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Ammonium recycling limits nitrate use in the oceanic subarctic Pacific

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Abstract

Seasonal and diel changes in nutrient concentrations and nitrogen assimilation rates were used to assess the effects of NH_4^+ on NO_3^- assimilation. Surface-water NO_3^- concentrations ranged from 6 to 17 μM while NH_4^+ concentrations ranged from 0 to 0.4 μM. Total N assimilation ranged from 84 to 732 nM d^{-1} but showed no seasonal trend. NH_4^+ and urea concentrations were <1% of total dissolved inorganic N, but use of this "regenerated" N still accounted for 44–89% of total N assimilation. Rates of NO_3^- assimilation were negatively correlated with ambient NH_4^+ concentrations, and concentrations of NH_4^+ between 0.1 and 0.3 μM caused complete inhibition of NO_3^- assimilation. NO_3^- was more important as a source of N in spring than in summer. We attribute this pattern to a summer increase in turnover rates for NH_4^+. Turnover times for the dissolved NH_4^+ pool were half as long in August as in May. Grazing and recycling in the euphotic zone apparently both play significant roles in preventing depletion of NO_3^- in the oceanic subarctic Pacific.

The most frequent forms of N taken up by phytoplankton are NO_3^- and NH_4^+. Dugdale and Goering (1967) introduced the distinction between "new production" (supported by NO_3^- oxidized from organic matter at depth in the water column) and "regenerated production" supported by NH_4^+ and urea which are produced in the euphotic zone as a result of grazing and other degradation of organic material. The relative use of NO_3^- is usually expressed as the *f* ratio, i.e. NO_3^- uptake/total N uptake (Eppley and Peterson 1979). Upward fluxes of NO_3^- balance downward fluxes of particulate N in steady state systems (Eppley and Peterson 1979). Similarly, any net increase in biomass in the euphotic zone or export of biomass from a particular region requires either net depletion of dissolved NO_3^- in the euphotic zone or an equivalent upward flux of dissolved NO_3^-.

NO_3^- assimilation, vertical transport of NO_3^- into the euphotic zone, and particulate N (PN) fluxes out of the euphotic zone should be equivalent if the system being described is in steady state and there is no net transport of dissolved organic N. Consequently, two definitions of new production have arisen in the literature, one based on the form of N being used for primary production and one based on the flux of particulate material out of the euphotic zone. Here, we restrict our attention to the former definition, i.e. the absolute and relative amounts of NO_3 supported production. Results are presented as *f* ratios, but we stress that they are not equivalent to the fraction of primary production that could leave the euphotic zone. The oceanic subarctic Pacific is not a steady state system for time scales <1 yr. Elsewhere (Wheeler et al. in prep.) we will compare various measures of new production and export production in the subarctic Pacific.

Phytoplankton use NH_4^+, urea, and NO_3^- as sources of N in that order of preference (McCarthy 1980). The physiological preference for NH_4^+ is presumably derived from an energetic advantage, although this explanation has recently been questioned (Thompson et al. 1989). Reduction of NO_3^- to the oxidation level of organic N requires the equivalent of 8 electrons $(\text{mol } NO_3^-)^{-1}$. NH_4^+, on the other hand, is already reduced. In most oceanic regions, NO_3^- concentrations are very low in surface water, and vertical transport of NO_3^- into the euphotic

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zone controls the availability of new N to phytoplankton. Since assimilation rates of NO_3^- in these regions are substrate limited, NO_3^- concentrations have been used to estimate the relative contribution of NO_3^- to primary production. To get reasonable results, however, Platt and Harrison (1985) had to eliminate stations with NH_4^+ concentrations >1 μM from the data used for their analysis.

A more generally applicable model for predicting f ratios requires knowledge of functional relationships for the effect of NO_3^- availability on uptake rates and the effect of NH_4^+ on NO_3^- uptake. McCarthy (1981) proposed a hyperbolic relationship between NH_4^+ concentrations and inhibition of NO_3^- uptake, and Harrison et al. (1987) assumed a hyperbolic relationship for evaluation of f ratios from coastal waters. Laboratory studies (Syrett 1981), however, show that NH_4^+ has multiple effects on NO_3^- uptake, including inhibition of membrane transport, inhibition of NO_3^- reductase, and repression of NO_3^- reductase synthesis. Thus, it is unlikely that kinetics of inhibition follow a simple hyperbolic relationship. The inhibitory effect of NH_4^+ on NO_3^- uptake in the Antarctic is well described by a linear relationship between NH_4^+ concentration and NO_3^- uptake rate (Olson 1980; Glibert et al. 1982), but in the New York Bight an inverse hyperbola provided a good fit to the data (Garside 1981).

Further analysis of these relationships for oceanic waters has been precluded by the difficulty of obtaining accurate estimates of both nutrient concentrations and uptake rates. High nutrient concentrations in the subarctic Pacific are an exception that provides an opportunity to evaluate the effects of NH_4^+ concentrations on NO_3^- assimilation for oceanic waters. NO_3^- concentrations in its surface water are high (>6 μM) throughout the year, but phytoplankton standing stocks (Chl levels) are low (<1 μg liter^{-1}) and relatively constant (Miller et al. 1988). The maintenance of low and constant phytoplankton stocks has been attributed to a balance between phytoplankton growth and grazing by copepods and microzooplankton (Frost 1987; Miller et al. 1988). A balance between primary production and grazing also implies significant regeneration of reduced N within the euphotic zone.

Several hypotheses can be considered for explanation of the persistently high NO_3^- concentrations in the subarctic Pacific: phytoplankton growth is slow due to limitation by some other nutrient, possibly Fe (Martin et al. 1989); frequent or continuous upwelling or wind mixing replenishes the NO_3^- supply to the euphotic zone and prevents depletion; nitrification just below the euphotic zone rapidly replenishes NO_3^- and prevents depletion; or, grazing by copepods and microzooplankton results in low phytoplankton standing stocks and rapid NH_4^+ regeneration, with NH_4^+ recycling, in turn, reducing NO_3^- uptake and preventing NO_3^- depletion. The goal of this study was to examine the potential role of NH_4^+ in regulating NO_3^- assimilation in this nutrient-rich oceanic region. The validity of alternate hypotheses for the persistently high NO_3^- concentrations in the subarctic Pacific is also examined.

The occanic subarctic Pacific is a gyre system bounded on the south by the Subarctic Current and on the north by the Alaska Current. Nutrient concentrations are maximal at the center of the gyre (Reid 1973). Interannual variations in volumetric flow rates of the major currents of the Pacific Ocean, e.g. ENSO (El Niño southern oscillation) patterns, are apparent as far north as the subarctic Pacific and may cause interannual variations in the exact location of the gyre's center (Emery et al. 1985). Following the 1983 ENSO event the depth of the permanent halocline in 1984 was also apparently shallower (80–90 m) than the 100–150-m depth of more normal years (Denman and Gargett 1988). Most sampling for the SUPER program was at 50°N, 145°W (Canadian Weather Station P) because a large database is available for nutrients and other biological parameters. Additional sampling at 53°N, 145°W was included when possible to allow spatial comparison and to have additional information for waters closer to the gyre center.

Ambient nutrient concentrations and N assimilation rates, measured with ^{15}N tracers, are presented and examined to deter-

mine if and when NH_4^+ availability influences NO_3^- uptake rates. Background information on physical parameters were given by Denman and Gargett (1988). An overview of biological parameters and interactions was given by Miller et al. (1988), and estimates of phytoplankton biomass and growth rates were reported by Booth et al. (1988).

Methods

Study site—Sampling was carried out as part of the subarctic Pacific ecosystem research (SUPER) program on six 1-month cruises in 1984 and 1987–1988. Sampling dates at 50°N were 1–21 May and 2–12 August 1984; 3–7, 14–19 June, and 19 September–1 October 1987; and 7–30 May and 10–13, 24–28 August 1988. Sampling dates at 53°N were 13–23 August 1984; 9–13, 24–28 June, and 13–17 September 1987; and 4–8, 15–22 August 1988.

Water sampling—Nutrient samples were taken twice daily (0600 and 1800 hours) from 12 depths with 5-liter Niskin bottles on a rosette sampler. Samples were withdrawn immediately from the Niskin bottles and stored in acid-washed polyethylene bottles on ice until analysis (within 1 h). Water for in situ and deck incubation experiments was collected in 20-liter, Teflon-lined GoFlo bottles. For in situ incubations, water was collected between 0300 and 0400 hours, and the in situ array was deployed between 0400 and 0500 hours. Retrieval of the array was ~24 or 48 h after deployment. For deck incubations, water was collected in the same manner between 0500 and 0800 hours, and incubations were started within 1 h.

Nutrient analyses—NO_3^- concentrations were determined by the standard diazo dye formation as modified for autoanalyzers. Coefficients of variation for duplicate NO_3^- analyses were typically <1.0%. NH_4^+ concentrations were determined either manually or by autoanalyzer with the phenolhypochlorite reaction (Grasshoff et al. 1983). Precision for duplicate manual NH_4^+ analyses was typically ±0.03 μM (SD). Autoanalyzer results were used when comparable precision could be achieved (i.e. 1984 and 1988). Urea was determined manually by the diacetyl monoxime method (Price and Harrison 1987).

Isotopic determinations of uptake rates—NO_3^- uptake rates were determined by 24- or 48-h "in situ incubations" in 1-liter polycarbonate bottles and 24-h "deck incubations" in 2.7-liter polycarbonate bottles with repeated sampling for time-course measurements. Bottles for deck incubations were screened with neutral density filters to approximate in situ light levels at appropriate depths, and surface seawater was used to cool the incubators. NO_3^- (98.8 atom% ^{15}N) was added at concentrations of 1–2 μM (for a 10% enrichment over ambient NO_3^- levels).

NH_4^+ and urea uptake rates at "trace" 0.1 μM concentrations (99.7 atom% $^{15}NH_4^+$ and 98.0 atom% [^{15}N]urea) were determined in separate bottles with repeated sampling for time-course measurements. For all deck incubations, samples were taken at 3–6 h, 7–12 h, and a final point at ~24 h. Particulate material from ~1 liter was collected on GF/F precombusted filters (500°C, 15 min), dried overnight at 60°C, and stored in Vacutainers before isotopic analysis. PN samples were taken at the beginning of each experiment by filtering 1 liter through Whatman GF/F filters. Persulfate digestion and subsequent analysis of NO_3^- (Grasshoff et al. 1983) was used for analysis of PN collected on the filters. We used this procedure in preference to CHN analysis because we ran parallel samples of material collected on polycarbonate filters (Nuclepore membranes) for fractionation studies.

Isotope dilution of dissolved NH_4^+ was measured during the deck incubation experiments. Dissolved NH_4^+ in GF/F filtrates was recovered at each sampling time by conversion of NH_4^+ to indophenol and recovery of indophenol with solid-phase extraction with a modified elution solvent (Wheeler et al. 1989). Isotopic enrichment of the indophenol was determined in the same manner as for PN (below). Significant isotope dilution occurred in most NH_4^+ incubations, and the weighted mean NH_4^+ enrichment for each time interval was used to calculate uptake rates. The weighted mean NH_4^+ enrichment for each interval was calculated assuming an exponential decrease.

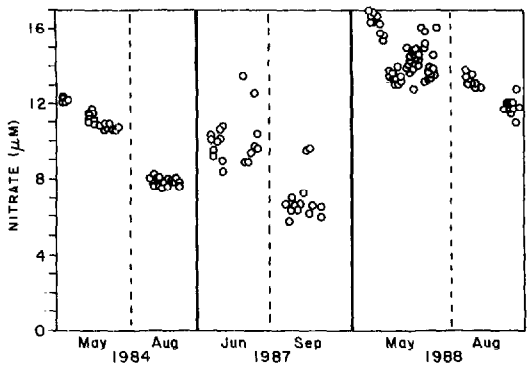

Fig. 1. NO_3^- concentrations in surface water at 50°N plotted as a function of day of month.

Assumption of a linear decrease (unweighted time mean) in enrichment would result in <10% difference in calculated NH_4^+ enrichments and in calculated uptake rates.

Analysis of ^{15}N enrichment—All ^{15}N samples were analyzed by emission spectrometry as described by Wheeler and Kirchman (1986). At least three peak scans were used for each ratio determination, and commercial isotope standards were used to calibrate the Jasco emission spectrometer. Coefficients of variation for the atom% enrichment values for triplicate combusted samples from a single time point were typically <1%. Carrier blanks for the NH_4^+ isotope dilution measurements were prepared in parallel with samples for each NH_4^+ regeneration experiment. These carrier values were used to correct for isotope dilution resulting from reagent additions and sample handling.

Calculations—Specific uptake rates were

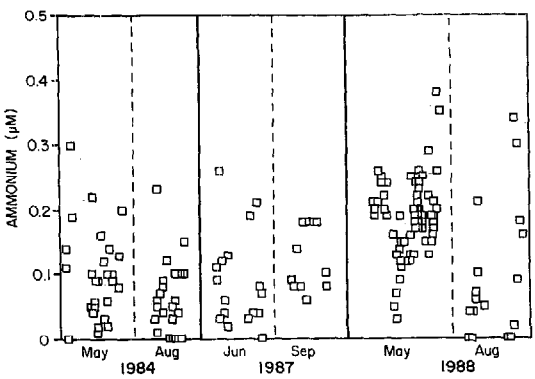

Fig. 2. As Fig. 1, but of NH_4^+.

Table 1. Net NO_3^- depletion in surface water at 50°N, 145°W.

	Rate ± SE (nM d^{-1})	n	r^2
May 84	98.3±7.3	29	0.87
Jun–Jul 84	18.3		
Jul–Aug 87	39.0		
May 88	412.6±77.8	38	0.74
Aug 88	159.9±12.7	15	0.91

calculated as the atom% excess of PN divided by the enrichment of the nutrient source and the length of the incubation. Absolute uptake rates were calculated as the specific uptake rates times the amount of PN. Initial values for PN were used since no detectable changes occurred during incubation periods of ≤48 h. Values are given as means ±1 SD unless otherwise noted. In order to check for possible underestimates of NH_4^+ uptake, we compared the amount of $^{15}NH_4^+$ removed from the medium to the amount recovered in the particulate fraction. Regression parameters for the effect of NH_4^+ concentrations on NO_3^- assimilation were calculated with model 2 regressions.

Results

Surface-water concentrations of nitrogenous nutrients—NO_3^- concentrations ranged from 5.8 to 17.0 μM and were highest on the earlier cruise for each year (Fig. 1). NO_3^- concentrations at 53°N were 1–3 μM greater than at 50°N. There were significant decreases in NO_3^- concentrations during both May cruises and in August 1988, and between May and August 1984 and June and September 1987 (Table 1). NH_4^+ concentrations were low and variable, ranging from undetectable (<0.03 μM) to 0.40 μM (Fig. 2). Mean NH_4^+ concentrations for each cruise ranged from 0.10 to 0.19 μM, but there were no significant differences (Student's two-tailed t-test, $P > 0.10$) among cruises or between stations. Urea concentrations were generally <NH_4^+ concentrations. At both stations NO_3^- concentrations exceeded NH_4^+ concentrations by at least one order of magnitude.

Diel periodicity in NH_4^+ concentrations—During calm weather (May 1988) strong diel periodicity in NH_4^+ concentration was detected. NH_4^+ concentrations decreased dur-

Table 2. Mean and standard error of ratio of $[NH_4^+]_{0600}:[NH_4^+]_{1800}$ for 5-d periods in 1988.

Sampling	0600:1800 Mean	SE	n
7–12 May	1.08	0.06	5
14–18 May	1.96	0.31	5
20–24 May	0.95	0.18	4
25–30 May	0.88	0.16	3
5–8 Aug	1.52	0.16	4
10–15 Aug	1.48	0.18	4
19–22 Aug	1.81	0.37	3

ing the day and increased at night on 9–10 and 15–17 May (Fig. 3A,B). The diel periodicity is less clear for 21–23 May (Fig. 3C). During May and August 1988, NH_4^+ concentrations were measured daily at 0600 and 1800 hours for 5-d sampling sequences. NH_4^+ was significantly greater at 0600 compared to 1800 hours for 4 out of 7 sequences (Table 2). We were unable to detect diel periodicity in NH_4^+ concentrations when net increases or decreases persisted for several days (e.g. Fig. 3C).

Diel changes in ambient NH_4^+ concentrations provide an estimate of net N assimilation during the day and net NH_4^+ remineralization at night. Net in situ NH_4^+ assimilation ranged from 3 to 10 nM h^{-1} (Table 3) and are within the range determined during deck incubations with ^{15}N tracers. Rates of net NH_4^+ remineralization at night ranged from 2 to 19 nM h^{-1} (Table 3). The diel patterns in NH_4^+ concentrations clearly illustrate both the rapid recycling of NH_4^+ and the disequilibrium between NH_4^+ uptake and remineralization within 24 h.

NO_3^-, NH_4^+, and urea uptake rates—Time-courses showed that uptake of NO_3^- was linear during the day, and that NO_3^- uptake at night was 2–10% of daytime rates (Table 4). NH_4^+ and urea uptake were both about linear over 24 h. Uptake rates at night were 82 and 69% of the 24-h uptake for NH_4^+ and urea respectively (Table 4). To compare relative use of each form of N on a 24-h basis, we present the uptake rates calculated from the atom% enrichment of PN at 24 h as a representative daily rate. Accuracy of our rate measurements for NH_4^+ uptake are further verified by the high recovery (67–85%) of $^{15}NH_4^+$ in the particulate fraction (Table 5). The label not recovered was probably in the bacterial fraction, a portion of which passes through GF/F filters (Kirchman et al. 1989).

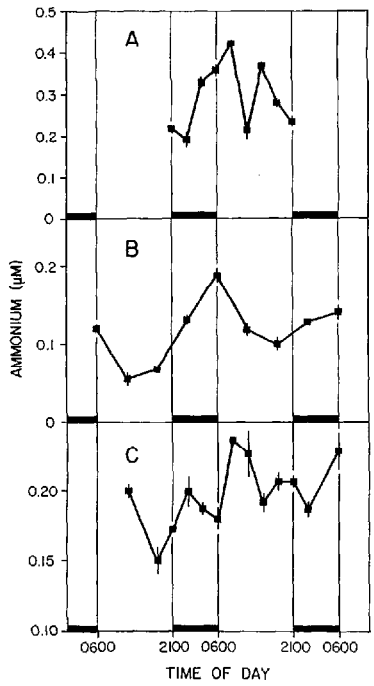

Fig. 3. Diel periodicity in mean ambient NH_4^+ concentrations for 0–30 m in May 1988. Results are aligned with respect to the light-dark cycle for ease of comparison. A. 9–10 May, 24-h sampling. B. 15–17 May, 48-h sampling. C. 21–23 May, 48-h sampling. Bars at bottom indicate dark period. Error bars give SE for mean, $n = 3$ or 4.

Total N assimilation ($NH_4^+ + NO_3^- +$ urea) in surface water ranged from 84 to 732 nM d^{-1} (Table 6). NH_4^+ uptake rates ranged from 18 to 54% of total uptake with an overall mean value of $39 \pm 9\%$. NO_3^- uptake rates ranged from 21 to 56% of the total with an overall mean of $37 \pm 10\%$ of the total. NO_3^- uptake rates determined isotopically compared well with rates of net NO_3^- depletion (Table 1). Urea uptake ranged from 10 to 43% of the total with an overall mean of $24 \pm 9\%$ of the total. The mean f ratio decreased from 0.41 ± 0.12 during May to 0.25 ± 0.05 during August 1988. There was no significant difference in f ratios between June and September 1987. The ranked proportional importance of the three N sources is $NH_4^+ \geq NO_3^- \geq$ urea.

Table 3. Rates (nM h^{-1}) of NH_4^+ uptake and regeneration calculated from in situ changes in NH_4^+ concentrations.

	Time (hours)	Uptake rate ± SE	*n*	Regeneration rate ± SE	*n*
9–10 May	2100–0900			19.3±4.1	5
	0900–2100	10.3±9.1	5		
15–17 May	0600–1200	10.0	2		
	1200–0600			2.3±1.1	4
	0600–1800	7.3±2.5	3		
	1800–0600			3.3±0.7	3
21–23 May	1200–1800	8.3	2		
	1800–0900			4.3±1.5	6
	0900–0000	2.8±1.0	6		
	0000–1500			6.7	2
Mean		7.7±3.0		7.2±7.0	

Turnover times for dissolved NH_4^+—NH_4^+ concentrations were highly variable both within and between cruises. In order to compare relative recycling rates, we divided uptake rates from deck incubations by NH_4^+ concentrations to estimate NH_4^+ turnover times (Fig. 4). The overall mean was 1.22 d, but a significant seasonal difference is apparent. Although NH_4^+ turnover time for May 1988 was 3.17±0.80 d (mean ± SE, $n = 4$), the mean for the June, August, and September samples was 1.48±0.61 (mean ± SE, $n = 14$).

Inhibition of NO_3^- uptake by NH_4^+—NO_3^- uptake rates decreased as a function of initial ambient NH_4^+, but the pooled data for all deck and in situ experiments showed considerable scatter (Fig. 5). Since ambient NH_4^+ concentrations can change significantly over a period of several hours, it is obvious that initial NH_4^+ concentration for incubation experiments is not representative of the mean NH_4^+ concentration during the incubation. To derive an accurate estimate of the functionality between NH_4^+ concentrations and daytime NO_3^- assimilation rates, we used the NH_4^+ concentration at the 6-h point and the NO_3^- uptake rates for the first 12 h of the 1987 and 1988 deck incubation experiments in model 2 regressions (Fig. 6A,B). NO_3^- assimilation rates during each cruise were inversely pro-

Table 4. Regressions for time-course measurements of uptake rates.

	Time period (h)	Coefficient of determination (r^2) (mean ± *SD*)	Dark uptake (mean ± *SD*)
NO_3^-*			Night/Day
Jun 87	0–7	0.957±0.044	NA
Sep 87	0–12	0.999	NA
May 88	*0–12*	0.927±0.054	0.02±0.02
Aug 88	0–12	0.996±0.004	0.10±0.07
NH_4^+			Night/(Day + Night)
Jun 87	0–24	0.982±0.036	0.92±0.13
Sep 87	0–24	0.989±0.005	0.84±0.16
May 88	0–24	0.938±0.104	0.92±0.07
Aug 88	0–24	0.950±0.020	0.62±0.05
Urea			
Jun 87	0–5	0.957±0.073	NA
Sep 87	0–24	0.971±0.041	0.87±0.08
May 88	0–24	0.906±0.091	0.50±0.36
Aug 88	0–24	0.964±0.017	0.70±0.10

* NO_3^- uptake rates were very low at night, so regressions for time-course cover only the light period.

Table 5. Recovery of ^{15}N during NH_4^+ incubations.

	Mean	SD
	(%)	
Jun 87	77.5	8.1
Sep 87	85.2	17.5
May 88	67.3	19.4
Aug 88	67.2	10.7
Overall mean	75.1	16.0

portional to NH_4^+ concentrations (Table 7). Maximal NO_3^- assimilation rates (Fig. 6, y-intercepts) ranged from 5.44 to 9.02 nM h^{-1}, while the NH_4^+ concentration resulting in 50% inhibition (k_i) ranged from 0.05 to 0.19 μM (Table 7).

In situ inhibition of NO_3^- assimilation—We had sufficient NH_4^+ data from the 1988 cruises to use the regression coefficients in Table 4 to obtain daily estimates of inhibition of NO_3^- assimilation by ambient NH_4^+. NH_4^+ inhibition decreased from 80 to 30% between 8 and 16 May, then increased continuously from 16 until 29 May, reaching 100% on the last day of sampling (Fig. 7A). During the latter part of May, f ratios decreased from 0.56 to 0.26. During August 1988, there was a decrease in inhibition by NH_4^+ from 80 to 30% at 53°N and an increase in inhibition from 10 to 80% at 50°N (Fig. 7B).

Table 6. N uptake rates and f ratio.

	NH_4^+	NO_3^-	Urea	Total	
	(nM d^{-1})				f ratio
Jun 87					
4	61	52	13	126	0.41
7	31	30	23	84	0.36
10	65	59	48	171	0.34
12	50	144	89	283	0.51
19	60	91	113	264	0.35
22	110	106	103	319	0.33
Mean	63	80	65	208	0.38
SD	24	38	39	86	0.06
Sep 87					
17	84	53	38	175	0.30
24	77	120	36	233	0.52
27	185	101	89	374	0.27
Oct 87					
3	58	72	24	154	0.47
Mean	101	86	47	234	0.39
SD	49	26	25	86	0.11
May 88					
8	61	110	26	198	0.56
14	86	110	31	228	0.48
20	84	59	36	179	0.33
27	70	34	26	130	0.26
Mean	75	78	30	184	0.41
SD	10	33	4	36	0.12
Aug 88					
5		29	53		
11	55	161	221	136	0.21
19	350	67	54	732	0.22
24	92	117	72	213	0.31
Mean	166	93	100	360	0.25
SD	131	50	70	264	0.05

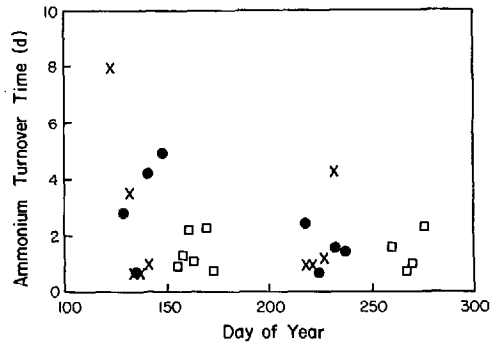

Fig. 4. Ammonium turnover times. Mean rate for each 5-d work cycle in 1984 (●), 1987 (□), and 1988 (×).

Correlation of NH_4^+ inhibition of NO_3^- uptake with phytoplankton biomass—The daily to weekly variations in inhibition of NO_3^- uptake by NH_4^+ are large and could be related to the relative abundance or activity of phytoplankton and microheterotrophs. We examined this possibility by comparing the mean percent inhibition and Chl *a* concentrations for each 6-d sampling

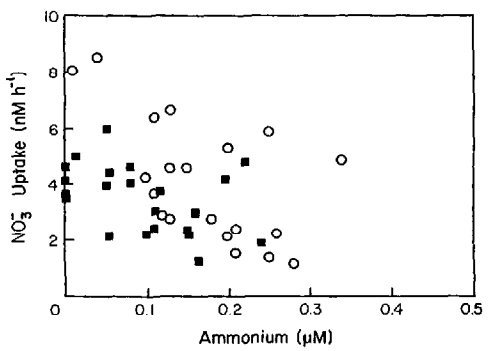

Fig. 5. NO_3^- uptake as a function of ambient NH_4^+ concentrations. Data from all in situ and deck incubations in 1987 (■) and 1988 (○).

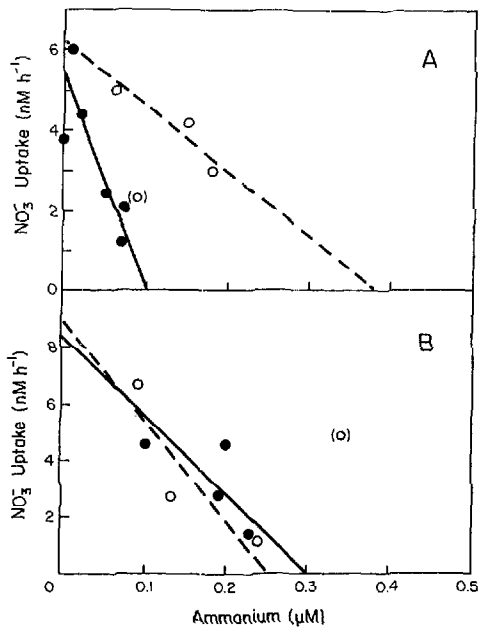

Fig. 6. NO_3^- uptake rates as a function of NH_4^+ concentrations measured at 6 h during deck incubation experiments. A. June (●) and September (○) 1987. B. May (●) and August (○) 1988. Points in parentheses were excluded from regression analysis.

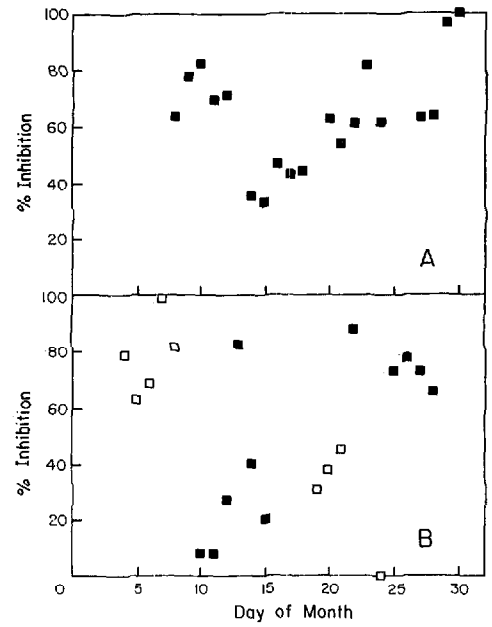

Fig. 7. Inhibition of NO_3^- uptake by NH_4^+. A. May 1988. B. August 1988. ■—50°N; □—53°N.

period during the 1988 cruises. The strong negative correlation between percent inhibition and Chl *a* concentration ($r = 0.70$, $P < 0.05$, Fig. 8), suggests that when autotrophic biomass is relatively high, NH_4^+ concentrations and inhibition of NO_3^- uptake are low. Conversely, when autotrophic biomass is relatively low, NH_4^+ concentrations are higher and cause significant inhibition of NO_3^- assimilation.

Discussion

In situ nutrient concentrations—The oceanic subarctic Pacific is a relatively stable region with respect to both vertical mixing and horizontal flows (Miller et al. 1988). As a consequence, regular patterns of changes in nutrient concentrations can be detected and can provide an in situ estimate of net uptake of NO_3^- and NH_4^+. For NO_3^-, significant decreases in surface-water concentrations were detected in May, but decreases were much slower or undetectable in August and September. Although net decreases in surface-water NO_3^- could be calculated for some portions of the data, the standard errors of the rates of decrease were rather large.

Table 7. Regression parameters for NH_4^+ inhibition of NO_3^- uptake. The inhibition constant, k_i, is the NH_4^+ concentration causing 50% inhibition.

	Max NO_3^- uptake (y-intercept) (nM h^{-1})	Slope	k_i (μM)	r^2
Jun 87	5.44	−52.3	0.05	0.83
Sep 87	6.17	−16.2	0.19	0.92
May 88	8.36	−27.8	0.15	0.68
Aug 88	9.02	−36.3	0.12	0.88

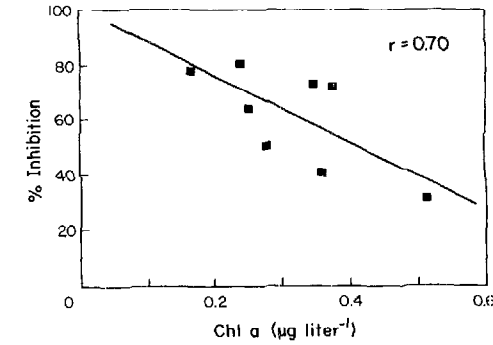

Fig. 8. Inhibition of NO_3^- uptake by NH_4^+ as a function of Chl *a* for May and August 1988.

Similar variability was evident in NO_3^- concentrations integrated for the upper 80 m of the water column (Wheeler unpubl. data), so we attribute departures from linearity to slight differences in water masses rather than to storm events.

Diel changes in NH_4^+ concentrations indicated net uptake during the day and net remineralization at night. This pattern agrees with our results from microcosm studies in 1987 (Wheeler et al. 1989). Both the small amplitude of changes in NH_4^+ concentration and net fluxes indicated that uptake and regeneration of NH_4^+ are closely compensating over a day. The similarity of assimilation rates determined from net changes in concentration to those determined with ^{15}N tracers suggests that there were no major incubation artifacts for the experiments reported here. Obviously, however, isotopic measures of uptake rates for a 24-h period would give a higher rate estimate than the net changes in NH_4^+ concentrations.

Although NH_4^+ concentrations in the oceanic subarctic Pacific are higher than in oligotrophic regions such as the Sargasso Sea (Glibert and McCarthy 1984) and the central Pacific gyre (Eppley et al. 1977), they are lower than the 1–4 μM levels reported for the Antarctic (Olson 1980; Glibert et al. 1982; Koike et al. 1986). NH_4^+ has been recognized as the dominant N source for phytoplankton in oligotrophic systems (e.g. Eppley et al. 1977; Glibert and McCarthy 1984) and occasionally in regions with persistently high NO_3^- concentrations (Glibert et al. 1982; Probyn and Painting 1985; Koike et al. 1986; Probyn 1988). In oligotrophic regions, however, NH_4^+ concentrations are close to detection limits and it is difficult to determine uptake rates accurately with ^{15}N techniques. Diel changes in NH_4^+ in the oceanic subarctic Pacific demonstrate unequivocally that NH_4^+ is recycled rapidly and that fluxes of $NH_4^+ \geq$ fluxes of NO_3^- into phytoplankton in the euphotic zone, despite a 100-fold greater concentration of NO_3^-.

Seasonal changes in NO_3^- uptake—NO_3^- assimilation accounts for 21–56% of the total measured N assimilation in the subarctic Pacific, and NO_3^- assimilation rates normalized to Chl *a* [7–35 nmol (μg Chl *a*)$^{-1}$ h^{-1}] are comparable to those reported in other waters high in NO_3^- (Kokkinakis and Wheeler 1987). Below ~30 m, NO_3^- assimilation and photosynthesis appear to be light limited (Miller et al. 1988), but in surface waters photosynthesis and N assimilation are light saturated. Seasonal decreases in NO_3^- assimilation can usually be attributed to depletion of NO_3^- in the euphotic zone (e.g. Sambrotto et al. 1986). This explanation, however, clearly does not apply in the subarctic Pacific where NO_3^- concentrations are always >6 μM.

Martin et al. (1989) postulated that high NO_3^- concentrations in the subarctic Pacific may be due to Fe limitation of phytoplankton growth. Phytoplankton are growing, however, at close to maximal rates (0.7–1.0 d^{-1}) expected for subarctic Pacific temperatures (5°–12°C) during both spring and summer (Booth et al. 1988). Furthermore, NO_3^- assimilation normalized to Chl *a* is the same in the subarctic Pacific in both spring and summer, despite a significant difference between in situ rates of net NO_3^- depletion. Thus, it is problematic to attribute continuously high NO_3^- levels in the subarctic Pacific to Fe-limited phytoplankton growth. It is possible, however, that Fe affects species composition and accumulation of phytoplankton biomass.

NH_4^+ inhibition of NO_3^- assimilation—Phytoplankton preference for NH_4^+ as a source of N is well documented for laboratory cultures (Syrett 1981) and natural populations (McCarthy 1981). For natural populations, the relative preference index introduced by McCarthy has been widely applied and clearly demonstrates that NH_4^+ is used preferentially relative to its availability. Physiological and biochemical processes regulating this preference, however, are not completely understood. NH_4^+ inhibits NO_3^- reductase activity and represses synthesis of NO_3^- reductase in phytoplankton (Syrett 1981), but the concentration at which this effect occurs is not clear. McCarthy (1981) suggests nearly complete inhibition of NO_3^- assimilation at NH_4^+ concentrations of ~1 μM, but also notes that it often occurs at lower NH_4^+ concentrations. Our results indicate that, in the subarctic Pacific, concentrations of 0.1–0.3 μM can completely inhibit NO_3^- assimilation.

The k_i (concentration resulting in 50% inhibition) for the subarctic Pacific is an order of magnitude lower than k_i values reported for coastal regions (Harrison et al. 1987) and for the Antarctic (Olson 1980; Glibert et al. 1982). We do not know what causes this apparent difference in sensitivity to NH_4^+.

Temporal scale for variations in inhibition—Our results suggest that NH_4^+ concentrations and turnover times vary significantly on several time scales: diel, weekly, and seasonal. We were unable to detect significant in situ diel variations in NH_4^+ concentrations during the 1987 cruises but did detect diel variations in NH_4^+ uptake and regeneration in large-volume (60 liter) microcosm experiments (Wheeler et al. 1989). During rough weather, any diel periodicity in NH_4^+ concentrations is difficult to detect due to limited frequency of sampling and increased mixing rates. Nonetheless, results presented here for May 1988 indicate that diel changes take place throughout the upper 30 m of the water column during calm weather. NO_3^- assimilation is light-dependent and NH_4^+ concentrations are highest at the end of the dark period, so rates of NO_3^- assimilation in the subarctic Pacific will depend on both light intensity and ambient NH_4^+ concentrations, and both have diel periodicities. At the beginning of the light period, residual NH_4^+ may cause a lag before onset of NO_3^- assimilation.

Variations in NH_4^+ concentration and inhibition of NO_3^- assimilation on temporal scales of days to weeks were negatively correlated with Chl *a* concentrations. We previously used Chl *a* : PN ratios, which ranged from 0.2 to 1.7 μg Chl *a* (μmol PN)$^{-1}$, as an index of autotrophic vs. heterotrophic biomass in coastal waters off Oregon and Washington (Kokkinakis and Wheeler 1987). For the oceanic subarctic Pacific, Chl *a* : PN ranges from 0.16 to 0.34 (Wheeler unpubl. data) suggesting a relatively low autotrophic biomass. Because PN is nearly constant (1.50±0.11 μM, mean ± SE, n = 18) in the subarctic Pacific, any relationship with Chl *a* : PN is dominated by variations in Chl *a*. Phytoplankton respond to changes in light intensity with a twofold to fivefold variation in cellular Chl *a* (Parsons et al. 1961; Chan 1980). Because light intensity was not limiting photosynthesis in the upper 30 m during our sampling (Miller et al. 1988), we suggest that changes in Chl *a* reflect variations in the relative abundance of autotrophic and heterotrophic biomass.

The diel to weekly variations in inhibition of NO_3^- assimilation by NH_4^+, then, may result from changes in the relative abundance and activity of phototrophic and heterotrophic microoganisms. High Chl *a* : PN reflects a plankton community dominated by phytoplankton with relatively low grazing pressure and low recycling rates. Low Chl *a* : PN reflects a community dominated by heterotrophs (or detritus or both) and relatively high grazing pressure. Our results suggest that the relative abundance or activity of the autotrophs and heterotrophs varies significantly on time scales of a few days in the subarctic Pacific.

In situ rates of NO_3^- depletion and ^{15}N measurements of NO_3^- uptake clearly decreased over the growing season in the subarctic Pacific. NH_4^+ concentrations were always relatively low and did not show large seasonal variation. NH_4^+ turnover rates, however, did vary, and we suggest that increased NH_4^+ availability is one factor that limits NO_3^- depletion in the subarctic Pacific. A similar seasonal decrease in the relative use of NO_3^- occurs in the Antarctic, where the *f* ratio decreases from 0.54 in early spring (Olson 1980) to 0.16 in late summer (Koike et al. 1986). In the Antarctic, however, the seasonal decrease in new production is correlated with a significant increase in NH_4^+ concentrations, i.e. from 0.3 μM in early spring (Olson 1980) to 2.0 μM in late summer (Koike et al. 1986). Koike et al. (1986) also attribute the increased availability and use of NH_4^+ to an increase in the relative abundance of heterotrophs.

In nutrient-rich regions, NO_3^- accounts for a large portion of the N assimilated during primary production, but there is some controversy over the maximal percent new production (*f* ratio). Eppley and Peterson (1979) estimate ~50% new production in upwelling regions, whereas Platt and Harrison (1985) estimate 80% new production for coastal regions. In the subarctic Pacific, grazing pressure (by calanoid copepods and microzooplankton) is sufficient to maintain

low phytoplankton stocks throughout the year. One consequence of this grazing pressure must be significant recycling of NH_4^+. The biomass of zooplankton equals or exceeds that of phytoplankton (Frost 1987), but most grazing is by microzooplankton (Miller et al. 1988). Standing stocks of heterotrophic flagellates and ciliates is about equal to the standing stock of phytoplankton (Booth unpubl. data); thus one could expect to find a steady state established in which the system is operating primarily on recycled nutrients. Excess inorganic N would remain in the system as NO_3^-. This type of situation has been modeled previously by Kiefer and Atkinson (1984) but seems not to have received much attention. An important consequence of grazing, NH_4^+ recycling, and the inhibitory effects of NH_4^+ on NO_3^- utilization is that it sets an upper limit on NO_3^- uptake in nutrient-rich, grazing-balanced ecosystems.

As argued above, persistently high levels of NO_3^- in the oceanic subarctic Pacific cannot be attributed to nutrient limitation of phytoplankton growth rates. Simple calculations also indicate that mixing and nitrification do not account for the high NO_3^- concentrations. The vertical flux of NO_3^- across the permanent halocline at 90 m in the subarctic Pacific is negligible. Maximal upward flux of NO_3^- across the halocline is <2 mg N m^{-2} d^{-1} (T. Powell pers. comm.), and this rate of NO_3^- input would have insignificant effect on net changes in NO_3^- concentrations in the euphotic zone seasonally. Rates of nitrification are on the order of 0.04 μM d^{-1} at the base of the euphotic zone (Ward et al. 1982). If it occurs in a 10-m layer of the water column, the supply rate of NO_3^- to the upper 80 m is 5 nM d^{-1}. It thus is unlikely that nitrification has any significant effect on either our isotopic rate measurements of NO_3^- assimilation or on the rate of net NO_3^- depletion between May and September in the subarctic Pacific.

In conclusion, grazing pressure in the subarctic Pacific has two effects on phytoplankton use of NO_3^-. Grazing maintains a low standing stock (Frost 1987; Miller et al. 1988), and, since uptake rates are proportional to biomass, it also reduces NO_3^- uptake. Maintenance of low standing stock, however, is not sufficient to explain high NO_3^- concentrations in the subarctic Pacific. As shown by the data presented here, phytoplankton use of N is about 250 nM d^{-1}. At these rates (in the absence of any N recycling), phytoplankton would deplete the N supply in 100 d. Our results clearly demonstrate an inverse relationship between NH_4^+ concentrations and rates of NO_3^- uptake. This regenerated N is used preferentially by phytoplankton and thus leads to a decrease in net NO_3^- depletion. Although physical factors undoubtedly influence the supply of NO_3^- to the euphotic zone, it is important to recognize that biological factors (grazing and physiological preferences for NH_4^+) can have dramatic effects on NO_3^- uptake rates and relative use of NO_3^--N for primary production.

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