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Bacterial chemolithotrophy in the ocean is associated with sinking particles

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The oceanic carbon cycle has traditionally been viewed as a reversible, one step reduction–oxidation reaction ($CO_2 \rightleftharpoons CH_2O$). Principle pathways were thought to involve eukaryotic photoautotrophy and oxygen-dependent bacterial respiration, respectively. However, prokaryotic (cyanobacterial) photoautotrophy is now well documented and has even been proposed as a major carbon pathway[1–6]. In a previous study of the mesopelagic zone in the North Pacific Ocean[7], the observed downward fluxes of organic carbon, nitrogen, ATP and RNA suggested production *in situ* of new particulate organic carbon at 700–900 m. Here we present evidence that this is indeed the case and that it is mediated by bacterial chemolithotrophy. Energy for this process may be in part provided by detrital NH_4^+ derived from the downward flux of large particles.

In order to reconcile the mesopelagic zone carbon budget, chemolithotrophic production of organic matter was proposed as a possible mechanism[7]. During the Vertex III sediment trap experiment, we had an opportunity to examine the possibility of bacterial chemolithotrophic production at a station, located in the eastern tropical North Pacific Ocean approximately 330 km off Manzanillo, Mexico (15°55′ N; 107°12′ W). The Vertex III free floating sediment trap array, used for 20.6 days, consisted of 168 individual collectors at 14 depths (50–2,000 m). Details of the trap construction are given elsewhere[8]. To test the chemolithotrophy hypothesis, we used five sediment trap collectors at each of five depths (100–750 m). Measurements included: (1) concentration of ATP extracted *in situ*[9] to estimate the biomass of living microorganisms associated with sinking particles, (2) shipboard-extracted ATP concentrations[7] in unpreserved traps to estimate the net *in situ* changes in biomass during the experiment (see Table 2), (3) concentrations of NH_4^+, NO_2^- (see Table 1), (4) organic carbon and nitrogen concentrations[8], (5) rates of microbial DNA synthesis *in situ*[7], (6) microbial incorporation of $^{14}CO_2$ (see Table 2), and (7) microscopic examination of glutaraldehyde-fixed sediment trap samples. The *in situ* ^{14}C-incubation experiments and ATP measurements were performed in paired sediment traps, with and without the addition of a mixture of potential chemolithotrophic substrates (see Table 2).

Several results suggest the activity of chemolithotrophic bacteria at these depths. First, the concentration of NH_4^+ measured in the sediment trap solutions (and hence, the corresponding downward fluxes of particle-associated NH_4^+) increased between 100 and 150 m (Table 1), indicating production of NH_4^+ in association with descending particles, scavenging of NH_4^+ from the water column during descent or horizontal advection of particle-associated NH_4^+. At greater depths both concentration and flux of NH_4^+ consistently decreased. The estimated NH_4^+ fluxes are considered to be minimum values as no attempt was made to remove associated or covalently bound NH_4^+ from the sinking particles. The flux of NH_4^+ thus calculated was 383 and 149 μmol m^{-2} day^{-1} at 150 and 550 m, respectively, indicating a loss of 234 μmol NH_4^+ m^{-2} day^{-1} over this depth interval (Table 1). Since NH_4^+ was undetectable (< 0.05 μmol l^{-1}) in this portion of the water column, we suggest that the NH_4^+ is consumed either in the water column or within the sinking particles at a rate which is equivalent to the measured rates of change (mean rate of 0.59 μmol m^{-3} day^{-1} between 150–550 m). The consumption of NH_4^+ (that is, $-\Delta NH_4^+/\Delta Z$) is associated with the appearance of NO_2^- (Table 1). These data suggest the presence of active bacterial nitrification.

Nitrifying bacteria of the genera *Nitrosomonas* and *Nitrosococcus* were counted using specific immunofluorescence techniques[10,11]. Nitrifiers were detected in high concentrations in all sediment trap collections and the majority were *Nitrosomonas*-type cells. Nitrifiers were frequently observed in clusters of up to 30 cells, but single and paired cells were also common. The mean cell size varied considerably, but large cells, up to 3 μm in diameter, were common.

The downward flux of particle-associated nitrifiers was consistently high (1.15 to 3.52×10^8 cells m^{-2} day^{-1}) throughout the depth range of our experiment (100–750 m). Sediment traps pre-charged with chemolithotrophic substrates and incubated *in situ* produced higher cell densities, with values (relative to the control) ranging from 118% (150 m) to 263% (550 m). If we assume a maximum nitrification rate of $1.4–2 \times 10^{-12}$ mol NH_4^+ per cell per day (refs 12, 13) the particles collected at 550 m are potentially capable of oxidizing between 160–230 μmol NH_4^+ m^{-2} day^{-1}, a value which agrees with the observed loss of NH_4^+ at 150–550 m (Table 1).

The comparison of *in situ* versus shipboard extracted ATP can be used to ascertain the extent to which net microbial growth had occurred in the unpreserved sediment materials during the experiment[7]. It is evident (Table 2) that the two surface traps (100 and 150 m) exhibited post-incubation decreases in total ATP (that is, shipboard ATP < *in situ* ATP) indicating net microbial death. However, for that region of the water column where NH_4^+ was consumed and NO_2^- was produced (550–750 m), there was an increase in total microbial ATP during the incubation period and hence, net carbon production. The greatest increase occurred at 550 m (Table 2). At all three depths where net growth had occurred (550, 650 and 750 m), we observed an increased biomass yield following the addition

Table 1 Nutrient concentrations, fluxes and average rates of change over the specified depth intervals

Sediment trap depth (m)	Nutrient concentrations (μmol per sediment trap)* NH_4^+	NO_2^-	Total flux (μmol m^{-2} day^{-1}) and average rates of change (μmol m^{-3} day^{-1}) for each depth interval† Organic C flux	$\Delta C/\Delta Z$	Organic N flux	$\Delta N/\Delta Z$	NH_4^+ flux	$\Delta NH_4^+/\Delta Z$
100	13.8	<0.005	3,900		609		172	
				(−34)		(−7.1)		(+4.2)
150	30.8	<0.005	2,192		254		383	
				(−3.7)		(−0.42)		(−0.59)
550	12.0	2.3	717		87		149	
				(+0.55)		(+0.04)		(−1.1)
650	3.4	1.4	772		91		42	
				(+1.5)		(+0.32)		(−0.28)
750	1.1	1.1	925		123		14	

* NH_4^+ and NO_2^- concentrations were measured in 0.22 μm filtrates of the unpreserved sediment trap solutions receiving no chemolithotrophic additions (see Table 2). Samples were analysed by S. Moore within 4 h after sediment trap recovery using a shipboard Technicon Autoanalyzer system.
† Organic C and N were measured in formalin preserved sediment traps as described by Knauer *et al.*[8].

Table 2 Effects of addition of exogenous reduced inorganic substrates *in situ* to sediment trap collected particles

Sediment trap depth (m)	ATP concentration, *in situ* extraction (ng per sediment trap)	Chemolithotrophic additions	ATP concentration, shipboard extraction (ng per sediment trap)	%ATP change*	^{14}C-HCO_3^- incorporation into organic matter (nCi per sediment trap)
100	1,760	No	705 ± 382	−60	11.0 ± 0.8
		Yes	1,434 ± 429	−19	16.1 ± 1.9
150	1,310	No	750 ± 6	−43	16.4 ± 0.5
		Yes	556 ± 37	−57	30.6 ± 5.3
550	110	No	748 ± 94	+580	42.7 ± 3.0
		Yes	4,004 ± 543	+3,540	48.7 ± 7.3
650	65	No	286 ± 106	+340	6.9 ± 0.2
		Yes	844 ± 271	+1,198	18.6 ± 0.7
750	220	No	662 ± 298	+201	18.3 ± 0.6
		Yes	1,198 ± 184	+445	14.5 ± 0.8

ATP associated with sinking particles was extracted *in situ* by precharging the sediment traps with a high density acid-salt solution (0.5 M H_3PO_4, 1.5 M NaCl $\rho = 1.05$ g cm^{-3}) as described by Fellows *et al.*[9]. For *in situ* incubations paired sediment traps were prepared for each depth by precharging with 2.5 l of an autoclaved high density solution consisting of 0.22 μm filtered seawater containing 33.8 g NaCl, 14.3 g $MgCl_2 \cdot 6H_2O$, 0.93 g KCl and 30 μCi $NaH^{14}Co_3$ (50 m Ci mmol^{-1}; New England Nuclear) per litre ($\rho = 1.065$ g cm^{-3}). At each depth, one of the paired traps received the chemolithotrophic mix consisting of NH_4Cl, $Na_2S_2O_3 \cdot 5H_2O$ and $MnCl_2$ to yield final concentrations of 400 μM NH_4^+, 400 μM $S_2O_3^{-2}$ and 100 μM Mn^{+2}. Shipboard ATP concentration refers to the value measured in the unpreserved sediment traps after recovery so as to distinguish it from the *in situ* extracted ATP. Details of the methods are presented by Karl and Knauer[7]. For ^{14}C incorporation, triplicate subsamples (100 ml each) were concentrated onto Whatman GF/F filters acidified with HCl, evaporated to dryness *in vacuo*, reconstituted with 1 ml deionized H_2O, mixed with 10 ml Aquasol (NEN) and radioassayed.

* Calculated as: [(shipboard ATP − *in situ* ATP)/*in situ* ATP] × 100%.

of potential chemolithotrophic substrates.

The mean total $^{14}CO_2$ incorporation (Table 2) also displayed a mid-depth (550 m) maximum and, at all depths but 750 m, the incorporation rates were stimulated by the addition of inorganic substrates. However, $^{14}CO_2$ incorporation data, by themselves, cannot be extrapolated directly to microbial carbon production without information on the proportion of autotrophic versus heterotrophic processes. Heterotrophic bacteria normally assimilate 2–10% of their total carbon cell quota as CO_2 (refs 14–16), whereas certain chemolithotrophs obtain potentially 100% of their cell carbon requirements from CO_2 (refs 17, 18). By assuming the ^{14}C-incorporation data represent strictly chemolithotrophic growth, the data can be extrapolated and compared with total microbial carbon production derived from estimates of DNA synthesis (Table 3). The DNA synthesis (^3H-adenine) technique does not discriminate between the two major pathways of carbon synthesis and hence provides an estimate of total microbial carbon production. In the surface sediment traps (100 and 150 m), total microbial production greatly exceeds the ^{14}C-derived estimate, indicating that chemoheterotrophic processes predominate (Table 3). This is consistent with the observed loss of organic carbon and production of NH_4^+ associated with the large sinking particles (Table 1). However, between 550 and 750 m chemolithotrophic carbon production was detected with estimates ranging from 7% (650 m) to 90% (550 m) of the total microbial production and may therefore be a significant, if not dominant pathway for carbon and energy flow at certain depths in the mesopelagic zone.

If we assume that nitrification is the dominant pathway we can calculate a minimum amount of energy required to sustain this observed mesopelagic bacterial production. The oxidation of NH_4^+ to NO_2^- proceeds with a ΔG of −66 kcal mol^{-1}, and with typical cell yields of 0.03–0.06 mol C produced per mol N oxidized[19]. Thus at 550 m, the estimated chemolithotrophic microbial production (425 μg C m^{-2} day^{-1}) would require a flux of 787 μmol NH_4^+ m^{-2} day^{-1}, if nitrifiers were exclusively responsible, which is a value approximately three times the minimum *in situ* NH_4^+ consumption rate estimated from the sediment trap data (Table 1). This rate of NH_4^+ oxidation could consume potentially 1.6 mmoles 0_2 m^{-2} day^{-1}, if completely oxidized to NO_3^-. Therefore, chemolithotrophy (bacterial reduction of CO_2 and concomitant O_2 consumption) rather than chemoheterotrophic utilization of surface-dervied organic mat-

Table 3 *In situ* microbial production of organic matter in association with sediment trap collected particles and estimates of the role of bacterial chemolithotrophy

Sediment trap depth (m)	Chemolithotrophic additions	Minimum ^{14}C-derived microbial production* (μg C m^{-2} day^{-1})	Total microbial production† (μg C m^{-2} day^{-1})	Estimated % chemolithotrophic based production‡
100	No	110 ± 8	15,900 ± 3,200	< 1
	Yes	160 ± 18		
150	No	163 ± 5.4	9,900 ± 750	2
	Yes	305 ± 53		
550	No	425 ± 30	480 ± 83	90
	Yes	485 ± 66		
650	No	69 ± 2.2	1,000 ± 17	7
	Yes	185 ± 6.6		
750	No	182 ± 5.7	870 ± 31	21
	Yes	144 ± 8.4		

See Table 2 for composition of chemolithotrophic mixture.

* Calculation assumes that CO_2 represents 100% of the carbon cell quota (that is obligately chemolithotrophic) and is based upon the measured initial ^{14}C specific radioactivity of 15 μCi mmol^{-1} of total inorganic C. Values are mean ± 1 s.d.

† Total carbon production extrapolated from *in situ* measurements of rates of DNA synthesis using the conversion factors of Karl and Winn[20]. Values are mean ± 1 s.d.

‡ Calculated from data obtained without chemolithotrophic additions.

ter may be an important mechanism for the consumption of O_2 in the mesopelagic zone of the ocean. Although we have singled out nitrification as an important metabolic process, other pathways such as the oxidation of CH_4, H_2S, H_2 and other reduced products of anaerobic decomposition may also contribute energy for the observed mesopelagic production.

These results suggest that the oceanic carbon cycle may be more complex than previously described. The flux of energy descending from the euphotic zone may greatly exceed that nominally contained in particulate organic detritus, which has previously been considered to be the principle factor controlling the rates of mesopelagic microbiological activity and O_2 utilization. Although the flux of energy (in the form of reduced products of bacterial decomposition) required to sustain the chemolithotrophic production measured in this study is probably derived from surface primary production and thus cannot exceed it in steady-state conditions, mesopelagic 'primary' production may have a major impact on the trophic organization of localized mid-water food webs and on the production and maintenance of the intermediate depth O_2 minimum.

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