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Carbon limitation of ammonium uptake by heterotrophic bacteria in the subarctic Pacific

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Abstract

The purpose of this study was to test the hypothesis that NH_4^+ uptake by heterotrophic bacteria in the subarctic Pacific is C limited. Addition of glucose (0.5–1.0 μM) stimulated NH_4^+ uptake in unfractionated water but had no effect on bacterial abundance. Glucose stimulation of NH_4^+ uptake was even greater in the bacterial size fraction (<0.8 μm). Regeneration of $^{15}NH_4^+$ from added [^{15}N] amino acids was measurable in the bacterial size fraction, but glucose additions prevented net NH_4^+ excretion because of increased uptake of NH_4^+. In the subarctic Pacific, NH_4^+ concentrations range from undetectable (≤0.05 μM) to 0.4 μM, whereas the maximal estimate of monosaccharide concentrations was 0.025 μM. These results indicate that the supply of C compounds like glucose can limit NH_4^+ uptake by heterotrophic bacteria.

Heterotrophic bacteria can process a large fraction of primary production (15–50%; Cole et al. 1988) and can dominate the biomass in the pelagic zone of the open oceans (Fuhrman et al. 1989). Because bacteria can be such a major component of the plankton and because the C : N ratio of bacteria is low (~4–5: Goldman et al. 1987), heterotrophic bacteria are likely to have a substantial impact on nitrogen cycling. In particular, a large fraction of NH_4^+ assimilation in marine waters can be attributed to heterotrophic bacteria (Wheeler and Kirchman 1986). NH_4^+ assimilation by heterotrophic bacteria could affect the phytoplankton, which generally use it in preference to NO_3^- (McCarthy 1981). Even in regions with seasonally or continuously high NO_3^- concentrations, NH_4^+ supplies a large percentage of the N used for phytoplankton growth, e.g. the Antarctic (Koike et al. 1986) and the oceanic subarctic Pacific (Wheeler and Kokkinakis 1990). The regulation of NH_4^+ assimilation by bacteria and potential effects of competition between bacteria and phytoplankton for NH_4^+ are not well understood.

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NH_4^+ assimilation by heterotrophic bacteria could be limited by its concentrations, which are often <0.05 μM in oligotrophic waters (Brzezinski 1988) and are usually <0.5 μM in other oceanic waters. Despite these low concentrations, however, the rate of NH_4^+ regeneration appears to be sufficient to support much of plankton production. Plankton may assimilate NH_4^+ from patches of high concentrations (McCarthy 1981) or simply have very efficient (low K_m) uptake systems for NH_4^+ (Koike et al. 1983). Suttle et al. (1990) found that relative NH_4^+ uptake by heterotrophic bacteria (percent uptake by the small size fraction) increased with decreasing NH_4^+ additions, implying that bacteria outcompete phytoplankton for NH_4^+ at low concentrations. Furthermore, several workers have argued that the high ratio of surface area to volume of bacteria is advantageous for assimilating compounds at low concentrations. Efficient transport systems coupled with high rates of NH_4^+ regeneration could preclude NH_4^+ limitation of NH_4^+ assimilation and growth by heterotrophic bacteria. There is strong evidence in support of this idea for the subarctic Pacific where NH_4^+ concentrations are relatively high (80% of samples >0.05 μM, n = 158; Wheeler and Kokkinakis 1990) and NH_4^+ additions have no effect on rates of bacterial production (Kirchman 1990).

Another possibility is that NH_4^+ assimi-

lation by heterotrophic bacteria is limited by the supply of dissolved organic C (DOC). Although bacteria have internal pools of NH_4^+ (Brown and Stanley 1972), eventually its uptake must be coupled with that of DOC. At low concentrations, NH_4^+ is assimilated via the glutamine synthetase system, which consumes one α-ketoglutarate for every NH_3 assimilated (Gottschalk 1986). The end result is that heterotrophic bacteria need to take up at least four atoms of organic C for every atom of NH_4^+-N assimilated. Although the concentration of DOC readily used by heterotrophic bacteria is known to be very low in oceanic waters, the impact of these low concentrations on NH_4^+ uptake has not been adequately explored. Sherr et al. (1986) found that addition of glucose increased NH_4^+ depletion, but concentrations of both glucose and NH_4^+ were high in their estuarine study.

The purpose of our study was to test the hypothesis that NH_4^+ assimilation by heterotrophic bacteria in the oceanic subarctic Pacific is limited by the supply of organic C. These experiments were conducted during the SUPER project (subarctic Pacific ecosystem research) as part of our effort to understand the role of heterotrophic bacteria in the N cycle. We found that glucose stimulated NH_4^+ depletion in unfractionated water and in the <0.8-μm size fraction, indicating that NH_4^+ assimilation by heterotrophic bacteria was limited by the supply rate of organic C.

Methods and materials

Experiments were conducted in September 1987 and May and August 1988 at Station P (50°N, 145°W). Water from 10 m was collected in 20-liter, Teflon-lined GoFlo bottles with Kevlar line. All experiments were conducted in 20-liter polyethylene carboys incubated on deck at surface seawater temperatures ($\sim$7°C in May, 12°C in August and September). There was one carboy per treatment for each experiment. The effect of glucose on NH_4^+ depletion was observed in five time-course experiments conducted on different days. Some treatments were darkened, while others were left exposed to ambient light.

The basic experiment consisted of measuring NH_4^+ concentrations over time with and without additions of glucose. The glucose additions were 1.0 μM (final concn), with one exception (*see below*). In order to ensure measurable NH_4^+ concentrations over the entire experiment, we added NH_4^+ to all incubations (0.4 μM final concn), except for one experiment (30 September 1987). In that experiment, no NH_4^+ was added and the glucose addition was 0.25 μM. The NH_4^+ additions did not influence how glucose affected NH_4^+ uptake.

Size fractionation—We examined NH_4^+ and amino acid uptake by heterotrophic bacterial assemblages during experiments designed to minimize (if not eliminate) potential bacterivory and excretion of DOC. Water was filtered through 0.8-μm Nuclepore filters (142-mm diam) by gravity and incubated at surface seawater temperatures in the dark. Kirchman et al. (1989) described in more detail the efficacy of both 0.8- and 1.0-μm pore-size filters for separating heterotrophic bacteria from other organisms. Bacterial abundance was measured by epifluorescence microscopy with acridine-orange staining (Parsons et al. 1984).

Nutrient concentrations—NH_4^+ concentrations were measured by standard methods (Parsons et al. 1984) or on a Technicon autoanalyzer. Particulate N was measured with a persulfate digestion of particulate matter (from 500-ml samples) collected on 25-mm Whatman GF/F filters, conversion of NO_3^- to NO_2^- by Cd reduction, and diazo dye formation from NO_2^- (Parsons et al. 1984). Dissolved primary amine concentrations were measured by the *o*-phthaldialdehyde (OPA) method (Parsons et al. 1984) with glycine as the standard. Fluorescence was measured with a Turner fluorometer as described by Kirchman et al. (1989). Fluorescence due to reaction of OPA to NH_4^+ was 3% of glycine fluorescence on a molar basis and was subtracted from total fluorescence with independently measured concentrations of NH_4^+ (*see above*). Kirchman et al. (1989) presented evidence that the primary amine measurement is almost entirely due to dissolved free amino acids.

We attempted to measure free carbohydrate concentrations with the MBTH spec-

trophotometric assay as described by Johnson et al. (1981). Glucose was used as the standard carbohydrate. Glucose concentrations were also estimated by the Wright-Hobbie approach (Wright and Hobbie 1966). Uptake of D-[6-^{3}H]glucose (final concn, 0.5–1 nM) was measured in the presence of the following (final) concentrations of unlabeled glucose: 0, 5, 10, 30, 50, and 100 nM. Each concentration was tested in duplicate. After 1–2-h incubations, uptake was stopped by filtration through 0.45-μm Millipore filters, which were rinsed twice with cold-filtered seawater and radioassayed. The [^{3}H]glucose was purchased from New England Nuclear, whereas all unlabeled compounds were from Sigma.

^{15}N analysis—We examined the effect of glucose on regeneration of NH_4^+ during uptake of a [^{15}N] amino acid mixture (MSD Isotopes) added to a final concentration of 0.2 μM. Uptake was measured by collecting particulate material on Whatman GF/F filters. NH_4^+ for ^{15}N analysis was extracted by converting it to indophenol which was recovered by a solid-phase extraction method (Kirchman et al. 1989), but with the following modifications: the indophenol solution was acidified with phosphoric acid, columns were rinsed with 2.5% methanol, and samples were eluted with 2 ml of acetone. The acetone-indophenol was dried onto Whatman 47-mm GF/F filters and analyzed for isotopic composition.

Particulate material or indophenol collected on Whatman GF/F filters was converted to dinitrogen gas prepared by a dry Dumas combustion with modifications described by Wheeler and Kirchman (1986). Samples were then analyzed for isotopic enrichment by emission spectrometry (Jasco). The emission spectrometer was calibrated with Jasco prepared standards, and the atom% enrichment of each sample was determined from the mean of three peak scans.

Results

Effect of glucose additions on NH_4^+ depletion—To test for possible C limitation of NH_4^+ uptake by heterotrophic bacteria, we added glucose to water samples from the subarctic Pacific. In September 1987, glucose additions of 1.0 μM significantly increased the depletion of NH_4^+ compared to control incubations (Figs. 1 and 2). In a dark incubation, NH_4^+ concentrations in unamended control samples remained above 0.4 μM for 55 h, while glucose additions caused NH_4^+ to decrease to unmeasurable levels (<0.05 μM) after 30 h (Fig. 1A). In a similar experiment but with surface light, NH_4^+ concentrations in both control and experimental containers decreased immediately (Fig. 2A). NH_4^+ depletion, however, was twofold faster with the glucose addition compared to the control (Table 1; Fig. 2A). Because heterotrophic bacteria dominate assimilation of dissolved organic matter (Azam and Hodson 1977), these results suggest that the rate of NH_4^+ uptake by het-

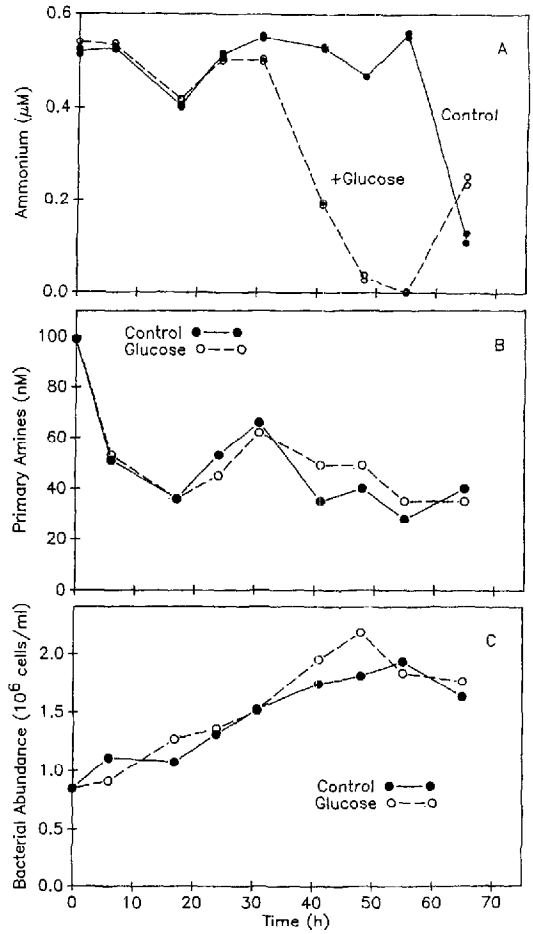

Fig. 1. A. Effect of glucose on NH_4^+ concentrations in unfractionated water. B. Primary amine concentrations. C. Bacterial abundance. Incubation was in the dark on 29 September 1987.

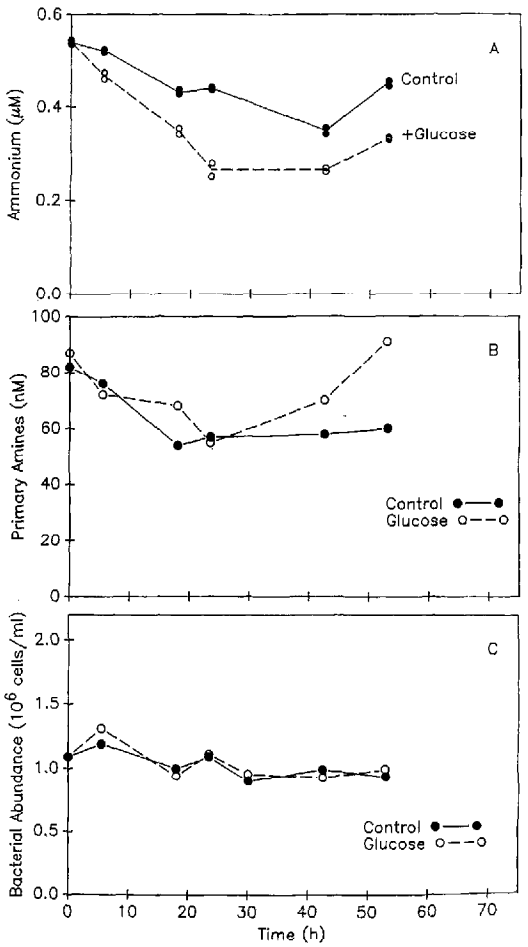

Fig. 2. As Fig. 1, but incubation was in surface light on 1 October 1987.

erotrophic bacteria was limited by the supply of C.

There was no consistent effect of glucose additions on net uptake of dissolved primary amines, which was dominated by dissolved free amino acids (DFAA; Kirchman et al. 1989). In the light experiment, concentrations of primary amines were higher at the end of experiments in samples with added glucose compared to controls (Fig. 2B), but this effect was not observed in the dark incubation (Fig. 1B). Other experiments showed no effect of glucose additions on DFAA uptake (*see below*).

Addition of glucose did not affect bacterial abundance in experiments with unfractionated water (Figs. 1C and 2C). In the dark

Table 1. Effect of glucose (1.0 μM, final concn) on the depletion rate (±SE) of NH_4^+. Glucose was not added to control incubations.

		NH_4^+ depletion (nM h^{-1})		
	Condition	Control	Glucose	*n*
1 Sep 87	Dark*	0.75±2.7	21.3±4.0	4
1 Oct 87	Light	5.0±1.0	11.4±0.6	4
27 May 88	Total†	5.0±3.9	7.1±1.2	6
27 May 88	Total	6.7±1.4	12.9±1.7	3‡
27 May 88	<0.8 μm	6.7±0.8	24.6±7.0	4

* In the dark experiment both the control and glucose treatment were totally covered; in the light experiment, carboys were exposed to surface sunlight. Time-course data given in Figs. 1 and 2.
† Total was unfractionated water, whereas "<0.8 μm" refers to the filtrate of a 0.8-μm Nucleopore filter. Total was incubated in 30% surface sunlight; the <0.8-μm size fraction was incubated in the dark. Time-course data given in Fig. 3.
‡ Last three time points.

incubation, bacterial abundance in both the control and glucose containers did increase (Fig. 1C). The net growth rates of the bacteria were 0.015±0.002 h^{-1} for the control and 0.020±0.001 h^{-1} (±SE; $n = 7$) for the glucose addition, which are not significantly different (Student's *t*-test; $P > 0.05$). The large net increase in bacteria observed in this experiment was very unusual, however, and was not repeated in the many other experiments that we have conducted with unfractionated water. For example, in the light incubation, bacterial abundance did not change substantially, and there was no difference between the control and glucose treatment (Fig. 2C).

Glucose additions to the bacterial size fraction—In order to examine NH_4^+ use specifically by the heterotrophic bacterial assemblage, we removed organisms >0.8 μm by filtration. Elsewhere we have shown that contamination of the <0.8-μm size fraction by autotrophic picoplankton was minimal because this size fraction contained 0.9±0.8% of total Chl (Kirchman et al. 1989) and 9.2±3.3% of $^{14}CO_2$ fixation (Kirchman unpubl. data). Glucose added (1.0 μM) to unfractionated water increased NH_4^+ depletion rates nearly twofold (Fig. 3A; Table 1), as shown above, but the effect was much greater (3.7-fold; Table 1) when glucose was added to the <0.8-μm size fraction (Fig. 3B). These results provide further evidence that glucose specifically stimulated NH_4^+ uptake by heterotrophic bacteria.

Effect of glucose on NH_4^+ regeneration by

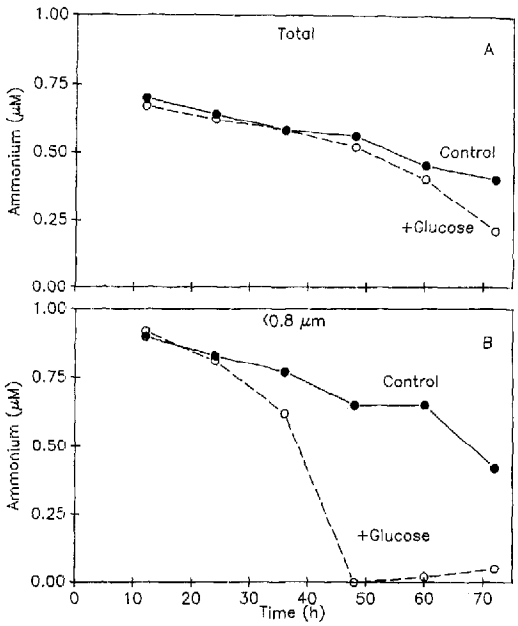

Fig. 3. A. Depletion of NH_4^+ with and without glucose. Unfractionated water, incubated at 30% of surface sunlight. B. Filtrate from 0.8-μm Nuclepore filter, incubated in the dark. Experiment was on 27 May 1988. Concentrations were measured with an autoanalyzer with a precision of 0.05 μM.

heterotrophic bacteria—We tested the effect of glucose on $^{15}NH_4^+$ regeneration from [^{15}N] amino acids in <0.8-μm size fractions. In the unamended control, NH_4^+ concentrations first decreased, then increased from 0.09 to 0.19 μM (Fig. 4A). The $^{15}NH_4^+$ released in the control during this same period was 0.05 μM (data not shown), so about half of the observed NH_4^+ regeneration was from the added [^{15}N] amino acids (*see also* Kirchman et al. 1989). In contrast, the glucose addition caused a continuous decrease in NH_4^+ concentration; there was no period of net NH_4^+ accumulation when glucose was added (Fig. 4A). Because the glucose addition increased the concentration of ^{15}N in particulate matter over that in the control (Fig. 4B), we think that glucose did not inhibit NH_4^+ regeneration directly, but stimulated its uptake and prevented its accumulation.

The amount of ^{15}N recovered in the particulate material in the glucose treatment was less than the amount of $^{15}NH_4^+$ released in the control (Fig. 4). We attribute this difference to inefficient trapping of the particulate matter by the Whatman GF/F filters (Kirchman et al. 1989).

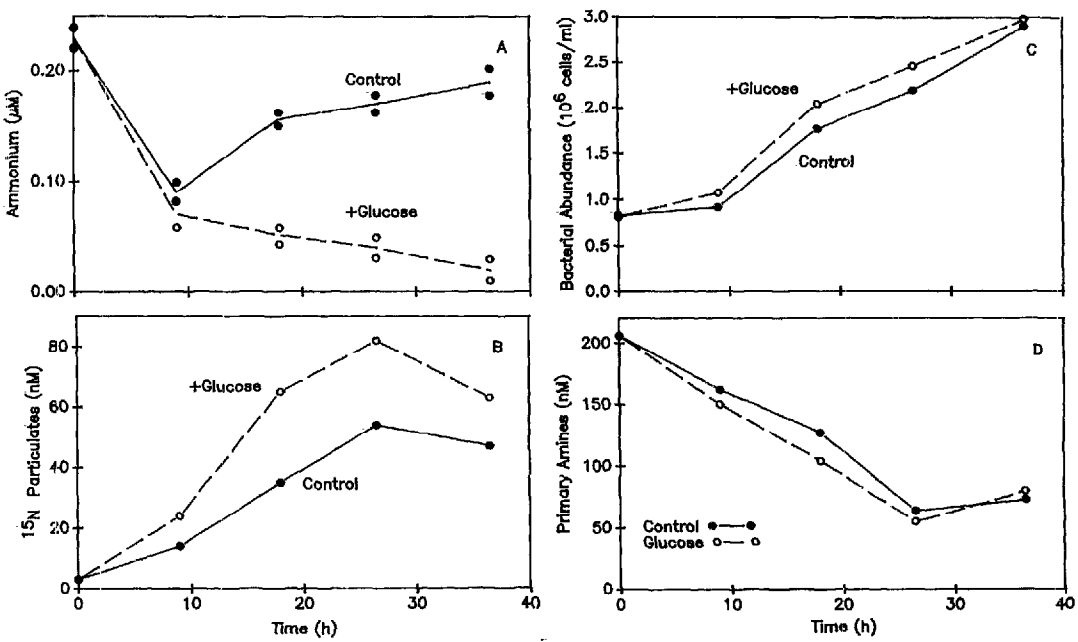

Fig. 4. Effect of glucose on regeneration of NH_4^+ from [^{15}N] amino acids in 0.8-μm filtrate. A. NH_4^+ concentrations. B. ^{15}N concentration in particulate matter. C. Bacterial abundance. D. Primary amine concentrations. Experiment was on 30 September 1987.

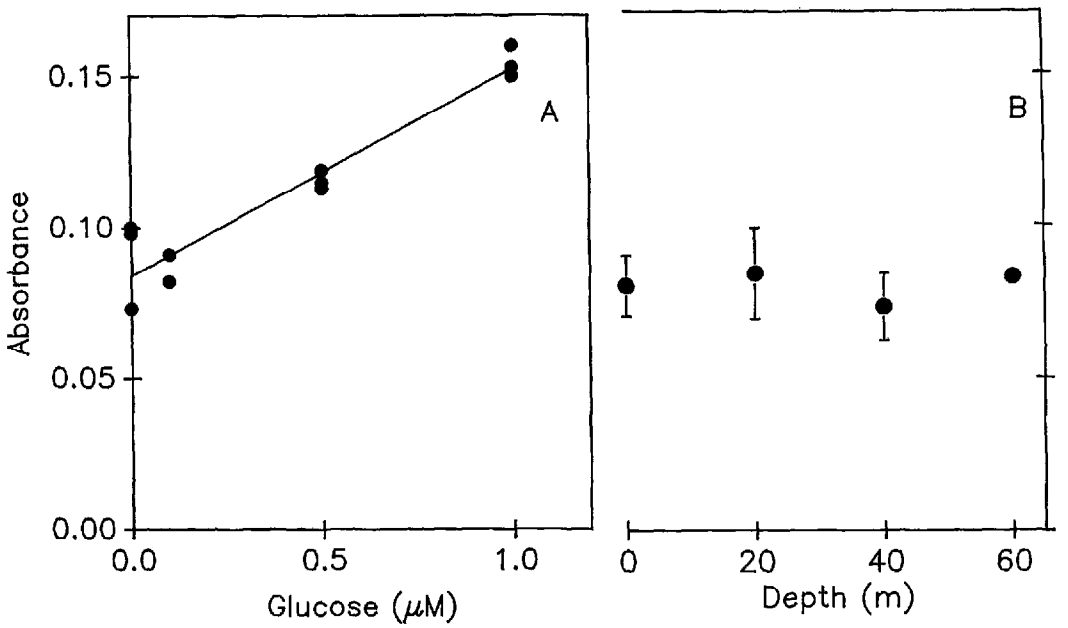

Fig. 5. MBTH assay for carbohydrates. A. Standard curve with glucose. B. Absorbance from MBTH assay on samples from various depths on 20 August 1988.

Bacterial abundance increased in both the control and glucose treatment due to the lack of grazing (Fig. 4C). Bacterial abundance was consistently higher in the glucose treatment, but the difference was small (0.1–0.2 × 10^6 cells ml^{-1}) and within measurement errors (Fig. 4C). Primary amine concentrations decreased at the same rate in both the glucose treatment and the unamended control (Fig. 4D), suggesting that glucose did not affect DFAA uptake.

Glucose concentrations in the subarctic Pacific—Free carbohydrate concentrations were unmeasurable by the MBTH method (Johnson et al. 1981). Values for samples from 0 to 60 m were similar to the blank (Fig. 5). The detection limit of the MBTH assay in our hands was ~0.2 μM, near that reported previously (Johnson et al. 1981). We tried the MBTH assay three times in May and twice in August. Carbohydrate concentrations were unmeasurable (<0.2 μM) each time.

To estimate glucose concentrations, we measured the kinetic parameters of glucose uptake by the Wright-Hobbie approach (Wright and Hobbie 1966). This approach can be used to estimate the sum of the half-saturation constant (K) and the in situ glucose concentration (S), which is thus an upper limit to the actual glucose concentration. The (K + S) values indicate that glucose concentrations did not exceed 25 nM in May and August (Table 2). The mean (K + S) was ~10 nM (Table 2).

Discussion

Our main finding was that adding glucose stimulates NH_4^+ uptake in samples from the subarctic Pacific. The additions affected

Table 2. Wright-Hobbie kinetics (±SE) of glucose uptake in the subarctic Pacific. Wright-Hobbie kinetics include the maximum uptake velocity (V_{max}), the turnover time of glucose, and the sum of the half-saturation constant (K) and the in situ concentration of glucose (S). Kinetics were determined with six glucose concentrations, each tested in duplicate.

1988	V_{max} (pM h^{-1})	Turnover time (h)	$K + S$ (nM)
14 May	34±9	267±316	9±11
18 May	31±2	434±216	13±7
23 May	28±6	120±316	3±9
28 May	21±3	1,601±243	5±1
20 Aug*	122±12	201±97	25±12
23 Aug*	49±6	68±24	3±1

* Measurements were at Station R (53°N, 145°W); all others were at Station P (50°N, 145°W).

mainly the heterotrophic bacteria in our experiments, since glucose stimulation of NH_4^+ uptake was greatest for the <0.8-μm size fraction which contains predominantly heterotrophic bacteria (Kirchman et al. 1989). Also, previous work has shown that bacteria are the primary sinks for dissolved organic compounds (Azam and Hodson 1977), even at high glucose concentrations (Parsons et al. 1981). In experiments with unfractionated water, NH_4^+ regeneration by >0.8-μm organisms (probably microflagellates and microzooplankton) could partially mask the effect of the glucose additions and helps explain why glucose enhancement of NH_4^+ depletion was greater for the <0.8-μm size fraction than unfractionated waters. These results indicate that the rate of NH_4^+ uptake by heterotrophic bacteria in the subarctic Pacific was limited by the supply of C.

Heterotrophic bacteria in the subarctic Pacific use DFAA in preference to NH_4^+, and DFAA additions inhibit NH_4^+ uptake (Kirchman et al. 1989). In ^{15}N studies with the <0.8-μm size fraction, Kirchman et al. (1989) showed that NH_4^+ can be regenerated during bacterial use of DFAA; the present study demonstrated that glucose can prevent this net NH_4^+ regeneration by stimulating NH_4^+ uptake (Fig. 4). The stimulation of NH_4^+ uptake by glucose and its inhibition by amino acids are consistent with consideration of C and N mass balance (Billen 1984; Goldman et al. 1987).

Our goal was to examine qualitatively the processes that affect NH_4^+ uptake by heterotrophic bacteria. Consequently, the glucose and NH_4^+ additions were chosen to ensure measurable changes in NH_4^+ concentrations. The advantages of this approach were that it was simple and that it allowed us to examine changes in *net* uptake rates. Our use of long incubation periods and elevated concentrations could conceivably compromise the application of our results to assessing in situ NH_4^+ cycling in the subarctic Pacific. We used, however, 20-liter carboys to minimize wall effects, and in some experiments the stimulation of NH_4^+ uptake by glucose was measurable within a day. Furthermore, our incubation times were short relative to mean bacterial generation times, which were about 1 week or longer in these waters (Kirchman unpubl. data).

We were interested in determining whether the stimulation of NH_4^+ uptake by glucose was reasonable and consistent with previous work on glucose utilization. This question can be addressed with results from experiments with the <0.8-μm size fraction because sources of organic compounds (e.g. phytoplankton) were removed. In Fig. 4, for example, there was a 0.19 μM difference in NH_4^+ concentrations between the control and the incubation with 250 nM glucose added. To balance that net N uptake into biomass requires a net C assimilation by bacteria of between 147 and 185 nM glucose, assuming that the C:N ratio (by weight) of bacteria is between 4 and 5 (Goldman et al. 1987). Thus, the efficiency of glucose uptake would have to be between 59 and 74%. These calculated efficiencies are not significantly different from the assimilation efficiency measured with radiolabeled glucose (e.g. Parsons et al. 1981).

Addition of glucose did not change bacterial abundance in our experiments with unfractionated water. Fuhrman et al. (1988) found that glucose increased bacterial growth in grazer-free "seawater cultures." We suspect that glucose additions did not increase bacterial abundance in unfractionated water in our experiments because grazing balances any change in bacterial abundance. In experiments with large "CEEs" bags in Saanich Inlet, Parsons et al. (1981) found that glucose additions caused as much as a two-fold increase in bacterial biomass, but they added at least 10-fold more glucose than we did and the incubation time was nearly a month. Perhaps the grazing response is limited so that very high organic additions increase steady state levels of bacterial abundance as well as bacterial production. There is a positive correlation between bacterial abundance and phytoplankton production (Cole et al. 1988), presumably because of changes in the supply rates of dissolved organic matter.

The stimulation of NH_4^+ assimilation by glucose additions suggests that bacterial production was C limited in these waters. Other experiments, however, indicate that DFAA additions have a much greater effect

on bacterial production than glucose additions (Kirchman 1990) and that it is misleading to state that bacteria are "C" or "N" limited. In fact, the greater stimulation by DFAA than by glucose plus NH_4^+ suggests that heterotrophic bacterial production in the subarctic Pacific was energy limited (Kirchman 1990).

Another line of evidence that supports the hypothesis that NH_4^+ assimilation by bacteria was C limited is based on the concentrations of glucose vs. NH_4^+ in the subarctic Pacific. Our upper bound on glucose concentration was 25 nM, and the actual concentration is probably <10 nM. Glucose is the most abundant monosaccharide in seawater (Mopper et al. 1980), although other types of organic compounds may also be important. In contrast to the apparent low carbohydrate concentrations in the subarctic Pacific, NH_4^+ concentrations can be relatively high, varying between undetectable and 0.4 μM (80% of samples were >0.05 μM; n = 158) (Wheeler and Kokkinakis 1990). NO_3^- concentrations never are <7 μM (Wheeler and Kokkinakis 1990). So at the ecosystem level, the subarctic Pacific cannot be considered N limited. This comparison of concentrations suggests that NH_4^+ uptake by heterotrophic bacteria is limited by the supply of organic C.

In N-limited systems, an increase in NH_4^+ uptake by bacteria, caused by increased carbohydrate supply, could inhibit primary production. Parsons et al. (1981) found that very high glucose additions did inhibit primary production by twofold to threefold and caused a threefold decrease in chlorophyll. They hypothesized that bacteria outcompeted the phytoplankton for NO_3^-. To date, however, there is no published evidence of NO_3^- uptake by heterotrophic bacteria in the euphotic zone (Wheeler and Kirchman 1986). What seems more likely is that the glucose additions of Parsons et al. (1981) allowed bacteria to outcompete phytoplankton for NH_4^+. N was the limiting nutrient in the experiments of Parsons et al. because both NH_4^+ and NO_3^- were unmeasurable (<0.05 μM) whereas PO_4 and silicate were >0.5 μM and >10 μM, respectively. The negative impact on primary production by heterotrophic uptake of NH_4^+ would have to be brief in the open ocean because bacteria ultimately depend on phytoplankton for organic C.

In environments with high NO_3^- concentrations, such as the subarctic Pacific, primary production may not be affected by NH_4^+ assimilation by bacteria because phytoplankton can use NO_3^-. A switch from NH_4^+ to NO_3^- utilization caused by increased inputs of organic C, however, could still have system-level effects. Parsons et al. (1981) found that the abundance of microflagellates in glucose-amended containers was lower than in controls while the abundance of large phytoplankton was higher. One explanation is that increased use of NH_4^+ by heterotrophic bacteria allowed large phytoplankton that use NO_3^- to outcompete small phytoplankton that use NH_4^+. In support of this idea, there is circumstantial evidence that large phytoplankton use disproportionately more NO_3^- than small phytoplankton. NH_4^+ uptake is predominantly by the small size fractions (Harrison and Wood 1988), and there is a correlation between the abundance of large phytoplankton species and high NO_3^- concentrations (Malone 1980). Because of the importance of NH_4^+ for phytoplankton growth and community structure, its uptake by heterotrophic bacteria needs to be considered for understanding both N-limited and N-sufficient ecosystems.

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