Acetate cycling in the water column of the Cariaco Basin: Seasonal and vertical variability and implication for carbon cycling

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Abstract

Acetate oxidation frequently has been used as proxy of organic carbon decomposition in marine anoxic sediments. However, the importance of acetate uptake in carbon cycling in marine anoxic water columns is less well studied. Acetate concentrations and uptake rate constants, together with total bacterial numbers, primary and chemoautotrophic production rates, and particulate organic carbon (POC) fluxes, were measured in the water column of the Cariaco Basin during upwelling and nonupwelling seasons between November 1995 and May 1999 as part of the international CARIACO (Carbon Retention In A Colored Ocean) program. Acetate uptake was found to vary strongly with depth and season. Zones of elevated acetate uptake were found in the surface waters and near the suboxic/anoxic interface. High acetate uptake in the surface oxic layer suggests that acetate cycling may be an important component of organic carbon oxidation in oxic environments as well as under anoxic conditions. Depth-integrated acetate uptake rates were correlated with the rates of organic carbon supply in the two zones ($r^2 = 0.37$, $P = 0.017$). Comparisons of acetate oxidation rates with rates of primary production, chemoautotrophic production, and POC flux show that, on average, acetate oxidation can account for respiration of between 16 and 46% of the organic carbon fixed in the water column.

During microbial degradation of organic matter, polymers are hydrolyzed to monomers (such as amino acids, sugars, and fatty acids) before they can be taken up by bacteria. In oxic environments, a single aerobic bacterium can completely oxidize monomers to carbon dioxide. In contrast, in anoxic environments, a consortium of microbes is required to successively decompose the organic substrates to carbon dioxide. Fermenting microbes usually transform the monomers into low molecular weight fatty acids (LMWFA), ethanol, and hydrogen. Among these fermentation products, acetate is the most important intermediate (Thauer et al. 1989; Fenchel and Finlay 1995). In the final stages of organic carbon remineralization under anoxic conditions, acetate is respired to carbon dioxide by the so-called terminal reducers such as denitrifiers, metal reducers, sulfate reducers, or methanogens (Thauer et al. 1989).

Acetate production and oxidation rates have been used as indicators of the decomposition rate of organic carbon in marine anoxic sediments. Sørensen et al. (1981) found that acetate accounted for at least 40–65% of the electron donors for sulfate reduction in coastal marine sediment. Similarly, Christensen (1984) reported that acetate represented at least 50–70% of the electron donors for sulfate reduction in the sediment of a shallow coastal lagoon. In Cape Lookout Bight sediment, the acetate oxidation rate was similar to both the sulfate reduction rate and the CO_2 production rate (Sansone and Martens 1982; Crill and Martens 1986, 1987). Wu et al. (1997) also reported that the rate of acetate oxidation was comparable to the rate of CO_2 production in the sediment of

Table 1. Comparison of depth-integrated acetate uptake rate with major organic carbon fluxes (mmol C m^{-2} d^{-1}).*

| Cruise No. | Date | Production | | POC flux (sediment trap depth, m) | | | | Depth-integrated acetate uptake rate† | |
		Primary (0–100 m)	Chemoautotrophic (200–450 m)	200	265	450	1,270	0–200 m	200–450 m
Upwelling									
CAR-05	Mar 96	175	nd	13	10	12	3	22–64	4–35
CAR-19	May 97	208	114	15	10	6	5	110–203	25–47
CAR-29	Mar 98	269	12	19	4	1	1	21–43	5–11
CAR-42	May 99	104	42	8	4	2	2	17–49	5–23
Nonupwelling									
CAR-01	Nov 95	nd	nd	nd	3	3	2	5–12	7–24
CAR-09	Jul 96	81	nd	6	nd	nd	nd	11–30	1–6
CAR-13	Nov 96	147	105	11	3	3	2	6–27	3–13
CAR-25	Nov 97	54	80	4	6	3	3	10–29	13–32
CAR-32	Jul 98	37	117	3	5	2	1	16–33	16–45
CAR-36	Nov 98	50	50	4	5	2	2	6–9	2–6

* Data sources: primary production (Muller-Karger et al. 2000); chemautotrophic production (Taylor et al. 2001); POC fluxes (Thunell et al. 2000, pers. comm.). POC flux at 200 m is estimated by the empirical function of Pace et al. (1987). nd, not determined.
† Lower limits of depth-integrated acetate uptake rates were calculated by using respiration rate constants; upper limits of the rates were obtained by using total uptake rate constants.

Long Island Sound. Although a number of studies of marine sedimentary systems have shown the importance of acetate cycling as a component of organic carbon oxidation, only a few have been carried out in marine anoxic water columns (Mopper and Kieber 1991; Lee 1992). Thus, the importance of this process for organic carbon oxidation in marine anoxic water columns is not well known.

In the present study, acetate concentrations and uptake rate constants were determined in the water column of the Cariaco Basin two to three times per year between November 1995 and May 1999 as a part of the CARIACO program. Bacterial abundance, rates of primary and chemoautotrophic production, and particulate organic carbon (POC) flux (all measured contemporaneously) are available for comparison.

Oxygen ─O─· (µM)

Primary production ─O─· (µM C d^{-1})

Hydrogen sulfide ─□─· (µM)

Chemoautotrophic production ─□─ (µM C d^{-1})

Fig. 1. Vertical profiles and schematic of the suboxic/anoxic interface and major organic carbon supplies in the water column of the Cariaco Basin (CAR-32). Left panel: oxygen and hydrogen sulfide. Right panel: primary production and chemoautotrophic production. Dashed line denotes the suboxic/anoxic interface.

The major goals of this study were to understand the temporal and vertical variability of acetate cycling, the relative importance of acetate uptake as a component of carbon cycling in the water column, and the response of microbial uptake of labile dissolved organic carbon (DOC) to changes in organic carbon supply.

Materials and methods

Study site and sampling—The CARIACO sampling site is located in the eastern subbasin of the Cariaco Basin in nearly 1,400 m of water (10.50°N, 64.66°W). The cruise numbers and their dates are listed in Table 1. The Cariaco Basin is an excellent site to study acetate cycling because the basin waters are truly marine and are permanently anoxic below depths between 220 and 350 m (Richards 1975; Muller-Karger et al. 2001). Upwelling occurs annually, usually between January and May (Muller-Karger et al. 2001), and photoautotrophic production and organic carbon flux vary dramatically with season (Muller-Karger et al. 2001). As a result of increased nutrient supply, both primary productivity and POC flux tend to be much higher between January and May than between June and December. Chemoautotrophic production is present at and below the suboxic/anoxic interface in the basin, and the rates are rapid (a factor of 0.1 to 3.3 of contemporaneous primary production), but chemoautotrophic production does not vary with season in a systematic way (Taylor et al. 2001). The strong seasonal and vertical fluctuations in organic carbon production, and in carbon flux to depth in the Cariaco Basin, provide an opportunity of examining responses of microbial uptake of acetate to variations in carbon cycling parameters. Typical profiles of oxic and anoxic zonation, and primary and chemoautotrophic productions, are shown in Fig. 1.

All samples were collected at the CARIACO site. Seawater was collected from 18 depths on each cruise using 8-liter Niskin bottles mounted on a rosette equipped with a
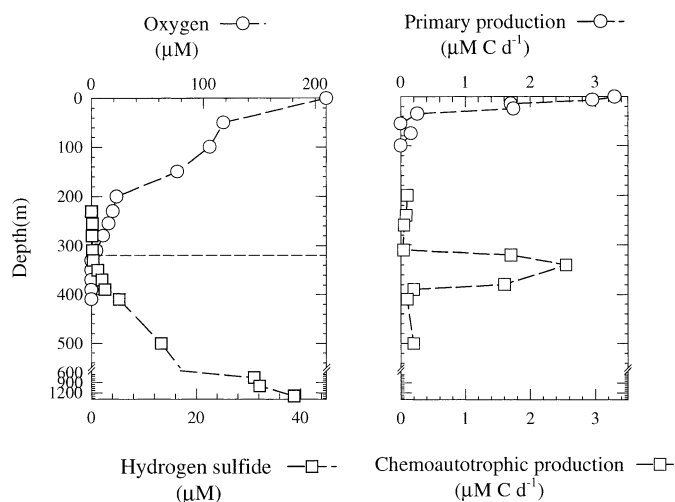
Seabird CTD. For the first three cruises (CAR-1, CAR-5, and CAR-9), seawater samples for acetate analysis were filtered using precombusted (450°C for 12 h) GF/F filters. For the remainder of the cruises, 0.2-μm Nuclepore polycarbonate filters were used. Because filtration rates were very slow with 0.2-μm Nuclepore filters alone, after cruise CAR-32, a sandwich composed of a GF/F filter over a 0.2-μm Nuclepore filter was employed to speed filtration. Filtration took place immediately after sampling at moderate (<20 psi) vacuum pressure (from CAR-1 to CAR-32) or under low N_2 pressure (CAR-36 and CAR-42). No significant acetate concentration differences were observed in comparisons between filters or filtration methods. To stop microbial activity, 0.5 ml 10 N KOH or NaOH was added to 250 ml of filtered sample in an acid-washed polypropylene bottle. Samples were returned to the U.S. at room temperature (about 5 d) and then refrigerated (5°C) prior to preconcentration and gas chromatography (GC) analysis.

Acetate concentration—Acetate samples were analyzed by GC using a flame ionization detector and an HP FFAP 530-μm fused silica column (Hordijk et al. 1990). The detection limit for acetate by this method is approximately 1–2 μM (Ho 2000). Therefore, the Cariaco samples were preconcentrated using the static membrane diffusion technique of Yang et al. (1993) to acquire sufficient sensitivity for quantification. This technique involves diffusion of acetate across a hydrophobic membrane from a dilute acid solution to a dilute basic solution. The recovery efficiencies were determined with internal ^{14}C-acetate standards. For Cariaco samples, a preconcentration factor of 50 was generally employed.

Acetate analysis at low micromolar levels is susceptible to contamination (Ansbaek and Blackburn 1980; Albert and Martens 1997), although the major sources of contamination in the blanks remain unclear (Albert and Martens 1997). One possibility is that acetate concentrations can be perturbed by degradation of DOC during storage and pretreatment (Ho 2000).

Previous studies have shown that acetate functional groups can represent as much as 8–10% of the carbon in ultrafiltered DOC in seawater (Aluwihare et al. 1997). However, these groups seem to be relatively nonlabile. To extract the acetyl groups on high molecular weight (HMW) DOC in seawater, the sample has to be digested for a long time at high temperature under strongly acidic conditions. Aluwihare et al. (1997) reported that acetate groups on ultrafiltered DOC from seawater were not extractable in aqueous solution at pH 1 but that samples had to be digested for 30 h in 4 N HCl at 90°C to recover most of the acetate on the HMW DOC. In contrast, the Cariaco samples were preconcentrated for 8–12 h at pH 2–3 at 70°C.

To evaluate the effect of the pretreatment procedures on labile low molecular weight (LMW) DOC, we chose *N*-acetyl-glucosamine (NAG) as a model form of LMW DOC. NAG was dissolved in Cariaco seawater and was processed under the same pretreatment procedures as the Cariaco samples. In these experiments, 1% of NAG-carbon (or 4% of acetyl groups on NAG) was released to the seawater as acetate. Ten percent of this acetate was released during an initial 1-month storage period, and the remaining 90% of the hydrolysis occurred during preconcentration. These data suggest that hydrolysis of DOC to acetate is most likely to occur during the preconcentration step but that only a small fraction of the acetyl groups were released as acetate.

Typical DOC values for the Cariaco water column range from 60 μM in deep waters to 200 μM in the euphotic zone. If 10% of this DOC is made up of acetate groups as suggested by Aluwihare et al. (1997) and 4% of the acetyl groups are released during the preconcentration step based on the incubation results of NAG, we would predict a release of 0.1–0.4 μM acetate during the analytical procedures. Because DOC is higher and more labile in the mixed layer than at greater depths, the blank probably is higher in surface water than in deep water.

A few studies were done to determine the blank value using subboiling, double-distilled water or Milli-Q water, which was taken in sealed vials to Venezuela, filtered on deck with the field samples, and subsequently treated like seawater. Double-distilled water or Milli-Q water prepared in this way typically initially contained about 10 μM total DOC. Typical measurements of acetate in these blanks were between 0.8 and 1.2 μM. Thus, 1 μM was taken as a typical overall blank for all the cruises. Because 15% of the seawater samples with DOC values of greater than 60 μM give similar or even lower acetate values to distilled water and Milli-Q water samples, it is clear that most of the acetyl groups on DOC in the Cariaco samples were not hydrolyzed to acetate during storage and preconcentration. All of the acetate analyses reported in this study have been corrected for a blank value of either 1 μM or the lowest acetate value measured for a water sample from the cruise if the lowest value was less than 1 μM.

Analytical precision for Cariaco samples was estimated by the samples from cruise CAR-13, which gave a relative standard deviation of 17% (relative standard deviation, $n = 6$). Comparison of data from all cruises showed that 70% of replicate acetate samples agreed within 30% rsd.

To evaluate the accuracy of acetate concentrations determined by gas chromatography, a reversed-phase high-performance liquid chromatography (HPLC) method (Albert and Martens 1997) was used for replicate samples from cruise CAR-48 (November 1999) in addition to the GC analysis. This comparison is shown in Fig. 2. The samples for HPLC analysis were filtered onboard with an 0.2-μm Nuclepore filter but were not fixed with any reagents. Samples for GC analysis were preserved with 10 N NaOH as before. All seawater samples were stored in a refrigerator at 5°C in Venezuela, transported back to the U.S. in a cooler with ice packs, then refrigerated once again at Stony Brook. The data shown in the figure have not been corrected for any blank. Results from the two methods show a similar trend and range of values for acetate, with highest concentrations for the samples at 235 and 930 m and with lowest concentrations (≤1 μM) for the samples from 255 to 295 m. Unfortunately, only a few samples were available on this cruise because of a breakdown of the CTD.

Acetate uptake rate constant—Acid-washed 1-liter glass bottles with Teflon stoppers were used to collect seawater
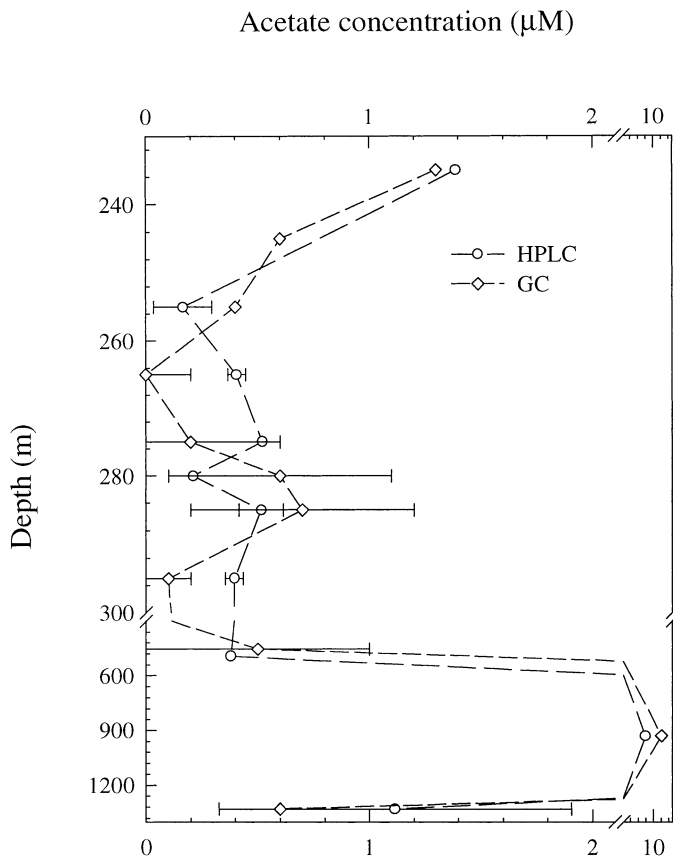
Acetate concentration (μM)

Fig. 2. Comparison of acetate concentration measured by HPLC and GC for C48 samples. Concentrations have been blank-corrected with the lowest acetate value. Error bar is ±1 average deviation for triplicate or duplicate samples. Data without error bars indicate a single sample ($n = 1$).

Table 2. Ratios of depth-integrated acetate uptake rates to organic carbon supplies in the surface (0–200 m) and the suboxic/anoxic interface (200–450 m) zones.

| Cruise No. | Depth-integrated acetate uptake rates to organic carbon supply (%) | | | |
| | Surface | | Suboxic/anoxic interface | |
	Respiration	Total uptake	Respiration	Total uptake
C5	12	37	nd	nd
C9	14	37	nd	nd
C13	4	18	3	13
C19	53	98	22	41
C25	18	53	16	40
C29	8	16	44	93
C32	44	88	14	39
C36	12	17	3	13
C42	16	47	11	54
Average	20	46	16	42

sample was preserved for measurement of the respiration rate constant by the addition of 0.5 ml of 10 N KOH. The amount of acetate respired to CO_2 was measured later in the laboratory by acidifying the basic sample in a closed flask containing a suspended filter soaked in 2 N KOH (Wu et al. 1997). Time zero controls were run for at least one depth in each 6-bottle cast. Correlation coefficients (r^2) for time course data were generally good, with 60% better than 0.9 and 82% better than 0.7. Most of the samples with the rate constants having $r^2 < 0.7$ were deep water samples with very low rate constants and were not used for the calculation of depth-integrated acetate uptake.

Incubation times of between 2 and 12 h were chosen to ensure first-order kinetics. The ^{14}C-acetate taken up was less than 10% of the ^{14}C-acetate added for more than 95% of samples ($n = 180$). Samples where more than 10% of added acetate was taken up were not included in the calculation of acetate rate constants.

Because organic substrates have to be taken up before being respired, during relatively short incubations, the rate constant is likely to be overestimated for microbial incorporation (operationally defined by filtration with a 0.2-μm filter), and underestimated for respiration, because of the absence of isotopic equilibrium. The true rate constants for respiration of acetate will lie between the rate constant for total uptake and the measured respiration rate constant. In this study, we used the total rate constant and assumed that all acetate taken up is eventually respired, giving an upper limit for the true uptake rate constant. In some cases, we have used data for the respiration rate alone as a lower limit for the rate of acetate oxidation (Tables 1, 2).

The uptake rate constant (k) can be estimated by plotting the fraction of ^{14}C-acetate taken up versus incubation time.

$$ k = \frac{-\ln\left[\dfrac{A - a}{A}\right]}{t} $$

k is the acetate uptake rate constant for either incorporation

from Niskin bottles. The glass sampling bottles were rinsed twice with seawater and were allowed to overflow from the bottom to exclude air bubbles. Water then was transferred to acid-washed 40-ml glass incubation vials under N_2 pressure. Each vial was filled from the bottom, allowed to overflow at least 10 ml of sample, and sealed with no air bubble using open screw caps and Teflon/butyl-rubber discs (Pierce). Acetate uptake rate constants were determined using a radiotracer method modified from Hobbie and Crawford (1969) as described by Billen et al. (1980) and Lee (1992). One hundred microliters of a nitrogen-purged solution of 1,2-^{14}C-acetate (55 mCi mmol^{-1}) with radioactivity of 4.4×10^6 to 8.8×10^7 dpm ml^{-1} were immediately added to each vial through the septum using a 500-μl gas-tight syringe. The final amount of acetate added to the incubation vials was equivalent to less than 1 nM. Incubations were conducted in the dark in water baths, where the temperature was maintained near in situ temperature, typically, 24–28°C for surface water and 17–18°C for bottom water. For each depth, three to four times between 0 and 12 h, vials were sacrificed by filtering two 5-ml subsamples through 0.2 μm polycarbonate Nuclepore filters. Each filter was placed in a separate 20-ml scintillation vial containing 5 ml Optiflour for determination of the incorporation rate constant. The remaining
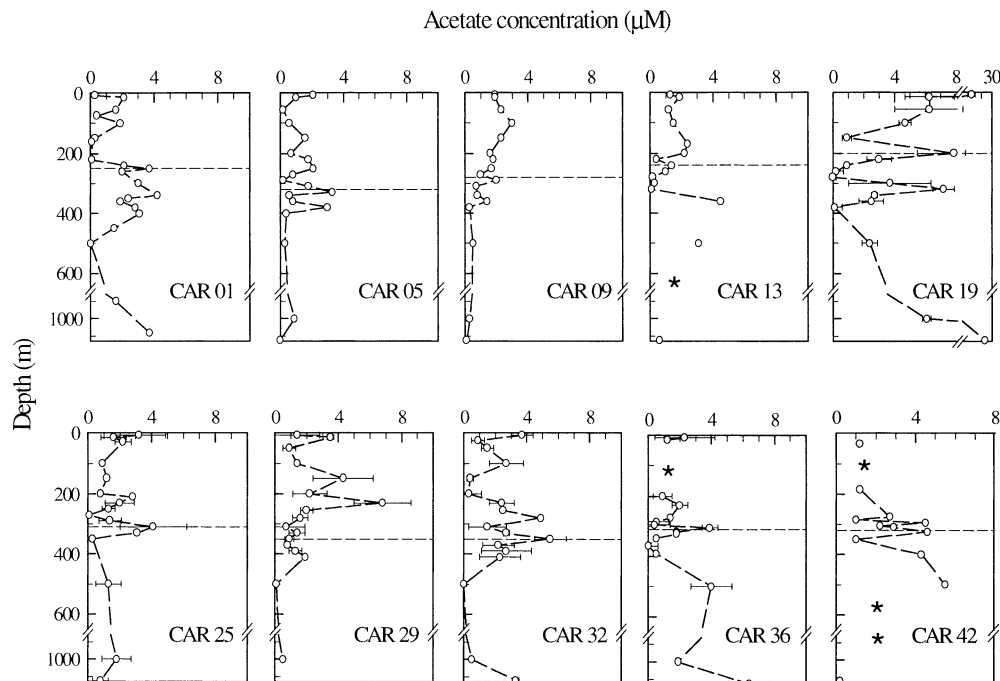
Acetate concentration (μM)

Fig. 3. Acetate concentration as function of depth. Concentrations have been blank-corrected. Dashed line denotes the suboxic/anoxic interface. Asterisk (*) denotes samples with trapping efficiency (<60%) in acetate preconcentration step or lost sample.

or respiration (d^{-1}), a is the amount of radioactivity incorporated, respired, or both to CO_2 (dpm ml^{-1}), A is the total radioactivity of ^{14}C-acetate added (dpm ml^{-1}), and t is incubation time (d).

The total uptake rate constant is calculated using the above formula, with a equal to the radioactivity incorporated plus radioactivity respired to CO_2. Acetate uptake rates are assumed to be first order and are obtained by multiplying the total uptake rate constant by the acetate concentration. Previous studies have found that not all LMW DOC (e.g., fatty acids, amino acids) determined by chemical methods like GC and HPLC is available for microbial uptake (Sansone and Martens 1982; Lee and Jørgensen 1995). In particular, a number of studies in marine anoxic sediments have shown that calculated acetate oxidation rates can exceed the rate of CO_2 production or sulfate reduction where sulfate reduction is known to be the major organic carbon oxidation pathway (Christensen and Blackburn 1982; Shaw et al. 1984; Sansone 1986; Shaw and McIntosh 1990; Wellsbury and Parkes 1995). Therefore, acetate concentrations estimated by chemical methods represent an upper limit for the amount of acetate available for microbial uptake.

Ancillary data—Sampling procedures and analytical methods for primary production rates, POC flux, chemoautotrophic production rates, and bacterial abundance are described by Muller-Karger et al. (2001), Thunell et al. (2000), and Taylor et al. (2001), respectively.

Results

Acetate concentrations in the water column of the Cariaco Basin ranged from less than 1 μM to 30 μM but mostly

varied from less than 1 μM to 4 μM with one or two maxima located at or near the suboxic/anoxic interface (Fig. 3). The surface water values were usually low (<2 μM), and the highest values observed were in the bottom water. Results are similar to those reported for the Black Sea, where several acetate peaks (1–4 μM) were observed near the O_2/H_2S interface, and a bottom maximum (60 μM) was found (Albert et al. 1995). No obvious seasonal pattern in acetate concentrations were observed (Fig. 3), although the data from July 1996 (CAR-9) and May 1997 (CAR-19) seemed different from those of other cruises. The CAR-9 profile had lower values at and below the suboxic/anoxic interface than other profiles, and acetate concentrations observed during CAR-19 were generally higher than on other dates.

Acetate uptake rate constants varied both with depth and season (Figs. 4, 5). Maxima were found both within surface waters and at the suboxic/anoxic interface, operationally defined here as the first appearance of H_2S or the first sampling depth with zero oxygen as determined by the Winkler method when H_2S was not measured. On some occasions, especially during upwelling periods (CAR-5, CAR-19, CAR-42), a maximum was also found in the suboxic zone (Fig. 5). The maximum in the suboxic zone was not observed during CAR-29, which took place during March, but because of the co-occurring El Niño, actual upwelling was minimal on this date (Ramon Varela pers. comm.).

Acetate uptake rate constants at the surface (0.1–1 d^{-1}) were usually a factor of two to seven higher than those at the suboxic/anoxic interface (Fig. 5). Below 500 m, uptake rate constants (usually <0.005 d^{-1}) were two orders of magnitude lower than in the rest of the water column (Figs. 4, 5). Uptake rate constants in the surface water during March
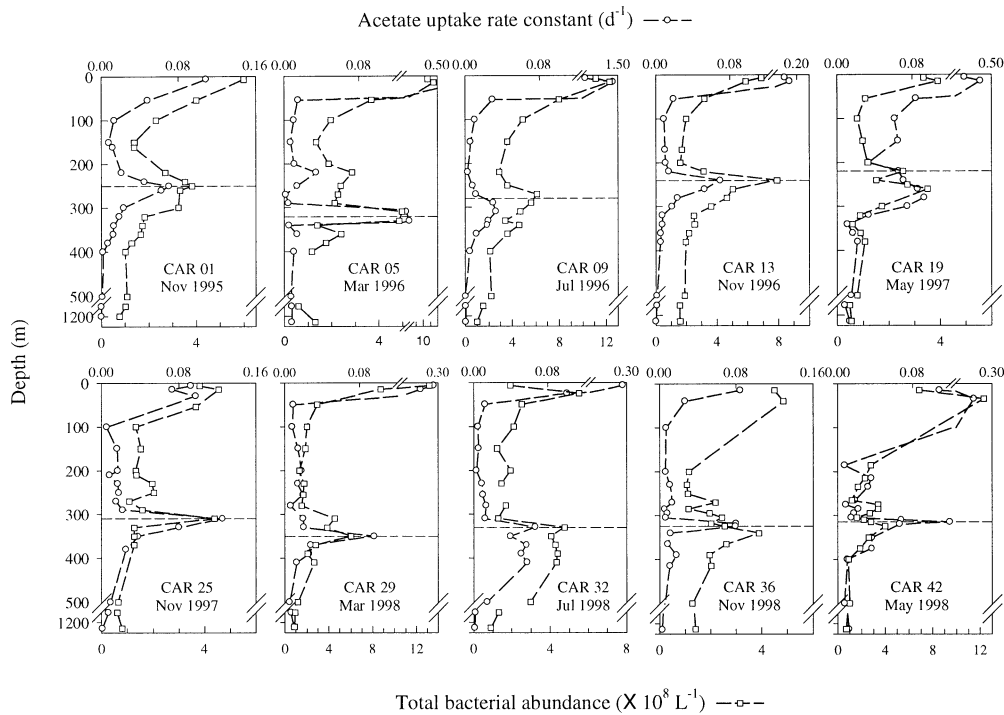
Acetate uptake rate constant (d⁻¹) — ○ —

Fig. 4.   Vertical profiles of acetate uptake rate constants and total bacterial abundance; dashed line represents the suboxic/anoxic interface. Note breaks in axes in some figures.

and May (upwelling) were two to eight times higher than during November (Fig. 5). Although seasonal variability of rate constants was less pronounced at the interface, and rate constants for November were consistent from year to year, the variability was higher during upwelling when the maximum rate constant varied by a factor of 10 (Fig. 5).

Profiles of acetate uptake rates seemed to be largely controlled by variations in rate constants (Fig. 5). Typically, two maxima were seen, one in the surface water and a second near the suboxic/anoxic interface. In surface waters, acetate uptake rates were between 0.1 and 3.0 μM d⁻¹, whereas values at the suboxic/anoxic interface ranged from 0.05 to 0.5 μM d⁻¹.

Discussion

Acetate concentrations were relatively constant from cruise to cruise. This pattern is similar to that seen by Billen et al. (1980), who reported little variation in the concentrations of labile organic substrates in different oxic aquatic environments, although uptake rates of substrates differed greatly.

Elevated acetate concentrations in the Cariaco Basin were usually found near the suboxic/anoxic interface and near the bottom (1,000–1,300 m). Occasional bottom and interface maxima can result from imbalances in a normally closely coupled production/uptake cycle. The maxima may mean that acetate production exceeds acetate consumption at some times, resulting in an accumulation of acetate. In anoxic sedimentary environments, transient acetate accumulations have been widely reported (Sansone and Martens 1982; Alperin

et al. 1994; Shannon and White 1996; Albert and Martens 1997; Chidthaisong et al. 1999). The concentration elevation appears to be due to abrupt changes in biological activity because of changes in temperature, changes in the supply of available electron acceptors (e.g., sulfate), changes in the supply of labile organic carbon, or shifts in methanogen population. In the Cariaco Basin, temperature is constant in deep waters, and sulfate, the most likely electron acceptor for organic carbon oxidation, is not expected to vary because sulfide levels are less than 80 μM (Scranton et al. 2001).

Organic carbon fluxes in this system, however, change markedly with season (Thunell et al. 2000). If the supply of labile organic carbon to a particular biological population abruptly increases, it could result in rapid production of acetate by fermenters or other acetate producers before terminal reducers begin to consume the acetate. Thus, acetate concentrations would increase.

The effect of transient acetate accumulations can be seen in the entire water column during the high production period. Acetate concentrations measured during CAR-19 were noticeably higher than those observed on all other cruises (Fig. 3), suggesting that cruise CAR-19 may have been sampled during a transient period. Both primary production (208 mmol C m⁻² d⁻¹) and chemoautotrophic production (114 mmol C m⁻² d⁻¹) were relatively high at this time (Table 1). POC flux to 1,270 m (5 mmol C m⁻² d⁻¹) also ranked highest among all cruises (Table 1). Fermentation might have been very active in response to this increased organic carbon supply, causing a period of acetate accumulation throughout the water column. A bottom maximum (60–80 μM) in acetate concentration observed in the Black Sea (Mopper and Kieber

Acetate uptake rate constant (d⁻¹)

Acetate uptake rate (μM d⁻¹)

Depth (m)

—○— CAR 01 (Nov 1995)   —○— CAR 05 (Mar 1996)
—□— CAR 13 (Nov 1996)   —□— CAR 19 (May 1997)
—△— CAR 25 (Nov 1997)   —△— CAR 29 (Mar 1998)
—⊙— CAR 36 (Nov 1998)   —⊙— CAR 42 (May 1999)
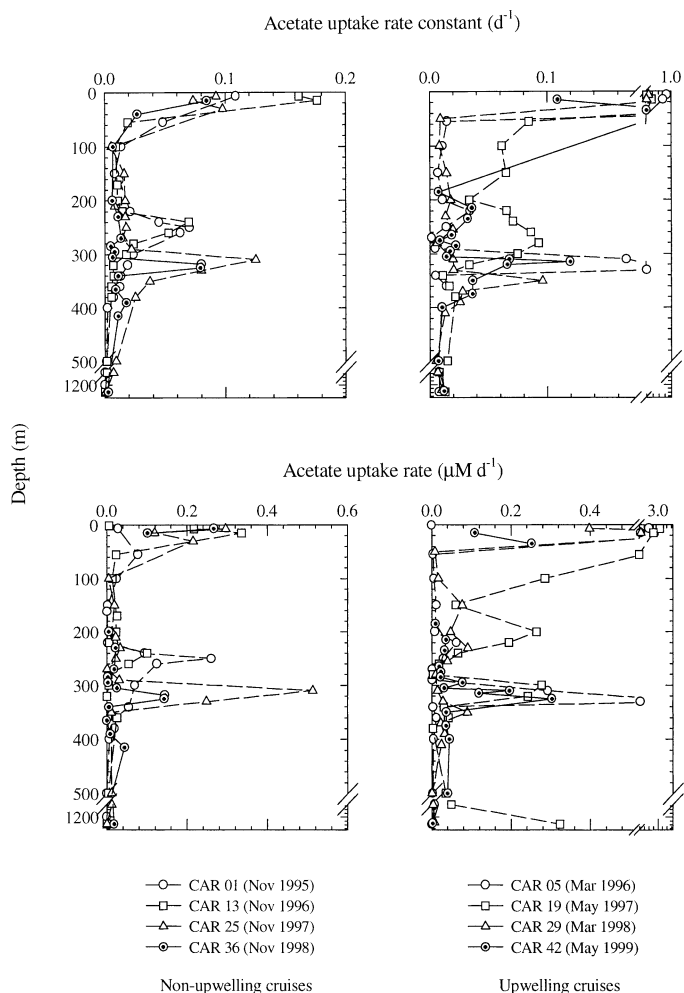
Non-upwelling cruises   Upwelling cruises

Fig. 5. Comparison of acetate uptake rate constants and acetate uptake rates for nonupwelling and upwelling seasons. Left panel shows November cruises (fall); right panel represents cruises during upwelling seasons (March and May). There are breaks in the *x*-axis scale in some profiles.

1991; Albert et al. 1995) might also be attributed to a period when acetate production rates were higher than acetate consumption rates. Once acetate uptake catches up with production, concentrations would decrease again.

The highest acetate uptake rate constants were found in the surface waters and near the suboxic/anoxic interface (Figs. 4, 5). These are the zones where organic carbon production (photoautotrophy and chemoautotrophy, respectively) is highest in the Cariaco water column (Fig. 1).

Billen et al. (1980) suggested that microbial uptake of labile organic substrates varies primarily because of variations in the physiological characteristics of bacteria and not in response to variations in substrate concentration. They also suggested the concentration of labile organic substrates usually remains low because of a rapid response of microbial uptake to changes in substrate production. Based on this argument, acetate uptake rates should depend mainly on changes in acetate uptake rate constants. In general, this was the case for the Cariaco Basin on most dates.

Acetate uptake rate constants varied with fluctuations in

supply of carbon by primary production, chemoautotrophic production, or both in both the surface and interface zones. For the four cruises carried out during periods of upwelling (CAR-5, CAR-19, CAR-29, and CAR-42), primary production averaged 189 ± 34 (1σ) mmol C m⁻² d⁻¹, compared with 74 ± 20 mmol C m⁻² d⁻¹ for cruises during nonupwelling periods (CAR-1, CAR-9, CAR-13, CAR-25, CAR-32, and CAR-36). The rate constants in surface water also were elevated during most cruises sampled at times of upwelling (Fig. 5). Similarly, across the suboxic/anoxic interface, when chemoautotrophic production was elevated (e.g., CAR-19 and CAR-32), a broad zone of high acetate rate constants was observed (Fig. 4). These results suggested that organic carbon supply was one of the major factors controlling microbial acetate uptake in the water column based on the correlation between organic carbon supply and acetate uptake ($r^2 = 0.37$, $P = 0.017$, $n = 18$).

A number of studies have reported the close coupling between heterotrophic activity and organic carbon supply by primary producers in oxic waters. Lancelot and Billen (1984) reported that bacteria respond rapidly to spring phytoplankton blooms. As primary production increases, there is an increase in production of labile organic substrates to fuel bacterial growth.

For the Cariaco Basin, changes in bacterial numbers alone cannot explain the observed acetate uptake features, although uptake rate constants and bacterial abundance are closely correlated ($r^2 = 0.5$–0.9, $n = 18$ for each cruise, $P < 0.01$), with both parameters exhibiting maxima at the same depths in the surface water and at the suboxic/anoxic interface (Fig. 4). However, after normalizing the uptake rate to bacterial abundance (data not shown), maxima were still found in both surface waters and the suboxic/anoxic interface. This suggests either that bacteria at these depths are more adapted for uptake of acetate than are the bacteria at other depths (e.g., they have a lower Michaelis–Menten half-saturation constant [Km] for acetate uptake) or that the bacteria at the interface represent specialized groups that can take advantage of the chemical gradients in this region (e.g., they can couple manganese oxide reduction with acetate oxidation).

To better understand the dependence of acetate uptake on supply of labile organic carbon, depth-integrated rates of acetate uptake were compared with carbon supply in two layers: the surface water and the interface region. Because 200 and 450 m were the upper and lower limits for active chemoautotrophic production in the basin (Taylor et al. 2001), we identified the two major carbon cycling zones as 0–200 m and 200–450 m. Data are inadequate to assess the influence of carbon supply on acetate uptake below 450 m.

The depth-integrated uptake of acetate was calculated for each layer using sublayers centered on the sampling depths, D_i (where $i = 1$–18 from surface to bottom). The thickness (m) of each sublayer (1) was calculated as $[(D_{i+1} - D_i) + (D_i - D_{i-1})]/2$. The depth-integrated uptake rate for each layer was obtained by multiplying the thickness of each layer by the acetate uptake rate for each depth. The major sources of uncertainty in estimates of depth-integrated acetate uptake are the analytical variability in acetate concentrations

(±30%) and the uncertainty in acetate uptake rate constants (10%), giving an overall uncertainty of (±33%).

Between 0 and 200 m, the major source of organic carbon is primary production in the surface water, whereas respiration and sedimentation are the major loss terms. Between 200 and 450 m, organic carbon is mainly supplied by chemoautotrophic production (Taylor et al. 2001) and sinking POC from surface waters (Thunell et al. 2000). Based on sediment trap data from 265 m, on average only 5% of primary production sinks to 265 m (Table 1). The chemoautotrophic production measured across the suboxic/anoxic interface (75 mmol C m^{-2} d^{-1} on average) was generally much larger than the POC flux to 265 m (Table 1). Loss of organic carbon from the 200–450 m zone is either by respiration or settling flux.

Thunell et al. (2000) have suggested that POC fluxes at any depth in the Basin can be estimated from the following relationship (Pace et al. 1987).

$$C_{flux}(Z) = 3.523Z^{-0.734}PP$$

$C_{flux}(Z)$ is the estimated POC flux at depth Z (m), and PP is the primary productivity depth-integrated over the euphotic zone. Depth-integrated acetate oxidation rates then can be compared to the organic carbon sources and sinks in the different layers (Table 1). The net organic carbon supply for the 0–200 m layer was taken to equal the rate of primary production minus the POC flux at 200 m. The POC flux was estimated from the exponential equation given above but constrained by the actual primary production and POC flux for each date. The net organic carbon source for the interface zone was calculated as equal to chemoautotrophic production plus POC flux into the layer at 200 m minus POC flux out of the layer at 450 m. This balance ignores any horizontal input of labile organic carbon.

As discussed above, the true acetate oxidation rate is likely to be somewhat less than the total uptake rate. Although we know our measurements of the respiration rate constant underestimate the true respiration rate because of isotopic disequilibrium, we have included values in Tables 1 and 2 based on the estimates of respiration alone as a lower limit to the amount of acetate respiration taking place. The ratios of depth-integrated acetate uptake to organic carbon supply in the surface and interface layers are presented in Table 2. In the zone bracketing the suboxic/anoxic interface, 16 ± 5% (based on respiration along) and 42 ± 15% (based on total uptake) of the sum of chemoautotrophic production and net POC supply may have been taken up as acetate (Table 2).

In a similar modeling effort, 20 ± 7% and 46 ± 15% of the net carbon supply was remineralized via acetate oxidation in the surface zone (0–200 m). These values are high, considering the known importance of other monomers and HMW organics in bacterial respiration under oxic and suboxic conditions. For CAR-19 and CAR-32, 90% of the organic carbon supply could have been respired through acetate, suggesting that acetate uptake rates might be considerably overestimated in the oxic zone.

Our poor understanding of the true blank value probably is the major reason for the overestimates of the depth-integrated acetate oxidation rate in the euphotic zone. One likely reason is that the analytical blank, because of hydrolysis during the dialysis step, is higher for surface water samples than for deeper waters because the surface samples likely contain more labile DOC than the samples from deep waters. In addition, the acetate concentrations of the surface samples (1–2 μM) were also generally lower than the concentrations of the samples across the suboxic/anoxic interface (4–5 μM), meaning that any blank makes up a larger fraction of the reported concentration in the surface water. In addition, we cannot be sure that all measured acetate is microbially available.

However, it was clear that acetate turnover times were shortest in the surface water of the Cariaco Basin. The rate constants ranged from 0.1 to 1 d^{-1} in the surface water; thus, the turnover times (the reciprocal of the rate constant) ranged from 1 to 10 d. Fast acetate turnover and high uptake rates were also found in various oxic aquatic environments, such as river water, estuaries, coastal waters, and the open ocean (Billen et al. 1980; Mopper and Kieber 1991; Wu et al. 1997; Yu 1999). Initially, it is surprising that acetate uptake is so rapid in surface waters because acetate is usually considered to be a fermentation product. However, there are several potential mechanisms for acetate production in oxic water systems. Photolysis is a major decomposition pathway for DOC in the euphotic zone (Kieber et al. 1989, 1990; Mopper et al. 1991), and acetate is known to be a major photolysis product in aquatic environments (Wetzel et al. 1995; Bertilsson and Tranvik 1998). Thus, photodegradation of DOC could be a major source of acetate in surface water. Suboxic or anoxic microenvironments are known to exist in oxic waters (Alldredge and Cohen 1987; Bianchi et al. 1992; Shanks and Reeder 1993), and acetate could be produced by fermentation in these systems. Excretion of intracellular acetate pools, or sloppy feeding of zooplankton on phytoplankton, also could be sources of acetate in oxic water. Moreover, the average concentrations of acetic and formic acids in dry aerosols are between 1 and 50 parts per billion by volume (Morikami et al. 1993; Khare et al. 1999). Acetic acid was found in precipitation, which usually contains several tenths micromolar of acetate and formate (Keene et al. 1989). Morikami et al. (1993) suggested that acetic and formic acids in the atmosphere could be one of the major organic carbon sources for the surface ocean. More studies are needed to better understand the role of acetate cycling in organic carbon cycle in surface waters. For the study to succeed, it is essential to be able to accurately determine acetate concentrations.

Conclusion—Marked vertical and temporal variations in acetate uptake occur in the Cariaco Basin. Acetate uptake typically increases at times of higher organic carbon supply and is most intense in the surface water and near the suboxic/anoxic interface. However, variations in carbon supply alone cannot fully explain the variability in acetate uptake. The high rates seen in the surface layers suggest that acetate uptake in the oxygen-containing waters is more important than previously thought.

Accurate quantification of acetate concentrations is critical for quantifying the importance of acetate cycling. In the suboxic/anoxic interface region, fermentation of a portion of the

chemoautotrophically produced biomass might be an important source of LMWFA. In both the surface layer and interface layer, as much as 46 ± 15% of carbon supply could be metabolized through acetate. Thus, microbial acetate uptake appears to be a major pathway for carbon cycling in the marine anoxic water column.

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