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Utilization of inorganic and organic nitrogen by bacteria in marine systems[1]

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Abstract

The relative contribution of various inorganic and organic forms of nitrogen to the nitrogen requirements of picoplankton was examined with ^{15}N tracers. Size fractionation was used to measure uptake by <1-μm size microorganisms, and inhibitors of protein synthesis were used to separate procaryotic from eucaryotic nitrogen uptake. Picoplankton utilized mainly ammonium and amino acids and only negligible amounts of nitrate and urea. Nearly all amino acid uptake was by procaryotes, while both procaryotes and eucaryotes utilized ammonium. About 78% of total ammonium uptake was by procaryotes, and a significant portion of this was due specifically to heterotrophic bacteria. Regeneration of ammonium was correlated with eucaryotic rather than procaryotic activity. Ammonium accounted for at least 20–60% of the summed ammonium plus amino acid utilization by bacteria. The results suggest that a significant portion of ammonium uptake in the euphotic zone was by heterotrophic bacteria rather than solely by phytoplankton. This may invalidate the use of the Redfield C:N ratio for estimating rates of nitrogen assimilation in the euphotic zone from carbon assimilation rates.

Historically, it has been assumed that phytoplankton is responsible for most of the uptake of inorganic nitrogen in the euphotic zone of the sea (e.g. Dugdale and Goering 1967). Exceptions to this generalization have been noted, and recent indirect evidence supports the hypothesis that heterotrophic bacteria may play a significant role in the utilization of inorganic nitrogen in the euphotic zone. Eppley et al. (1977) found that uptake rates of inorganic nitrogen were high compared with $^{14}CO_2$ fixation rates during winter in the central North Pacific Ocean and attributed this discrepancy to nitrogen uptake by heterotrophic bacteria. Laws et al. (1985) compared ammonium uptake rates with rates of nitrogen uptake estimated from light-dependent $^{14}CO_2$ incorporation into protein. They observed that ammonium uptake was substantially higher than estimated from $^{14}CO_2$ incorporation rates and concluded that ammonium uptake by heterotrophic bacteria must have been high.

Several studies have examined the uptake of inorganic nitrogen by particles of different sizes. Ammonium uptake by the <1-μm fraction was small (<20%) in three marine environments (Harrison et al. 1977; Harrison 1978; Glibert 1982; Probyn 1985). However, Probyn and Painting (1985) found that ammonium uptake by the <1-μm organisms was 50% of total uptake at two out of five stations in Antarctic surface waters; nitrate uptake was negligible at two stations and <50% of total uptake at four stations. Uptake by picoplankton in the <1-μm size fraction is a maximum estimate of uptake by trapped heterotrophic bacteria, since this size fraction also contains procaryotic (cyanobacteria) and eucaryotic phototrophs (Johnson and Sieburth 1982). The relative use of inorganic and organic forms of nitrogen by picoplankton has not been compared for procaryotes and eucaryotes or for phototrophs and heterotrophs.

Various experimental approaches have been used to demonstrate that heterotrophic bacteria are the major sink for dissolved organic nitrogen (DON) in marine environments (*see* Billen 1984). However, the sufficiency of the supply of DON for meeting the nitrogen requirements for growth of heterotrophic bacteria has not been established. Bacterial production appears to be substantially higher than the maximum measured uptake rates of dissolved free amino acids. Bacterial produc-

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tion is in the range of 0.2–178 nmol N liter^{-1} h^{-1}, when expressed in terms of nitrogen with a C : N ratio of 3.5 (*see* Williams 1983). Amino acid uptake rates range from 0.005–0.012 nmol N liter^{-1} h^{-1} in oligotrophic waters to 5.0–20 in eutrophic waters (Billen 1984). Ferguson and Sunda (1984) used "clean" techniques and estimated that maximum amino acid uptake rates were 0.02–1.7 nmol N liter^{-1} h^{-1} at oligotrophic and eutrophic sites. Since amino acid uptake is very low compared with bacterial production, it seems likely that heterotrophic bacteria must obtain nitrogen from additional sources.

The purpose of our study was to examine ammonium utilization by procaryotes and eucaryotes and the relative contribution of inorganic nitrogen and dissolved free amino acids to the nitrogen requirements of the procaryotic picoplankton—cyanobacteria and heterotrophic bacteria. Using three different approaches, we found that ammonium was an important nitrogen source for heterotrophic bacteria and that much of the ammonium uptake was by procaryotic picoplankton.

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Methods

Experimental approaches—We measured the uptake of ^{15}N-labeled nitrogenous compounds by the <1-μm microorganisms. Additional experiments involved the use of procaryotic and eucaryotic inhibitors of protein synthesis during uptake experiments with unfractionated microbial assemblages. Finally, we also examined the effect of various nitrogen additions on net growth of heterotrophic bacteria.

Study sites and experimental conditions—Experiments were conducted with water samples collected from Marsh Landing, Sapelo Island, Georgia, between February and November 1984 and from the Gulf Stream off the coast of Georgia during the RV *Cape Hatteras* cruise from 2 to 11 May 1984. Unless indicated otherwise, samples were taken 0.3 m below the surface with plastic buckets or polyethylene carboys, and experiments were started within 1 h after collection. Incubations were at ambient temperatures (18°–27°C) and at 50–100 μEinst m^{-2} s^{-1} of "cool-white" fluorescent lighting.

Uptake measurements—Uptake was determined by time-course sampling during an incubation lasting 4–9 h. Subsamples of 300–1,000 ml were collected on precombusted (500°C, 15 min) 47-mm Whatman GF/F glass-fiber filters. Uptake rates of six different nitrogen sources were compared for picoplankton: the inorganic sources were ammonium and nitrate, the organic sources were urea, alanine, glycine, and an algal amino acid mixture. For experiments with particles <1 μm, water samples were filtered (<7 cm of Hg vacuum) through 1-μm Nuclepore filters before incubations. We chose prefiltration rather than postfiltration for the size fractionation to avoid the potential artifact of collecting labeled fragments of large cells and thus overestimating uptake by small cells. Filters for the prescreening were changed frequently (as soon as flow rates started to decrease) to maintain a reasonably constant sieve size.

The effects of chloramphenicol and cycloheximide on ammonium and amino acid uptake were examined. The inhibitors were added 1–2 h before the ^{15}N tracers. At low concentrations, chloramphenicol inhibits the function of 70S ribosomes in procaryotes and prevents procaryotic protein synthesis; cycloheximide inhibits the function of 80S ribosomes of eucaryotes, thus preventing cytoplasmic protein synthesis (Vazquez 1974). Concentrations for both inhibitors were 10–15 mg liter^{-1} for the Gulf Stream experiments and 100 mg liter^{-1} for the Sapelo Island experiments.

Isotopes were obtained from MSD (Montreal). All ^{15}N additions were at final concentrations of 1.0 μM (95–99 atom%). This concentration was chosen to obtain po-

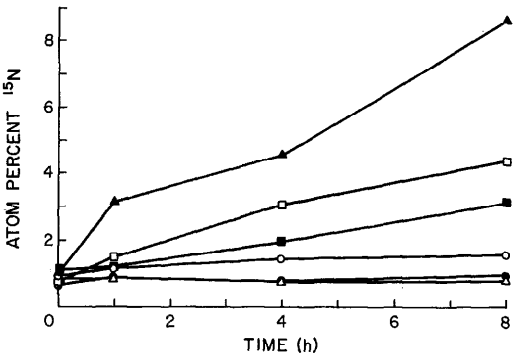

Fig. 1. Time-course of accumulation of ^{15}N in organisms of <1-μm size during incubations with ^{15}N-labeled nitrogen sources. Results from experiment at Sapelo Island, 23 February; amino acid mixture (▲), ammonium (□), glycine (○), alanine (■), urea (●), and nitrate (△). High time-zero intercepts are from nonbiological adsorption on particulate material. Standard errors on duplicate ^{15}N analyses were smaller than the symbols.

tential or maximum rates and to minimize the error in estimates of specific activity.

The amino acid mixture used was a commercial preparation extracted from labeled algal cells. After lipid and carbohydrate components had been removed, the protein fraction was subjected to acid hydrolysis. The mixture of free amino acids was then isolated and provided as a dried powder. The free amino acid content was 95.9% after correction for loss on drying; the remaining 4.1% was nonhydrolyzed protein. The preparation also contained ~7% ammonium (as percent total N).

^{15}N analysis—Filters were dried at 50°C overnight and stored under vacuum until isotopic analysis. Preparation of samples for ^{15}N analysis was adapted from LaRoche (1983). Filters were ground by hand with a mortar and pestle or with a mechanical grinder (Spex Industry Mixer Mill) with 0.5–1.0 g of Coleman Cuprox reagent containing a platinum catalyst. A subsample equivalent to 10 μg of N was then placed in a 1–2-ml precombusted Pyrex glass tube containing about 0.5 g of precombusted (900°C, 3 h) CaO. Tubes were sealed under vacuum (<2 μm Hg) then combusted at 500°C for ~2–24 h. After they cooled, the relative ^{15}N abundance was determined by at least three scans of the light emission spectra on a Jasco emission spectrometer. The mean coefficient of variation for replicate samples is <1% at ^{15}N enrichments of >1.0 atom%. The instrument was calibrated with commercial standards prepared from samples analyzed by mass spectrometry.

Specific uptake rates (v) were calculated from the slope of the linear portion of the change in atom% ^{15}N as a function of time divided by the atom% ^{15}N of the dissolved nitrogen source. Concentrations of free amino acids and nitrate were estimated from published data for the sites (*see footnotes to tables*) to determine the approximate specific activity used in the experimental incubations. Since the tracer additions were large (1.0 μM), ambient concentrations had very little effect on the specific activity for either nitrate or amino acids. The specific activity of all nitrogen sources was assumed to be constant during these relatively short incubations with high substrate additions. Changes in specific activity of ammonium during all regeneration experiments were small (<10%), and the rates of uptake have not been corrected for isotope dilution since the data were not available for every experiment. The interval used for each rate determination was chosen to include only the linear portion of the time-course and is indicated in Tables 1 and 4. Absolute uptake rates (ρ) were calculated by multiplying the specific uptake rate by the particulate nitrogen concentration.

Nutrients and biomass—Ammonium concentrations were determined by the phenolhypochlorite reaction (Strickland and Parsons 1972). Particulate nitrogen was determined with a Coleman nitrogen analyzer.

Bacteria were counted by epifluorescent microscopy of Formalin-preserved samples after staining with acridine orange and collection on Nuclepore filters (Hobbie et al. 1977). All cells counted by this technique were considered to be heterotrophic bacteria. The mean size of these cells (~0.6 μm) was smaller than the known size of coccoid cyanobacteria (~1.5 μm). Furthermore, most of the cells in our preparations fluoresced green, whereas cyanobacteria have a characteristic red fluorescence.

Ammonium regeneration—Rates of ammonium regeneration were determined by isotope dilution (Harrison 1978). Ammo-

Table 1. Uptake of nitrogen sources (μmol liter^{-1} h^{-1}) by organisms in the <1-μm size fraction at Marsh Landing, Sapelo Island, on 23 February 1984. Slopes and standard errors (SE) are from the linear regression of atom% ^{15}N of the particulate material vs. time for the interval indicated. (NA—Not applicable, only two time points used.)

N source	Concn* (μM)	Time interval (h)	Slope	SE	Uptake rate
Ammonium	1.50	0–1	0.733	NA	0.064
		0–4	0.570	0.040	0.050
		0–8	0.444	0.050	0.039
Alanine	1.01	0–1	0.228	NA	0.014
		0–4	0.127	0.025	0.008
		0–8	0.081	0.019	0.005
Glycine	1.01	0–1	0.135	NA	0.008
		0–4	0.235	0.025	0.015
		0–8	0.261	0.013	0.016
Amino acid mix	1.30	0–1	2.139	NA	0.167
		0–4	0.795	0.333	0.062
		0–8	0.878	0.116	0.068
Urea	2.00	0–1	0.020	NA	0.002
		0–4	−0.015	0.009	−0.002
		0–8	−0.001	0.006	0.000
Nitrate	1.30	0–1	0.017	NA	0.001
		0–4	0.008	0.041	0.001
		0–8	0.021	0.014	0.002

* Estimated ambient concentration plus 1.0 μM ^{15}N addition. Ambient nitrate was taken from seasonal values for these waters (Haines 1979). Ambient urea was assumed to be equivalent to twice ambient ammonium (Remsen et al. 1974). In both cases uptake rates were low, and the exact specific activity has little effect. Individual amino acids were estimated as 0.10 μM, and total free amino acids as 0.30 μM (Crawford et al. 1974).

nium in the filtrates from the uptake samples was converted to indophenol by the phenolhypochlorite reaction and then concentrated by extraction into dichloromethane and subsequent evaporation of the solvent (Dudek et al. 1986). Rates of regeneration were calculated from equations described by Blackburn (1979).

Results

Uptake by picoplankton—In two experiments at Sapelo Island and two off the coast of Georgia, we found significant uptake of ammonium, individual amino acids, and the amino acid mixture by organisms <1 μm. In the same experiments, we were unable to detect uptake of either urea or nitrate nitrogen by these organisms. Typical results are presented in Fig. 1. The amino acid mixture was taken up most rapidly, followed by ammonium, and then the individual amino acids. The rate of accumulation of ^{15}N decreased with time for ammonium, alanine, and the amino acid mixture, with the most noticeable decreases occurring for ammonium and the amino acid mixture (Table 1). We conclude from these experiments that both ammonium and amino acids were being utilized as nitrogen sources by microorganisms of <1 μm in size. In contrast, neither urea nor nitrate was used to a significant extent.

Inhibitor experiments—Chloramphenicol, which caused a decrease in bacterial abundance in these samples (Fig. 2), strongly inhibited amino acid uptake (Fig. 3). In all six experiments, uptake was inhibited by >58%. Cycloheximide, on the other hand, caused an increase in bacterial abundance (Fig. 2) and had little effect on amino acid uptake (Fig. 3). In one experiment, uptake was inhibited by 20%, but in the other five experiments amino acid uptake was either only slightly inhibited or was actually stimulated by the addition of cycloheximide. These results indicate that nearly all of the amino acid uptake measured at these high (1 μM) concentrations is by procaryotes rather than eucaryotes.

Chloramphenicol also inhibited ammonium uptake (Fig. 4). In all six experiments, uptake was inhibited by >25%, and in three experiments inhibition was >50%. Cycloheximide either slightly inhibited or ac-

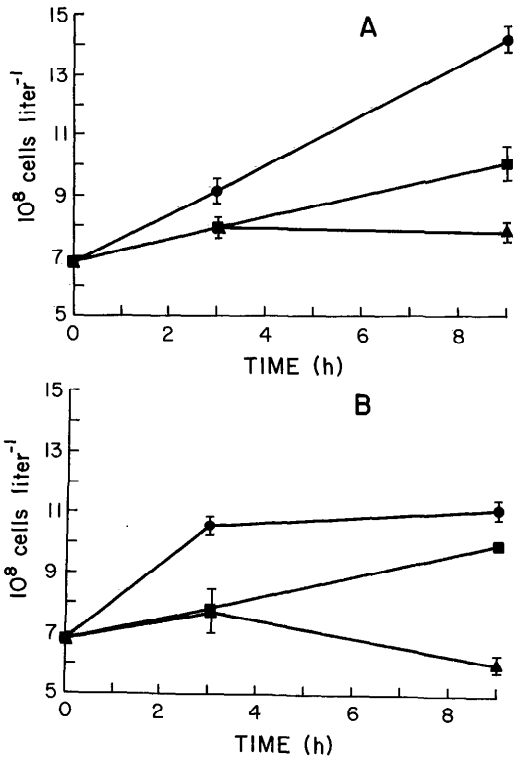

Fig. 2. Changes in bacterial abundance in Sapelo Island water during amino acid uptake (A) and ammonium uptake (B) experiments with protein synthesis inhibitors; control (■), chloramphenicol (▲), and cycloheximide (●). Bars—SE of the mean when it was larger than the symbol.

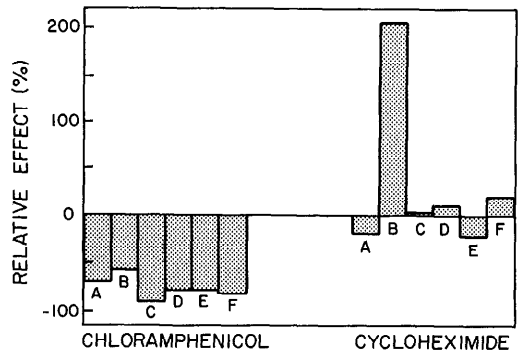

Fig. 3. Effect of inhibitors on amino acid uptake. Relative effect was calculated as [(treatment − control)/control] × 100. A—9 May; B—11 May; C—22 August; D—29 August; E—18 October; F—20 October. May experiments are from the Gulf Stream. All other experiments were from Sapelo Island with water collected at high tide, except D which was low tide.

tually stimulated ammonium uptake in three experiments (Fig. 4). In the other three experiments, cycloheximide caused a significant inhibition (36–98%) of ammonium uptake. The effect of the inhibitors indicates that both procaryotes and eucaryotes are utilizing ammonium.

Both chloramphenicol and cycloheximide contained some ammonium as a contaminant. Less than 0.1 μM ammonium was introduced as a contaminant for the Gulf Stream incubations; however for the Sapelo experiments the addition of higher concentrations of the inhibitors was necessary and resulted in 0.86 μM increases in ammonium for chloramphenicol and 0.20 for cycloheximide. For the Sapelo experiments, chloramphenicol caused a further net increase in ammonium both before and after tracer addition, while cycloheximide always caused a net decrease (Fig. 5 and Table 2). These changes are discussed below with the ammonium regeneration results. Ammonium uptake rates were corrected for the initial changes in the specific activity of the ammonium in all inhibitor experiments.

Growth experiments—In order to examine exclusive heterotrophic utilization of ammonium, we measured the effect of nitrogen additions on net growth of heterotrophic bacteria in water samples from the Gulf Stream. The addition of glucose plus ammonium (100 nM) stimulated net heterotrophic bacterial growth after a 4-h incubation. The net growth rate observed with the addition of glucose plus ammonium (0.07 ± 0.02 h^{-1}) was significantly higher than the rate with an amino acid mixture (100 nM total) added (0.03 ± 0.01 h^{-1}) and the rate of net bacterial growth in the control (0.001 ± 0.004). Since the addition of ammonium or glucose alone had no significant effect on bacterial growth (Fig. 6), both carbon and nitrogen were needed to stimulate net growth of heterotrophic bacteria. Furthermore, the lack of a significant effect by ammonium alone indicates that the stimulation by glucose plus ammonium was not due to stimulation of phytoplankton production.

Regeneration of ammonium—Regeneration of ammonium was measured during some ammonium and amino acid uptake

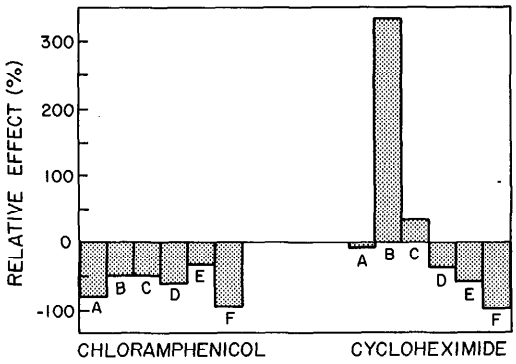

Fig. 4. As Fig. 3, but on ammonium uptake. C—27 August; all Sapelo Island experiments were with water collected at high tide.

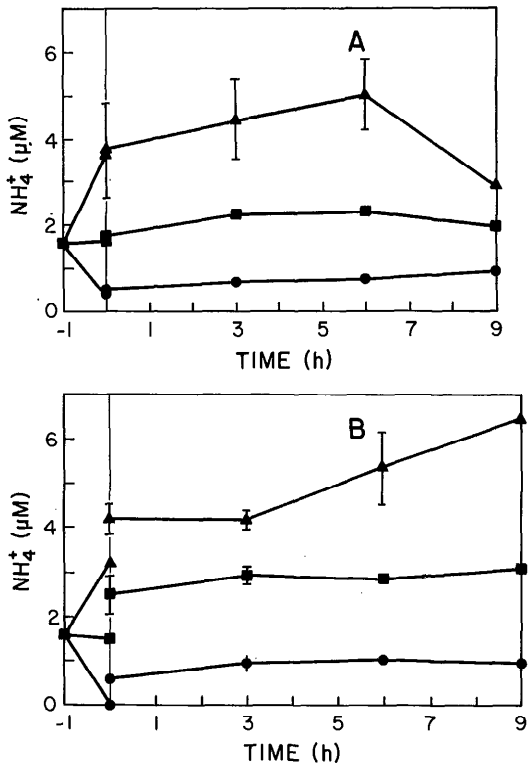

Fig. 5. Changes in ammonium concentration during inhibitor experiment for amino acid uptake (A) and ammonium uptake (B). Results from experiment at Sapelo Island, 20 October. Time interval to the left of zero represents incubation with inhibitor before tracer addition. At time zero, the ^{15}N-labeled substrates were added. Symbols as in Fig. 2.

experiments by the isotope dilution technique. Rates of regeneration were low (0–0.04 μmol liter^{-1} h^{-1}) in the Gulf Stream, but roughly equivalent to uptake rates (Table 3). Regeneration of ammonium was usually an order of magnitude higher in the Sapelo Island water and often exceeded ammonium uptake rates (Table 3).

Attempts to measure ammonium regeneration during the inhibitor experiments failed due to interference of the inhibitors with the organic extraction procedure used for isotope dilution experiments. Nonetheless, we have minimum estimates of rates of ammonium production from changes in ammonium concentrations. Chloramphenicol usually caused a net increase in ammonium concentration while cycloheximide caused a large initial decrease in ammonium before tracer addition and smaller decreases afterward (Fig. 5, Table 2). The rates of ammonium production in the chloramphenicol treatments were similar to the rates of ammonium regeneration measured in the controls by the isotope dilution technique. In three of four experiments, cycloheximide caused a net decrease in ammonium during the incubation period when a net increase occurred in the controls

Table 2. Changes in ammonium concentrations (μmol liter^{-1} h^{-1}) during incubations with inhibitors.

	Initial $[NH_4^+]$ (μM)	Change before tracer addition			Change after tracer addition		
		Control	Chloramphenicol	Cycloheximide	Control	Chloramphenicol	Cycloheximide
27 Aug	2.74	0.240	−0.700	−0.130	0.045	−0.075	0.118
29 Aug	1.30	0.424	−0.072	−0.208	−0.210	−0.193	−0.060
18 Oct*	1.12	0.120	0.000	−0.370	−0.008	−0.069	0.157
18 Oct†	1.12	−0.412	−0.452	−0.528	0.117	−0.030.	0.377
20 Oct*	1.58	−0.080	0.820	−1.780	0.025	0.000	0.156
20 Oct†	1.58	0.040	1.290	−1.360	−0.047	0.037	0.202

* Ammonium incubation.
† Amino acid incubation.

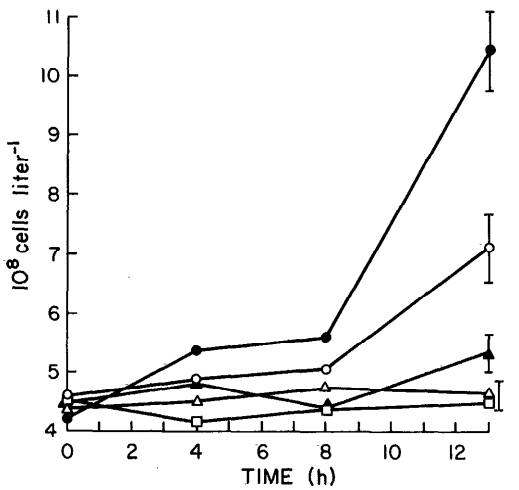

Fig. 6. Bacterial growth in Gulf Stream water samples with and without organic and inorganic additions. The concentration of the compounds added was 100 nM. Growth rates were calculated from the change in bacterial numbers over the entire interval (13 h). Growth rates and standard errors are: control (□) 0.001±0.004; NH_4^+ (△) 0.004±0.001; glucose (▲) 0.010±0.010; amino acids (○) 0.030±0.020; glucose + NH_4^+ (●) 0.070±0.020 h^{-1}. Bars—SE of the mean of replicate counts for the final samples.

(Table 2). These results suggest that eucaryotes were responsible for ammonium regeneration.

We did not detect a release of labeled ammonium from [^{15}N]amino acids. In fact, the apparent dilution of the initial [^{15}N]ammonium in the amino acid mixture (~7.0 atom% ^{15}N) was less than that occurring during the ammonium incubations (Table 3). This result suggests that amino acid nitrogen is assimilated directly without significant regeneration of ammonium nitrogen.

Discussion

In a review of heterotrophic utilization of nitrogen, Billen (1984) concluded that bacteria primarily use amino acids as a nitrogen source while phytoplankton primarily use ammonium, nitrate, and urea. Laboratory studies have shown that both groups of organisms can use either organic or inorganic forms of nitrogen as their sole nitrogen source (Brown 1980; Wheeler et al. 1974). Our major interest from an ecological viewpoint was to determine the major sources

Table 3. Uptake and regeneration (μmol liter^{-1} h^{-1}) of ammonium during incubations with [^{15}N]ammonium and [^{15}N]amino acids (AA). May experiments are from the Gulf Stream; all other experiments are from Sapelo Island. (Dash—no measurements made; nd—not detectable.)

	Initial $[NH_4^+]$ (μM)	Uptake	Regeneration	
			$+^{15}NH_4^+$	+[^{15}N]AA
4 May	0.01	—	0.012	—
5 May	0.08	—	—	nd
7 May	0.13	0.028	0.037	0.028
8 May	0.22	0.004	nd	—
27 Aug	2.93	0.057	0.262	—
20 Oct	2.58	0.109	0.112	0.024
15 Nov*	2.33	0.040	0.169	0.058
15 Nov†	2.33	0.096	nd	—

* For time interval 0–4 h.
† For time interval 4–6 h.

for heterotrophic bacteria in natural populations.

It is difficult to separate the heterotrophic bacterial and phytoplankton components of natural assemblages completely, since there is some overlap in classes of particle size. Furthermore, cyanobacteria are common in marine environments and are functionally part of the phytoplankton component, despite closer structural affinity with heterotrophic bacteria. We tried several methods with nonoverlapping problems in an attempt to separate phototrophic and heterotrophic components of natural plankton assemblages. Each approach has its merits and drawbacks, which are discussed below. None of these approaches alone provides conclusive evidence of exclusively heterotrophic bacterial activity. However, taken together, the results provide a strong indication of the importance and extent of ammonium utilization by heterotrophic bacteria in natural microbial populations.

The size fractionation studies indicate that ammonium and amino acids were the primary nitrogen sources for organisms <1 μm. Neither nitrate nor urea was taken up at significant rates. Nitrate concentrations were very low, and it is not an important nitrogen source in the areas sampled (Haines 1979). Probyn and Painting (1985) also found negligible nitrate uptake by organisms in the <1-μm size fraction. However, in the Antarctic waters sampled in the latter study, nitrate was a significant nitrogen source for

larger phytoplankton. Urea is a significant nitrogen source in some coastal waters, but either is not utilized in the waters we sampled or is utilized only by larger phytoplankton, as has been found in other areas (Billen 1984).

In waters with relatively small cyanobacterial populations, the procaryotic vs. eucaryotic inhibitor experiments provided an alternate means of separating phytoplankton and bacterial activities. Cycloheximide has no effect on bacterial protein synthesis (Vazquez 1974), and only very high concentrations of chloramphenicol have an inhibitory effect on algal protein synthesis (Morris 1966). The decrease in bacterial numbers and growth rate in our chloramphenicol treatments indicates the effectiveness of the inhibitor on heterotrophic growth, as well as the apparent absence of an inhibitory effect on grazing by eucaryotes. Similarly, the increase in bacterial numbers and growth rate in the cycloheximide treatment indicates the effectiveness of cycloheximide in inhibiting eucaryotic bacterivores as well as the absence of an inhibitory effect on bacterial growth. These results validate the effectiveness of the inhibitors for separation of procaryotic and eucaryotic activity in our study.

The strong inhibitory effect of chloramphenicol on uptake of both amino acids and ammonium suggests a major bacterial component in both activities. At times, cycloheximide had a strong inhibitory effect on ammonium uptake, which suggests a significant phytoplankton contribution. Cycloheximide had very little effect on amino acid uptake, which was expected since amino acids in these waters are utilized predominantly by bacteria (Kirchman et al. 1985). If we assume that all of the amino acid uptake is by bacteria and that chloramphenicol has an equivalent effect on amino acid and ammonium uptake by bacteria, we can then use the difference between the inhibitor effects on uptake of the two substrates in each set of parallel incubations to estimate the amount of bacterial ammonium uptake. For the six experiments shown in Figs. 3 and 4, 78±22% of ammonium uptake was by bacteria.

Several lines of evidence suggest that a significant portion of the procaryotic ammonium uptake is due specifically to heterotrophic bacteria. The relative importance of phototrophic picoplankton (as indicated by Chl *a* levels and primary production) tends to be much less in nearshore than oceanic waters (Azam and Hodson 1977; Herbland et al. 1985; Probyn 1985; Fuhrman et al. 1986). Furthermore, we can estimate the amount of nitrogen required for heterotrophic bacterial growth and compare this with measured uptake rates in estuarine waters off Sapelo Island. Heterotrophic bacterial production, as measured by both the thymidine (Fuhrman and Azam 1980) and leucine (Kirchman et al. 1985) techniques, is of the order of 1×10^8 cells liter^{-1} h^{-1} in these waters (Fallon et al. 1986; Kirchman et al. 1985). From this rate of production and the nitrogen composition of bacteria, we estimate that heterotrophic bacteria in the waters off Sapelo Island should be taking up 0.18 μmol N liter^{-1} h^{-1}. Our summed maximum (potential) uptake rates for ammonium plus amino acids were 0.076–0.37 μmol N liter^{-1} h^{-1} for the Sapelo experiments. Thus, either heterotrophic bacteria are responsible for a major portion of the uptake observed in the <1-μm size organisms, or a major bacterial nitrogen source was not included in the compounds studied (ammonium, nitrate, urea, and amino acids). In addition, since ammonium plus glucose stimulated growth of heterotrophic bacteria but had no effect when supplied singly, we conclude that heterotrophic bacteria required and were utilizing both substrates. Finally, we found that both ammonium and amino acid uptake increased as heterotrophic bacterial abundance increased during the cycloheximide experiments.

Clearly, of the nitrogen sources studied, the most important ones in the coastal area off Georgia and the Gulf Stream were ammonium and amino acids. Using the estimates derived above for the percent of ammonium uptake due to bacteria and assuming again that all amino acid uptake is by bacteria, we can calculate the relative contribution of each nitrogen source to meeting bacterial nitrogen requirements for growth. The potential or maximum uptake

Table 4. Utilization of ammonium and amino acids (AA) as nitrogen sources.

	Particulate N (μM)	N source	Concn* (μM)	Time interval (h)	Uptake rate†	N uptake	Bacterial N uptake‡
						$NH_4^+/(NH_4^+ + AA)$	
23 Feb	19.30	Ammonium	1.50	0–4	0.050	0.23	0.19
		Amino acids	1.30	0–1	0.171		
9 May	1.43	Ammonium	1.20	0–6	0.007	0.47	0.41
		Amino acids	1.10	0–4	0.008		
11 May	0.67	Ammonium	1.20	0–6	0.004	0.31	0.26
		Amino acids	1.10	0–6	0.009		
22 Aug	12.80	Ammonium	11.00	0–4	0.116	0.31	0.26
		Amino acids	1.30	0–2	0.257		
23 Aug	8.80	Ammonium	8.25	0–4	0.061	0.56	0.50
		Amino acids	1.30	0–4	0.049		
29 Aug	7.90	Ammonium	2.30	0–6	0.051	0.49	0.43
		Amino acids	1.30	0–6	0.054		
18 Oct	7.06	Ammonium	2.12	0–1	0.059	0.62	0.56
		Amino acids	1.30	0–1	0.037		
20 Oct	5.03	Ammonium	2.58	0–6	0.049	0.66	0.60
		Amino acids	1.30	0–9	0.026		
15 Nov	6.96	Ammonium	2.33	0–2	0.055	0.67	0.61
		Amino acids	1.30	0–2	0.028		

* Concentration used for uptake measurement.
† μmol liter^{-1} h^{-1}.
‡ Estimated assuming that 78.4% of ammonium uptake and all of amino acid uptake is by bacteria; *see discussion.*

rates reported here were used to compare the relative utilization of ammonium and amino acid nitrogen (Table 4). The use of the potential uptake rates overestimates the importance of amino acid nitrogen for both study sites since ambient amino acid concentrations are likely to be less than ambient ammonium concentrations (the difference is most extreme for the Sapelo Island experiments where ambient ammonium was usually >1.0 μM). Nonetheless, the potential uptake rates indicate that ammonium accounted for at least 20–60% of the summed ammonium plus amino acid nitrogen used by bacteria (Table 4).

Various other small- and large-molecular-weight compounds could be utilized as nitrogen sources by heterotrophic bacteria. Alkylamines are present in seawater at nanomolar concentrations (C. Lee pers. comm.) and can be metabolized by bacteria. However, addition of polyamines does not stimulate bacterial growth (Höfle 1984), suggesting that other nitrogen sources are more important. Nucleosides and nucleotides are also taken up and metabolized (e.g. Karl and Winn 1984), but rates of utilization as nitrogen sources have not been determined. Dissolved combined amino acids are more likely to be an important nitrogen source for bacteria. Some low-molecular-weight peptides are taken up and assimilated by bacteria in preference to individual amino acids (Kirchman and Hodson 1984), and oligopeptide concentrations may be a significant fraction of combined amino acids (R. Coffin pers. comm.). Hollibaugh and Azam (1983) reported turnover rates of 3.0% h^{-1} for proteins in coastal water based on degradation rates for labeled protein. Using combined amino acid concentrations reported by Billen (1984) and the 3% h^{-1} turnover rate, we estimate the potential contribution of combined amino acids to be 7–53 nmol liter^{-1} h^{-1} in coastal waters. This rate of utilization is similar to Billen's estimate of free amino acid uptake (5–20 nmol liter^{-1} h^{-1}). Thus, free amino acids and combined amino acids may contribute equally to bacterial nitrogen requirements. However, the total utilization of free and combined amino acid nitrogen is still low compared with bacterial requirements for growth. This further supports the argument for the need of additional nitrogen sources for bacterial growth.

Bacteria can utilize amino acids as sources of both C and N. Schell (1974) found preferential retention of N from glycine, but of C from glutamate during parallel ^{15}N- and ^{14}C-labeling experiments. Zehr et al. (1985) found a similar preferential retention of glutamate C in a freshwater system. Hollibaugh et al. (1980) estimated that amino acid degradation accounted for 60% of the flux into the ammonium pool in enclosed water columns. However, in our study free amino acids were not a significant source of nitrogen for remineralization. The relative availability of C and N sources should have a dominant effect on the preferential retention of C vs. N from compounds that contain both elements. Our estimates of the contribution of dissolved amino acids to bacterial nitrogen requirements suggest that amino acid production rates are low relative to rates of bacterial production. Thus, low ambient concentrations and low production rates of free amino acids in marine waters (Andersson et al. 1985) may force bacteria to utilize ammonium as a nitrogen source. Ammonium was taken up more rapidly than amino acids in our experiments, and ammonium regeneration rates were equivalent to or greater than uptake rates. Both were sufficient to meet the requirements for growth of heterotrophic bacteria.

The effect of the inhibitors on ammonium production suggests that eucaryotes rather than procaryotes are responsible for a major portion of the ammonium regeneration. We do not have an explanation for the large initial decrease of ammonium in the cycloheximide treatment, however the later sustained decreases in ammonium suggest that inhibition of eucaryotic activity results in an inhibition of ammonium regeneration. This agrees with results from culture studies indicating that protozoans rather than bacteria are responsible for a major portion of ammonium regeneration (Goldman et al. 1984). The rates of ammonium production in our chloramphenicol treatments were similar to rates of regeneration in the controls as measured by the isotope dilution technique. This similarity suggests that most of the ammonium accumulation observed in the chloramphenicol treatment is due to inhibition of ammonium utilization and that procaryotes are not responsible for the major portion of ammonium regeneration. Furthermore, the correspondence between chemical and tracer measurements of uptake and regeneration in the inhibitor experiments (Tables 2 and 3) increases confidence in the rate determinations.

Our evidence for the importance of bacterial ammonium utilization has some important implications for the analysis of C and N cycling in the ocean. A significant portion of particulate nitrogen production in the euphotic zone may be through incorporation by nonphototrophic microorganisms. Typical phytoplankton C : N ratios are used as conversion factors for estimating net particulate production rates and vertical flux rates for these two elements (e.g. Knauer and Martin 1981). The C : N ratio for nutrient-sufficient phytoplankton (7.1) is close to the Redfield ratio (6.6) (Goldman et al. 1979). Bacteria, on the other hand, generally have a much lower C : N, about 3–4. Hence, if ammonium accounts for a significant amount of total nitrogen use (the range is 50–90%) and bacteria account for a significant portion of ammonium utilization, then C production rates divided by the assumed C : N of phytoplankton could result in serious underestimates of particulate nitrogen production.

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