
26 July	906 ± 71	50.2 ± 0.7	0.97 ± 0.03	934	52	18
9 Aug	920 ± 38	48.0 ± 0.8	0.74 ± 0.12	1243	65	19

dant in the experimental bottles and averaged 903 µM. Element ratios of the dissolved pool suggest that even though P was increased 20-fold over the ambient concentration, P was still in short supply compared to the availability of C and N.

Initial cell C:N ratios (see 'Discussion') ranged between 8.1 and 15.1 (Table 2) and were at the high end of the range of values reported elsewhere (Bratbak 1985, Goldman et al. 1987, Lee & Fuhrman 1987, Brinch-Iversen & King 1990, Tezuka 1990). Initial cell C:P ratios were typically above 100:1 but approached or exceeded 200:1 by late July-early August. There was a net increase in the element content of cells following enrichment and incubation. Cells tended to accumulate proportionately more N and P than C, consequently, C:P and C:N ratios generally decreased during incubation. However, with the exception of the 2 experiments conducted in late July, N:P ratios changed only slightly as a result of nutrient enrichment.

C:P and C:N ratios of newly synthesized biomass were generally lower than the initial or final ratios for the entire bacterial assemblage. However, N:P ratios of newly synthesized biomass were clearly lower than the initial and final N:P ratios for the entire assemblage only for the 2 experiments conducted in late July. When averaged, new biomass had a C:P ratio of 44.4, N:P of 8.5, and C:N of 5.5 (C:N:P = 44:9:1).

Element ratios and bacterial dynamics

Total bacterial abundance in L240 was determined weekly between May and September. Fig. 2 presents the temporal distribution of bacterial abundance and the N:P and C:P ratios of bacterial biomass (see Table 2, initial values). Bacteria increased from 5×10^5 cells ml⁻¹ in May to approximately 2.4×10^6 cells ml⁻¹ in mid-July. Cell abundance fell dramatically between

Table 2. Element content and element ratios of bacteria prior and subsequent to nutrient enrichment. Element ratios of newly synthesized bacterial biomass were calculated from the difference between initial and final element content. Each estimate is the mean from 3 experimental bottles

Date	Status	Element concentration ($\mu\text{M} \pm \text{SE}$)			Element ratios (atomic)					
		C	N	P	Assemblages			New biomass		
					C:P	N:P	C:N	C:P	N:P	C:N
13 June	Initial	18.01 \pm 0.24	1.20 \pm 0.04	0.16 \pm 0.01	112.6	7.5	15.1			
	Final	32.10 \pm 1.04	4.80 \pm 0.30	0.60 \pm 0.06	53.5	8.0	6.7	32.0	8.2	3.9
20 June	Initial	15.09 \pm 0.25	1.24 \pm 0.01	0.15 \pm 0.01	100.6	8.3	12.1			
	Final	60.35 \pm 1.29	9.11 \pm 0.23	0.87 \pm 0.07	69.4	10.5	6.6	62.9	10.9	5.8
28 June	Initial	18.39 \pm 0.50	1.95 \pm 0.16	0.29 \pm 0.06	63.4	6.7	9.4			
	Final	41.38 \pm 2.68	4.27 \pm 0.25	0.77 \pm 0.23	53.7	5.5	9.7	47.9	4.8	9.9
5 July	Initial	29.26 \pm 1.69	1.99 \pm 0.15	0.27 \pm 0.02	108.4	7.4	14.7			
	Final	35.90 \pm 1.75	5.73 \pm 0.27	0.71 \pm 0.03	50.6	8.1	6.3	15.1	8.5	1.8
14 July	Initial	49.32 \pm 0.10	5.56 \pm 0.07	0.46 \pm 0.01	107.2	12.1	8.9			
	Final									
19 July	Initial	24.58 \pm 0.42	2.64 \pm 0.14	0.14 \pm 0.02	175.6	18.8	9.3			
	Final	67.32 \pm 1.13	9.27 \pm 0.13	0.76 \pm 0.09	88.6	12.2	7.3	68.9	10.7	6.5
26 July	Initial ^a	52.96	6.50	0.3 \pm 0.06	176.5	21.7	8.1			
	Final	64.41 \pm 0.70	8.72 \pm 0.06	0.59 \pm 0.12	109.2	14.8	7.4	39.4	7.7	5.2
9 Aug	Initial	56.05 \pm 1.03	5.61 \pm 0.21	0.26 \pm 0.01	215.6	21.6	10.0			
	Final									

^aC and N from single bottle assayed in duplicate

mid- and late July and remained below 1.2×10^6 cells ml^{-1} . During the period when the cell abundance was increasing, N:P ratios were below 12:1 and C:P ratios were below 115:1. Immediately prior to the rapid decline in abundance, the C:P and N:P ratios rose to 176:1 and 19:1, respectively. The remaining 2 samples were taken following the rapid decline in abundance during the period when abundance remained depressed. N:P and C:P ratios remained elevated at these times compared to those obtained when cell abundance was actively increasing.

DISCUSSION

Element content of cells

Gently filtering lake water through 1.0 μm filters apparently disrupted cell microaggregates because cell abundance in 1.0 μm filtrates averaged 10% greater (range -8 to +34%) than cell abundance in unfiltered water. We considered material passing a 1.0 μm pore-size filter to be bacteria. However, some detrital material may also pass this filter and thereby bias measures attributed to cells. Estimating the magnitude of this source of error is difficult as we must be concerned with the element composition of detritus, and the proportion of labile elements remaining in detritus that may subsequently be incorporated into biomass.

In a previous study using L240 water (Elser et al. 1995), we estimated that 34% of the C and 60% of the N in the <1.0 μm fraction was bacterial. Similar estimates were, and are, not possible for P as conversion factors are not available. However, it is reasonable to assume that element proportions in detritus are $C \gg N > P$, and thus we reach the conclusion that most of the P (>60%, from above) in the <1.0 μm filtrates was cellular P and that C:N and C:P ratios for cells (particularly for 'initials') are likely to be somewhat less reliable than are N:P ratios. Finally, we assume that the elements associated with detrital material are not readily available for metabolism and thus represent a constant portion of the elements captured on GF/F filters. GF/F filters were approximately 90% efficient in retaining bacteria.

C:N ratios in initial samples from 1.0 μm filtrates were within the range of C:N ratios reported for aquatic bacteria, but tended towards the high end of this range. It seems likely that the elevated C:N ratios were due to C associated with microdetritus passing the 1.0 μm filter. Nutrient enrichments brought about a decrease in the C:P, and to a lesser extent the C:N, ratios of the bacteria in the 1.0 μm filtrates. With the exception of experiments conducted in late July, the N:P ratio of cells changed only slightly (see below). Newly synthesized bacterial biomass had an average C:N ratio of 5.5 (range 1.8 to 9.9) and this ratio is typical of the C:N ratio expected of bacteria (Nagata 1986, Lee & Fuhrman 1987, but see below). That newly syn-

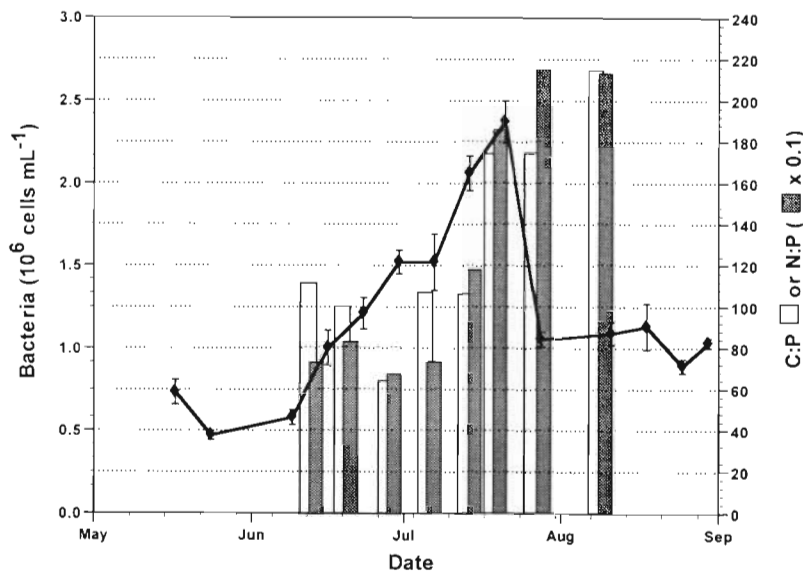


Fig. 2. Temporal distribution of bacterial abundance and the N:P (shaded bars) and C:P ratios (open bars) (ratios are atomic) of bacteria biomass in oligotrophic L240. Error bars are \pm SE from triplicate samples

thesized biomass had C:N ratios lower than either the initial or final C:N ratios suggests that despite our attempts to develop a 'natural' enrichment from seston, we could not meet the nutrient demands of the entire bacterial assemblage. Obviously only a portion of the assemblage was responding to the nutrient enrichment since the C:N ratios of new biomass did not match the C:N ratios of the entrained assemblage at the end of incubation. The nutrients derived from L227 seston may have been of inappropriate molecular composition or may have lacked sufficient P to meet the needs of the entire entrained assemblage.

Our lowest C:N ratio (1.8) as well as the lowest C:P ratio (15.1) for newly synthesized biomass occurred on the same sampling date (5 July). These ratios are low to the point of being unrealistic and seem to have resulted from low measures of C incorporation rather than high measures of N or P incorporation (Table 2). Since C and N were determined from the same filters (CHN analysis), we have no adequate explanation for the low value of C incorporation. If data from this date are removed from consideration, the average C:P, N:P and C:N ratios for newly synthesized biomass become 50:1, 8.5:1, and 6.3:1, respectively (C:N:P = 50:9:1). Our estimates of C:N:P ratios of newly synthesized bacterial biomass (for the complete or reduced data) are very similar to the ratio calculated by Reiners (1986) (46:7:1) from data compiled by Bowen (1979) and to that of Fagerbakke et al. (1996) (55:10:1) who directly determined the element content (X-ray diffraction analysis) of both marine and freshwater bacteria.

Integrating experimental and field data

In the second portion of this work, we compared the dynamics of a natural bacterial assemblage to the element composition of the cells (Fig. 2). In the absence of biochemical (thymidine, leucine incorporation) or direct (dilution bioassays) indicators of growth, it is difficult to assess whether a decline in abundance is due to increased rates of mortality or to decreased growth rates. Relying on the physiological relationship between bacterial element composition and growth rate (theory, Fig. 1, and below), we are able to infer that the decline in abundance in L240 was due to a decrease in bacterial growth rate.

An upper limit to the bacterial N:P ratio of between 20 and 24 is suggested from the data in Fig. 1 and from data collected from a wide variety of cells grown at an equally wide variety of rates (summarized in Kyle 1994). Further, N:P ratios in this range (20 to 24) appear to represent slow-growing cells (generation times >24 h) whereas lower ratios appear to represent faster-growing cells. Nutrient enrichments brought about a decrease in the C:P ratio in each experiment, but caused a noticeable decrease in the N:P ratio only in the experiments conducted in late July. From early to mid-summer, bacterial cell abundance in L240 was increasing and at least some portion of the assemblage was obviously growing (Fig. 2). N:P ratios of the assemblage were low during this period (Fig. 2) and were in the range expected of growing cells (Fig. 1). Since the N:P ratio of the cells changed little as a result of enrichment, we speculate that the cells had a greater need for C or N than for P. Cell ratios reflect the net accumulation of elements, so for C, the demand was much greater than indicated by net accumulation. Kristiansen et al. (1992) estimated that C growth efficiencies may be as low as 30%, thus C flux through the cells (hence demand) must have been at least double and as high as triple the net accumulation.

During the enrichment experiments conducted in late July, initial bacterial N:P ratios were high and decreased as a result of nutrient enrichment. These experiments correspond to the time when the bacterial abundance in L240 fell and remained low. The N:P ratios of the initial biomass suggest that the bacterial assemblage entered a period of slow growth (Fig. 2) and the decline in bacterial abundance appears to have been the result of decreased growth rate coupled with losses to bacterivores.

Our data also suggest that P turnover in L240 was extremely rapid. TDP concentration in experimental bottles that received nutrients derived from L227 were near the target concentration of 1 μM (Table 1). This implies that the ambient P concentration in L240 must have been extremely low. However, since bacterial abundance was increasing for much of the time covered by the enrichment experiments, there must have been enough nutrient (P) flux to allow for the synthesis of new biomass (see Sterner et al. 1995).

Conclusions

Clearly the element content of the bacterioplankton (growing and non-growing) undergoes change during the course of a season. Shifts or seasonal trends in the N:P or C:P ratio of the bacteria may not only corroborate other indices of bacterial activity (such as thymidine or leucine incorporation) but may also indicate a shift in the nature and/or availability of resources supporting bacterial growth. This has implications for understanding planktonic nutrient dynamics. There is compelling evidence indicating that bacteria have the potential to compete with phytoplankton for P (Currie & Kalff 1984a, b, Currie et al. 1986, Vadstein et al. 1993). Thus during periods when bacteria are actively increasing and characterized by low C:P or N:P ratios, they should directly compete with phytoplankton for P (see above discussion on nutrient flux). At the same time, but dependent on their own element demands, bacterivores grazing on these bacteria would seem likely to regenerate a greater proportion of P than N from their 'P-rich' prey (Sterner 1990, Sterner et al. 1992, Urabe 1993). In P-depauperate oligotrophic waters this source of P may promote the turning of Goldman's (1984) 'spinning wheel'. [It has been shown recently that bacteria in L240 do in fact rely on regenerated nutrients to meet a portion of their metabolic demand (Chrzanowski et al. 1995, Sterner et al. 1995).] During periods of bacterial senescence (high bacterial C:P or N:P ratios), bacteria incorporate less P and bacterivores, again contingent on their own element composition, would seem likely to regenerate a greater proportion of N than P. Dependent upon the reason for bacterial growth to slow (limitation by C?), bacteria may shift from a position of competing with phytoplankton for nutrients (as P) to one of dependence upon phytoplankton for nutrients (C). Knowledge of the element composition of bacteria and how it relates to growth thus assumes importance not only as a means of defining those factors that may regulate bacterial nutrient demand, but also as a means through which we may further understand the potential for nutrient regeneration by both the bacteria and their predators.

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