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RACER: Microbial uptake and regeneration of ammonium during the austral spring bloom

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Recent studies on the dynamics of heterotrophic marine bacteria have shown the importance of this component of the microbial food web on the uptake of dissolved organic nitrogen and ammonium (Wheeler and Kirchman 1986; Kirchman, Keil, and Wheeler 1989). Since marine bacteria are capable of regenerating ammonium through dissolved organic nitrogen mineralization as well, their activity in the water column would influence the cycling of ammonium and, consequently, other nitrogen substrates. As part of the Research on Antarctic Coastal Ecosystem Rates (RACER) study on nitrogen dynamics, the uptake and regeneration of ammonium by bacterial heterotrophs were examined to determine the extent of their role as consumers or mineralizers of ammonium and to assess their possible impact on nutrient dynamics in this highly productive area.

Surface seawater from the RACER stations (see Huntley et al., *Antarctic Journal*, this issue) was collected by a clean bucket, filtered through a 0.8 micrometer Millipore filter, and dispensed into 2-liter polycarbonate bottles. The 0.8-micrometer filter greatly reduced the concentration of chlorophyll-*a*-containing organisms (approximately 1 percent or less of the total chlorophyll *a* was found in the filtrates) but allowed about 95 percent of the original bacterial population to pass through. The number of protozoans after filtration was less than 3 per milliliter so the filtered seawater was essentially composed of the bacterial population. The number of bacteria of the different waters sampled in the Gerlache Strait ranged from 4.0 to 6.0×10^5 bacteria per milliliter.

To five pairs of bottles were added individually nitrogen-15 labeled glutamate (0.040 micromole of nitrogen per liter), glycine (0.050 micromole of nitrogen per liter), leucine (0.040 micromole of nitrogen per liter), lysine (0.050 micromole of nitrogen per liter), and ammonium (0.2 micromole of nitrogen per liter). One bottle from each pair was filtered through a glass fiber filter (Whatman, GF/F) and the filter and filtrate frozen for initial time measurements. The remaining five bottles were placed in black paper bags and incubated for 24 hours on the ship's deck in a tank continuously supplied by surface seawater. Incubation temperature was generally 0 °C. After incubation, the water from each bottle was filtered as above, and the filter and filtrate frozen for storage.

Chlorophyll *a* concentrations were determined using the method of Holm-Hansen and Riemann (1978). Bacteria and protozoan numbers were estimated by epifluorescence microscopy using 4,6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980). A quadropole mass spectrometer was used to measure the particulate nitrogen and nitrogen isotope ratios according to the method of Ohtsuki, Ino, and Fujii (1983) and Saino (1982). The nitrogen isotope ratio of the ammonium in the filtrate was determined after steam distillation (Keeney and Nelson 1982). Dissolved free amino acid concentrations were determined by reverse phase high-performance liquid chromatography using a modification of the method of Lindroth and Mopper (1979). Ammonium concentrations were measured according to Koroleff (1976) on a Technicon autoanalyzer. Uptake estimates of ammonium and amino acids were calculated according to Dugdale and Goering (1967) and normalized to a 24-hour day. Ammonium regeneration was estimated using the model of Blackburn (1979).

Estimates of ammonium uptake and mineralization from station A301 are shown in the table. An independent estimate of ammonium uptake (u) was derived from the isotope dilution model (value in parenthesis below ammonium uptake value p). The close agreement of these two values shows that the ammonium consumed from the seawater used in the incubation was largely converted into bacterial biomass. This implies that there were no other significant pathways of ammonium metabolism except that of assimilation into particulate organic nitrogen.

According to previous concepts of nitrogen uptake by bacterial heterotrophs, the active assimilation of ammonium implies an insufficiency of organic nitrogen substrates in their environment, as a consequence, there is no regeneration of ammonium (Goldman, Caron, and Dennot 1987). As was seen from the dilution of the nitrogen-15 isotope in the ammonium substrate, however, there was active production of ammonium from the mineralization of dissolved organic nitrogen by the bacterial population. These results show that the microbial

Uptake of labelled substrates and the production of ammonium at station A301, November 13

Parameter (unit)	Nitrogen-15 labelled substrate				
	Glutamate	Glycine	Leucine	Lysine	Ammonium
Ambient concentration[a]	0.113	0.064	0.021	0.016	0.63
Substrate concentration[a,b]	0.153	0.114	0.061	0.066	0.83
Uptake (p)[c,d]	0.046	0.028	0.011	0.008	0.103
					(0.123)[e]
Mineralization[d,f]	0.032	0.008	0.002	0.001	0.123[g]
M index[h]	40.7	22.5	14.6	11.1	—[i]

[a] In micrograms of nitrogen per liter.
[b] Ambient concentration + tracer additions.
[c] Uptake rate (p) according to Dugdale and Goering (1967).
[d] In micromoles of nitrogen per liter per day.
[e] Uptake rate (u) from isotope dilution (Blackburn 1979).
[f] Mineralization rate from isotope dilution (Blackburn 1979).
[g] No change in NH_4^+ concentration thus uptake (u) = mineralization.
[h] M index = Mineralization/(Uptake (p) + Mineralization) × 100.
[i] Not applicable.

populations are assimilating both organic and inorganic nitrogen, at the same time regenerating ammonium.

Experiments using nitrogen-15 labeled amino acids further show the mineralization of such substrates even with the assimilation of both amino acids and ammonium. The table shows the estimated uptake of four amino acids, and their production of ammonium. These estimates have some uncertainty because of possible perturbations caused by large substrate addition and the inability to account for isotope dilution in the substrate. Some interesting patterns of amino acid metabolism, however, can be inferred.

The labeled amino acids used in the experiments were assimilated to various degrees, with glutamate being the largest and lysine the smallest among the four. These results clearly show that the marine bacterial populations are actively taking up ammonium together with dissolved amino acids to fulfill their nitrogen demands. These amino acids were mineralized to varying degrees as well, again with glutamate being the largest and lysine the smallest. A relative index (M index) based on the ratio of the amount of amino acid mineralized and the total amount of amino acid utilized compares the relative production of ammonium from the four amino acids. It was found that in general, glutamate had the highest M index followed by glycine, leucine, and lysine.

These findings on the dynamics of the microbial population in the Gerlache show that this component of the microbial food web plays a significant role in the cycling of available dissolved organic nitrogen and ammonium in the area, and that will influence the growth and abundance of both autotrophic and heterotrophic organisms.

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