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Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles

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Abstract

We have used a model food chain composed of a natural bacterial assemblage, a pennate diatom and a bacterivorous microflagellate to investigate the factors controlling the relative importance of bacteria and protozoa as sources for regenerated nitrogen in plankton communities. In bacterized diatom cultures in which diatom growth was nitrogen-limited, the carbon:nitrogen (C:N) ratio of the bacterial substrate greatly affected which population was responsible for the uptake of nitrogen. When nitrogen was added as NH_4^+ and the cultures were supplemented with glucose, the bacteria competed successfully with the algae for NH_4^+ and prevented the growth of algae by rapidly assimilating all NH_4^+ in the cultures. Bacterivorous protozoa inoculated into these cultures grazed the bacterial population and remineralized NH_4^+, thus relieving the nitrogen limitation of algal growth and allowing an increase in algal biomass. In contrast, bacteria in cultures supplemented with the amino acid glycine (C:N = 2) were major remineralizers of nitrogen, and the influence of protozoan grazing was minimal. We conclude that the relative importance of bacteria and protozoa as nutrient regenerators in the detrital food loop is dependent largely on the overall carbon:nutrient ratio of the bacterial substrate. The role of bacterivorous protozoa as remineralizers of a growth-limiting nutrient is maximal in situations where the carbon:nutrient ratio of the bacterial substrate is high.

Introduction

Our understanding of the roles of bacteria and bacterivorous protozoa has evolved rapidly in recent years. Traditionally, it has been assumed that the bacteria were the primary agents for the remineralization of major nutrients contained in dissolved and particulate organic material, and that the main role for bacterivorous protozoa was to maintain rapid bacterial metabolic rates (Barsdate *et al.*, 1974). This conception of detrital food webs has changed, however, and it is now recognized that natural bacterial assemblages often compete with primary producers for a growth-limiting inorganic nutrient. Consequently, a significant fraction of the nutrient remineralization taking place in these communities may be a direct result of protozoan activity (Azam *et al.*, 1983).

The ability of bacteria to compete successfully with algae for a growth-limiting nutrient has been demonstrated repeatedly in laboratory-based studies with cultured species of bacteria and phytoplankton (Rhee, 1972; Mayfield & Inniss, 1978; Currie & Kalff, 1984c; Bratbak & Thingstad, 1985; Gude, 1985). This competitive ability is a result of high affinity uptake systems for inorganic nitrogen and phosphorus which have been demonstrated with cultured species of bacteria (Brown *et al.*, 1972; Currie & Kalff, 1984a). In addition, a net flux of inorganic nitrogen or phosphorus into the bacterial assemblage often occurs in natural systems (Faust & Correll, 1976; Horstmann & Hoppe, 1981; Krempin *et al.*, 1981; Currie & Kalff, 1984b; Berman, 1985; Wheeler & Kirchman, 1986) indicating that this competition is a common phenomenon in aquatic environments.

A major factor for determining whether bacteria are sources or sinks for regenerated nutrients in the

plankton is the carbon:nutrient ratio of the assimilable bacterial substrates (Fenchel & Blackburn, 1979). During growth, bacteria use major nutrients (nitrogen and phosphorus) and organic carbon in a defined stoichiometry to form bacterial biomass. For example, when nitrogen is present in organic compounds used for growth (e.g. amino acids) in greater amounts than required for the production of bacterial biomass, the excess nitrogen is released as NH_4^+. On the other hand, if nitrogen in the organic substrate is insufficient for growth, then the bacteria will assimilate additional nitrogen from the pool of inorganic nutrients (NO_3^-,NH_4^+) in the surrounding medium. This results in a predictable relationship between the amount of bacterial nutrient regeneration for a given substrate and the carbon:nutrient ratio of the substrate (Hollibaugh, 1978; Parsons *et al.*, 1981; Billen, 1984; Lancelot & Billen, 1985). Therefore, in the case of nitrogen, the uptake of inorganic nitrogen by natural bacterial assemblages indicates that the availability of low C:N substrates (proteins, peptides, amino acids) often is not sufficient to supply all of the nitrogen required for bacterial growth (Ferguson & Sunda, 1984; Hagström *et al.*, 1984; Wheeler & Kirchman, 1986).

The possibility that bacteria may be important sinks for inorganic nutrients in the plankton implies that the major consumers of this biomass, the protozoa, at times may be the primary agents for nutrient regeneration in detrital food webs. It has been shown in recent studies that protozoa are abundant in planktonic ecosystems (Sorokin, 1981; Fenchel, 1982; Davis *et al.*, 1985; Sherr *et al.*, 1984, 1986), are voracious consumers of bacteria (Sieburth, 1984; Fenchel, 1986), and typically regenerate a substantial fraction of the nutrients in their food (Gast & Horstmann, 1983; Sherr *et al.*, 1983; Goldman *et al.*, 1985; Gude, 1985; Wambeke & Bianchi, 1985; Andersen *et al.*, 1986). Therefore, in situations where the bacteria are consumers rather than producers of inorganic nutrients, the bacterivorous protozoa may be instrumental in the regeneration of nutrients limiting the growth of the primary producers.

Previously, we have used model systems (manipulated combinations of bacteria, protozoa and microalgae) to test traditional hypotheses concerning the roles of these microorganisms in nutrient cycling in plankton communities (Caron *et al.*, 1985; Goldman & Caron, 1985; Goldman *et al.*, 1985; Caron *et al.*, 1986; Anderson *et al.*, 1986; Caron & Goldman, in press). Our goal in this study was to use a similar approach to demonstrate some of the factors controlling the relative importance of bacteria and bacterivorous protozoa for the remineralization of a growth-limiting nutrient. Growth of the diatom *Phaeodactylum tricornutum* in a nitrogen-limited medium was compared when specific microbial populations (bacteria and bacterivorous protozoa) were present or absent, and with different types of organic compounds added to serve as bacterial substrates. Bacteria in these cultures reduced or augmented the concentration of inorganic nitrogen depending on the carbon : nutrient ratio of the bacterial substrate. When bacterized cultures were enriched with glycine (C:N = 2), bacterial nitrogen remineralization was high, the production of bacterial biomass was low, and protozoan nutrient regeneration was insignificant compared to regeneration by the bacteria. However, when supplemented with glucose, the bacteria competed for NH_4^+ with the alga, and bacterivorous protozoa were instrumental in remineralizing some of the nutrient contained in the bacterial biomass thereby partially relieving nutrient limitation of algal growth.

Material and methods

Microbial populations

The pennate diatom *Phaeodactylum tricornutum* (clone TFX-1) was used as the test alga in all experimental manipulations. We chose this species because our preliminary work with this diatom indicated that it grew well in laboratory culture on a synthetic seawater medium with NH_4^+ as a nitrogen source, and was sufficiently large to prevent phagocytosis by the protozoan. In addition, although *P. tricornutum* can grow using some amino acids as a sole nitrogen source (Flynn & Syrett, 1986a, b), we tested the ability of our strain to do so on several amino acids and observed that diatom growth was very poor when glycine was the only source of nitrogen. We could therefore be assured

that utilization of amino acid nitrogen in our study would be mediated by the bacterial assemblage.

A natural bacterial assemblage was prepared by passing natural seawater from Vineyard Sound, Massachusetts through a sterile 0.8 μm pore size Nuclepore filter into a sterile filter flask. Yeast extract (10 μg) was added to approximately 100 ml of filtrate and the flask was incubated in the dark for 3 days to ensure that no living bacterivorous protozoa had passed through the filter.

The protozoan used in this study is a small (3–4.5 μm diameter), spherical chrysomonad microflagellate in the genus *Monas*. A clonal culture of *Monas* sp. was started by micropipetting individual cells grown in seawater samples from Buzzards Bay, Massachusetts which were enriched with sterile rice grains to promote growth of the natural bacterial assemblage. This protozoan grew readily on a variety of bacterial species (Caron, 1984) but our preliminary studies indicated that it was too small to prey on *P. tricornutum*.

Experimental design

A summary of the different experimental treatments and combinations of the microbial populations is given in Table 1. Basically, *P. tricornutum* was cultured in three ways: in axenic cultures, with the natural bacterial assemblage present (bacterized cultures), and with bacteria and the microflagellate present (3-member cultures). These combinations were grown in synthetic seawater medium (Goldman & McCarthy, 1978) in which nitrogen was the element limiting phytoplankton growth. Three types of nutrient enrichments were used to culture each of the microbial combinations. Ammonium was added to all treatments at a concentration of 50 μg atoms of nitrogen liter^{-1}. One set of cultures received no further enrichment while a second set also received 100 μg atoms of nitrogen liter^{-1} as glycine (C:N ratio = 2) and a third received 1 000 μg atoms of carbon liter^{-1} as glucose for a total of nine treatments and microbial combinations (Table 1). In addition, the bacterial assemblage was cultured alone on 100 μg atoms of nitrogen liter^{-1} as glycine, or 50 μg atoms of nitrogen liter^{-1} as NH_4^+ plus 1 000 μg atoms of carbon liter^{-1} as glucose. We calculated that this concentration of glucose was sufficient to promote uptake of all of the NH_4^+ by the bacterial assemblage assuming conservative values for gross growth efficiency (30%) and the carbon:nitrogen ratio of bacterial biomass (C:N = 6).

The cultures were grown in 1 liter Erlenmeyer flasks (~750 ml of culture) in continuous light at the same intensity (~200 μEinsteins m^{-2} sec^{-1}) at 20 °C. All cultures were continuously mixed with magnetic stirrers. Samples were taken initially and

Table 1. Summary of the experimental manipulations and combinations of microbial populations.

Microbial population(s) present			Nitrogen and Organic Carbon constituents added		
Diatoms	Bacteria	Protozoa	Ammonium	Glucose	Glycine
+			+		
+			+	+	
+			+		+
+	+		+		
+	+		+	+	
+	+		+		+
+	+	+	+		
+	+	+	+	+	
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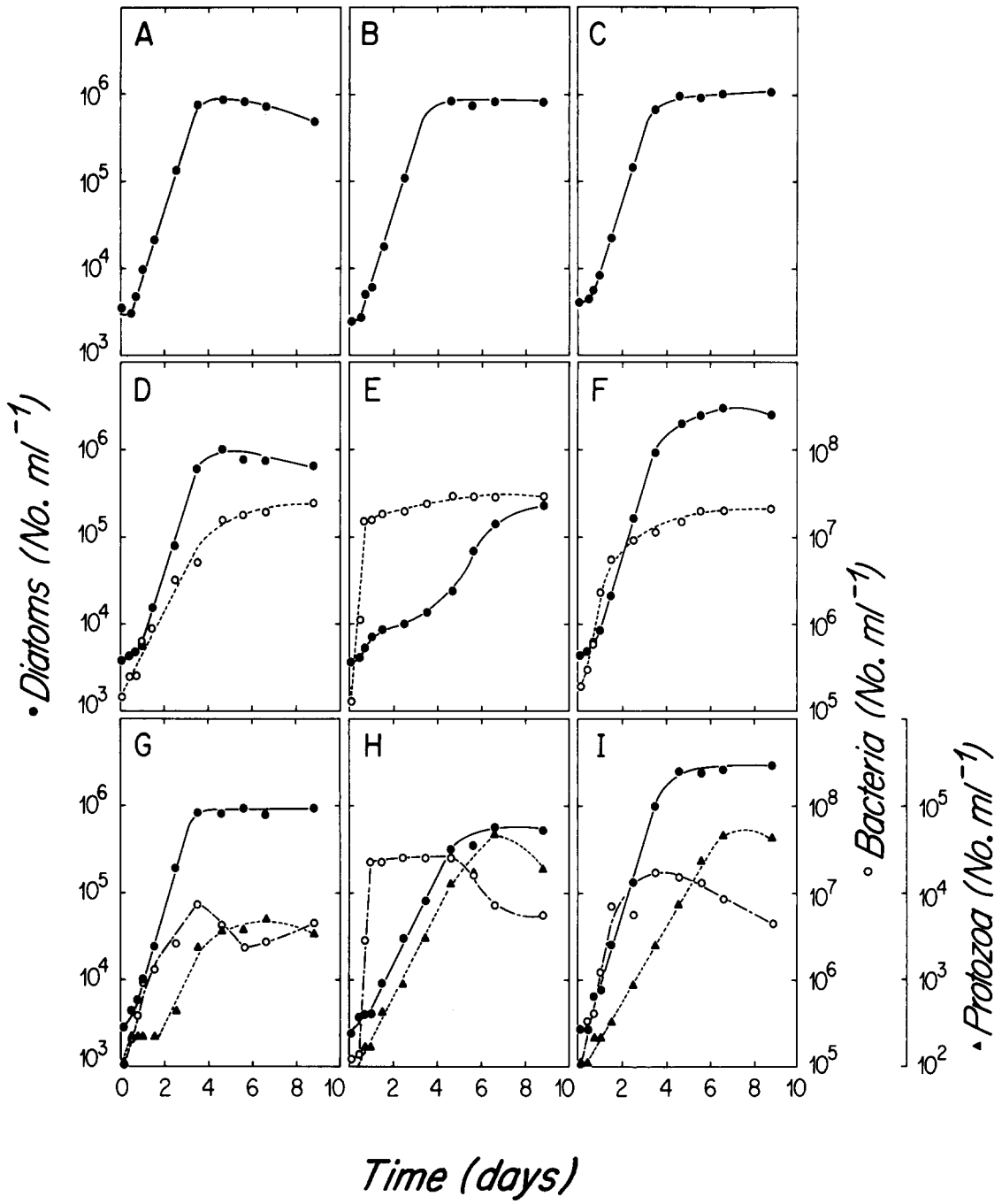

Fig. 1. Cell counts of the microbial populations in axenic cultures of the diatom *Phaeodactylum tricornutum* (A–C), bacterized (mixed natural bacterial assemblage) *P. tricornutum* cultures (D–F), and bacterized *P. tricornutum* cultures inoculated with the microflagellate *Monas* sp. (G–I). The growth medium for the cultures was a synthetic seawater medium with nitrogen as the element limiting phytoplankton growth. Cultures A, D and G contained 50 μg atoms of nitrogen liter^{-1} as NH_4^+, cultures B, E, and H contained 50 μg atoms of nitrogen liter^{-1} as NH_4^+ and 1000 μg atoms of carbon liter^{-1} as glucose, and cultures, C, F and I contained 50 μg atoms of nitrogen liter^{-1} as NH_4^+ and 100 μg atoms of nitrogen liter^{-1} as glycine.

four more times during the first 1.5 days of incubation and then at approximately daily intervals for a 9 day period. The axenic diatom cultures were sampled aseptically through a sterile siphon. The bacteria-free status of the cultures containing only diatoms was confirmed by epifluorescence microscopy at the end of the experiment.

The three microbial populations were enumerated in all samples using epifluorescence microscopy by the acridine orange (Watson *et al.*, 1977; Davis & Sieburth, 1982) or DAPI (Porter & Feig, 1980) techniques. Particulate nitrogen (PN) and particulate carbon (PC) were measured on 25 or 50 ml samples filtered onto pre-combusted Whatman GF/F glass-fiber filters using a Perkin Elmer 240 Elemental Analyzer. Ammonium concentration was measured on the filtrate using the procedure of McCarthy and Kamykowski (1972).

Results and discussion

Axenic diatom cultures

The growth curves for axenic cultures of the diatom *P. tricornutum* were unaffected by enrichment with glucose or glycine (Fig. 1A–C). The growth rates for these three populations were virtually the same, and the maximum densities of diatoms obtained in the three treatments were not significantly different (confidence level of 95%; Table 2). These results are in agreement with our conclusion that direct utilization of glycine as a nitrogen source by the diatom was insignificant.

Changes in the concentration of NH_4^+ and PN also were similar in all three axenic cultures (Fig. 2A–C). Ammonium concentrations decreased exponentially and were undetectable at 3.5 days in all axenic cultures, coinciding with maxima in PN concentrations and the beginning of the stationary growth phase of the diatom populations. The initial NH_4^+ concentration in the glycine-enriched cultures was less than the 50 μg atoms N l^{-1} added to these cultures ($\sim$37 μg atoms N l^{-1}). We speculate that some of the NH_4^+ may have bound to the glycine. However, this process apparently was reversible because the maximum diatom density and PN concentration obtained in this treatment were similar to the values obtained in the other axenic cultures.

Bacterized diatom cultures

The presence of a natural bacterial assemblage in the *P. tricornutum* cultures had a significant effect on the growth of the diatom (relative to axenic growth) only when organic carbon was added to the culture (Fig. 1D–F). Growth of *P. tricornutum* in the bacterized culture where NH_4^+ was the only nitrogen

Table 2. Maximum densities (cells $ml^{-1} \times 10^5 \pm 1$ S.D.) and growth rates (day^{-1}) of the diatom *Phaeodactylum tricornutum* in nitrogen-limited cultures. Ammonium was added at 50 μg atoms N l^{-1}, glycine at 100 μg atoms N l^{-1}, and glucose at 1000 μg atoms C l^{-1}. Growth rates were calculated from the linear portions of the population growth curves in Fig. 1 (5–6 data points each).

Nitrogen and organic carbon constituents added		Microbial population(s) present		
		Diatoms only	Diatoms + bacteria	Diatoms + bacteria + protozoa
NH_4^+	density	8.56 ± 1.56	10.3 ± 2.1	9.37 ± 1.60
NH_4^+ + glucose	density	8.28 ± 0.95	2.34 ± 0.32	5.61 ± 0.69
NH_4^+ + glycine	density	10.7 ± 1.8	31.0 ± 3.3	29.4 ± 3.1
NH_4^+	growth rate	1.8	1.7	1.8
NH_4^+ + glucose	growth rate	1.8	0.5	1.1
NH_4^+ + glycine	growth rate	1.7	1.8	1.9

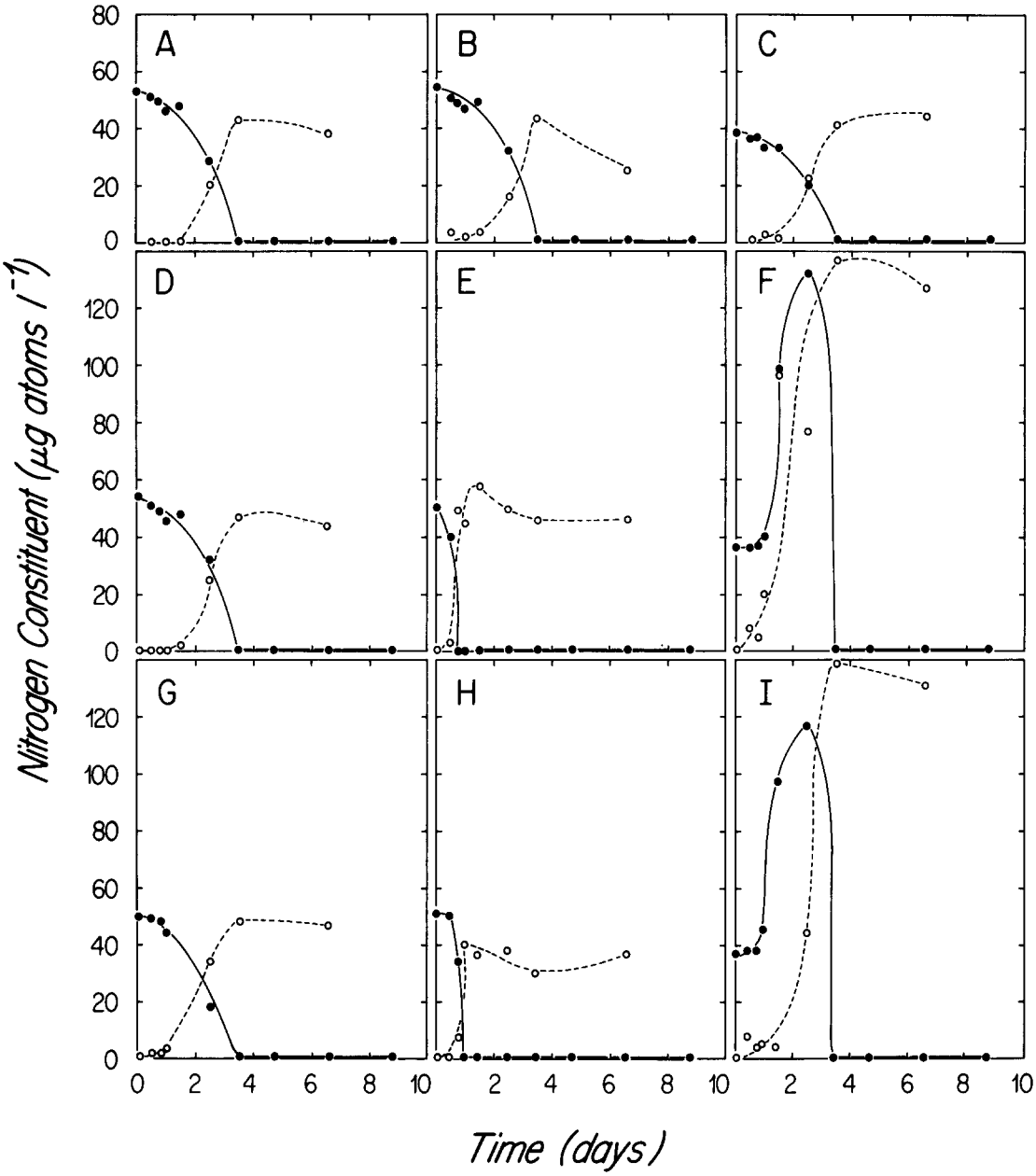

Fig. 2. Particulate nitrogen (o) and NH_4^+ (•) concentrations in cultures of the diatom *Phaeodactylum tricornutum*. See legend for Fig. 1 for the microbial populations present, and nitrogen and organic carbon supplementation in the cultures.

source and no organic carbon was added was indistinguishable from growth in the axenic cultures (Fig. 1A–D). Diatom growth rate and maximum population density (Table 2) and the pattern of change in NH_4^+ and PN concentrations (Fig. 2D) in this culture were similar to the changes in these parameters in the axenic cultures. Bacterial growth in the unenriched diatom culture was dependent on assimilable organic carbon compounds produced and released by the algae. The production of these compounds apparently constituted only a small fraction of the total organic carbon produced by the

diatom over the experimental period because bacterial growth did not affect the growth of the alga.

The addition of glucose to the bacterized diatom culture caused a significant reduction in diatom growth rate and the maximum population density of diatoms relative to algal growth in the axenic cultures (Fig. 1E, Table 2). Ammonium concentration decreased rapidly in this culture and was undetectable after 1 day (Fig. 2E). PN concentration and bacterial abundance increased to a maximum during this same period (Figs. 1E, 2E). Changes in the bacterial density and the concentration of NH_4^+ and PN in this culture were the same as the changes observed when only bacteria were present (Fig. 3B, D). The addition of an assimilable source of organic carbon containing no nitrogen resulted in the rapid uptake of NH_4^+ by the bacteria and the production of bacterial biomass, making nitrogen unavailable for diatom growth. Measurable amounts of NH_4^+ were not observed in the glucose-enriched culture containing only bacteria until the fourth day of incubation (Fig. 3D). Overall, only a minor fraction of the total nitrogen was released as NH_4^+ in this culture throughout the course of the study ($\sim$16%) resulting in stunted growth of the algal population.

The addition of glycine to the bacterized diatom culture did not alter the growth rate of *P. tricornutum*, but it did result in a significantly greater maximum population density of the diatom relative to the axenic cultures (Fig. 1F; Table 2). Remineralization of nitrogen from glycine by the bacterial assemblage resulted in a rapid increase in the NH_4^+ concentration of this culture to a maximum of 132 μg atoms N l^{-1} at 2.5 days, followed by a rapid decrease in the concentration of NH_4^+ to undetectable levels (Fig. 2F). A comparison of nitrogen incorporation and remineralization by the bacterial assemblage alone when glycine was the sole nitrogen and organic carbon source indicated that most of the nitrogen contained in the glycine was remineralized and only a small fraction was used to produce bacterial biomass (Fig. 3A, C). Consequently, more of the growth-limiting nutrient was available for diatom growth in the bacterized glycine-enriched cultures than in the axenic glycine-enriched culture or cultures not receiving glycine.

3-Member cultures

The presence of bacteria and bacterivorous protozoa did not significantly affect the growth of the diatom when NH_4^+ was the only nitrogen source and no organic carbon was added (Fig. 1G). Diatom growth rate and maximum population density (Table 2), and changes in the concentrations of NH_4^+ and PN in this culture (Fig. 2G) were similar to these parameters in axenic cultures. Likewise, diatom growth rate and changes in nitrogen constituents in the glycine-enriched culture with bacteria and protozoa were indistinguishable from these parameters in bacterized diatom cultures without protozoa (Figs. 1I, 2I, Table 2).

The pattern of diatom growth in the glucose-enriched culture with bacteria and protozoa, however, differed significantly from the patterns in the axenic and bacterized cultures (Figs. 1H; 2H). The initial pattern of rapid bacterial growth accompanied by an increase in PN concentration and a decrease in NH_4^+ concentration from the time of inoculation to day 1 was similar to changes in the bacterized culture without protozoa. As previously stated, these changes were a result of bacterial uptake of NH_4^+. However, subsequent grazing by bacterivorous protozoa reduced the density of the bacterial assemblage and supported more (and faster) diatom growth than in the ungrazed system by remineralizing some of the nitrogen tied up in the bacterial biomass. The growth rate of the diatom in this culture (1.1 day^{-1}) was identical to the growth rate of the microflagellate (slopes in Fig. 1H) which presumably indicates that growth of the diatom was directly related to growth (and concomitant nutrient regeneration) of the microflagellate.

The maximum population density of *P. tricornutum* in this culture was intermediate to, and significantly different from, the population densities observed in the axenic and bacterized glucose-enriched cultures (confidence level of 95%; Table 2). This result is a consequence of the fact that some of the nitrogen was still tied up in residual bacterial biomass and in protozoan biomass. Therefore, even though the protozoa were instrumental in remineralizing some of the growth-limiting nutrient, they

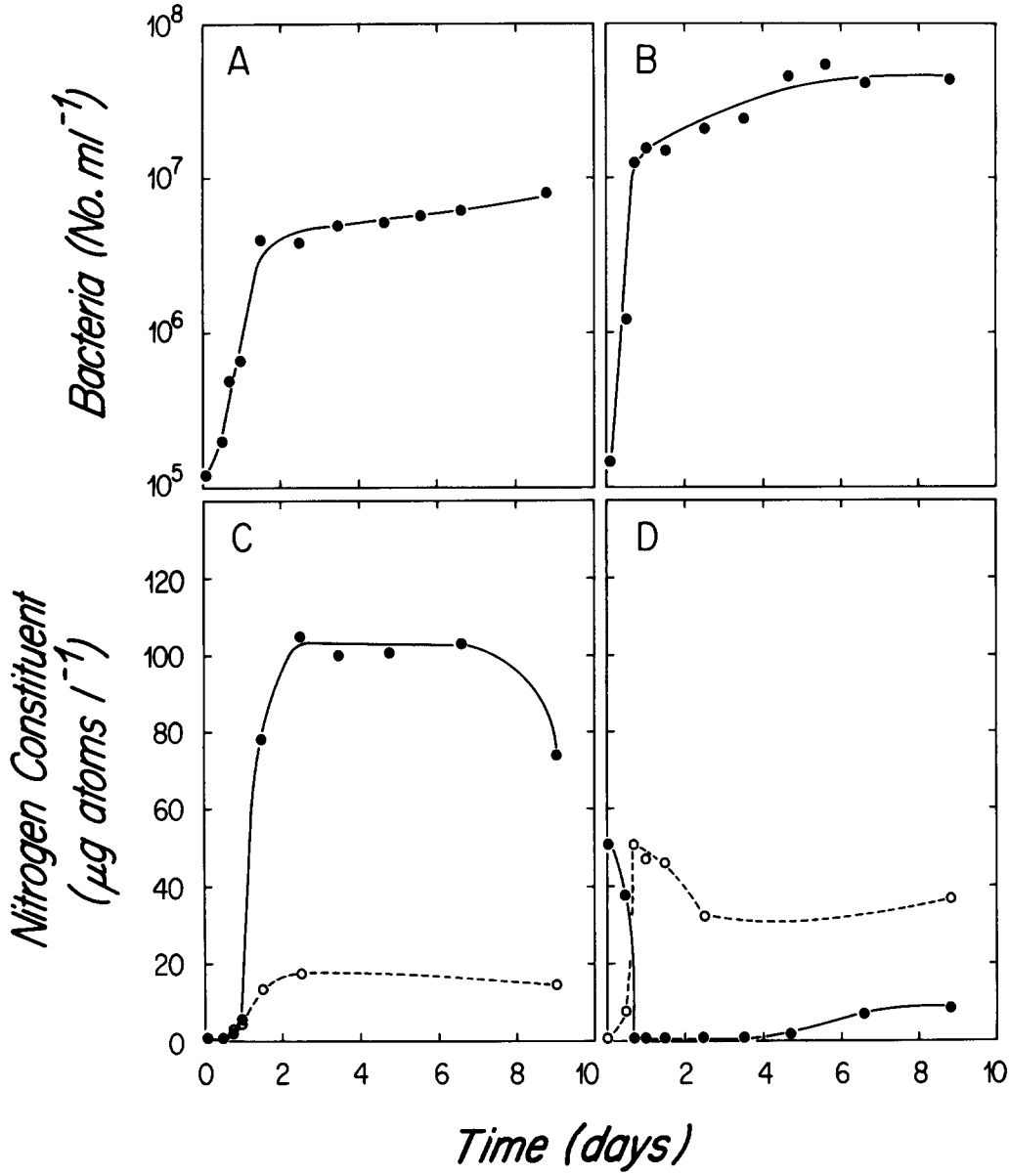

Fig. 3. Cell counts (A, B) and particulate nitrogen (o) and NH_4^+ (•) concentrations (C, D) in two cultures of a mixed natural assemblage of bacteria in a synthetic seawater medium containing 50 μg atoms of nitrogen liter^{-1} as NH_4^+ and 1000 μg atoms of carbon liter^{-1} as glucose (B, D) or 100 μg atoms of nitrogen liter^{-1} as glycine (A, C).

also effectively reduced the flow of some of this nutrient back to the primary producer by virtue of the production of their own biomass. The degree to which they reduced the flow of nutrient was dependent on their gross growth efficiency (i.e., their ability to convert bacterial biomass into protozoan biomass).

Changes in the morphological types of bacteria present in the grazed cultures also may have reduced the amount of nitrogen remineralized by the protozoan. Short rod-shaped cells and cocci were dominant in all bacterized cultures prior to a significant grazing effect by the protozoa (Fig. 4A). These morphological forms continued to dominate the bacteri-

al assemblage throughout the experimental period in cultures not inoculated with microflagellates (Fig. 4C) but filamentous and aggregated bacteria became important in the cultures inoculated with the microflagellate (Fig. 4B, D). This phenomenon presumably was due to the selective removal of unaggregated non-filamentous bacteria by the protozoa thus favoring the growth of morphologies which could not be phagocytized (Gude, 1979). This "predation resistant" assemblage constituted a poten-

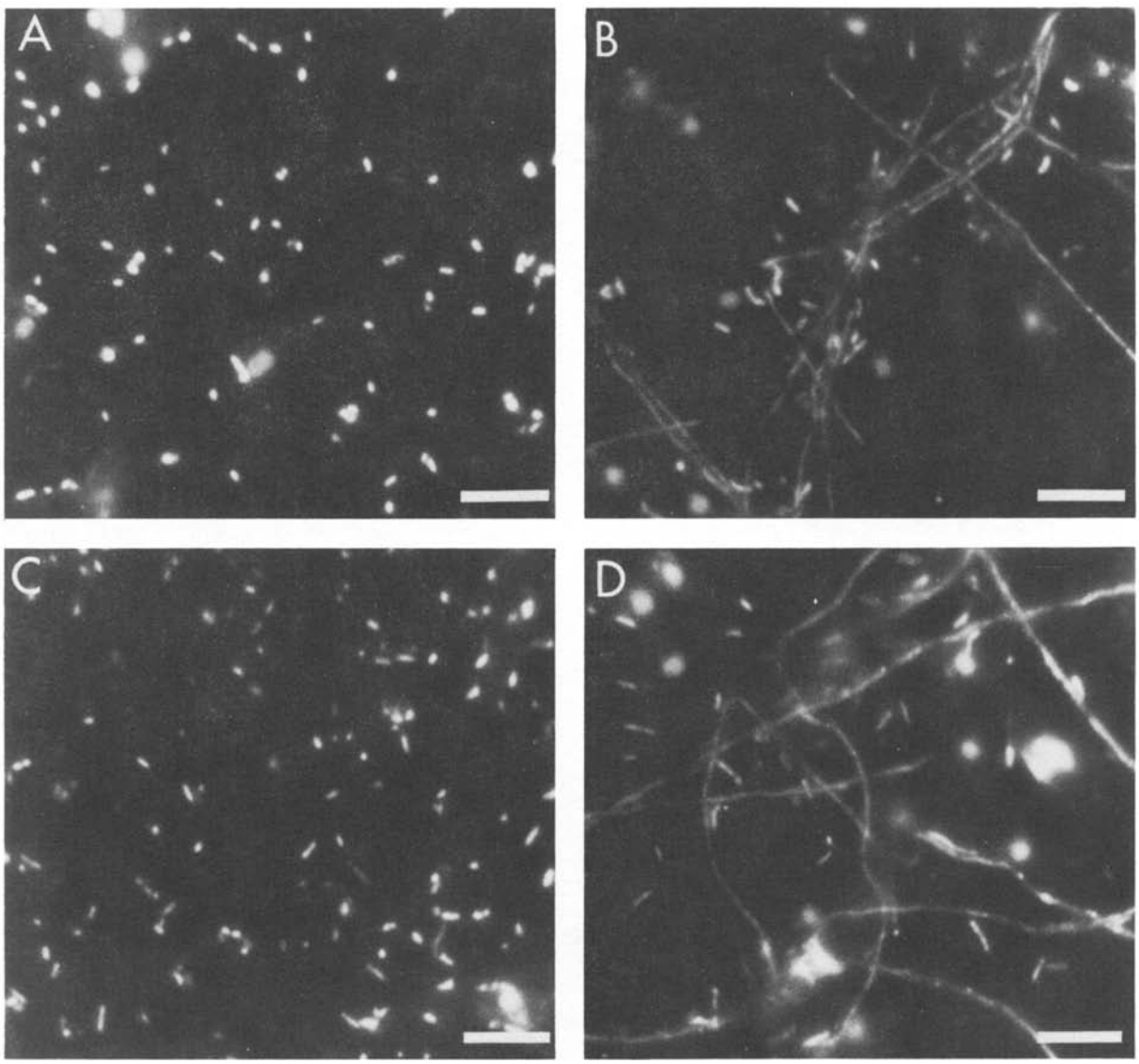

Fig. 4. Epifluorescence photomicrographs of DAPI-stained bacteria showing the changes in morphological forms as a result of grazing by the microflagellate *Monas* sp. A, B) Bacterial morphological forms present in an ammonium and glycine-enriched culture of *P. tricornutum* and inoculated with bacteria and protozoa. A) 3.5 days after inoculation but prior to a significant reduction in bacterial density due to grazing. B) Filamentous bacteria in the same culture at 8.8 days, subsequent to microflagellate grazing on bacteria. C, D) Bacterial morphological forms present in two cultures with ammonium as the only nitrogen source and without organic carbon added. C) 8.8 days after inoculation with *P. tricornutum* and bacteria but without protozoa present. D) 8.8 days after inoculation with *P. tricornutum*, bacteria and microflagellates. Marker bars = 5.0 μm. Large round fluorescing objects (1–2 μm diameter) are the nuclei of *P. tricornutum*. Other cell contents of the diatom are barely visible as poorly-stained "ghosts".

tially important sink for nitrogen in these cultures. We may have been able to increase the efficiency of transfer of nitrogen to the diatom population in the 3-member cultures by the use of cultured species of bacteria which would not form predation resistant morphologies. In this way, the residual bacterial biomass in these cultures would have been minimal, and a larger percentage of the growth-limiting nutrient would have been available for phytoplankton growth.

Conversely, the addition of more glucose to the bacterized cultures (protozoa absent) throughout the experiment probably would have resulted in more efficient retention of nitrogen by the bacterial assemblage and even less diatom growth than that observed (Fig. 1E). The appearance of NH_4^+ after 4 days of incubation in the glucose-enriched culture with bacteria only (Fig. 3B, D) presumably was an indication of organic carbon limitation at that time. Interestingly, an increase in diatom growth rate in the bacterized cultures of *P. tricornutum* enriched with glucose (Fig. 1E) was observed between 3.5 and 6.6 days ($\mu = 0.8$ day^{-1}) relative to the growth rate between 1.5 and 3.5 days ($\mu = 0.3$ day^{-1}). Alleviation of organic carbon limitation for the bacterial assemblage by the addition of more glucose might have prevented the release of NH_4^+ and subsequent growth of the diatom in this culture at day 4.

Particulate carbon and C:N ratios

The concentrations of PC and the carbon:nitrogen (C:N) ratios in the various cultures were affected by the predominance of bacterial or diatom biomass. For example, in the glucose-enriched culture inoculated with only bacteria, PC concentration was 4.1 mg C l^{-1} at 6.6 days. In comparison, the two axenic diatom cultures with the same amount of nitrogen had an average concentration of 8.8 mg C l^{-1} (Table 3). Production of PC by the alga during stationary growth resulted in more organic carbon per unit nitrogen in the algal biomass than in the bacterial biomass. Particulate carbon in the glucose-enriched diatom culture inoculated with bacteria had a PC concentration at 6.6 days which reflected the dominance of bacterial biomass in this culture (4.4 mg C l^{-1}). Particulate carbon concentration was somewhat higher in the glucose-enriched culture with bacteria and protozoa (5.4 mg C l^{-1}) but was not comparable to cultures without glucose added. Glycine enrichment resulted in the most striking contrast in PC concentration. Diatom cultures containing NH_4^+ and glycine which were inoculated with bacteria (with or without protozoa) averaged 38 mg C l^{-1} at 6.6 days compared to 12.2 mg C l^{-1} in the glycine-enriched axenic culture and 1.0 mg C l^{-1} in the glycine-enriched culture inoculated with only bacteria.

Differences in the amount of PC per unit PN in bacterial and diatom biomass were reflected in the C:N ratios of the cultures. The two cultures containing only bacteria had C:N ratios (by weight) of 6.9 and 8.0 at 6.6 days. In contrast, the C:N ratios of all the diatom cultures except the two glucose-enriched cultures inoculated with bacteria ranged from 18 to

Table 3. Particulate carbon concentration (mg l^{-1}) in nitrogen-limited cultures after 6.6 days of incubation in continuous light at 20 °C. Ammonium was added at a concentration of 50 μg atoms N l^{-1}, glycine at 100 μg atoms N l^{-1}, and glucose at 1000 μg atoms C l^{-1}.

Nitrogen and organic carbon constituents added	Microbial population(s) present			Bacteria only
	Diatoms only	Diatoms + bacteria	Diatoms + bacteria + protozoa	
NH_4^+	9.7	12.6	12.9	
NH_4^+ + glucose	7.9	4.4	5.4	4.1
NH_4^+ + glycine	12.2	38.5	37.6	1.0*

* Glycine was the sole nitrogen and organic carbon source added to the culture.

22 at that time. These C:N values are in agreement with C:N values observed for nitrogen-limited cultures of *P. tricornutum* in other studies (e.g. Terry *et al.*, 1985). The glucose-enriched diatom culture with bacteria had a C:N ratio similar to that of the bacteria (6.7), while the glucose-enriched 3-member culture had a ratio intermediate to that of the bacteria and the diatom (C:N = 10), reflecting the fact that some of the bacterial nitrogen had been converted to diatom nitrogen via the grazing activity of the protozoa.

Qualitatively, nitrogen and carbon assimilation by the bacteria were complementary for glucose- and glycine-enriched cultures. High carbon assimilation efficiency (> 50%) and low nitrogen assimilation efficiency (< 20%) were observed for glycine additions, while low carbon assimilation efficiency (< 30%) and high nitrogen assimilation efficiency (uptake of nearly 100% of the NH_4^+) were observed for glucose additions. A more detailed study of the interaction of substrate carbon:nutrient ratios and carbon and nutrient assimilation/regeneration efficiencies of bacteria will be presented elsewhere (Goldman *et al.*, in prep.).

Implications for nutrient regeneration

The processes that we have demonstrated in this study represent highly simplified (i.e., 1-, 2-, or 3-member cultures) or extreme situations (growth on glycine only or glucose only) designed to define the major roles of the microbial populations and the chemical constituents. In nature, all components of the detrital loop are, of course, present, and bacterial growth is undoubtedly dependent on a mixture of natural substrates. In addition, protozoan herbivory and omnivory complicate the already difficult task of elucidating pathways of nutrient flow among microorganisms. However, the principles governing the activity of these microorganisms and the utilization of these compounds should be applicable, albeit in more complicated configurations, to natural systems. We conclude, based on the results of this study, that the relative importance of nutrient regeneration by bacterivorous protozoa is dependent to a large extent on the activity (incorporation vs. remineralization) of the bacterial assemblage. In turn, bacterial nutrient regeneration is affected by the carbon:nutrient ratio of the assimilable substrates, the carbon:nutrient ratio of the bacterial biomass, and the efficiency of conversion of substrate into bacterial biomass.

A considerable amount of literature now exists on the latter two parameters. In general, conversion efficiencies (i.e., gross growth efficiencies, the conversion of substrate carbon into bacterial carbon) for bacteria are relatively high and a value of 50% is commonly used (Fuhrman & Azam, 1982; Hagström *et al.*, 1984). On the other hand, bacterial C:N ratios generally are lower than other heterotrophic organisms. Nagata (1986) recently reported the average C:N ratio (by weight) for natural assemblages of aquatic bacteria to be approximately 5.

These values indicate that the overall C:N ratio for natural bacterial substrates musts be relatively low (overall C:N < 10) in order to support nitrogen remineralization by the bacterial assemblage. For example, assuming a C:N ratio of ~4.0 for bacterial biomass and a gross growth efficiency for carbon assimilation of 50%, it can be calculated that for each mole of glucose metabolized one mole of substrate with a C:N ratio of 2.0 (e.g., glycine) must be co-metabolized in order to provide enough nitrogen to maintain the C:N ratio in the cell. This calculation assumes 100% efficiency for nitrogen assimilation. Even at a much lower growth efficiency, a substantial amount of low C:N substrate must be metabolized (e.g., at 25% efficiency, 0.4 M of glycine must be co-metabolized for 1 M of glucose). For amino acids with higher C:N ratios, more of these substrates must be co-metabolized to lower the C:N ratio of the total substrate mixture (e.g., at a growth efficiency of 50%) 2 M of leucine (C:N = 6) must be co-metabolized with 1 M of glucose).

It seems unlikely that in nature this low C:N requirement for net bacterial nitrogen regeneration will always be realized. The major sources of low C:N ratio substrates in nature (dissolved free amino acids, peptides, proteins and perhaps nucleic acids) must "counterbalance" the intake of high C:N ratio substrates (e.g., carbohydrates, lipids) if net nitrogen remineralization by the bacteria is to occur. Estimates of the turnover rates of dissolved combined

and free amino acids in natural waters indicate that these compounds are taken up readily by bacterial assemblages (Williams *et al.*, 1976; Hollibaugh *et al.*, 1980; Amano *et al.*, 1982; Jorgensen, 1982; Keller *et al.*, 1982; Hollibaugh & Azam, 1983; Carlucci *et al.*, 1984; Ferguson & Sunda, 1984). However, it would appear that most of these substrates are used for protein synthesis (Kirchman & Hodson, 1984) and, therefore, relatively little nitrogen may be remineralized. Based on the data that are presently available, it appears that the flux of total amino acids (free and combined) is small compared to the flux of high C:N ratio substrates (e.g., carbohydrates; Burney, 1986a, b). In addition, a comparison of the rates of assimilation of total amino acids with estimates of bacterial productivity has shown that the fluxes of organic nitrogen compounds in natural systems generally are less than the amount of nitrogen required to meet the observed bacterial production, and that inorganic sources of nitrogen must be utilized (Ferguson & Sunda, 1984; Hagström *et al.*, 1984). More data are needed on the flux of these material to verify this speculation.

Carbohydrates are undoubtedly important bacterial substrates in the plankton. In addition to large flux rates in natural waters (Burney, 1986a, b), these compounds (along with glycollate) are known to be major components of the organic carbon compounds released by natural phytoplankton populations (Mague *et al.*, 1980; Nalewajko *et al.*, 1980; Cole, 1982; Lancelot & Billen, 1985). The interactions of dissolved carbohydrates, bacteria and bacterivorous protozoa have been observed and modelled in several studies (Burney *et al.*, 1981, 1982; Laacke *et al.*, 1983). Bratbak & Thingstad (1985) have observed that when competing with bacteria for a growth-limiting nutrient, phytoplankton tend to release organic compounds which are depleted in that nutrient. Subsequent uptake of these compounds by the bacteria resulted in increased competition by the bacteria for the growth-limiting nutrient, and, thus, a more severe inhibition of phytoplankton growth. Based on these results and the results of the present study, it is probable that competition between the bacteria and the phytoplankton for a growth-limiting nutrient may be a common phenomenon of aquatic ecosystems and that grazing activity by the bacterivorous protozoa may be instrumental in relieving nutrient limitation of the primary producers.

In summary, it is clear from the discussion above and the information discussed in the Introduction that natural aquatic bacterial assemblages may act as significant nutrient "sinks" of inorganic nitrogen. These arguments strengthen the probability that bacterivorous protozoa are important remineralizers of major nutrients in plankton communities. We have shown that in these situations, where bacteria compete with the phytoplankton for inorganic nitrogen, grazing by bacterivorous protozoa may be the primary mechanism for the remineralization of nitrogen contained in the bacterial biomass. Protozoan grazing under these circumstances prevents the paradoxical situation of bacteria outcompeting the algae for a growth-limiting nutrient (as described by Bratbak & Thingstad, 1985) by releasing the nutrient from the bacterial biomass, thus making it available for the primary producers.

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