Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significant midwater source of organic carbon production

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Abstract
During the CARIACO time series program, microbial standing stocks, bacterial production, and acetate turnover were consistently elevated in the redox transition zone (RTZ) of the Cariaco Basin, the depth interval (~240–450 m) of steepest gradient in oxidation-reduction potential. Anomalously high fluxes of particulate carbon were captured in sediment traps below this zone (455 m) in 16 of 71 observations. Here we present new evidence that bacterial chemosynthesis fueled by reduced sulfur species, supports an active secondary microbial food web in the RTZ and is potentially a large midwater source of labile, chemically unique, sedimenting biogenic debris to the basin’s interior. Dissolved inorganic carbon assimilation (27–159 mmol C m⁻² d⁻¹) in this zone was equivalent to 10%–33% of contemporaneous primary production, depending on the season. However, vertical diffusion rates to the RTZ of electron donors and electron acceptors were inadequate to support this production. Therefore, significant lateral intrusions of oxygen, mixing processes, or intensive cycling of C, S, N, Mn, and Fe across the RTZ are necessary to balance electron equivalents. Chemoautotrophic production appears to be decoupled temporally from short-term surface processes, such as seasonal upwelling and blooms, and potentially is more responsive to long-term changes in surface productivity and deep-water ventilation on interannual to decadal timescales. Findings suggest that midwater production of organic carbon may contribute a unique signature to the basin’s sediment record, thereby altering its paleoclimatological interpretation.

The permanently anoxic Cariaco Basin on the northern continental margin of Venezuela (Fig. 1) has been treated by oceanographers, paleoceanographers, and paleoceanologists as a natural sediment trap, recording climatic changes

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in the tropical Atlantic region over timescales varying from seasons to the last 12,000 yr (Hughen et al. 1996). The basin’s varved sediments consist of alternating layers of light biogenic debris (formed by upwelling-driven plankton blooms during the dry, windy season) and dark continental materials deposited during the wet season (Overpeck 1989; Peterson et al. 1991; Hughen et al. 1996). Relative thicknesses of biogenic layers are believed to reflect the intensity and duration of upwelling, its accompanying plankton blooms, and vertical export to the seabed. Stable isotope and biomarker signatures in sediments have been interpreted as further indicators of upwelling intensity, planktonic community structure, and trophic status (Werne et al. 2000). However, inferences from the sedimentary record are based on the assumptions that delivery of biogenic debris to the seabed is exclusively driven by surface processes and that source and decay terms are well known.

In open waters, deposition of biogenic debris is adequately understood as a function of depth, surface productivity, epipelagic community structure, and aerobic remineralization of material in transit (Pace et al. 1987: Michaels and Silver 1988; Taylor 1989). However, lateral advection of surface or intermediate waters in open systems and bioturbation by the
benthos may corrupt the sedimentary record's representation of overlying epipelagic processes. In contrast, the enclosed and stratified Cariaco Basin would appear to be an ideal site to examine the relationship between export flux and sediment deposition, because of the basin's limited lateral exchange and its anoxia. This large, tectonically formed basin (approximate volume below 180 m, $5.2 \times 10^{11}$ m$^3$) is 1,400 m deep, is surrounded by a sill (90–150 m in depth), and has remained anoxic below depths of 250–350 m for centuries (Richards 1975). In effect, its geomorphology confines lateral advection to the surface layer, and biogenic, locally generated debris primarily accumulates in the basin's sediments (Thunell et al. 2000). Thus, these sediments potentially represent excellent integrators of surface processes for the region. Furthermore, integrity of depositional layers is exceptionally good because of the system's physical quiescence and the absence of bioturbation caused by metazoan infauna.

Stratified water columns prone to anoxia have long been known to support multiple layers of biological production (Sorokin 1972; Indrebo et al. 1979; Jorgensen et al. 1979, 1991; Sorokin et al. 1995). Chemical gradients of electron donors and acceptors are established at depth by anaerobic mineralization of biogenic debris exported from the photic zone. Large and productive microbial communities at the oxic-anoxic interface capitalize on the residual chemical energy (H$_2$S, NH$_3$, CH$_4$, H$_2$, low-molecular-weight organics, etc.) emanating from anoxic waters. Enrichments in bacterial abundances, microbial adenosine triphosphate (ATP), protozoa, elemental cycling, and chemooautotrophic production have been reported for the oxic-anoxic interface of the Cariaco Basin and the Black Sea, the two largest examples of such systems (Sorokin 1972; Karl et al. 1977; Karl 1978; Hastings and Emerson 1988; Bird and Karl 1991). The extent to which activity in these layers is coupled to surface processes, the magnitude of energy recovery, and the contribution of this zone to a secondary, midwater flux of biogenic debris are unknown. These processes could have a significant impact on the sediment record and its interpretation.

The present investigation reports on midwater carbon-flux anomalies and the microbial processes that may be their source in the Cariaco Basin. In the past, this system has received sporadic study, but it recently (Nov 1995 to the present) has been the site of an intensive time-series program. The cooperative U.S.-Venezuelan Carbon Retention in a Colored Ocean (CARIACO) program consists of monthly Joint Global Ocean Flux Study–style productivity cruises and seasonal process cruises, continuous meteorological and remote sensing monitoring, and sediment flux measurements from an array of moored sediment traps. Results from this time series confirm that midwater enrichments in microbiological standing stocks and activities are a persistent feature in this dynamic system. Moreover, our observations suggest that bacterial chemooautotrophic production within the redox transition zone (RTZ) contributes significantly to overall biological productivity, governs elemental cycling across the oxic-anoxic interface, and may influence the chemical quality of the sedimentary flux.

Materials and methods

Site description and sampling—The CARIACO time-series station is located in the eastern subbasin of the Cariaco system (Fig. 1) in nearly 1,400 m of water (10.50°N, 64.66°W). All results presented are from this single site. Monthly sampling was conducted aboard the B/O Hermano Gines, operated by Estacion de Investigaciones Marinas, Fundacion la Salle de Ciencias Naturales, located on Margarita Island, Venezuela. During process cruises, conducted 2–3 times per year, water samples were collected at 18 depths with a SeaBird rosette accommodating 12 TFE (tetrafluoroethylene, Teflon)-lined, 8-liter Niskin bottles. For
hydrographic profiling, the rosette included a SeaBird conductivity, temperature, depth (CTD) probe, a Yellow Springs Instruments, Inc. oxygen probe, a Chelsea Instruments fluorometer for chlorophyll a estimates, and a Sea Tec c-beam transmissometer (660 nm). To resolve relatively narrow features, vertical sampling intervals were 10–20 m across the oxic-anoxic interface and greater than that in shallow and deep waters. Peaks in beam attenuation from the transmissometer were found to be reliable proxies for bacterial maxima near the interface, and sampling depths were adjusted accordingly, to resolve these features. Time, manpower, and the ship’s motion, however, constrained sampling resolution, so finer-scale features were sometimes missed. Samples were withdrawn from Niskin bottles under N₂ atmosphere, to prevent oxygenation of samples. All samples used for biological rate measurements were transferred from Niskin bottles to HCl-washed 1-liter TFE-stoppered glass bottles and sealed without head space after overflowing ~1–2 volumes. Samples for biological incubations were then dispensed under N₂ pressure from these 1-liter bottles into acid-washed, 40-ml septa vials (laminated TFE-butyl rubber septa; Pierce) or into 40-ml glass-stoppered bottles and sealed without head space after overflowing.

Microbial abundances and heterotrophic production—At each depth, whole-water samples (200 ml) were preserved with 2% (final concentration) borate-buffered formaldehyde and stored at 5°C. In the laboratory, standard DAPI (4’6-diamidino-2-phenylindole)-stained slides were prepared on dark 0.2 or 0.8 μm Poretics polycarbonate membranes for enumeration, by epifluorescence microscopy, of bacteria or flagellated protozoa, respectively (Porter and Feig 1980). Viral-like particles (VLPs) were enumerated by epifluorescence microscopy according to the methods of Noble and Fuhrman (1998).

Bacterial net production (BNP) was estimated from incorporation of ³H-leucine into protein, according to the methods of Kirchman (1993). At each depth, triplicate samples in 40-ml septa vials were immediately spiked with 50 μl of N₂-purged ³H-leucine [10 nM final concentration; L-(4,5-²H[N])-leu; 52 Ci mmol⁻¹] using a gas-tight syringe. Samples were incubated in on-deck water baths for 8–12 h and maintained at ambient temperature in darkness. Samples from the interface and below were incubated in sulfidic deep waters maintained at 17–19°C and shallow samples in surface water at 25–28°C. After incubation, samples were fixed with cold trichloroacetic acid (TCA; 5% final concentration) and refrigerated until processing immediately after the cruise according to the methods of Kirchman (1993). ³H-leucine incorporated into protein over time was corrected with filter blanks (T₀) from samples fixed with 5% TCA immediately after ³H-leucine was added. BNP was calculated using a conversion factor of 3.1 kg C mol⁻¹ of leucine incorporated (Kirchman 1993).

Acetate turnover—Acetate uptake rates were estimated by calculating rate constants of ¹⁴C-acetate incorporation and respiration and multiplying their sum by ambient acetate concentrations (Wright and Hobbie 1966; Hobbie and Crawford 1969). Acetate uptake rate constants were determined by immediately spiking samples in septa vials (as described above) with 100 μl of N₂-purged ¹⁴C-acetate (<1 nM final concentration; 100 Ci mmol⁻¹, uniformly labeled). During time course (0–12 h), individual vials were sacrificed, and duplicate subsamples (5 ml) were collected on 0.2 μm Nuclepore polycarbonate membranes, to measure incorporation. The remaining sample was alkalized with 0.5 ml 10N KOH, to kill biota and minimize ¹³CO₂ loss. Respired ¹³C-acetate was measured later in the lab by acidification of the alkalized sample in a closed flask containing a suspended filter soaked in 2N KOH. Filters and ¹³CO₂ traps were radioassayed in Optiflour scintillation liquid cocktail. Rate constants were calculated as the sums of the slopes of incorporated and respired ¹³C plotted against time. This approach corrects for passive sorption of ¹³C-acetate to particles or membranes, background ¹⁴CO₂ in the spike, and any volatilization of ¹¹C-acetate during trapping of ¹³CO₂, which should be greatest at the initiation of experiments.

Immediately after hydrocasts (usually within 30 min), samples (250 ml) for ambient acetate concentration were passed through 0.2-μm Nuclepore membranes at moderate (<200 mm Hg) vacuum pressure. To stop microbial activity, 0.5 ml of 10N KOH was added to each filtered sample. Acetate and other low-molecular-weight fatty acids were preconcentrated by use of a static diffusion technique (Yang et al. 1993). Concentrations were corrected for recovery efficiency, which was determined by addition of a ¹³C-acetate internal standard, and for reagent blanks. Preconcentrated samples were analyzed with gas chromatography by using an HP FFAP 530μm bore fused silica column and FID (flame ionization detection) (Hordijk et al. 1990).

Vertical carbon flux—A sediment trap mooring was located in the deepest portion of the eastern basin (~1,400 m) and consisted of four automated traps positioned at depths of ~275, 455, 930, and 1,255 m (Thunell et al. 1999). Traps have a 0.5-m² opening at the top and 13 collection cups at the bottom, each programmed to sequentially collect samples over 2-week intervals. Prior to deployment, collection cups were filled with buffered formalin (2%) in filtered seawater, to preserve accumulating organic matter. Upon retrieval, collection cups were sealed and refrigerated. Particulate organic carbon concentrations were measured in a Perkin-Elmer 2400 Elemental Analyzer according to the methods of Foeleich (1980).

Dark carbon assimilation—Chemoautotrophic assimilation of inorganic carbon was measured by ¹³C-bicarbonate incorporation into particles. After dispensing samples into 40-ml ground glass–stoppered bottles, 200 μl of chilled N₂-purged ¹³C-bicarbonate in an alkaline brine (pH 9.5; S = 60 on the practical salinity scale) was injected into the bottom before sealing (Tuttle and Jannasch 1973a). Samples were incubated in parallel with BNP and ¹³C-acetate turnover samples for 14–20 h. Time-course experiments showed rates were linear up to 30 h (not presented). Particles were collected on 0.22 μm cellulose membranes (Osmonics), which were then rinsed twice with 5 ml of filtered seawater. Filters were purged of unassimilated ¹³C in a saturated HCl atmosphere for >1 h, then dried and suspended in Hionic-Fluor
scintillation cocktail and radioassayed. Data were corrected for isotopic fractionation (×1.06) and for nonbiological sorption by use of samples processed immediately after introduction of the radiotracer. Rates of dark $^{14}$C-assimilation were normalized to $\mu$M C d$^{-1}$ by use of values of dissolved inorganic carbon (DIC) derived from pH, temperature, and alkalinity measurements (courtesy of Richard Bohrer, USF).

Primary production—Photosynthetic assimilation of inorganic carbon was measured by standard $^{14}$C-bicarbonate protocols (UNESCO 1994). Samples were routinely collected from 1, 7, 15, 25, 35, 55, 75, and 100 m before dawn. Subsamples were dispensed under subdued light into 300-ml polycarbonate bottles—one dark and three transparent. Each bottle was spiked with 3.2–3.8 $\mu$Ci of $^{14}$C-bicarbonate and sealed. Bottles were deployed at ~0700 h local time on a buoyed array at their collection depth for a 4-h exposures to ambient light fields, equivalent to 33% of total daily irradiance at this latitude. After recovery, samples were filtered through Whatman GF/F filters, which were rinsed with 0.25 ml of 0.48N HCl, placed in scintillation vials, and radioassayed in Cytoscient scintillant. Data were corrected for isotopic fractionation (×1.06) and dark assimilation. Daily photosynthetic rates were estimated from measured hourly rates, photoperiod, and DIC concentrations (UNESCO 1994).

Dissolved inorganic chemical species—In addition to continuous dissolved O$_2$ concentration profiles obtained from the rosette’s YSI electrode, O$_2$ in discrete samples was measured by standard Winkler titrations after samples were fixed in the field (Aminot 1983). Samples for H$_2$S were collected by syringe, avoiding atmospheric contact, and immediately transferred to vials containing zinc acetate or zinc chloride, to form ZnS precipitate. ZnS was derivatized and measured colorimetrically according to the methods of Cline (1969). NO$_2^-$, NO$_3^-$, and NH$_4^+$ concentrations were determined, by standard colorimetric methods, from frozen samples (Strickland and Parsons 1972). Mn and Fe were measured by graphite furnace atomic absorption spectrometry in filtered (0.2 $\mu$m) and unfiltered acidified samples to calculate particulate (presumably mostly oxidized) and dissolved (presumably mostly reduced) metal by difference (Balistrieri et al. 1992).

Results and discussion

Anomalies in vertical fluxes of biogenic debris—In open ocean and continental margin systems, carbon fluxes generally can be described by a power function of the form $f = b_0Z^{-b_1NPP^{b_2}}$, where $f$ is the carbon flux (g C m$^{-2}$ d$^{-1}$), $b_0$, $b_1$, and $b_2$ are empirically derived coefficients, Z is depth (in meters), and NPP is the net primary production (g C m$^{-2}$ d$^{-1}$) (Pace et al. 1987) (curves in Fig. 2a). Vertical profiles of carbon fluxes at the CARIACO time-series station usually conform to these open-water models, and the hatched bars in Fig. 2a serve as an example. However, anomalies in sedimentation of biogenic debris are common, whereby carbon fluxes to the 455-m sediment trap exceed those to the 275-m trap by a significant margin, as exemplified by the solid bars in Fig. 2a. To illustrate the central tendencies and anomalies in depth-dependent decay, carbon fluxes to 455, 930, and 1,255 m were normalized by contemporaneous fluxes to 275 m for data collected from 8 Nov 95 to 24 Apr 99 ($n$ = 71). Compare these data with curves derived from four published models, for open meso- to oligotrophic waters (Suess 1980; Betzler et al. 1984; Pace et al. 1987; Taylor and Karl 1991) (Fig. 2b). These models predict that fluxes to 455, 930, and 1,255 m should decrease to between 62% and 75%, 32% and 50%, and 24% and 43% of fluxes to 275 m, respectively.

The median values of our observations (vertical lines within box plots, Fig. 2b) agree well with predictions, demonstrating that, contrary to Demaison and Moore’s (1980) assertion, decomposition in anoxic systems is not necessarily slower than that in oxic systems. Relatively high bottom temperatures in the Cariaco Basin (>17°C) may compensate to some extent for the absence of oxygen. Being temperature dependent, bacterial metabolism increases by factors of 2–3 for every 10°C increment ($Q_{10}$) until maximal rates are reached. Therefore, anaerobes in the Basin are expected to remineralize carbon at least twice as fast as anaerobes at comparable depths outside the basin, where temperatures are ~5°C. In fact, the mean (solid profile, Fig. 2b) and ~75% of all observations fall within the uncertainty of existing models’ predictions (boxes, Fig. 2b). However, fluxes to 455 m exceeded fluxes to 275 m in 16 of the 71 observations, sometimes doubling between these depths (circles, Fig. 2b). These anomalies illustrate that vertical fluxes of biogenic debris leaving the RTZ may exceed export from surface waters (~275 m). A similar anomaly was suggested by a single set of observations in the Black Sea (Karl and Knauer 1991). The three most plausible mechanisms that could produce these observations (vertical migrators, lateral advection, and in situ production) are explored below.

Vertical migrators—Vertically migrating mesozooplankton and nekton feeding in surface waters, then defecating, molting, or dying below 275 m would enrich observed fluxes to 455-m traps. However, significant activity of metazoans, especially vigorous swimmers, is unexpected in this sulfidic system. For example, Vinogradov et al. (1985) found that O$_2$ concentrations of 18–22 $\mu$M in the Black Sea represented the lower boundary for vertical distributions of a variety of zooplankton, including copepods, ctenophores, and chaetognaths. O$_2$ concentrations usually fall below 20 $\mu$M near 200 m in the Cariaco Basin, which is well above the shallowest sediment trap. Nonetheless, diel vertical migrations of the Gadiformes fish, Bregmaceros nectabantus, across the Cariaco Basin’s O$_2$-H$_2$S interface to depths of 800 m have been documented (Baird et al. 1973). During the CARIACO time series, the ship’s fish finder repeatedly recorded an acoustic scattering layer that disappeared from surface waters at dawn, descended to depths >600 m by midday, and returned to surface waters after dusk (CARIACO program, unpubl. data). Presumably, the migrating scattering layer is caused by nekton or mesozooplankton. However, the identity of these populations has not been confirmed, and their abundance and behavior at depth remain unexamined. Recognizable feces or remnants from mesozooplankton or fish have
never been observed in sediment trap materials below 275 m, so the impact of vertical migrators to flux anomalies is not immediately evident.

Lateral advection—A second possibility is that lateral advection may cause sedimentation anomalies. Lateral advection of water could bias sediment-trap observations by two independent mechanisms. If current speeds exceed a threshold value characteristic of the sediment trap’s design, then traps would undersample as a result of internal turbulence and resuspension of material from the cone (Baker et al. 1988). If currents at 275 m sporadically exceed this undefined threshold, then traps at this depth would collect at lower efficiencies than traps at 455 m and below, which presumably reside in quiescent water. This would result in an apparent flux anomaly. However, currents sufficient to generate turbulence have not been documented below sill depth, and hydrographic profiles are inconsistent with strong currents.

Lateral advection of biogenic debris from a more productive region to depths below 275 m would also produce flux anomalies. The extent of lateral intrusions of water masses below 275 m is constrained by a sill whose mean depth is 100 m (Richards 1975), and waters must sink at least 175 m prior to arriving at our station. Distributions of physical properties, salinity, temperature, and water density, do not provide evidence of intrusions, being nearly homogenous below 200 m for most of our monthly observations. For example, on the 7 Jul 98 (CAR-32) cruise, increased monotonically from 26.390 to 26.446 between 200 and 455 m (Fig. 3a). However, water outside the basin is oxic at all depths, and if it were to spill over the sill and sink along isopycnals, then an oxygen anomaly would be predicted. Shallow O₂ anomalies (<200 m) are quite common (see Fig. 3b) and are evidence of lateral intrusions. Occasionally, such O₂ anomalies have been observed as deep as 340 m (Scranton et al. in press), but this is atypical, and their timing does not correspond with vertical flux anomalies (discussed below). Typically, dissolved O₂ distributions immediately above the interface (250–350 m) are smooth, as if they are controlled by diffusive supply from above and in situ consumption. Occasional intrusions of particle-rich waters may contribute to the basin sediment budget along the margin, but loadings of particles apt to sink in waters passing over our sediment traps are probably quite attenuated in comparison with near the sill, >50 km away. We conclude that the likelihood of lateral transport of biogenic debris contributing to detectable flux anomalies is remote.

In situ microbial production—The third possible source of flux anomalies is midwater microbiological production.
At least one peak in the transmissometer’s beam attenuation, an indication of high particle concentrations, is typically present in the vicinity of the \( \text{O}_2 \)-H\(_2\)S interface (see Fig. 3b). Given the uniformity of water density in this zone, accumulation of settling particles along weak isopycnals is highly improbable. A more plausible explanation is that discrete particle layers are formed by in situ microbiological activity in response to chemical gradients or formed by transformations of redox-sensitive elements, such as Mn and Fe, which precipitate when oxidized (Tuttle and Jannasch 1973a; Neelson and Myers 1992). We have repeatedly observed elevated concentrations of bacteria and high heterotrophic activity at depths where H\(_2\)S first appears. During all cruises analyzed to date, maxima in concentrations of not only bacteria, but also VLPs and protozoans (flagellates and ciliates), are observed in the vicinity of the \( \text{O}_2 \)-H\(_2\)S interface.

Frequently, as exemplified by observations from CAR-32 (Fig. 4a), two or more peaks in microbial biomass occur immediately above and below the interface. Peaks in microbial biomass below the interface, including bacteria, VLPs and flagellates, correspond to the deep particle maximum detected by the transissimeter (Fig. 3b). Biomass in these secondary peaks is always within the same order of magnitude as in the primary maximum of the photic zone (\( \leq 60 \) m). Furthermore, these peaks almost always occur at depths between the shallowest two sediment traps (275 and 455 m). This distribution of microbiological biomass argues against the use of strictly one-dimensional vertical models of carbon flux with depth, in which export production from the photic zone is progressively decomposed (Fig. 2a) and becomes increasingly energy depleted and, hence, capable of supporting diminishing biomasses of heterotrophs with depth (Cho and Azam 1988; Karl et al. 1988). Secondary maxima in BNP and acetate uptake have been consistently observed within the RTZ on 11 cruises, residing below a zone of low activity in intermediate waters (100–250 m), as exemplified by CAR-32 (Fig. 4b). Concordant with biomass distributions, heterotrophic activity profiles strongly suggest a midwater source of labile organic matter that supports an active microbial food web, complete with bacterivores and viral pathogens.

**Chemoautotrophic production**—The preceding observations demonstrate that, unlike the layers immediately above and below, the RTZ maintains a prolific microbial food web. We propose that chemoautotrophic production is the foundation of this food web, providing new labile organic matter. In this zone of the Cariaco Basin and Black Sea, significant rates of dark DIC assimilation and H\(_2\)S oxidation have been reported elsewhere (Sorokin 1972; Tuttle and Jannasch...
In the aphotic zone, DCA can not be ascribed exclusively to chemosynthetic bacteria, because it is the sum of chemosynthetic and anaplerotic reactions by heterotrophs and can be expressed as, DCA = BAP + aBHP, where BAP is bacterial autotrophic production, a is proportion of DIC in total heterotrophic C incorporation, and BHP is bacterial heterotrophic production (Karl and Knauper 1991). The proportion of total heterotrophic production supported by DIC assimilation (a) through tricarboxylic acid cycle–associated pathways (via phosphoenolpyruvate carboxylase) is growth-dependent, varying from 0.04 to 0.08 over the range of growth rates relevant to this study (Li 1982). If most of the DCA were attributable to heterotrophy, as suggested by Morris et al. (1985) for the Cariaco Basin, then bacterial production within the RTZ during our study would be in the range of 338–1988 mmol C m⁻² d⁻¹ or equivalent to 510%–1,160% of overlying primary production.

Such disparities could only be sustained if large amounts of allochthonous organic matter were continuously advected horizontally into the system below 250 m. However, CARIACO sediment-trap samples show little evidence of allochthonous input of organic matter (Thunell et al. 2000). Parallel measurements of heterotrophic BNP during the CARIACO time series demonstrate that, even if \( a = 0.08 \), the mean aBHP only amounts to 2.2% of total DCA. Therefore, we conclude that >97% of DCA below 250 m can reasonably be ascribed to chemosynthetic bacteria whose metabolism is presumably fueled by upward fluxes of inorganic reductants, such as reduced sulfur species and possibly \( \text{NH}_4^+ \), from anoxic waters.

Depth distributions and rates of chemosynthetic activity suggest that this process is central to the microbial ecology of waters below the \( \text{O}_2 - \text{H}_2 \text{S} \) interface. The depth of maximum chemosynthetic activity occurred 10–30 m below secondary maxima in total bacterial abundance and BNP in all observations (Figs. 4 and 5). However, peaks for microbial abundance and heterotrophic activity always overlapped with those of chemosynthetic activity. To illustrate che-
Fig. 5. Example of vertical profiles for contemporaneous measurements of 14C-bicarbonate assimilation rates in light (photosynthesis) and dark (chemoautotrophic + anaplerotic reactions) incubations of samples from Sta. CARIACO. Dark carbon assimilation and primary production were measured on 7 and 9 Jul 98 (CAR-32), respectively. Presented as means of triplicate incubations ±1 SD. In many instances, errors of measurement are no wider than symbols. Profiles of O2 and H2S concentrations illustrate redox gradients. Horizontal dotted line defines the oxic-anoxic interface.

Chemoautotrophy’s importance in local microbiological production, heterotrophic bacterial production, integrated from 310 to 410 m, only amounted to 2.8, compared with 123.2 mmol C m⁻² d⁻¹ produced autotrophically over the same depth interval in July 1998. Commonly, peaks in chemoautotrophy coincided with those of flagellated protozoans and VLPs (Figs. 4a and 5). Elevated concentrations of predators and pathogens can only be sustained if the prey/hosts are actively growing. Therefore, we infer that chemoautotrophy leads to elevated bacterial production locally, thereby stimulating trophic transfer of newly assimilated DIC to predators and pathogens and to heterotrophic bacterioplankton through mortality processes. The degree of coupling between these processes and secondary vertical export to the sediments is unknown but could be significant.

**Temporal variability in DIC assimilation**—Although DCA is a persistent feature below the interface, its magnitude and depth distribution varied significantly over time (Fig. 6). Chemoautotrophy is usually defined by a single peak, 60–100 m wide, but on two occasions two peaks were evident, 10 Mar 98 (CAR-29) and 7 Nov 98 (CAR-36). The shallower peak observed during CAR-29 at 255 m may indicate chemoautotrophic activity of nitrifying bacteria (discussed below). The split peak observed during CAR-36 may represent a remnant feature created by lateral intrusions of oxygenated water or by a mixing event. In most cases, the depth of maximum DCA was below the O₂-H₂S interface, which has migrated on the order of 75 m in response to physical forcing during the CARIACO program (Fig. 6).

Carbon assimilation within the chemoautotrophic layer varied between 27 and 159 mmol C m⁻² d⁻¹, compared with contemporaneous measurements of primary production, varying from 29 to 392 mmol C m⁻² d⁻¹ (Fig. 7). The rates of net primary production varied by a factor of 13.5, which is indicative of strong seasonal differences between upwelling (Jan–May) and nonupwelling (Jun–Dec) seasons. In contrast, rates of chemoautotrophic production only varied by a factor of 5.9 during the same sampling period (Fig. 7). Averaged over the observation period, chemoautotrophic production accounts for 62% of photoautotrophic production; 82 versus 120 mmol C m⁻² d⁻¹. No statistically significant relationship between surface and midwater autotrophy is apparent from existing data. Nor can we correlate variations in chemoautotrophy with vertical flux anomalies (Fig. 7). The unreasonably high ratio of chemo- to photoautotrophy and the absence of any correlation between these two processes or sedimentation anomalies underscores two facts—the basin is not in steady state (Scranton et al. 1987; Holmen and Rooth 1990; Zhang and Miller 1993), and our temporal sampling was insufficient to fully resolve amplitudes of variances in both forms of production. We also note that observed intrusions of water (Fig. 7) do not correlate with vertical flux anomalies. Therefore, horizontal advection below sill depth does not appear to directly contribute to the enrichments in particles observed in the RTZ. Furthermore, effects of upwelling and its relaxation on chemoautotrophy are not evident from our observations.

Our chemoautotrophic production estimates were equivalent to between 10% and 33% of contemporaneous measurements of primary production, compared with 10%–32% in the Black Sea in 1988 (Jørgensen et al. 1991; Karl and Knauer 1991) and 17%–58% in the Caribco in 1973 (Tuttle and Jannasch 1979). However, simultaneous measurements of primary production were performed only by Karl and Knauer (1991) and Jørgensen et al. (1991), whereas Tuttle and Jannasch (1979) and Sorokin et al. (1995) based their comparisons on historical measurements and indirect estimates that do not account for temporal variability.

We expect that maximum rates of chemoautotrophy would be higher than those reported by Tuttle and Jannasch (1979) (0.23 μM C d⁻¹ or 11–18 mmol C m⁻² d⁻¹), because H₂S concentrations in Caribco bottom waters, which presumably fuel chemoautotrophic processes, were lower in 1973 than they are at present (30 vs. ≤ 76 μM). Furthermore, although present and past Caribco studies share the same experimental protocols, we used 0.22-μm filters rather than the 0.45-μm filters used by Tuttle and Jannasch (1979) to assay assimilated ¹⁴C. The 0.45-μm filters probably captured fewer la-
dark DIC assimilation (μM C d\(^{-1}\))

![Graph showing dark DIC assimilation over time with depth](image)

**Fig. 6.** Temporal variability of dark DIC assimilation (dca) rates in the chemosynthetic layer and distributions of \(O_2\) (electrode data) and \(H_2S\) (discrete samples) at Sta. CARIACO. Mean and 1 SD of triplicate incubations presented for dark DIC assimilation. In many instances, errors of measurement are no wider than symbols. \(H_2S\) data not available for CAR-13, 9 Nov 96 = CAR-13, 8 May 97 = CAR-19, 14 Nov 97 = CAR-25, 10 Mar 98 = CAR-29, 7 Jul 98 = CAR-32, 7 Nov 98 = CAR-36, and 7 May 99 = CAR-42.

**Fig. 7.** Temporal variability in areal net primary and chemosynthetic production and carbon export from RTZ at Sta. CARIACO from Jun 1996 to May 1999. Production values represent 14C-incorporation into >0.22 μm particles in light and dark incubations, integrated over eight depths in the upper 100 m (photic zone usually ≤55 m) and over 5–7 depths in the upper 60–100 m of the RTZ. Carbon export from RTZ expressed as organic carbon fluxes to 455 m normalized to contemporaneous measurements at 275 m. Values ≥1 are considered to be flux anomalies. Solid triangles are dates of known midwater intrusions over the sill (Y. Astor pers. comm.).

beled cells. It is also possible that they did not sample the zone of highest activity because of the lower vertical resolution of their sampling.

**Comparisons to the Black Sea**—Maximum chemosynthetic rates for the Black Sea (0.32–1.50 μM C d\(^{-1}\)) are similar to those we measured in the Cariaco Basin (Jorgensen et al. 1991; Karl and Knauser 1991; Sorokin et al. 1995), with the lowest rates being reported for the 1988 expedition, when a wide suboxic zone (\(O_2\)- and \(H_2S\)-free) replaced an interface where \(O_2\) and \(H_2S\) overlap. Integrated chemosynthetic production reported for the Black Sea varied from 2 to 27 mmol C m\(^{-2}\) d\(^{-1}\), depending on the station and depth intervals included (14–90 m).

Lower areal chemosynthetic production rates are expected for the Black Sea than for the Cariaco. Even though \(H_2S\) concentrations in the Black Sea are ~7 times greater than those in the Cariaco Basin (Table 1), the zone of dark DIC assimilation is usually ≤40 m thick in the former, compared with 60–100 m thick in the Cariaco Basin. The relative narrowness of the RTZ in the Black Sea (45–95 m in 1988) and steepness in the \(H_2S\) gradients results from a much higher degree of thermohaline stratification. Sulfide gradients in the Black Sea are 5–6 times steeper, and
Table 1. Comparison of variables relevant to chemosynthesis in the Black Sea and Cariaco Basin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Black Sea</th>
<th>Cariaco Basin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum C-assimilation rate (µM C d⁻¹)</td>
<td>0.32–1.50</td>
<td>0.40–2.52</td>
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<td>Chemoautotrophic zone (m)</td>
<td>14–90</td>
<td>80–100</td>
</tr>
<tr>
<td>Areal C-assimilation rate (mmol C m⁻² d⁻¹)</td>
<td>2–27</td>
<td>26–157</td>
</tr>
<tr>
<td>Maximum H₂S concentration (µM)</td>
<td>~550</td>
<td>76</td>
</tr>
<tr>
<td>H₂S gradient (µM m⁻¹)</td>
<td>0.51–0.83</td>
<td>0.08–0.18</td>
</tr>
<tr>
<td>Density gradient (Δσ, m⁻² × 10⁶)</td>
<td>8.80–13.30</td>
<td>0.15–0.32</td>
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<tr>
<td>Diffusive H₂S flux (mmol m⁻² d⁻¹)</td>
<td>0.61–0.99</td>
<td>0.71–1.35</td>
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<td>H₂S required (mmol m⁻² d⁻¹)</td>
<td>14–189</td>
<td>186–1101</td>
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<tr>
<td>O₂ flux (mmol m⁻² d⁻¹)</td>
<td>ND</td>
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<tr>
<td>O₃ required (mmol m⁻² d⁻¹)</td>
<td>2–27</td>
<td>26–157</td>
</tr>
<tr>
<td>NO₃ flux (mmol m⁻² d⁻¹)</td>
<td>0.12–0.28</td>
<td>0.37–1.59</td>
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<td>NO₃ required (mmol m⁻² d⁻¹)</td>
<td>22–302</td>
<td>297–1762</td>
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</table>

* Unless otherwise noted, data from Jørgensen et al. (1991) and Sorokin et al. (1995) were used for the Black Sea, and data from this study were used for the Cariaco Basin.
† Karl (1978).
‡ Scraton et al. (in press).
§ Flux estimated as product of concentration gradient and vertical eddy diffusivity, F = K₂ ΔC/Δz (Scraton et al. 1987).
∥ Assumes yield of 0.14 mol CO₂ assimilated per mol H₂S oxidized based on field observations of Jørgensen et al. (1991) and laboratory studies of Kelly (1989).
ND, not determined.
# Theoretical stoichiometry of 1C:1O₂ for aerobic chemosynthesis.
†† Estimated from Murray et al. (1995).
‡‡ Assumes 1.6 mol NO₃ required for complete oxidation of H₂S to SO₄²⁻ through dissimilatory denitrification, 2 NO₃⁻ + 10e⁻ → N₂.

Water density gradients (8.8–13.3 Δσ, m⁻² × 10⁶) are 42–59 times greater than comparable zones in the Cariaco Basin (Table 1). Density gradients calculated between 200 and 500 m at Sta. CARIACO only varied from 0.15 to 0.32 Δσ, m⁻² × 10⁶, indicative of comparatively subtle changes in water density (Table 1). The relative physical homogeneity of the Cariaco’s anoxic zone presents a considerably smaller barrier to vertical diffusive and mixing processes. Therefore, higher fluxes of H₂S and O₂ to the interface, and broader features are expected for the Cariaco Basin. When vertical eddy diffusivity, K₂, were the only operative transport process and the sediments (>1 km from the interface) are the primary H₂S source (Scraton et al. 1987), then H₂S fluxes estimated as the product of the concentration gradient and K₂ would be slightly higher for the Cariaco Basin than it is for the Black Sea; 0.71–1.35 and 0.61–0.99 mmol m⁻² d⁻¹, respectively (Table 1). Trends in H₂S diffusive fluxes in both basins are consistent with observed rates of DIC assimilation. However, diffusive fluxes alone are insufficient to explain observations from either basin (Murray et al. 1995; this study).

Flux balance conundrum—In order for chemical redox reactions to proceed, the supply of electron donors must balance that of electron acceptors. In the Cariaco Basin, however, we have been unable to balance fluxes of electron donors and acceptors (H₂S, O₂, and NO₃⁻) in electron equivalents with DIC assimilation rates on the basis of observed chemical gradients, estimates of vertical eddy diffusion, and typical stoichiometries. Measured rates of DIC assimilation surpass estimated rates of supply of electron donors and acceptors to the RTZ by a significant margin. This has been the case for studies of a number of other anoxic basins (Tuttle and Jannasch 1979; Jørgensen et al. 1991; Murray et al. 1995). Jørgensen et al. (1991) suggested that 7–9 mol of H₂S are required for mixed chemosynthetic communities to assimilate 1 mol of CO₂ under field conditions. Culture studies with several species of marine sulfide oxidizers report that between 2.4 and 7.2 mol of H₂S are required to assimilate 1 mol of CO₂ by chemolithotrophy, with demand for reductant depending on species and environmental conditions (Tuttle and Jannasch 1977; Kelly 1989). When a molar requirement of 7 is used, fluxes of H₂S into the Cariaco’s RTZ only account for between 0.1%–0.4% of the chemosynthetic demand (Table 1). The flux imbalance is only slightly smaller in the Black Sea, where the supply of H₂S accounts for 0.5%–4.4% of the demand. Even under the assumption of the theoretical maximum efficiency (where stoichiometry is 1H₂S : 1CO₂ : 1O₂ for aerobic sulfide oxidation), the Cariaco’s flux balance is improved only minimally, with diffusive H₂S fluxes accounting for 0.7%–2.8% of the demand for reductant.

Aerobic sulfide oxidation is constrained by low O₂, diffusive fluxes to the interface, potentially accounting for 2.5%–4.3% of estimated demand in the Cariaco Basin (Table 1). Nitrate can also serve as an electron acceptor for denitrifying sulfide oxidizers, like Thiomicrospora denitrificans, Thioploca spp. or Beggiatoua spp. (McHatton et al. 1996; Jørgensen and Gallardo 1999) and typically penetrates deeper in the water column than O₂. However, eddy diffusion can only deliver on the order of 0.4–1.6 mmol NO₃⁻ m⁻² d⁻¹ to the interface, satisfying an additional 0.1% of the estimated demand for oxidant (Table 1). Even under the assumption of reduction of NO₃⁻ to NH₄⁺, as has been suggested for Thioploca communities (Farias 1998), and ideal stoichiometry, 1H₂S : 1CO₂ : 1NO₃⁻, diffusive fluxes of NO₃⁻ could only supply 1.0%–1.4% of the demand for oxidant. Similar analyses for the Black Sea suggest that NO₃⁻ supplies 0.1%–0.6% of the oxidant demand by diffusion (Table 1). Clearly, diffusive fluxes of electron donors and acceptors are insufficient to balance observed productivity across the interface in either anoxic basin.

Adveective processes—One problem with the preceding estimates is that horizontal and vertical advectional processes in the Cariaco Basin are ignored, mainly because they are so poorly understood. Although lateral intrusions are probably not energetic enough to import biogenic debris into the central basin as discussed above, they do import dissolved (e.g., O₂), and probably colloidal, materials. O₂ anomalies, which are indicative of lateral intrusions, were detected between 225 and 330 m on 8 May 97, 14 Nov 97, 10 Mar 98, and 7 Jul 98, four dates when dark carbon fixation was measured (Fig. 6). Horizontal advection would deliver O₂ to the interface at much higher rates than vertical eddy diffusion. Unfortunately, the magnitude, frequency, and velocity of these intrusions is unknown, because appropriate physical measurements are lacking. The fact that O₂ remains after the water transits from the margin to the eastern basin’s center,
Fig. 8. Vertical profiles of oxidized and reduced forms of nitrogen (a), manganese (b), and iron (c) at Sta. CARIACO on 7 Jul 98 (CAR-32). Although undetermined experimentally, the redox state of particulate Mn and Fe (p-Mn and p-Fe) is assumed to be mostly oxidized above the interface and dissolved to be mostly reduced. Dotted line represents oxic-anoxic interface in all panels.

despite oxygen utilization by chemical and biological reactions in transit, argues that lateral advective fluxes could be relatively large. Whether advective $O_2$ fluxes are sufficient to balance requirements for oxidant remains to be demonstrated and will depend on the relative demands of aerobic heterotrophy, nitrification, abiotic oxidation of redox-sensitive elements (S, Mn, and Fe), and chemosyntheticotrophy.

The ultimate energy source ($e^-$ donor) for observed chemosyntheticotrophy most likely is $H_2S$, whether by direct utilization or through intermediate oxidation products ($S^0$, $S_2O_3^{2-}$, or $SO_4^{2-}$) not measured in this study. As demonstrated above, vertical diffusive fluxes of $H_2S$ are insufficient to meet demand, so advective processes or intensive cycling of redox pairs must play an important role in meeting this demand, as has been suggested for the Black Sea (Lewis and Landing 1991; Murray et al. 1995). Density ($\sigma$) profiles and T-S diagrams clearly illustrate that water in the basin is relatively homogenous below 200 m (Fig 3a). Therefore, only minimal energy needs to be applied to induce vertical mixing. Mechanisms that would provide that energy, however, are not immediately obvious. The surface mixed layer and pycnocline are quite shallow, usually <50 and 120 m, respectively, so wind forcing at the interface is unlikely. Sporadic turbidity flows created by mass wasting of unstable sediments on the basin’s walls would cause mixing and upward displacement of water. This process can be catalyzed by seismic activity (Thunell et al. 1999) but may also be induced by other processes, such as gravity flows when gravitational forces exceed the cohesive forces of sediments collecting on inclined surfaces. Lateral intrusions above the $O_2$–$H_2S$ interface could contribute to deep convective circulation driven by interfacial stress. This could entrain deeper sulfidic waters from the basin’s leading margin and advect it into the basin’s interior beneath the interface. Such intrusions could also contribute to shear stress–driven vertical mixing. Both processes (advection and mixing) associated with intrusions could deliver $H_2S$ to the interface far more rapidly than back- ground eddy diffusion. The frequency and areal extent of these advective events is unknown. A more elaborate evaluation of mixing terms is premature, given existing data.

**Elemental cycling**—One of the surprising features of the chemosyntheticotrophic activity in the Cariaco Basin is that the vast majority of DCA occurs in regions lacking detectable $O_2$, which implies that bacterial populations use terminal electron acceptors other than $O_2$. Previous field observations suggest that chemosyntheticotrophy in the absence of $O_2$ is common in stratified anoxic basins, such as the Cariaco Basin and the Black Sea (Tuttle and Jannasch 1973a, 1979; Jørgensen et al. 1991; Karl and Knauer 1991; Sorokin et al. 1995). In our study, peak chemosyntheticotrophic production coincided with the disappearance of $NO_3^-$ and particulate Mn and Fe, as well as enrichments of dissolved $Mn^{2+}$ and $Fe^{2+}$ (Fig. 8). These distributions suggest that denitrifying and metal-reducing sulfide-oxidizing populations may be important in DIC assimilation. Anaerobic chemosyntheticotrophs that oxidize $H_2S$ and $S_2O_3^{2-}$ at the expense of $NO_3^-$ have been isolated from the Cariaco Basin, the Black Sea, and suboxic mat communities dominated by *Beggiaota*, *Thioploca*, or *Thiomargarita* spp. in upwelling regions (Tuttle and Jannasch 1973a; McHatton et al. 1996; Jørgensen and Gallardo 1999; Schulz et al. 1999). As described above, the estimated
fluxes of $\text{NO}_3^-$ appear to be insufficient to support high rates of denitrification below the $\text{O}_2$-$\text{H}_2\text{S}$ interface.

Distributions of $\text{NH}_4^+$, $\text{NO}_3^-$, and $\text{NO}_2^-$ suggest that dissimilatory nitrogen cycling is consistently operative in two layers in the Cariaco Basin (Fig. 8a) and may account for some portion of observed chemosynthesis. In all profiles analyzed ($n = 5$), nitrogen speciation suggests that nitrifying bacteria are active between 25 and 75 m, where slight enhancements of DCA are also evident (not presented). The shallow $\text{NO}_3^-$ peak (Fig. 8a) may be indicative of $\text{NH}_4^+$ oxidation in this layer, where $\text{NH}_4^+$ concentrations generally varied from 0.1 to 0.7 $\mu$M. Subsequent in situ oxidation of $\text{NO}_2^-$ contributes to $\text{NO}_3^-$ accumulation in waters from 25 to 200 m, and phytoplankton assimilation accounts for total depletion in shallower waters. A nitrifying community may also reside slightly above the $\text{O}_2$-$\text{H}_2\text{S}$ interface, where trace concentrations of $\text{O}_2$ are available and a strong $\text{NH}_4^+$ gradient exists. For example, on 10 Mar 98 the upper peak in DCA at 255 m (Fig. 6) corresponds to a peak in $\text{NO}_3^-$ (0.1 $\mu$M) and $\text{NH}_4^+$, $\text{NO}_2^-$, and $\text{O}_2$ concentrations of 0.2, 4.5, and $\sim 10$ $\mu$M, respectively, whereas the deeper peak occurred in sulfidic waters devoid of $\text{NO}_2^-$, $\text{NO}_3^-$, or $\text{O}_2$ (only $\text{O}_2$ data are presented). Nitrifiers are expected only in sulfide-free zones, because these strictly aerobic chemosynthetic organisms are strongly inhibited by $\text{H}_2\text{S}$ (B. B. Ward pers. comm.).

The sinking of particulate oxides across the interface may be an alternative transport mechanism to eddy diffusion and lateral advection. Although concentrations are relatively low (10–600 nM), distributions of Mn and Fe are consistent with the hypothesis that these metals serve as “redox shuttles.” As applied to metal-respiring heterotrophs in the Black Sea by Nealon and Myers (1992), dissolved and reduced metals diffuse up to oxic waters and rapidly oxidize, abiotically forming colloids and particulates (oxides and oxyhydroxides). Newly-formed particulates sink back into the RTZ and are biologically reduced through dissimilatory respiration by bacteria, then diffuse back up to the interface for reoxidation. This mechanism permits deeper and faster penetration of oxidant into the RTZ and repetitive cycling of the same redox pairs. For example, particulate phases of Mn and Fe in the Cariaco Basin are most abundant in oxic waters, especially just above the interface (Fig. 8b, c). Particulate metals that occur in anoxic waters are most likely metal-sulfide precipitates, carbonates, or detrital phases (Lewis and Landing 1991). Dissolved Mn$^{2+}$ and Fe$^{2+}$ phases, which may also include colloidal metal sulfides (Lewis and Landing 1991) or oxides, are enriched in the upper 75 m of the anoxic zone, coincident with peaks in chemosynthetic activity and bacterial abundances (Figs. 4, 5). These distributions suggest a zone of intensive redox nickel activity just below the interface, which must be supplied from above. On 7 July 98, the particulate Fe and one of the particulate Mn peaks coincided with $\text{O}_2$ anomalies and may indicate either lateral injection of colloidal metals or in situ oxidation of metals (Fig. 8). The deeper particulate Mn peak could be a remnant of an earlier intrusion in which the $\text{O}_2$ has been depleted below detection limits by any number of oxidative processes. Thus, supply of metal oxides to the interface may be controlled by both lateral intrusion and settling of metal oxide particles, both of which are likely to be orders of magnitude faster than diffusive processes.

Jørgensen et al. (1991) speculated, on the basis of distribution profiles, that anaerobic chemosynthetic bacteria oxidize $\text{H}_2\text{S}$ or $\text{SO}_4^{2-}$ at the expense of oxidized Mn and Fe in the Black Sea. Nealon and Myers (1992) have isolated a heterotrophic bacterium, *Shewanella putrefaciens*, capable of reducing metal oxides at the expense of several simple organic substrates. A chemosynthetic bacterium, capable of disproportionating $\text{S}^0$ or $\text{SO}_4^{2-}$ and dependent on extracellular ferrihydrite [Fe(OH)$_3$] to scavenge reactive $\text{H}_2\text{S}$, has recently been isolated from anoxic sediments (Finster et al. 1998). Although freshwater species have been isolated (Prønke et al. 1992), we are unaware of any reports documenting facultative or obligate anaerobes capable of directly reducing oxidized Mn and Fe at the expense of $\text{H}_2\text{S}$, $\text{S}^0$, or $\text{SO}_4^{2-}$ while supporting chemosynthetic growth under marine conditions. Considering both thermodynamic and kinetic arguments, Mn oxide, and, to a lesser extent, Fe oxide, should be as energetically favorable as $\text{NO}_3^-$ in both the Cariaco Basin and the Black Sea (Nealon and Myers 1992).

Amendment experiments were performed to assess the roles of selected electron donors and acceptors in chemosynthetic metabolism. On two occasions, experiments were conducted with samples collected from six to seven depths, spanning the RTZ. In addition to $^1\text{C}$-bicarbonate, replicate samples were spiked with relatively high concentrations of alternative electron donors or acceptors (N$_2$-purged), then incubated and processed in the same way as standard samples. DCA by suboxic and anoxic communities was stimulated by amendments of $\text{SO}_4^{2-}$ in six of nine observations by factors of 1.2–16.4 over parallel unamended samples (Table 2). Stimulation by $\text{S}^0$ amendments was unconvincing, showing slight stimulation ($\times 1.6$) at one suboxic depth only (295 m). Depending on redox conditions, $\text{SO}_4^{2-}$ and $\text{S}^0$ may be used as either reductants or oxidants or, in some cases, as both, through disproportionation (Finster et al. 1998). Results may have been influenced by the low bioavailability of the crystalline $\text{S}^0$ used, or our sample depths may have omitted a narrow layer where $\text{S}^0$ turnover is important, as has been suggested by Hastings and Emerson’s (1988) data.

Stimulation of DCA by addition of $\text{NH}_4^+$ was detected in only two of eight observations and was equivocal (1.1–1.8$\times$) at best, suggesting that $\text{NH}_4^+$ provides very little of the reducing power for DIC assimilation in this zone. However, $\text{NH}_4^+$ may have stimulated activity at depths shallower than those tested. In deeper anoxic samples, equimolar MnO$_2$ and Fe$_2$O$_3$ amendments stimulated DIC assimilation rates by factors of 1.1–4.3 and 1.9–8.5, respectively while having no effect on the shallower samples, perhaps because of trace ambient concentrations of $\text{NO}_3^-$ or $\text{O}_2$. In deeper samples, addition of $\text{NO}_3^-$ and air (deliberate headspace) stimulated DIC assimilation rates by factors of 1.5–15.7 and 6.7 over unamended samples, respectively (Table 2). These results suggest that chemosynthetic communities are dominated by bacteria capable of oxidizing thiosulfate, and presumably sulfide, and capable of facultative respiration of metals and $\text{NO}_3^-$ (denitrifiers). In agreement with earlier observations from the Cariaco Basin and the Black Sea (Tuttle and Jan-
Table 2. Stimulation of dark DI$^1$C assimilation by amendments with alternate electron donors or acceptors. Other than amendments, experimental conditions same as described in Materials and Methods. Results are expressed as ratio of $^1$C-bicarbonate biologically fixed in amended samples to that fixed in parallel unamended samples. Ranges relative to mean are presented in parentheses for treatments that were duplicated. Depth ranges of dark DIC assimilation: 7 Jul 98 = 320–390 m, peak = 350 m; 7 Nov 98 = 310–390 m, peak = 318 m.

<table>
<thead>
<tr>
<th>Date</th>
<th>Depth (m)</th>
<th>$S_2O_5^-$ (50 μM)*</th>
<th>Range (m)</th>
<th>S*†</th>
<th>$NH_4^+$ (50 μM)</th>
<th>Range (m)</th>
<th>$MnO_2$ (9.0 μM)</th>
<th>Range (m)</th>
<th>$FeO_2$ (4.5 μM)</th>
<th>Range (m)</th>
<th>$NO_3^-$ (50 μM)</th>
<th>Air (5 ml)</th>
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</thead>
<tbody>
<tr>
<td>7 Jul 98</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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* Final concentrations in samples.
† Saturated suspension.
‡ ND, not determined.

nasm of Taylor et al. 1979; Sorokin et al. 1995), nitrifiers do not appear to be responsible for much DIC reduction near the oxic-anoxic interface.

Our stimulation experiments verify only the metabolic potential of bacterial communities and provide no indication of in situ rates of various redox reactions. However, these results are consistent with selective enrichment cultures derived from the RTZ during this study, in which autotrophic $H_2S/SO_2^–$-oxidizing denitrifiers, $S_2O_3^2–$-oxidizing/Mn$^{4+}$ reducers, $S^0$ disproportionaters, and $S^2$ reducers were obtained (Madrid 2000). In our stimulation experiments, one could argue that Mn$O_2$ and Fe$O_2$ oxidized $H_2S$ to $S^0$ abiotically and thereby stimulated $S^0$-disproportionating bacteria, rather than metal-respiring bacteria. However, Madrid (2000) demonstrated that cultures from the RTZ reduced Mn$O_2$ autotrophically at the expense of $S_2O_3^2–$ in a mineral medium, whereas $S_2O_3^2–$ did not reduce Mn$O_2$ abiotically under experimental conditions. This supports the hypothesis that metal-reducing chemosynthrophs are indigenous to the RTZ. The importance of autotrophic $H_2S/SO_2^–$-oxidizing denitrifiers below the interface is further underscored by the frequency of their occurrence in 16S rDNA clonal libraries established from polymerase chain reaction (PCR)-amplified nucleic acids (Madrid 2000). It appears that this physiological group dominated the RTZ, if, in fact, PCR amplified genomes in proportion to their natural abundances.

A variety of physiological types of chemosynthetic and heterotrophic bacteria probably inhabit the region immediately above and within the RTZ, many coexisting syntrophically (Tuttle and Jannasch 1973b). Fermentative bacteria appear, on the basis of $H_2$ profiles (Scranton et al. 1984), enrichment cultures, and 16S rDNA libraries (Madrid 2000), to be particularly important just below the interface and throughout the anoxic zone. Sulfate-reducing bacteria associated with anoxic microenvironments above the interface may also be important, as suggested by Hastings and Emerson (1988). Heterotrophs, which require organics for growth but also assimilate CO$_2$ at the expense of $S_2O_3^2–$, may play a role in energy flow and sulfur cycling as well (Tuttle and Jannasch 1977). Furthermore, facultative anaerobes capable of reducing sulfate, thiosulfate, and tetrahionate to sulfide and thiosulfate at the expense of low-molecular-weight fatty acids may also be present (Tuttle and Jannasch 1973b). Sulfur cycling may be very intensive within the RTZ, with chemolithotrophy facilitated by sequential oxidations and reductions of sulfur species by different physiological groups, and demands closer examination in the future.

**Symbiotic associations**—The above discussion assumes that all motion of chemicals and bacteria is controlled by advection or diffusion. Participation of migratory organisms is also possible. Migration of free-swimming chemosynthetic bacteria across chemical gradients to sequester electron acceptors, analogous to acquisition of NO$_3^–$ by Beggiatoa spp., *Thiothrix* spp., or *Thiomargarita* namibiensis (McHatton et al. 1996; Jorgensen and Gallardo 1999; Schulz et al. 1999), seems unlikely, because the small, free-living bacteria in this system are theoretically only capable of migration rates on the order of 0.15 m h$^{-1}$ (Khan 1990). They would not be able to span distances (≥30 m) between the productivity maximum and detectable NO$_3^–$ in less than 8 d, which is energetically unfeasible. However, symbiotic associations with larger, faster-swimming organisms, such as protozoa, could provide a possible adaptive strategy to circumvent this problem. Anaerobic ciliates have been documented to migrate along O$_2$ and light gradients at rates of up to 5 m h$^{-1}$ (Finlay et al. 1987), which is sufficiently fast
to complete a round trip in the Cariaco in 12 h. Significant populations of anaerobic protozoa have been reported for the Black Sea, anoxic regions of the Baltic Sea, and a variety of smaller bodies of marine and freshwater (Fenchel et al. 1990; Setälä 1991; Zubkov et al. 1992). Bird and Karl (1991) reported a peak the abundance for a symbiont-bearing ciliate centered at the depth at which H₂S first appears in the Black Sea.

Some anaerobic species of phagotrophic ciliates lack mitochondria and are known to ferment substrates derived from prey, supplying volatile fatty acids and H₂ to endosymbiotic methanogens and ectosymbiotic sulfate-reducers (Fenchel et al. 1990). However, results from our amendment experiments (Table 2), methane distributions (Scranton 1988), and the absence of Archaea in the 16S rDNA library compiled from this layer (Madrid 2000) suggest that methanogens (symbiotic or free-living) are not major consumers of DIC in the Cariaco’s RTZ. Anaerobic protozoa are known to form symbiotic associations with algae (zoocchlorellae) and purple sulfur bacteria (Fenchel and Finlay 1994). Perhaps undescribed symbiotic associations of chemosynthetic bacteria, capable of sequestering NO₃⁻, like the free-living T. namibiensis (Schulz et al. 1999), occur with migratory ciliates. We have repeatedly observed maxima in abundances of ciliates and flagellates within the Cariaco’s RTZ (unpubl. data). The presence of symbionts in some of these protozoa is expected but needs to be confirmed. Such an association would help explain how anaerobic sulfide oxidizers could acquire sufficient oxidant from shallower horizons and return to sulfidic waters to oxidize sulfur species and assimilate DIC.

Larger-scale implications—Observed cruise-to-cruise variations in rates of chemosynthesis do not correlate with changes in H₂S or O₂ gradients or with primary production. In fact, midwater carbon production appears to be either decoupled from surface processes or is responsive over much longer timescales than our record. H₂S concentrations have varied significantly over the latter half of this century, with a steady increase until a dramatic drop in 1997 (Scranton et al. 1987, in press; Zhang and Millero 1993). Current levels of chemosynthetic activity appear to be driven by upward fluxes of H₂S. These fluxes are controlled by organic carbon delivery to the seabed, diagenetic processes within the sediments, externally-forced vertical and horizontal advection, and upward diffusion. Processes contributing to basin ventilation vary over decadal timescales in response to fluctuations in regional wind stress (Holmen and Roeth 1990). Therefore, current chemosynthetic production may reflect return of stored energy derived from surface export production from the recent past (seasonal, or annual to decadal timescales) and, in essence, represents the basin’s memory. The delay between the delivery of fresh particulate organic matter to the basin’s interior and arrival of dissolved chemical reductant derived from its remineralization at the interface is not known, nor are the processes that control them fully described.

Werne et al. (2000) used δ¹⁸O and δ¹⁵N signatures in their interpretation of the Cariaco’s varved sediments to reconstruct paleoclimate in the tropical western Atlantic region. Their interpretation employs a model based on open ocean oxygenated water in which phytoplankton typically have δ¹⁸O of −8‰ to −24‰ (Ruby et al. 1987; Fry et al. 1991). On the basis of departures in δ¹⁸O and δ¹⁵N from expectations in the varves, they draw inferences about DIC limitation and upwelling intensity. An alternative explanation is possible if a significant portion of sedimentary organic matter is derived from midwater production by chemosynthetic bacteria. It is known that chemosynthetic bacteria fractionate stable isotopes to a higher degree than photoautotrophs and produce biomass depleted in ¹³C, ¹⁵N, and ³²S, resulting in isotopically light biomass (Fry et al. 1991). For example, two species of mesophilic, aerobic, chemolithotrophic bacteria have been found to be ~25‰ depleted in ¹³C relative to their medium (Ruby et al. 1987). Consequently, their δ¹³C could be −26‰ or lower in the Cariaco, where the DIC is −1‰ to −2‰ (Fry et al. 1991). Preuß et al. (1989) have reported even greater ¹³C fractionation for non–sulfur-oxidizing autotrophic bacteria (Δδ¹³C of −26.1‰ to −39.7‰, depending on the DIC fixation pathway). Although isotopic signatures of anaerobic, chemolithotrophic bacteria are unknown, it seems likely that chemosynthetic production in the RTZ will be isotopically light and that the isotopic signature of bacterivorous organisms will be even more depleted in ¹³C and ¹⁵N (Ruby et al. 1987).

If the RTZ has a significant export flux with a distinctively light isotopic signature, which varies out of phase with surface production, then bulk fluxes and isotopic composition of the sediment record may not directly reflect epipelagic and atmospheric processes. Current models interpret isotopically light organic matter (relatively negative) in the sedimentary record as reflecting a period when surface waters were nutrient replete and photoautotrophy was rapid (Werne 2000). Our data suggest that, instead, these may be periods when flux of chemosynthetically derived material was quantitatively more important. If true, then current paleoceanographic and paleoclimatological interpretations of the Cariaco Basin’s sediment record may require reevaluation.

References


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