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Importance of heterotrophic bacterial assimilation of ammonium and nitrate in the Barents Sea during summer

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Abstract

In a transect across the Barents Sea into the marginal ice zone (MIZ), five 24-h experimental stations were visited, and uptake rates of NH_4^+ and NO_3^- by bacteria were measured along with their contribution to total dissolved inorganic nitrogen (DIN) assimilation. The percent bacterial DIN uptake of total DIN uptake increased substantially from 10% in open Atlantic waters to 40% in the MIZ. The percentage of DIN that accounted for total bacterial nitrogen production also increased from south to north across the transect. On average, at each of the five 24-h stations, bacteria accounted for 16–40% of the total NO_3^- uptake and 12–40% of the total NH_4^+ uptake. As a function of depth, bacteria accounted for 17%, 23%, and 26% of the total NH_4^+ assimilation and 17%, 37%, and 36% of the total NO_3^- assimilation at 5, 30, and 80 m, respectively. Bacteria accounted for a higher percentage of total NO_3^- uptake compared to total NH_4^+ uptake in 12 out of 15 samples. Bacterial productivity explains a substantial amount of the variability associated with bacterial DIN uptake, but the relationship between bacterial production and bacterial DIN uptake is best explained when the data from the open Atlantic water stations are grouped separately from the MIZ stations. The percentage of DIN that accounts for bacterial N production is approximately four-fold higher in 24 h MIZ stations compared with open Atlantic stations. This suggests that bacteria play a larger role in NO_3^- utilization, particularly in the MIZ, than previously hypothesized and that bacterial uptake of NO_3^- should not be ignored in estimates of new production. Understanding processes that affect autotrophic based new production, such as heterotrophic bacterial utilization of NO_3^-, in polar oceans is of particular significance because of the role these regions may play in sequestering CO_2.

Keywords: Nitrogen cycle; Nitrate; Arctic; New production; Heterotrophic bacteria; Microbial loop; ^{15}N

1. Introduction

The primary role of heterotrophic bacteria is classically considered to be the decomposition and mineralization of dissolved and particulate organic

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nitrogen (DON and PON) (Pomeroy, 1974). The role of heterotrophic bacteria in the consumption of a significant fraction of the total NO_3^- or NH_4^+ flux in marine environments is seldom considered in pelagic carbon and nitrogen cycling models (Fasham et al., 1990; Bissett et al., 1999; Haupt et al., 1999).

Several studies have documented high rates of NO_3^- utilization by bacteria (Parker et al., 1975; Parsons et al., 1980; Horrigan et al., 1988; Kirchman et al., 1991), but only a few have attempted to measure bacterial inorganic nitrogen utilization directly by separating the bacterial size fraction after incubation in the presence ^{15}N tracers (Harrison and Wood, 1988; Probyn, 1990; Kirchman, 1994; Kirchman and Wheeler, 1998). One such study in the Barents Sea estimated that 53% and 48% of NH_4^+ and NO_3^-, respectively, was attributable to organisms <0.8 μm (Kristiansen et al., 1994). A recent review of marine studies that have measured NH_4^+ and NO_3^- uptake by heterotrophic bacteria reports that bacteria account for 42% and 16% of NH_4^+ and NO_3^- uptake, respectively (Kirchman, 2000).

Significant heterotrophic bacterial utilization of dissolved inorganic nitrogen (DIN) would have profound effects on the fluxes of N and C in the water column (Kirchman et al., 1992; Kirchman, 1994). In particular, NO_3^- uptake by heterotrophic bacteria is important because of its potential impact on estimates of new production and on the relationship between new production and carbon export out of the euphotic zone (Legendre and Grosselin, 1989; Kirchman, 2000). The magnitude of the vertical flux is thought to be determined by new production, and biogenic matter vertical flux dynamics in the Barents Sea has been investigated previously (Wassmann et al., 1990, 1993; Andreassen et al., 1996, 1999).

The traditional view of the marine nitrogen cycle holds that the downward flux of particulate nitrogen would have to equal NO_3^- uptake at steady state regardless of which group of microbes is using the NO_3^- (Bronk et al., 1994; Kirchman, 2000). What is not appreciated or understood, however, is the extent to which bacteria might cause the system to deviate from steady state or to at least complicate this picture by substantial utilization of NO_3^- in the euphotic zone. NO_3^- uptake by bacteria could potentially uncouple new and export production by directing more carbon into the microbial loop where more trophic transfers are required to produce large amounts of sinking particles (Kirchman, 2000).

Understanding the variability and magnitude of new production in the polar regions is of particular interest because of the role these regions may play in sequestering CO_2 (Sarmiento and Toggweiler, 1984; Walsh, 1989; Erickson et al., 1990). Studies in the Northern Bering Sea, the Chukchi Sea, and the Barents Sea have measured some of the highest primary production rates in the world's oceans (Sambrotto et al., 1984; Olsson et al., 1999; Luchetta et al., 2000). Although there is considerable disagreement over the role of bacteria in these cold waters (Pomeroy and Deibel, 1986; Thingstad and Martinussen, 1991), Arctic bacterioplankton appear to be important consumers of dissolved organic matter (Cota et al., 1990; Rivkin et al., 1996; Steward et al., 1996), but their contribution to total DIN utilization remains unclear.

Kirchman et al. (1989) found that about 50% of heterotrophic bacteria from subarctic waters will pass through GF/F filters (nominal pore size 0.7 μm). The purpose of this study was to more accurately estimate bacterial and eukaryotic uptake by using 0.8 and 0.2 μm silver membrane filters (see Materials and methods section). The rate and amount of DIN utilized by bacterioplankton was determined along a transect that began in the Southern Barenst Sea, crossed the Polar Front, and ended in the marginal ice zone (MIZ) dominated by Arctic water.

2. Materials and methods

Experiments were conducted during a cruise (ALV-3) aboard the R/V Jan Mayen in July 1999. Five stations (Fig. 1) were sampled over a 24 h period along a transect beginning in 80% ice cover on July 1 (78°13.67′ N, 34°23.02′ E) and ending in the southern Barents Sea on July 9 (73°47.99′ N, 31°44.10′ E). The stations are referred to as Stns. I–V from south to north. Water samples were collected using 10 L Niskin bottles.

2.1. Uptake of inorganic nitrogen experiments

At each station, ^{15}N tracer techniques were used to estimate uptake rates of NO_3^- and NH_4^+ into the >0.8 and <0.8 μm size-fractions at 5 m, 30 m and 80 m.

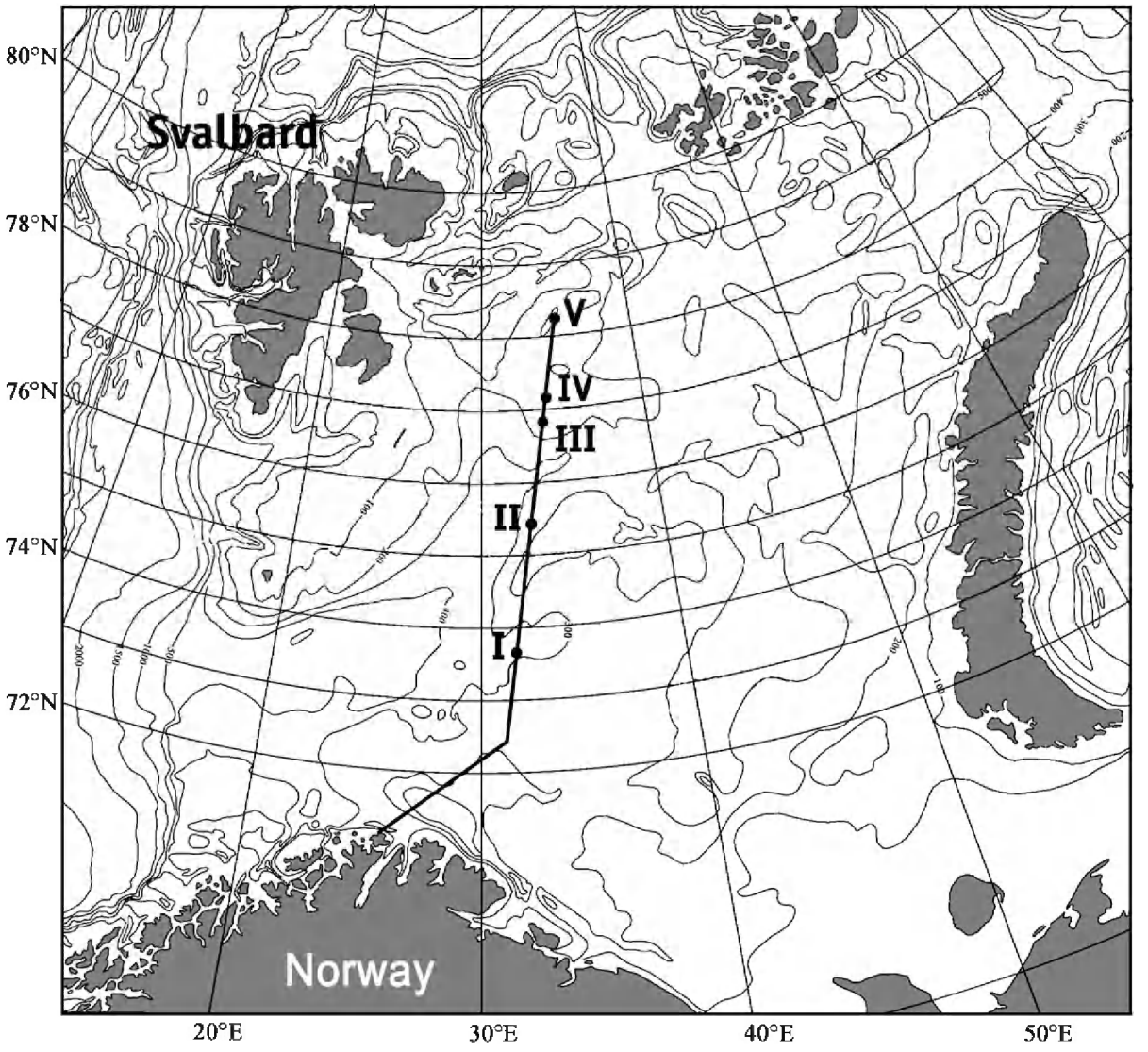

Fig. 1. A map of the cruise track of the R/V Jan Mayen and locations of the five 24-h experimental stations (I–V) in July 1999.

Nutrient concentrations from the literature were used to estimate ^{15}N additions that would yield approximately 10% enrichment over ambient levels. For NO_3^- and NH_4^+, the enrichments were 0.01, 0.1, and 0.6 μM and 0.01, 0.01, and 1 μM at 5, 30, and 80 m, respectively. For the most part, target enrichment levels were achieved to within 1.0 μM. At no time, however, did the ^{15}N additions result in enrichments of over 10% of ambient NH_4^+ or NO_3^- concentrations.

Incubations were done in 1 liter polycarbonate bottles in on-deck flow-through incubators under simulated in situ light and temperature conditions. Whole water (unfractionated) and fractionated treatments for NO_3^- and NH_4^+ uptake experiments were prepared from samples collected at each depth. The fractionated treatment consisted of water that was gently filtered (<5 in. Hg) through a 1.0-μm filter prior to incubation. Incubations lasted 3 h in order to minimize the risk of substrate depletion.

The incubations were terminated by passing the whole-water samples through a 0.8-μm silver filter (Osmonics, Minnetonka, MN) to collect the ^{15}N labeled particulate nitrogen in the >0.8-μm size class. The filtrate from these samples was collected and subsequently passed through a 0.2-μm silver filter to measure the ^{15}N labeled particulate nitrogen in the bacterial size class. In the case of the pre-incubation 1.0 μm filtered treatments, the incubation was terminated by passing the sample through a 0.2-μm silver filter. The purpose of measuring ^{15}N uptake in filtered and whole water treatments was to compare bacterial uptake in the presence of grazing and nutrient regeneration (the whole water treatments) and in samples where these processes were not occurring (the 1.0 μm fractionated treatments). Filters were immediately frozen at −20 °C onboard the ship. Upon returning to the lab, filters were dried at 50 °C, ampulated and analyzed by mass spectrometry using a Europa GEO 20/20 with a ANCA prep. unit. Uptake rates were calculated as described by Bronk et al. (1998).

2.2. Incorporation of ^{3}[H]-leucine

Rates of incorporation were determined by modifications of the micro-centrifugation method for ^{3}[H]-leucine uptake (Sherr et al., 1999; Smith and Azam, 1992). Triplicate water samples were incubated with leucine (final concentration 20 nM) from 1 to 3 h at in situ temperatures in a flow through incubator on board the ship. For each sample, 1.7 ml aliquots were pipetted into 2 ml micro-centrifuge tubes. A fourth aliquot in each set, a kill sample, was amended with 190 μl of 50% TCA as a control for abiotic uptake. After incubation, leucine incorporation was terminated by adding 190 μl of 50% TCA to each of the live replicates. All of the samples (kill and live) were processed on board the ship immediately, and stored in 1.5 ml scintillation cocktail (EcoScint, National Diagnostics) at 4 °C for the duration of the cruise. Upon returning to the laboratory, the micro-centrifuge tubes containing the samples were placed in 20 ml scintillation vials and activity was determined with a scintillation counter.

Killed controls were subtracted from the average of triplicate live DPM values after values for molar incorporation rates of leucine were determined. Incorporation rates of leucine were converted to bacterial production assuming 1.15×10^{17} cells mol^{-1}, which is the mean of all open oceanic studies (Ducklow and Carlson, 1992; Kirchman, 1992). Bacterial carbon content for Barents Sea bacterioplankton was assumed to be 15 fg C cell^{-1} (Fagerbakke et al., 1996). These two assumptions lead to a 1.725 kg C mol^{-1} conversion factor, which results from assuming no isotope dilution, 7.3% leucine in protein, and that protein is 61.8% of total cellular carbon (Simon and Azam, 1989). Prior work in cold-water environments (Carlson et al., 1999; Fagerbakke et al., 1996) has determined that the more commonly used 3.1 kg C mol^{-1} conversion factor (Simon and Azam, 1989) may overestimate production in these waters. Our conversions of leucine incorporation rates lead to lower production rates and therefore are likely to provide conservative estimates of heterotrophic bacteria productivity. Biomass production was expressed in N units by assuming 0.25 N/C ratio by weight for Barents Sea bacterioplankton (Fagerbakke et al., 1996).

2.3. Seawater culture enrichment experiments

Two batch experiments were conducted to determine whether DIN limited bacterioplankton production in two different water masses. Batch experiment (1) was initiated with seawater collected at a coastal fjord station and batch experiment (2) was prepared with seawater collected at 24-h station IV in the MIZ. Seawater for batch experiments was collected from the surface with a bucket. Seawater cultures were prepared by inoculating 0.22 μm filter sterilized seawater with a 0.8-μm filtered inoculum at a dilution of 10%. Experiments were conducted in 2 liter Teflon bottles. Each experiment consisted of eight bottles: two controls (no nutrient amendment), a 2 μM NH_4^+ amendment, a 2 μM NO_3^- amendment, two 5 μM NH_4^+ amendments, and two 5 μM NO_3^- amendments.

Bottles were incubated in the dark at in situ temperatures. At six time points over the course of 96 h, the bottles were sampled to determine bacterial abundance and nutrient concentrations. For nutrient samples, 50 ml were filtered through a precombusted GF/F filter and the sample collected in an acid washed bottle and stored at −20 °C. For bacterial cell counts, 20 ml samples were stored in 25% glycerol (final concentration) at −20 °C. Cell densities were deter-

mined directly by epiflourescent microscopy after staining with DAPI (Williams et al., 1998).

2.4. Nutrient concentrations and statistical analysis

Concentrations of NO_3^-, and NH_4^+, were measured with a Flow Solutions IV segmented flow analyzer (OI Analytical, College Station, TX) according to analytical chemistries provided by the manufacturer. The data were analyzed by analysis of variance and regression models with Systat (Wilkinson, 1990).

3. Results

3.1. Nutrient concentrations

The concentration of DIN at the five stations reflected the origin of the water mass and the extent of ice cover (Table 1). Stations I and II had low concentrations of NO_3^-, suggesting significant utilization by the plankton community and post bloom conditions (Verity et al., 2002). Station III, in the MIZ near the Polar Front, had 10–20% ice cover and much reduced NO_3^- concentrations associated with a larger sized phytoplankton community dominated by chained diatoms and *Phaeocystis pouchetti* single cells (Verity et al., 2002). Stations IV and V, which had 40–50% and 70–80% ice cover, respectively, had the highest NO_3^- concentrations. NO_3^- concentrations in surface waters were approximately 5 μM and between 6 and 9 μM at 30 m, suggesting early bloom to prebloom conditions. There was less variability associated with NH_4^+ concentrations, which were equal to or less than 1.0 μM at 5 and 30 m at all stations. For an overview of the hydrography, suspended biomass and nutrients along the transect, see Reigstad et al. (2002).

3.2. Uptake of DIN by heterotrophic bacteria

Total DIN uptake and DIN uptake by bacteria (<0.8 μm size-fraction) varied approximately two-fold (Fig. 2A,B). The rate of total DIN uptake decreased slightly across the transect from Atlantic water into the MIZ, and the rate of bacterial DIN uptake increased gradually across the transect.

To compare relationships between bacterial DIN uptake at the different stations, the 5, 30, and 80 m data were averaged. The <0.8 μm size fraction accounted for an average of between 10% and 40% of total DIN uptake (Fig. 3A) at the five stations. A significantly higher percentage of total DIN uptake was attributed to bacteria at Stns. IV and V than Stns. I, II, or III ($P<0.001$) (Fig. 3A).

As a percentage of the total NO_3^- or NH_4^+ uptake, averaged for each station, bacteria accounted for between 16% and 40% and 10% and 40%, respectively (Fig. 3B). In general, between stations, there was less variability associated with the percentage of total NO_3^- uptake by bacteria than there was for the percentage of total NH_4^+ uptake. The difference between the percentage of total NO_3^- uptake by bacteria at Stns. IV and V (40% and 36%, respectively) and Stn. I (17%) was significant ($P<0.05$). The difference between the percentage of total NH_4^+ uptake by bacteria at Stns. IV and V (41% and 34%, respectively) and Stns. I, II, and III (12%, 14%, and 10%, respectively) was highly significant (ANOVA, $P<0.001$).

When the percentage of total NO_3^- and NH_4^+ uptake by bacteria is compared (both were measured in the same sample), NO_3^- uptake by bacteria was

Table 1
Concentrations of NH_4^+ and NO_3^- (μM) at 5, 30, and 80 m (depths where ^{15}N experiments were conducted) at the five stations

Depth (m)	I		II		III		IV		V	
	(open water)		(open water)		(10–20%)		(40–50%)		(70–80%)	
	$[NH_4^+]$	$[NO_3^-]$	$[NH_4^+]$	$[NO_3^-]$	$[NH_4^+]$	$[NO_3^-]$	$[NH_4^+]$	$[NO_3^-]$	$[NH_4^+]$	$[NO_3^-]$
5	0.95	0.03	0.69	0.75	0.93	0.06	0.19	4.83	0.33	4.44
30	0.56	0.44	1.05	1.69	0.59	3.90	0.33	9.01	0.65	6.61
80	2.46	9.30	2.60	6.65	3.80	7.67	3.85	8.52	0.20	9.89

Ice cover extent is given in parentheses below the stations.

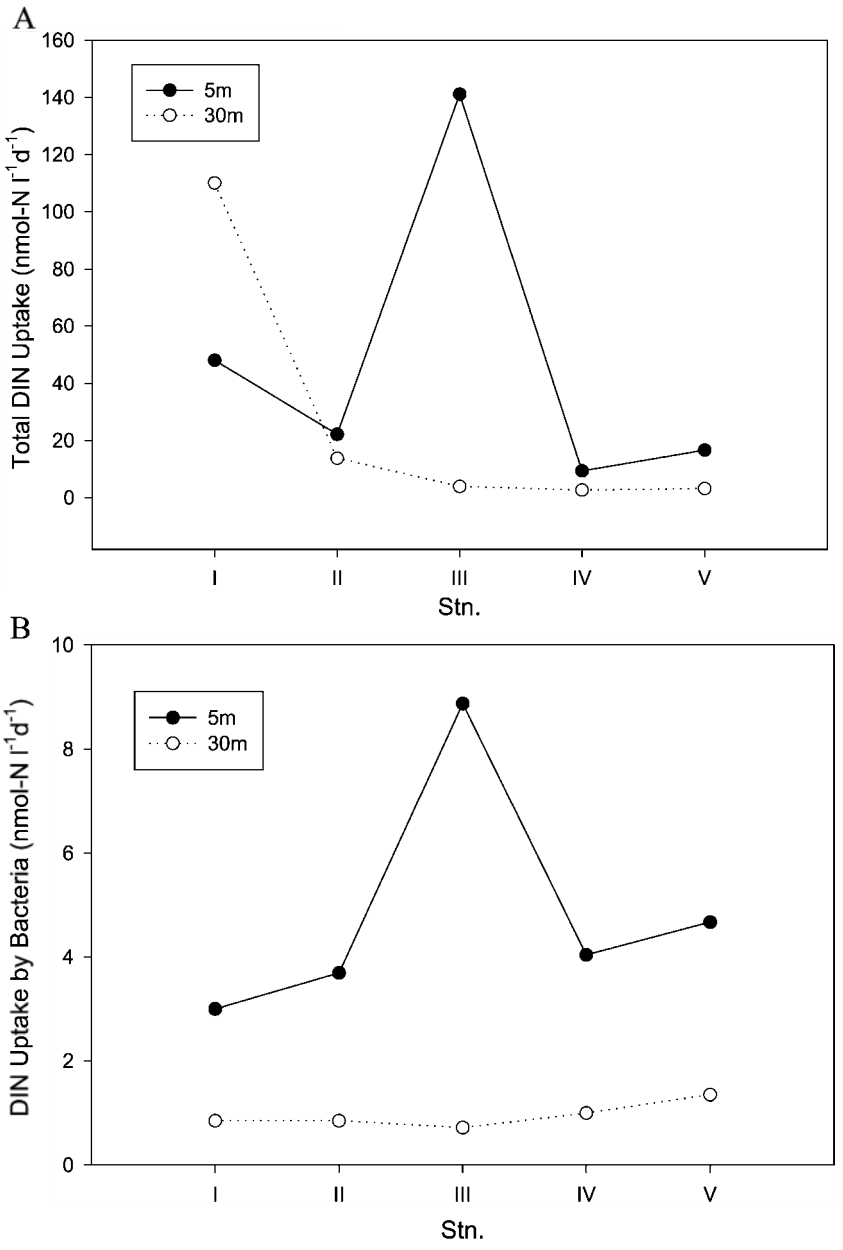

Fig. 2. Uptake of dissolved inorganic nitrogen (DIN) at 5 and 30 m at five stations during July 1999. (A) Total DIN by both size fractions (>0.8 μm size fraction + < 0.8 μm size fraction). (B) Total DIN uptake by bacteria (< 0.8 μm size fraction only).

much higher (nearly two-fold) than NH_4^+ uptake (Fig. 4). The percentage of total NO_3^- uptake by bacteria was higher than the percentage of total NH_4^+ uptake by bacteria in 12 out of 15 samples (Fig. 4).

Bacteria accounted for between 22% and 36%, and 19% and 24% of the total NO_3^- and NH_4^+ uptake, averaged for 5, 30, and 80 m, respectively (Fig. 5A). Because these data represent the averages from all five stations, the standard deviations are quite large, how-

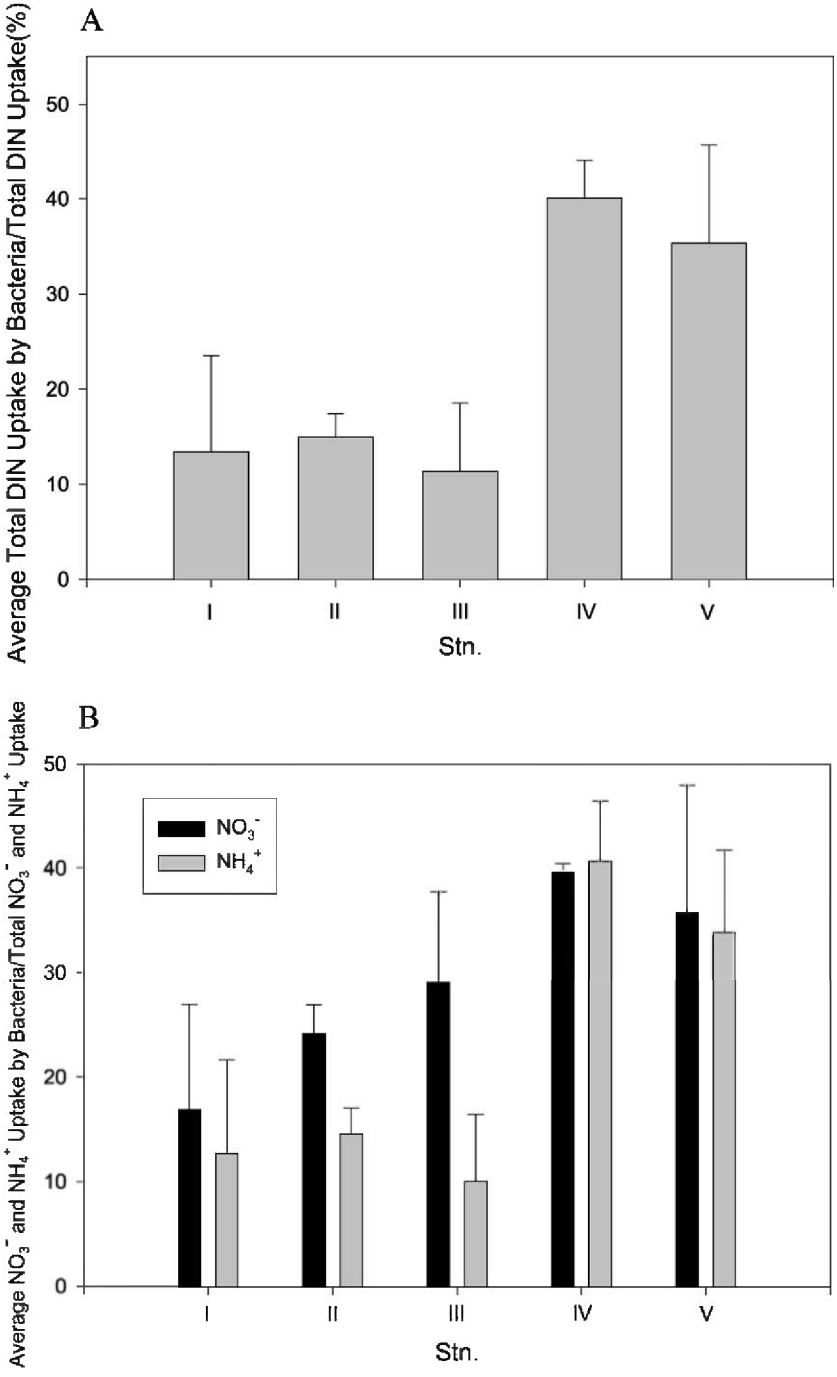

Fig. 3. Average percentage of total DIN uptake by bacteria (<0.8 μm size fraction) averaged between 5, 30, and 80 m at five stations (I–V). (A) Percentage of total DIN uptake attributable to bacteria at Stns. I–V. (B) Percentage of total NO_3^- or NH_4^+ uptake that is bacterial at Stns. I–V.

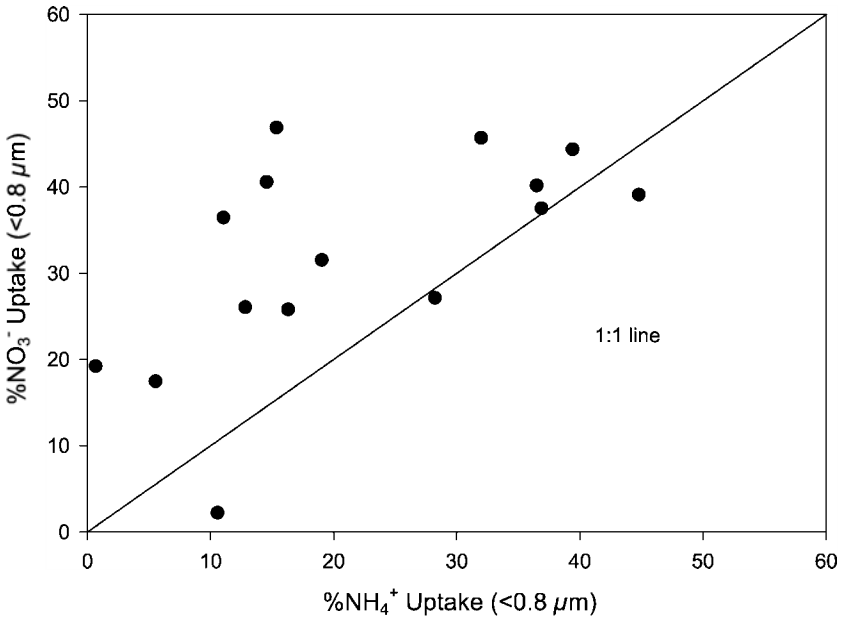

Fig. 4. Relative uptake of NO_3^- or NH_4^+ by heterotrophic bacteria (<0.8 μm size fraction) for each sample where both uptake rates were measured simultaneously.

ever, there is a trend of increasing percentage of NO_3^- uptake by bacteria at 30 and 80 m compared to 5 m. The difference between average percent NO_3^- uptake by bacteria at 5 m (22%) and 30 m (37%) was significant ($P<0.05$).

3.2.1. DIN uptake in fractionated (pre-filtered) vs. unfractionated treatments

The ratio of NO_3^- or NH_4^+ uptake in fractionated samples (where the >0.8 μm community was removed prior to incubation), compared to unfractionated samples were almost always less than one, indicating that bacterial NO_3^- or NH_4^+ uptake was higher in the unfractionated treatments (Fig. 5B). The ratio of fractionated to unfractionated DIN uptake averaged for 5, 30, and 80 m increased with depth. The fractionated/unfractionated ratio for total DIN, NO_3^-, and NH_4^+ uptake was significantly higher at 80 compared to 5 m (Fig. 5B) ($P<0.005$). Therefore, the effect of fractionating prior to the incubation decreased with depth and the ratio of fractionated/unfractionated uptake approached one. The amount of total particulate nitrogen collected on the filter for the fractionated and unfractionated treatments, however, was not different, which implies that a significant amount of bacterial biomass was not caught on the filter during the fractionation prior to the incubation. The variability of particulate nitrogen collected on the filters was not significantly correlated with treatment, tracer ($^{15}NO_3^-$ or $^{15}NH_4^+$), depth, or station ($P>0.1$).

3.3. Relationship between bacterial DIN uptake and biomass production

The ratio of DIN uptake to bacterial production (expressed in N units) was highly variable between Stns. I–II and Stns. III–V. DIN accounts for between 5% and 10% of bacterial nitrogen production at Stns. I–II, and between 39% and 54% of bacterial nitrogen production at Stns. IV–V (Fig. 6A). The average percentage of bacterial nitrogen production which resulted from DIN uptake tended to increase with depth at most of the stations. Consequently, there was a large degree of variability associated with the average percentage of DIN that accounted for total bacterial N at each of the stations. Nevertheless, the percentage that DIN contributes to total bacterial N demand increased from south to north in the transect, and a significantly higher percentage of bacterial N

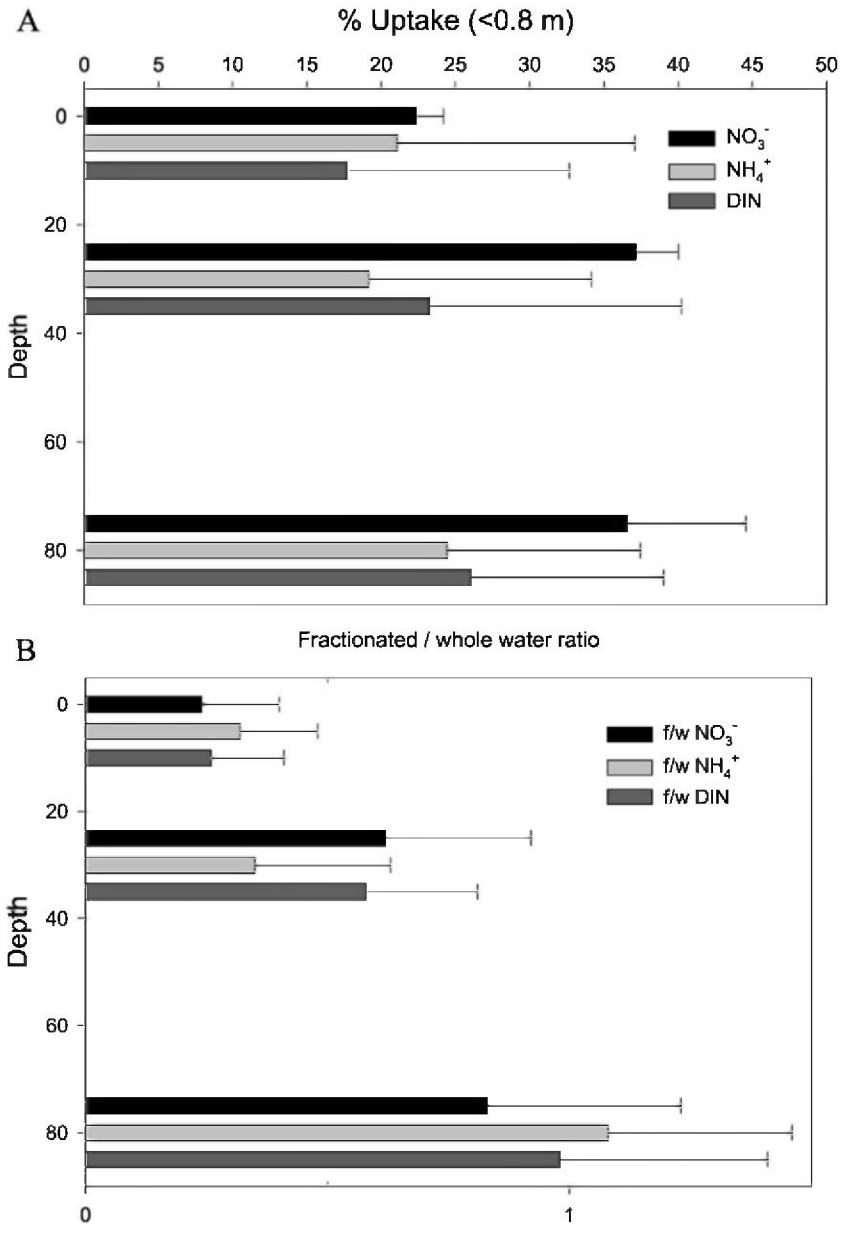

Fig. 5. Percentage of total DIN, NO_3^-, and NH_4^+ uptake by bacteria in unfractionated and fractionated treatments (<0.8 μm size fraction) averaged between each station for 5, 30, and 80 m. (A) Average percentage of total DIN, NO_3^-, and NH_4^+ uptake in unfractionated treatments at 5, 30, and 80 m. (B) Average percentage of total DIN, NO_3^-, and NH_4^+ uptake in fractionated treatments at 5, 30, and 80 m.

resulted from DIN at Stns. IV–V compared to I–II (ANOVA, $P<0.05$).

Interestingly, the correlation between bacterial production and DIN uptake was significant only if data from Stns. I–II and Stns. III–V are considered separately (Fig. 6B). At Stns. I and II, where DIN accounts for between 5% and 10% of total bacterial N, the correlation between bacterial production and DIN uptake is high ($r^2=0.87$; $n=6$; $P<0.005$); the slope of the regression line is 0.07. At Stns. III–V,

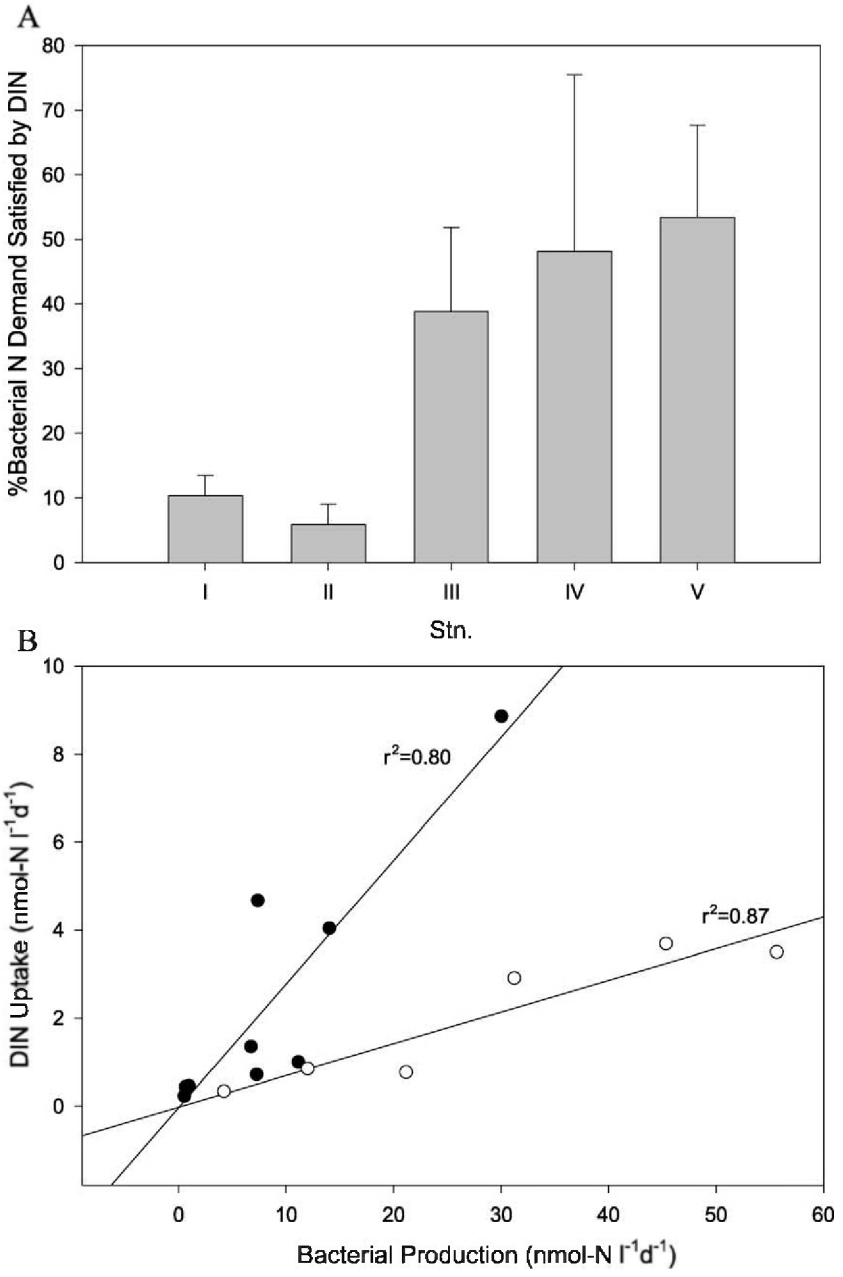

Fig. 6. Relationship between bacterial production and bacterial DIN uptake. (A) Average percentage of bacterial production (converted to N units) accounted for by bacterial (<0.8 μm size fraction) DIN utilization. (B) Uptake of DIN by bacteria vs. bacterial production. Stns. I and II (○), Stns. III, IV, and V (●).

where DIN accounts for between 39% and 54% of estimated bacterial N demand, the correlation between bacterial production and DIN is also high ($r^2=0.80$, $n=9$, $P<0.001$); the slope of the regression line is 0.28. Although, the total number of samples is low for both of these relationships, these

Table 2
Percentage of bacterial NH_4^+ or NO_3^- uptake of total bacterial DIN uptake at 5, 30, and 80 m at the five stations

Depth(m)	I		II		III		IV		V	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
5	99.5	0.5	90.1	9.9	82.7	17.3	70.6	29.4	78.9	21.1
30	87.0	13.0	81.5	18.5	43.7	56.3	16.6	83.4	18.6	81.4
80	81.1	18.9	94.2	5.8	82.0	18.0	51.0	49.0	31.2	68.8

data suggest that bacterial production can account for much of the variability in bacterial DIN uptake and that approximately four times as much bacterial N resulted from DIN at stations III–V compared to Stns. I–II.

As a percentage of total bacterial DIN uptake, NO_3^- and NH_4^+ varied in terms of their contributions to the total (Table 2). The percentage of bacterial NH_4^+ uptake of total bacterial DIN utilization ranged between 16% and 99%. On average, NH_4^+ accounted for 67% of bacterial DIN utilization and NO_3^- was responsible for 33%. However, the relative contribution of bacterial NO_3^- uptake to total bacterial DIN uptake increased, as averaged for each station, from 10% at station I to 57% at Stn. V (Table 2). Also, the contribution of bacterial NO_3^- uptake to total bacterial DIN uptake increased, as averaged for each depth, from 15% at 5 m to 50% and 32% at 30 m and 80 m, respectively (Fig. 7). The increase in NO_3^- uptake as a contribution to total bacterial DIN uptake from 15% to 50% between 5 and 30 m was significant (ANOVA, $P<0.05$).

3.4. Relationship between bacterial NO_3^- uptake and new production

The contribution of NO_3^- uptake to total DIN production provides a reasonable estimate of the *f*-ratio and the importance of new vs. regenerated production. The ratio of total NO_3^- uptake to total DIN uptake for each experiment is given in Table 3. In 11 out of 15 cases, the total NO_3^- uptake to total

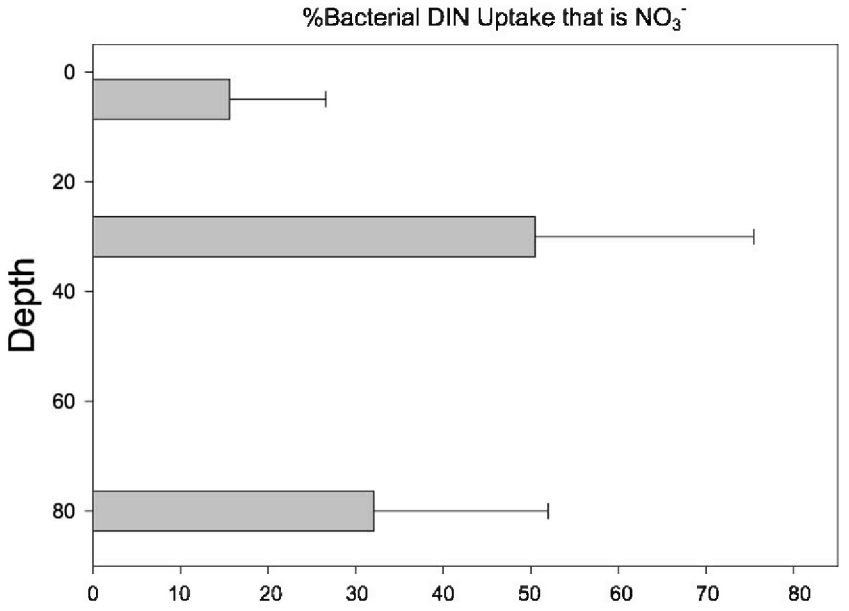

Fig. 7. Percentage of bacterial DIN uptake that is NO_3^- at 5, 30, and 80 m averaged over all five stations.

DIN uptake ratio was equal to or below 0.28. At Stns. I–III, the total NO_3^- uptake to total DIN uptake ratio was generally <0.1, which indicates that the relative importance of NO_3^- based production was not very significant compared to regenerated production. NO_3^- availability at Stns. I–III was also low and might partially explain this observation. At Stns. IV–V, however, NO_3^- based production was much more important and the total NO_3^- uptake to total DIN uptake ratio was often >0.5.

3.5. Seawater culture experiments

Ambient inorganic nitrogen concentrations in the controls and the treatments at the beginning of the experiments and the number of doublings of the bacterial community in each bottle are given in Table 4. In the control treatments for batch experiment 1, DIN concentrations were near the limit of detection throughout the 96-h incubation, and the 5 μM NH_4^+ and NO_3^- additions appear to have relieved DIN limitation and resulted in at least two doublings of the bacterial populations. In batch experiment 2, where the ambient NO_3^- concentration was approximately 5 μM, the DIN additions did not have any effect relative to the controls.

Table 3
The percentage of total NO_3^- uptake to total DIN uptake (both rates measured by combining the >0.8 μm fraction and the <0.8 μm fraction) for each experiment, and the percentage of bacterial NO_3^- uptake (<0.8 μm fraction only) to total NO_3^- uptake

	Depth (m)	*f*-ratio[a]	%Total NO_3^- uptake bacterial
Stn. I	5	2.4	2.2
	30	10.3	19.2
	80	12.7	31.5
Stn. II	5	7.1	25.8
	30	7.2	46.9
	80	3.6	26.1
Stn. III	5	6.5	17.5
	30	28.9	36.5
	80	7.4	40.6
Stn. IV	5	32.2	39.1
	30	83.1	37.5
	80	21.5	40.2
Stn. V	5	22.3	27.2
	30	75.2	45.7
	80	66.9	44.4

[a] *f*-ratio is calculated as the ratio of total NO_3^- uptake/total DIN uptake. *f*-ratios are expressed as percentages.

Table 4
Results of batch experiment (1) and batch experiment (2)

	Number of doublings	NH_4^+, μM	NO_3^-, μM
Batch experiment 1			
Control (A)	0	0.11	0.81
Control (B)	0	0.12	0.79
(5 μM) NH_4^+ (A)	2.39	5.50	0.76
(5 μM) NH_4^+ (B)	1.95	5.61	0.70
(5 μM) NO_3^- (A)	1.62	0.10	5.62
(5 μM) NO_3^- (B)	2.95	0.10	5.75
Batch experiment 2			
Control (A)	2.68	0.51	4.91
Control (B)	2.24	0.46	4.99
(5 μM) NH_4^+ (A)	2.16	5.61	4.79
(5 μM) NH_4^+ (B)	1.83	5.77	4.81
(5 μM) NO_3^- (A)	3.53	0.49	10.12
(5 μM) NO_3^- (B)	2.44	0.55	10.01

For each treatment, the number of doublings of the bacterial community and the initial NH_4^+ and NO_3^- concentration in each of the bottles is reported. Batch experiment (1) was initiated in North Atlantic waters at a coastal fjord station (not one of the 24-h stations). Batch experiment (2) was initiated at 24-h Stn. IV in the MIZ.

4. Discussion

4.1. Bacterial NO_3^- uptake in polar waters

Although the bacterial size fraction (0.2–0.8 μm) could contain both small phytoplankton and heterotrophic bacteria, chlorophyll *a* was not detected. Onboard microscopic examination of representative samples did not indicate the presence of autotrophic cells in the <0.8 μm size-fraction. Previous findings also support the observation that photosynthetic picoplankton are scarce in the Barents Sea (Throndsen and Kristiansen, 1991). Therefore, the <0.8 μm fraction is dominated by heterotrophic bacteria.

High rates of NH_4^+ utilization by marine bacteria have been observed several times and it is known that heterotrophic bacteria are capable of assimilating NO_3^- (Kirchman, 2000). The substantial levels of NO_3^- uptake by bacteria observed during this study, however, are noteworthy in that they are comparable to only one other study (Kirchman and Wheeler, 1998).

Small cells such as bacteria and picophytoplankton usually account for relatively more NH_4^+ uptake than NO_3^- uptake (Lipschultz, 1995). It is likely that the high levels of NO_3^- utilization observed in the sub-Arctic Pacific by Kirchman and Wheeler (1998), and in the MIZ (this study), result from the high ambient NO_3^- concentrations (5–20 μM), where only low per cell nitrogen uptake rates are necessary to supply bacteria with substantial nitrogen. A modeling study suggested that, for a given DIN uptake rate, fewer resources have to be allocated to NO_3^- uptake than to NH_4^+ uptake when the former is at a higher concentration (Vallino et al., 1996).

4.2. Bacterial productivity and inorganic nitrogen utilization

Correlations between rates of nitrogen uptake and bacterial production provide insights into the variability associated with bacterial DIN dependence. Across the transect from the Atlantic water into the MIZ, bacteria utilization of DIN increased significantly. The relationship between bacterial production and DIN uptake at open Atlantic Stns. I–II compared to MIZ Stns. III–V indicates that at the latter stations bacterial nitrogen production was supported by four times as much DIN. According to nutrient data and plankton composition data (Ratkova and Wassman 2002; Reigstad et al., 2002) Stns. I and II, showed signs of nitrogen limitation and were in postbloom phases and Stn. III was probably in declining bloom phase. Stns. IV and V, however, were not nutrient depleted. Although station III was nutrient depleted, the microbial food web was very diverse and *Phaeocystis* single cells were abundant. As a result there were likely very high levels of NH_4^+ regeneration, which contributed to a large spike in bacterial DIN uptake. Interestingly, however, NO_3^- accounted for 56% of bacterial DIN utilization in the experiment at 30 m, but only 17% and 18% at 5 and 80 m, respectively.

Although bacterioplankton populations at Stns. I–II and at Stns. III–V exhibited different levels of bacterial DIN dependence, bacterial productivity accounts for much of the variability observed in each case. The bioassay batch experiments demonstrated similarly DIN limited bacterial production in Norwegian Coastal Current waters (which are similar to open Atlantic waters), but not limiting bacterial production in the MIZ. Not surprisingly, ^{15}N data indicated that bacterial DIN utilization is much higher in the MIZ where DIN was not limiting, compared to N limiting open Atlantic waters. It is possible that dissolved free amino acid (DFAA) production accounted for much of the bacterial nitrogen production at Stns. I and II or that the bacteria were limited by carbon availability. Because bacterial production at Stns. I–II is higher on average compared to Stns. III–V, the former hypothesis is plausible.

It has been suggested that bacterial affinity for inorganic nutrients, particularly NO_3^-, is reduced at low temperatures (Reay et al., 1990). Therefore, it was surprising that such high levels of bacterial NO_3^- utilization were detected in the northern portion of the transect considering that there was a 9 °C drop in surface water temperature across the five stations. The decrease in temperature did not appear to have any discernable effect on bacterial DIN utilization, although it may have influenced phytoplankton production and microzooplankton grazing (Verity et al., 2002).

Bacterial activity, as measured by the modified vital stain and probe technique (mVSP), indicates that the percentage of physiologically active bacterial cells at Stns. I, II and IV is on average 75 to 50%, compared to 25 to 50% for Stns. I and III (Howard-Jones et al., 2002). Higher rates of bacterial DIN uptake and higher percentages of total bacterial NH_4^+, NO_3^-, and DIN uptake were observed at Stns. IV, and V. Therefore, it appears that physiologically active cells, which are not DOC limited or inhibited by high concentrations of DFAA, will readily assimilate NH_4^+ and NO_3^- simultaneously with a preference for NH_4^+ that can be offset by high NO_3^- concentrations.

The observed DIN utilization by bacteria in the fractionated compared to unfractionated treatments also provides insight into some of the factors that are likely to control bacteria DIN uptake. It has previously been demonstrated that bacteria are likely to exhibit a higher growth efficiency and assimilate more DIN in the presence of remineralization processes such as grazing compared to situations where nitrogen remineralization is absent (Hopkinson et al., 1989). Results from this study indicate a similar result for the case of Barents Sea bacterioplankton. Bacteria

in the fractionated treatments, on average, took up less NH_4^+ and NO_3^- compared to bacteria in the unfractionated treatment. The difference between DIN uptake in the different treatments decreases with depth, where it is expected that bacterial microzooplankton grazing processes are not as important. These results indicate that the removal of grazing processes reduced bacterial dependence on NO_3^- as well as NH_4^+. A potential artifact of these experiments is that, during prefractionation, DON could be released into the fractionated samples and as a result bacterial DIN affinity is decreased. The removal of phytoplankton and production of carbon substrates could limit bacterial production as well, however, the incubations were probably not long enough for this to occur. Nevertheless, the striking relationship between the ratio of bacterial DIN uptake in the fractionated compared to unfractionated treatments with depth strongly suggests that the removal of grazing processes in surface waters decreases bacterial activity and growth as well as DIN uptake.

4.3. Autotrophic production and new production estimates

A previous study reported that new production as a percent of total production (*f*-ratio) was hyperbolically related to NO_3^- concentration in the Barents Sea (Kristiansen et al., 1994). Results presented in this study suggest that high levels of bacterial NO_3^- assimilation can inflate estimates of new production, and that high *f*-ratios should be interpreted cautiously if bacterial NO_3^- utilization is not accounted for, especially if NO_3^- concentrations are relatively high. One result from this study is that in experiments where the total NO_3^- uptake to total DIN uptake ratio is >0.5, bacteria accounted for approximately 40% of the NO_3^- utilization. This implies that in regions where apparent high *f*-ratios are observed, bacteria may be partially responsible for the new production measurements, but do not result in increased new autotrophic production.

It has been demonstrated previously and in the present study using ^{15}N techniques that bacteria can account for a substantial portion of the NO_3^- uptake in the euphotic zone. Recently, it has also been shown with molecular techniques that bacteria that are metabolically capable of assimilating NO_3^- are common and widely distributed in the world's oceans (Allen et al., 2001). Because bacteria can be responsible for close to 40% of the observed NO_3^- uptake, their contribution cannot be ignored in estimates of new production. Since Eppley and Peterson (1979), it has been assumed that the downward flux of nitrogen has to equal NO_3^- uptake at steady state regardless of which group of microbes is using the NO_3^- and therefore bacterial uptake of NO_3^- is thought to have a negligible effect on *f*-ratios (Kirchman et al., 1992). However, substantial NO_3^- utilization by bacteria is one mechanism which might cause the system to deviate from steady state because bacterial carbon production, which results from NO_3^- utilization, represents POC that is more likely to remain in the water column and not to sink. Therefore, additional trophic transfers are required for the small bacterial particles to be transformed into sinking material. Also, it is clear that substantial bacterial NO_3^- utilization can lead to the observation of high *f*-ratios, which should be interpreted cautiously.

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