Part III

The Carbon Isotope Biogeochemistry of Methane Production in Anoxic Sediments 1. Field Observations

Neal E. Blair*, Susan E. Boehme and W. Dale Carter, Jr.[1]

Department of Marine, Earth and Atmospheric Sciences,

North Carolina State University

Raleigh, NC 27695-8208

[1] Present address: Archer Daniels Midland Company

P.O. Box 10640

Southport, NC 28461

ABSTRACT

The natural abundance $^{13}C/^{12}C$ ratio of methane from anoxic marine and freshwater sediments in temperate climates varies seasonally. Carbon isotopic measurements of the methanogenic precursors, acetate and dissolved inorganic carbon, from the marine sediments of Cape Lookout Bight, North Carolina have been used to determine the sources of the seasonal variations at that site. Movement of the methanogenic zone over an isotopic gradient within the dissolved CO_2 pool appears to be the dominant control of the methane $^{13}C/^{12}C$ ratio from February to June. The onset of acetoclastic methane-production is a second important controlling process during mid-summer. An apparent temperature dependence on the fractionation factor for CO_2-reduction may have a significant influence on the isotopic composition of methane throughout the year.

INTRODUCTION

Methane, like other carbon-containing materials, is comprised of approximately 99% ^{12}C and 1% ^{13}C (20). Small but distinct source-related variations in the $^{13}C/^{12}C$ ratio are well documented (52,59). It is important to understand the processes which control the isotopic composition of methane for a variety of reasons. The $^{13}C/^{12}C$ of biogenic methane may provide information concerning the methanogenic ecosystem and the relative rates of methane-producing pathways as a function of season (7,9,15,44) or environment (59). The increasing concentration of tropospheric methane and its potential impact on global temperature and the stratospheric mixing ratios of water vapor and chlorine radicals (8,16,25) require a better understanding of the sources and sinks of that gas. A carbon isotope budget of atmospheric methane which includes seasonal effects would be a powerful constraint on source estimates (44,55). An understanding of the controls of the isotopic composition of biogenic methane would also aid hydrocarbon exploration as approximately 20% of the world's natural gas resources are biogenic in origin (50).

Attempts to model the carbon isotopic composition of methane have been hampered by insufficient information concerning the rates of the methanogenic processes and the isotopic signatures of the methanogenic precursors. Early models used a Rayleigh distillation calculation to simulate the isotope effects associated with methane production in a marine sediment (17,46). These models had three

major assumptions: 1. Methane was produced only via CO_2-reduction, 2. Net CO_2 consumption occurred in the methanogenic zone, and 3. The system was closed to material fluxes of CO_2 and CH_4. No information was available concerning the importance of acetate dissimilation or other pathways. Ignoring the CO_2-production which occurs during methanogenesis (57) led to a significant error in the models. Subsequent treatments, which involved freshwater systems, considered both CO_2-reduction and acetate dissimilation pathways as well as net CO_2-production in the methanogenic zone (28,36). It was assumed that the acetate was utilized only by methanogens in the methanogenic zone, a point which is contradicted by ^{14}C-tracer studies (12,37,60). The isotopic composition of sedimentary acetate was not measured in those studies.

This report reviews our attempts to model the carbon isotopic composition of biogenic methane (5; N.E. Blair and S.E. Boehme, submitted). The ultimate goal of this project is to determine the source of the seasonal variations observed in the $^{13}C/^{12}C$ ratio of methane from the organic-rich marine sediments of Cape Lookout Bight, North Carolina (44). In general, the $^{13}C/^{12}C$ ratio increased in the summer months when methane production rates were highest (Table 1). Similar seasonal variations have been observed in freshwater environments (15) and may be a common phenomenon. The general approach in this project has been to measure the natural abundance $^{13}C/^{12}C$ ratios of methanogenic precursors, CO_2 and acetate, and combine those values with estimates of the relative rates of CO_2-reduction and acetate dissimilation, to simulate the

seasonal variations with an open system model. In doing so, hypotheses concerning the controls of the isotopic composition of methane are tested. In a second study reported in this volume, results from a laboratory microcosm experiment are used to test the model (1).

Field Site

Cape Lookout Bight, North Carolina, is a 1-2 km^2 coastal basin located 115 km SW of Cape Hatteras on the Outer Banks (Fig. 1;40,43). Fine-grained sediment, with an organic content up to 4% dry weight (41), accumulates at a rate of 8-12 cm/yr (11,13) at the sampling station, A-1. The organic matter appears to be derived from phytoplankton and seagrass debris (32).

The rapid flux of metabolizable organic matter to the seabed results in a high rate of organic carbon remineralization (41). Sulfate reduction, occurring in the upper 10 cm of sediment during summer months, and methanogenesis, which occurs in the underlying zone (21), are the dominant diagenetic processes at this site and respectively account for 68$\pm$20% and 32$\pm$16% of the organic carbon remineralization (41). Approximately 20-30% of the methane is produced via acetate dissimilation and the remainder of the gas is formed primarily by CO_2-reduction (22).

METHODS

All $^{13}C/^{12}C$ ratios (R) are reported in the $\delta^{13}C$ notation which is defined (20) as:

$$\delta^{13}C = [(R_{sample} - R_{PDB})/R_{PDB}] \times 10^3 .$$

R_{PDB} is the carbon isotopic ratio of the international standard, Peedee Belemnite, and has the accepted value of 0.0112372 (33). The preparation of samples for isotopic analysis is discussed below in the appropriate sections.

Diver-collected cores were obtained at A-1 with 9.5 cm diameter lucite tubes. Pore water samples for sulfate and ΣCO_2 measurements were collected with a sediment press (49). Pore water samples for acetate $\delta^{13}C$ measurements were isolated with the press or by centrifugation. The porewater samples were frozen immediately after collection and stored at -86^0C until analysis.

The acetate samples were treated as described previously (5,6,7). The acetate fraction was isolated by a series of cryogenic distillations coupled with a preparative liquid chromatography step. The acetate was converted to CO_2 for isotopic analysis with a gas chromatograph - combustion system (24,39).

The isotopic analysis of the acetate methyl group was accomplished by the pyrolysis of sodium acetate (6,45,47). A 200:1 mixture of NaOH and acetate (from the last distillation) was dried under N_2 at 135^0C in a quartz tube (9 mm i.d. x 20 cm long). The tube was evacuated after drying and heated to 500^0C. Methane, which

is derived from the methyl group, was quantitatively collected, measured and injected into the gas chromatograph-combustion system via a Toepler pump.

One to two milliliter subsambles of porewater were injected into evacuated 120 ml serum bottles (Wheaton) sealed with crimped 20 mm rubber stoppers (Alltech Assoc.) and frozen until analysis for ΣCO_2 concentrations and $\delta^{13}C$ values. Immediately prior to analysis, one ml of 1M phosphoric acid saturated with cupric sulfate was added to the thawed sample. The cupric sulfate was added to precipitate sulfide. The resulting CO_2 was removed from the bottle through a 23 gauge hypodermic needle connected to a vacuum line via a 1/4" Ultratorr union (Cajon). The CO_2 was purified cryogenically, quantitated with a manometer and sealed in a 6 mm o.d. pyrex tube for isotopic analysis. The analytical precision and accuracy of the ΣCO_2 extraction procedure were $\pm$4% and $\pm$0.5mM respectively as determined by the measurement of CO_2 and bicarbonate standards (S.E. Boehme, M.S. thesis, North Carolina State University, Raleigh, 1989). The accuracy of the $\delta^{13}C$ measurements, as determined by the analysis of the NBS-20 carbonate standard (19), was $\pm$0.02 per mil.

The methane bubbles were collected from stirred sediment and stored in sealed bottles (44). The methane was converted to CO_2 at 780^0C with CuO and purified cryogenically for isotopic analysis (24,39).

The $\delta^{13}C$ measurements of the CO_2 from the various preparations were analyzed on either a modified Nuclide 6-60 RMS (NASA-Ames

Research Center) or one of two Finnigan MAT 251 mass spectrometers (NCSU Stable Isotope Laboratory and the University of Georgia Center for Applied Isotope Studies). A cross-calibration of a CO_2 standard by the three facilities produced results consistent to within 0.25 per mil. Procedural blanks were collected and used to correct the results of all analyses.

Dissolved sulfate was measured on 5 mL of pore water by the gravimetric analysis of the precipitated barium salt (J.P. Chanton, Ph.D. thesis, Univ. North Carolina, Chapel Hill, 1985; 14). Sulfide was removed immediately after the recovery of the pore water sample by the addition of $ZnCl_2$ followed by the filtration of the zinc sulfide precipitate. The accuracy of this procedure is typically $\pm$0.5 mM.

RESULTS AND DISCUSSION

Ebullition is the primary mode of transport of methane from the sediments of Cape Lookout Bight, accounting for approximately 86% of the total flux of 7.4±2 mol-m^{-2}-yr^{-1} (41,42, S.E. Boehme et al., in prep.). The rapid bubble transport from the methanogenic zone through the overlying sediments limits the exposure of the methane to oxidizing conditions which could alter its isotopic composition (2,18). Concordance between the $\delta^{13}C$ values of naturally- and diver-released bubbles and pore water methane supports that conclusion (44). Thus the $\delta^{13}C$ value of the methane is controlled primarily by its production.

In organic-rich marine sediments, methane is formed by CO_2-reduction (17,22,46) and acetate dissimilation (22,35) with the latter process accounting for 20-50% of the total production. Accordingly, the isotopic composition of methane produced will be the result of a mass balance of material from those two sources. The isotopic composition of the methane from each pathway is dependent on the isotopic composition of the precursor, CO_2 or acetate, and the fractionation factor, α, (k_{12}/k_{13}) associated with each process.

Acetate Dissimilation

Acetoclastic methanogenesis is accomplished by the *Methanosarcina* and *Methanothrix* genera (58). Acetate is converted

to acetyl-CoA, after which the carbon-carbon bond of the acetyl moiety is cleaved (31,58). The methyl is reduced to methane and the carboxyl is oxidized to CO_2 (10,54,61). The fractionation factor associated with the formation of methane via acetate dissimilation by cultures of *Methanosarcina barkerii* is 1.02-1.03 (34; J.B. Risatti and J.M Hayes, Geol. Soc. Am. Abstr. Progr.,1983, 15:671).

The $\delta^{13}C$ value of the acetate methyl group and the <u>in situ</u> fractionation factor for acetate dissimilation in Cape Lookout Bight sediments have been determined via intramolecular carbon isotope measurements of acetate isolated from pore water samples (5,7). The $\delta^{13}C$ value of the total acetate molecule ranges from -17.6 per mil in non-methanogenic surficial sediments to -2.8 per mil in methane-producing sediments (Fig. 2; 5). Near the sediment surface, the similarity of the $\delta^{13}C$ value of the acetate to that of the average particulate organic carbon fraction (-19.1 ± 0.3; 5,7) indicates that little net fractionation occurs during acetate cycling in the sulfate-reducing zone. However, a large fractionation occurs in the methanogenic zone which leaves the acetate enriched in ^{13}C.

Isotopic analysis of the methyl group and a mass balance calculation of the $\delta^{13}C$ value of the carboxyl group indicates that the fractionation affects both carbon atoms of acetate (Fig. 2). The magnitude of the ^{13}C-enrichment correlates well with the parameter f, which is defined by the equation

$$f = r_{CH4}/(r_{CH4}+r_{CO2}) \qquad (1)$$

where r_{CH4} and r_{CO2} are the respective rates of the conversion of the acetate methyl group to CH_4 and CO_2 (5). The downcore profile of f (Fig. 2) was determined from the turnover rate of U-^{14}C acetate in Cape Lookout sediments (22,51). On average, 38±11% (f=.38) of the methyl group is reduced to methane in the uppermost sediments of the sulfate-depleted zone (10-20 cm) at this site.

The *in situ* fractionation factor for the dissociation of the methyl group from acetate (α_{diss}) was estimated with the equation

$$\alpha_{diss} = 1 + (\delta_{obs} - \delta_{syn})/[f(\delta_{syn} + 10^3)] \qquad (2)$$

where δ_{obs} is the average $\delta^{13}C$ value of the methyl group in the 10-20 cm interval (-11.2±3.0) and δ_{syn} represents the $\delta^{13}C$ value of the newly synthesized acetate (5). The average $\delta^{13}C$ value of the methyl group in the 0-5 cm interval (-23.2±2.2) was used as an estimate for δ_{syn} because, as noted earlier, the similarity of the isotopic composition of the total acetate from that interval with the particulate organic fraction suggests that little fractionation occurs during the synthesis or uptake in the surficial sediments. The assumption is made that the synthetic isotope effect is also small in the methanogenic zone. It is assumed that the fractionation factor for the conversion of acetate to CO_2 and other non-methane products is 1.000. Using f=.38±.11, α_{diss} was calculated to be 1.032±0.014. The excellent agreement between our estimate of the *in situ* α_{diss} with the culture-derived values of 1.02-1.03 (34; J.B. Risatti and J.M. Hayes, Geol. Soc. Am. Abstr. Progr. 15:671)

indicates that our assumptions are reasonable to a first approximation.

Under steady state conditions (33), the $\delta^{13}C$ value of the methane produced from acetate is given by

$$\delta^{13}C(CH_4/Acet) = \delta_{syn} - (1-f)(\alpha_{diss}-1)10^3. \quad (3)$$

Using the same values and assumptions as above for the appropriate parameters, the $\delta^{13}C$ value of the methane is calculated to be -43±10 per mil. A similar ^{13}C-enrichment relative to methane produced via CO_2-reduction (see below) has been observed in the laboratory microcosm experiment (1).

Approximately, 20 and 26% of the methane is derived from acetate in the upper 30 cm of sediment at Cape Lookout in July and August, respectively, with the remainder formed via CO_2-reduction (22). Direct measurements of the relative rates of the two methanogenic processes are not available for other months. The $\delta^{13}C$ value of the acetate methyl group in the 8-16 cm interval in June, 1984 was -26 per mil (7), which is a value more similar to that found in the sulfate reducing sediments than in the underlying methanogenic zone. That suggests that little of the acetate was dissimilated to CH_4 and CO_2 (f=0) at that time. On the other hand, one must consider the possibility that the June value is the combined result of a synthetic isotope effect, similar to that associated with acetogenesis (30,48), and a methanogenic isotope effect. For the purposes of the model, the simpler scenario is

assumed, i.e. the synthetic isotope effect remains small and unchanged throughout the summer and f=0 in June. Accordingly, when f=0, then the proportion of methane produced from acetate relative to total methane production (F) must be zero. There is no information concerning F for any other months. For the purposes of the model F=0 for all months except July and August, where it equals .20 and .26 respectively (Fig. 3b).

CO_2-Reduction

$^{12}CO_2$ is selectively converted to CH_4, creating an isotopic gradient in the dissolved inorganic carbon (ΣCO_2) pool as buried sediment encounters and passes through the methanogenic zone (46, Fig. 4; Table 2). At Cape Lookout Bight, the methanogenic zone moves along the $\delta^{13}C$ gradient in response to the seasonal changes in the depth of sulfate penetration (Fig.4). In addition, the $\delta^{13}C$ profiles of ΣCO_2 respond to the seasonal changes in organic matter remineralization rates. Thus, the methanogens are exposed to different isotopic compositions of CO_2 throughout the year because of the two phenomena. The temporal CO_2 signal has been estimated by calculating the isotopic composition of CO_2 in equilibrium with HCO_3^- at the peak of the CO_2-reducing zone for each month that ΣCO_2 profiles were available (Blair and Boehme, submitted; S.E. Boehme, Ph.D. thesis, North Carolina State University, Raleigh, in prep.). The peak of the CO_2-reduction zone approximately coincides with the shallowmost depth where the sulfate concentration is less than

1.0±0.5 mM (21,22). The relative contributions of the CO_2, HCO_3^- and $CO_3^=$ to the ΣCO_2 pool were estimated, assuming mutual chemical equlibrium (53), for the average pore water pH of 6.95 (J.P. Chanton, Ph.D. thesis, Univ. North Carolina, Chapel Hill; N.E. Blair, unpublished results). The isotopic composition of the CO_2 was estimated by solving the following equations simultaneously,

$$\delta^{13}C(\Sigma CO_2) = x\delta^{13}C(CO_2) + y\delta^{13}C(HCO_3^=) + z\delta^{13}C(CO_3^=) \quad (4)$$

$$\alpha(HCO_3^-/CO_2) = [10^3+\delta^{13}C(HCO_3^-)]/[10^3 + \delta^{13}C(CO_2)] \quad (5)$$

$$\delta^{13}C(HCO_3^-) = \delta^{13}C(CO_3^=), \quad (6)$$

where x,y,z represent the fractions of each of the dissolved components. The $\delta^{13}C$ values of the HCO_3^- and $CO_3^=$ ions are assumed to be equivalent (equation 6) to simplify the calculations. Theoretical studies indicate that the HCO_3^- ion may be enriched in ^{13}C by 1.4-1.7 per mil for the temperature range involved (23). For the given pH, the $CO_3^=$ ion represents less than 1% of the ΣCO_2 pool, thus the small isotopic difference is considered insignificant. The equilibrium fractionation factor is given by

$$\ln\alpha(HCO_3^-/CO_2) = (9.552/T) - 0.0241 \quad (7)$$

where temperature (T) is in Kelvin (26). The resulting CO_2 $\delta^{13}C$ values as a function of time are shown in Fig. 3c. The seasonal

isotopic variations are large and clearly must have a significant effect on the methane $\delta^{13}C$ values.

The fractionation factor for CO_2-reduction ranges from 1.03 to 1.06 in cultures (3,4,27,29). The evidence for a temperature effect on the fractionation factor, while expected, is equivocal and may be dependent on culture conditions. We have attempted to estimate the *in situ* fractionation factor using data associated with the two temperature extremes at this site. At 7.8°C (Feb.), the $\delta^{13}C$ values of the CH_4 and CO_2 were -61.7 and -3.6, respectively. Using the equation

$$\alpha_{CO2} = [\delta^{13}C(CO_2) + 10^3]/[\delta^{13}C(CH_4) + 10^3], \qquad (8)$$

α_{CO2} was calculated to be 1.062, during a period of time when it has been assumed that methane was produced predominantly via CO_2-reduction. In August (T=26.5°C), the $\delta^{13}C$ of the methane was -57.7. Given that 26% of the methane is derived from acetate dissimilation, and $\delta^{13}C(CH_4/Acet) = -43$, then the $\delta^{13}C$ value of the methane produced via CO_2-reduction ($\delta^{13}C(CH_4/CO_2)$ should be approximately -62.1. Finally, with $\delta^{13}C(CO_2) = -9.8$, we estimate $\alpha_{CO2} = 1.056$ at 26.5°C. Fitting the two estimates to an Arrhenius temperature dependence, one obtains

$$\ln \alpha_{CO2} = (25.0/T) - 0.029 \qquad (9)$$

where temperature is in Kelvin. The seasonal variation of α_{CO2} is

shown in Figure 3d. The isotopic composition of the methane produced from the CO_2 for the other months was calculated using

$$\delta^{13}C(CH_4/CO_2) = [[\delta^{13}C(CO_2) + 10^3]/\alpha] - 10^3 \qquad (10)$$

and is shown in Figure 5a.

$\delta^{13}C(CH_4)$

The isotopic composition of the methane produced at A-1 is described simply by the mass balance relationship,

$$\delta^{13}C(CH_4) = F\delta^{13}C(CH_4/Acet) + (1-F)\delta^{13}C(CH_4/CO_2). \qquad (11)$$

The calculated monthly $\delta^{13}C$ values, using the parameters in Figures 3a-d, are in excellent agreement with measured values for the period February to September (Fig. 5b). The movement of the methanogenic zone over the ΣCO_2 $\delta^{13}C$ gradient and the temporal variation of α_{CO2} are responsible for the gradual depletion of ^{13}C in the methane between February and June. The onset of acetate dissimilation in July-August coupled with the change in $\delta^{13}C$ value of the CO_2 within the methanogenic zone results in a dramatic enrichment of ^{13}C in those months. The trend is reversed when acetate dissimilation ceases in late August. The subsequent ^{13}C-

depletion in October and November is caused by changes in α_{CO2} and the $\delta^{13}C$ value of the CO_2. The relatively poor fit of the model to the observed values in October and November may be because the model estimates instantaneous $\delta^{13}C$ values and the measured values represent a pooled product. The largest deviation between modelled and observed values would be expected at this time when the reservoir of methane is large and bubbling rates are low.

Implications for Freshwater Sediments

Methane produced in freshwater environments is often enriched in ^{13}C relative to biogenic gas from marine sediments (59). It has been hypothesized that this is due to the relatively greater importance of acetate dissimilation as a methane-producing pathway in freshwater systems (59). ^{14}C-tracer studies indicate that 50-70% of methane production is via acetoclastic processes (F=0.5-0.7; 12,37,53,60). Our calculations indicate that the methane derived from the acetate methyl group is enriched in ^{13}C relative to that from CO_2-reduction, thus apparently confirming the hypothesis. The ^{13}C-enrichment is a consequence of the smaller fractionation factor and large degree of conversion of the methyl group to methane (f=0.4, see equation 3). In freshwater sediments, where f=0.7-0.9 (12,37,53,60), the potential ^{13}C-enrichment could be greater if the synthetic pathways of acetate and the associated isotope effects

are comparable to those at Cape Lookout. Other investigators, using culture and modelling results, have proposed that acetate synthesis in freshwater sediments occurs by a very different process, i.e. acetogenic CO_2-reduction (30). Acetate produced in this manner would be significantly depleted in ^{13}C because the acetogenic process exhibits a large ($\alpha \cong 1.06$) isotope effect (30,48). However, ^{14}C-tracer studies indicate that <2% of the acetate in eutrophic lake sediments is produced by CO_2-reduction (38). The results from Part 2 (1) suggest that the ecological niche of the acetogenic bacteria may be an opportunistic one. Application of the approach summarized in this report to freshwater systems should resolve the issue. Such work is currently underway.

SUMMARY

The isotopic composition of methane varies seasonally at a variety of sites (15,44). In the organic-rich marine sediments of Cape Lookout Bight, North Carolina, the carbon isotopic variations appear to be the result of three factors. During the period February-June, CO_2-reduction is the dominant methanogenic pathway and the $\delta^{13}C$ variations are driven by the movement of the methanogenic zone along an isotopic gradient within the dissolved CO_2 pool. Changes in the relative rates of CO_2-reduction and acetate dissimilation become the dominant factor from July to September. Throughout the whole time period, a temperature dependence on the fractionation factor for CO_2-reduction may play a role controlling the methane $\delta^{13}C$ value.

The Cape Lookout model is the first to combine measured isotopic compositions of both methanogenic precursors, CO_2 and acetate, with measured rates of methanogenic processes. The agreement between model predictions and observed $\delta^{13}C$ values of methane verifies estimates of *in situ* fractionation factors associated with acetate cycling and methanogenesis. The model also indicates that methane produced by acetate dissimilation should be enriched in ^{13}C relative to that produced via CO_2-reduction, thus verifying, in general terms, earlier hypotheses concerning the isotopic differences commonly observed between methane from freshwater and marine sediments (59).

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TABLE 1. Carbon isotopic compositions of methane bubbles from Cape Lookout Bight, North Carolina

Month	$\delta^{13}C$ 1983[1]	1984[1]	1986[2]
February		-63.4	-60.0
April		-63.8	-61.7
May		-66.2	-60.8
June	-64.5	-64.1	
July	-62.2	-60.0	-58.5
August	-59.6	-57.6	-55.9
September	-60.3	-58.0	-58.0
October	-60.0		-58.3
November	-62.2		-59.4

[1] Data from (44).
[2] Data from S.E. Boehme, Ph.D. dissertation, North Carolina State University, Raleigh, in progress.

TABLE 2: Carbon isotopic composition ($\delta^{13}C$) of porewater ΣCO_2 from Cape Lookout Bight, North Carolina.

	2/20/86		7/1/86	
Depth (cm)	core 1	core 2	core 1	core 2
bottom water	0.7		-10.4	-9.4
0-1	-2.6		-11.6	-10.3
1-2	-1.0	0.4	-12.0	-11.2
2-3	-1.0	1.2	-11.8	-9.5
3-4		-1.0	-11.5	-9.6
4-5	-1.9		-11.1	
5-6	-2.7			
6-7	-2.2	-2.9	-9.7	-9.2
7-8		-3.5		
8-9		-3.2	-7.5	-7.2
9-10	-1.6	-2.4		
10-12				
11-12	0.0			
12-14		-2.2		
16-18		-0.5	-1.9	
20-22		1.4		
24-25	4.6		3.8	2.8
24-26		5.1		
28-30				
29-31	6.6	6.7		7.0
32-34				
35-36	8.1	8.0		
36-38				
37-39	7.8			

FIGURE CAPTIONS

Figure 1: Cape Lookout Bight, North Carolina. The sampling station is designated A-1.

Figure 2: The $\delta^{13}C$ values of acetate and its methyl group as a function of depth. The $\delta^{13}C$ values are averages of results from 8/86 and 7/87 (5). The fraction of the methyl group which is converted to methane (f) as a function of depth (22,51).

Figure 3: Model parameters as a function of time.

3a: Average monthly temperature at Cape Lookout (40,41,44 this study).

3b: The fraction of methane derived from the dissimilation of acetate (F).

3c: The $\delta^{13}C$ value of dissolved CO_2 in the methanogenic zone.

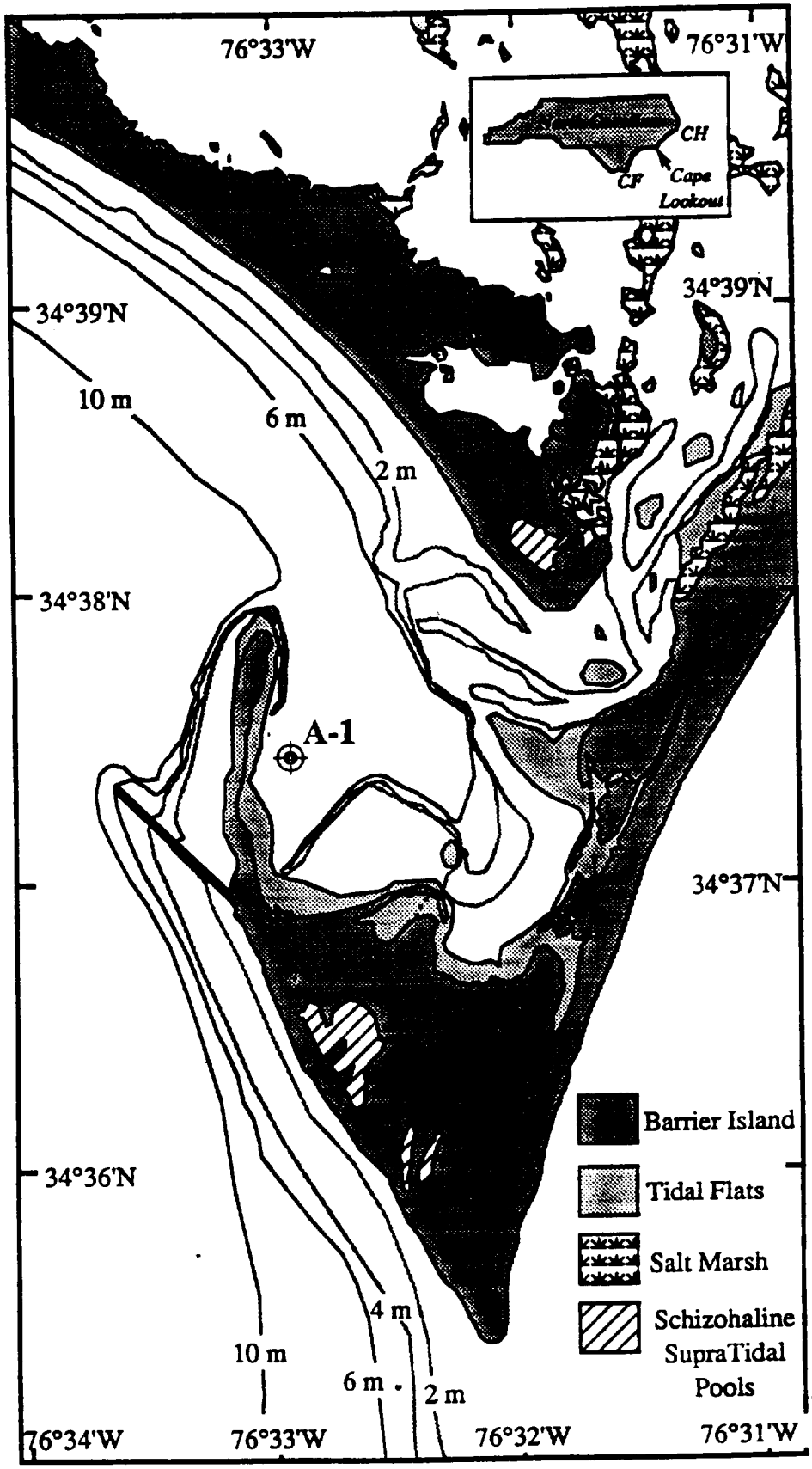
3d: The fractionation factor (α) for CO_2-reduction.

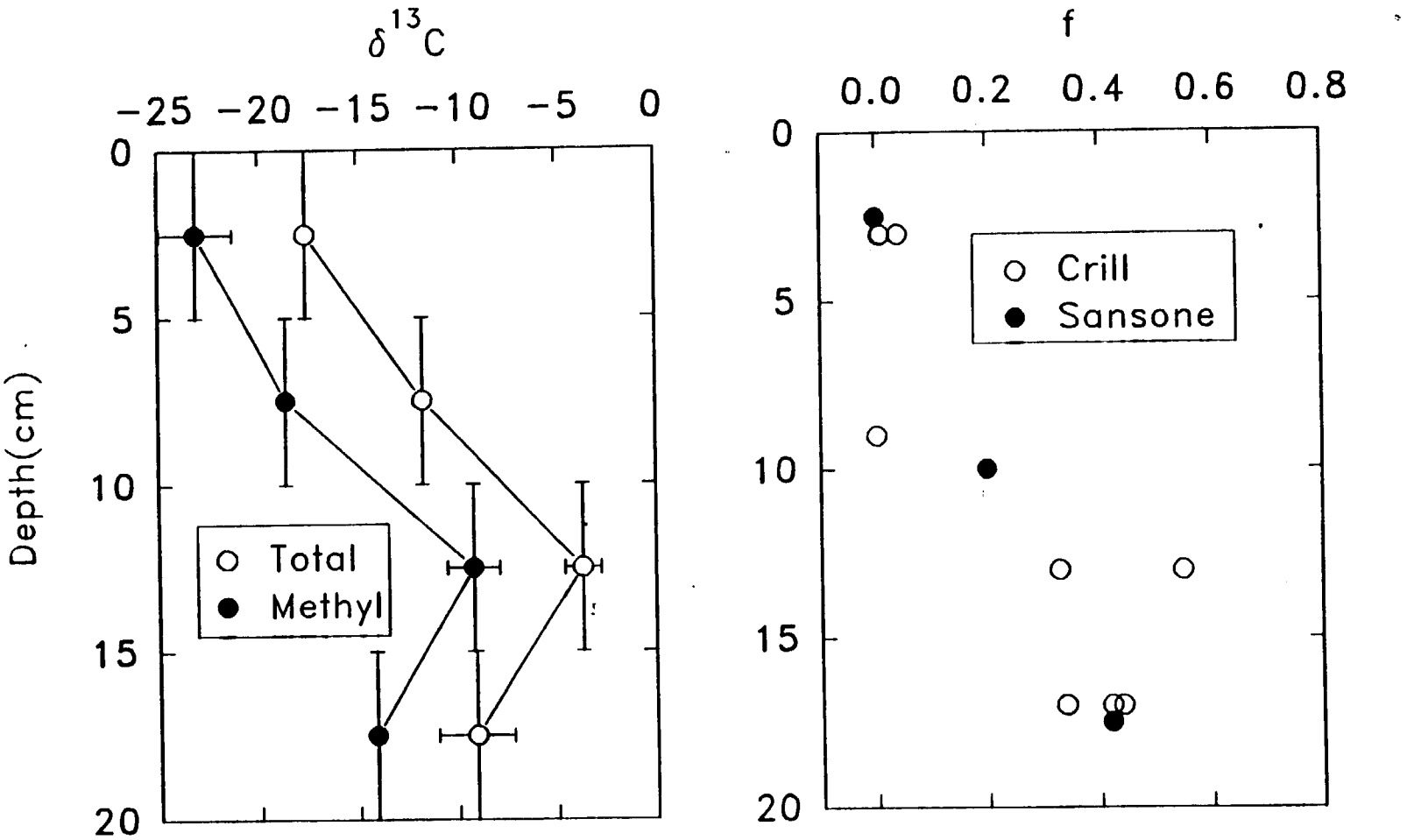
Figure 4: The $\delta^{13}C$ values of ΣCO_2 as a function of depth within the sediment from Cape Lookout. Profiles from February and July, 1986 are shown. The dotted horizontal lines represent the depths at which dissolved sulfate concentrations are equal to or less than 1.0 ± 0.5 mM in February and July.

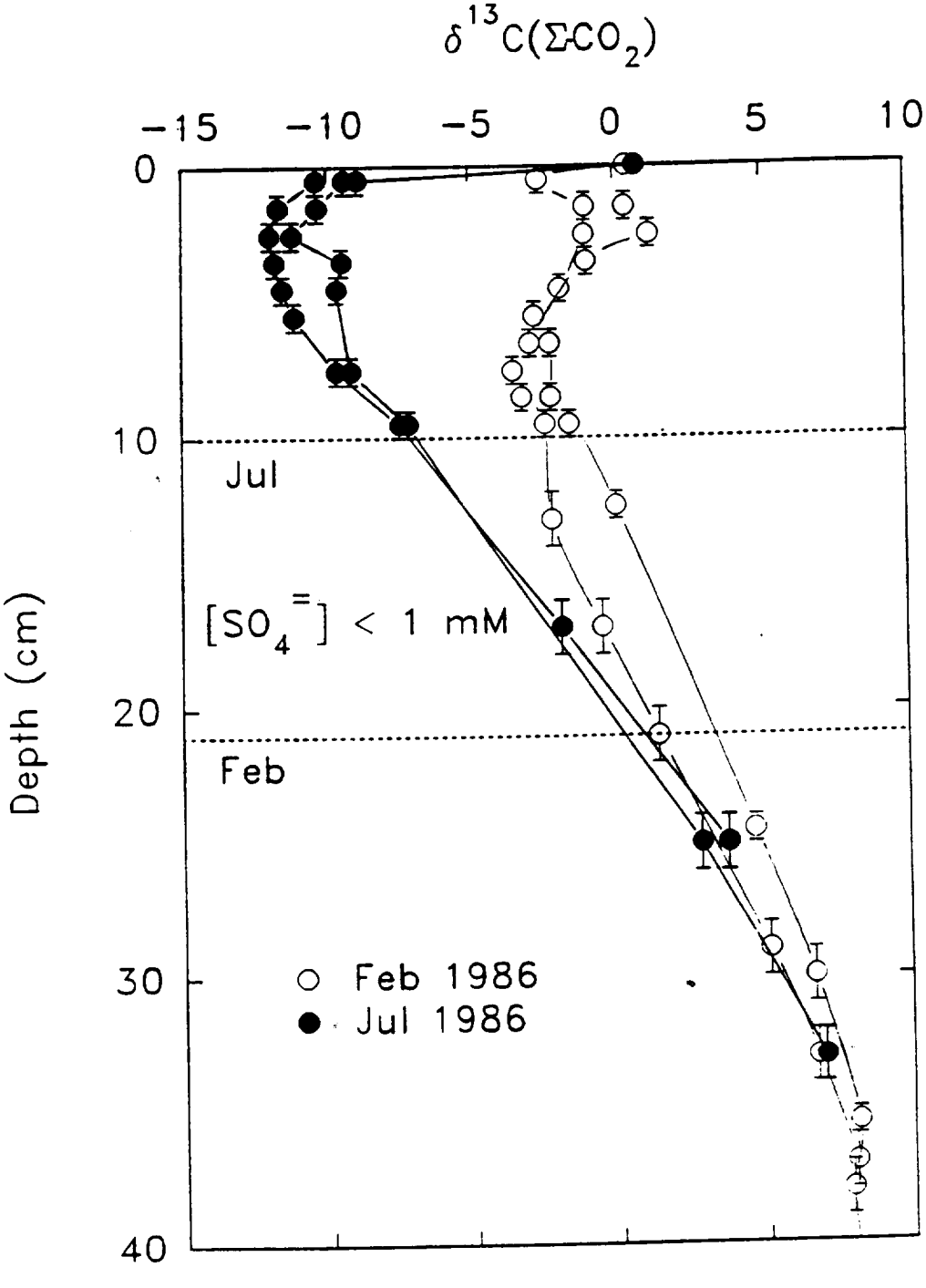
Figure 5: The measured and calculated $\delta^{13}C$ values of methane from Cape Lookout.

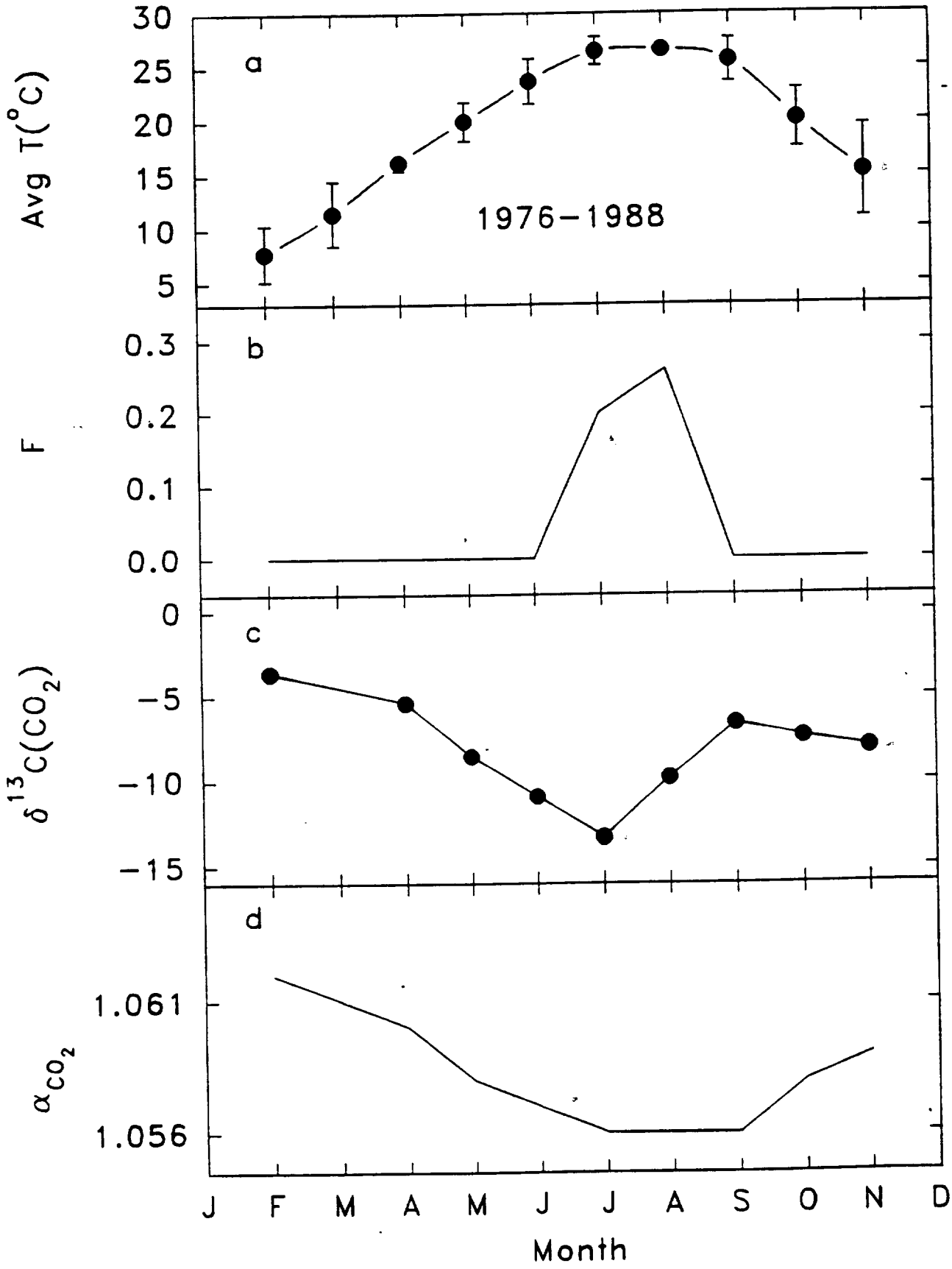
5a: Calculated $\delta^{13}C$ values of methane produced from CO_2-reduction.

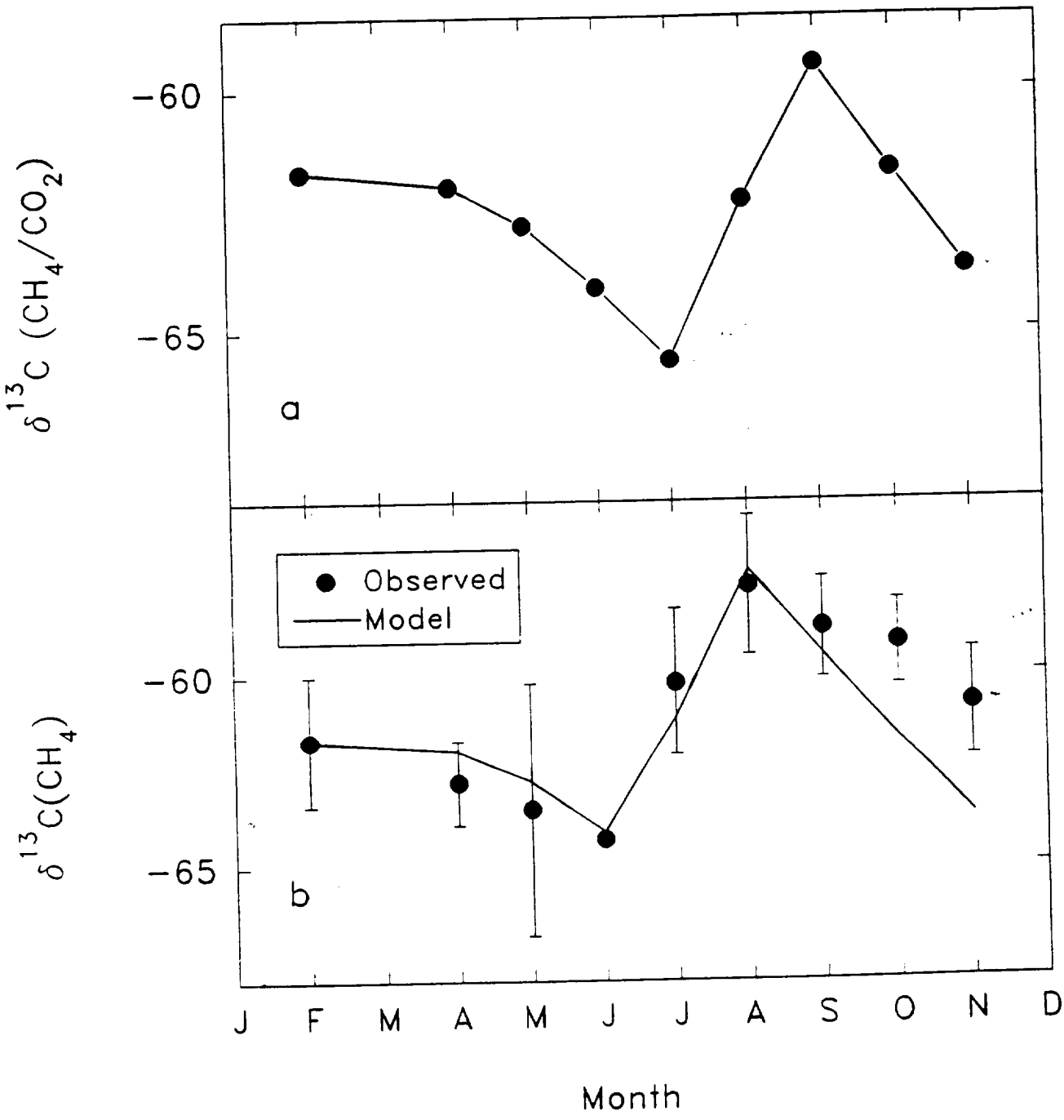
5b: The monthly average measured $\delta^{13}C$ values of methane bubbles for the period 1983-1984 and 1986 (●). The calculated $\delta^{13}C$ values using the model described in the text (——).


76°33'W
76°31'W
CH
CF
Cape
Lookout
34°39'N
34°39'N
10 m
6 m
2 m
34°38'N
A-1
34°37'N
34°36'N
4 m
10 m
6 m
2 m
Barrier Island
Tidal Flats
Salt Marsh
Schizohaline
SupraTidal
Pools
76°34'W
76°33'W
76°32'W
76°31'W


δ13C
−25 −20 −15 −10 −5 0
0
5
10
15
20
Depth(cm)
Total
Methyl
f
0.0 0.2 0.4 0.6 0.8
0
5
10
15
20
Crill
Sansone


$\delta^{13}C(\Sigma CO_2)$
−15
−10
−5
0
5
10
0
10
20
30
40
Depth (cm)
Jul
Feb
$[SO_4^{=}] < 1$ mM
Feb 1986
Jul 1986


a
1976–1988
Avg T(°C)
30
25
20
15
10
5
b
F
0.3
0.2
0.1
0.0
c
$\delta^{13}C(CO_2)$
0
−5
−10
−15
d
α_{CO_2}
1.061
1.056
J F M A M J J A S O N D
Month


−60
−65
$\delta^{13}C$ (CH_4/CO_2)
a
Observed
Model
−60
−65
$\delta^{13}C(CH_4)$
b
J F M A M J J A S O N D
Month

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The carbon isotope biogeochemistry of acetate from a methanogenic marine sediment

N. E. BLAIR and W. D. CARTER JR.
Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695-8208, USA

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The carbon isotope biogeochemistry of acetate from a methanogenic marine sediment

N. E. BLAIR and W. D. CARTER JR.*

Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695-8208, USA

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Abstract—The $\delta^{13}C$ value of porewater acetate isolated from the anoxic sediments of Cape Lookout Bight, North Carolina, ranged from −17.6‰ in the sulfate reduction zone to −2.8‰ in the underlying methanogenic zone. The large ^{13}C-enrichment in the sulfate-depleted sediments appears to be associated with the dissimilation of acetate to CH_4 and CO_2. Fractionation factors for that process were estimated to be 1.032 ± 0.014 and 1.036 ± 0.019 for the methyl and carboxyl groups. A subsurface maximum in $\delta^{13}C$ of the total acetate molecule, as well as the methyl and carboxyl carbons at 10–15 cm depth within the sediment column indicates that changes in the relative rates of acetate cycling pathways occur in the methanogenic zone. The methyl group of the acetate was depleted in ^{13}C by 7–14‰ relative to the carboxyl moiety. The intramolecular heterogeneity may be the result of both synthetic and catabolic isotope effects.

INTRODUCTION

ACETATE IS A KEY INTERMEDIATE in the early diagenesis of organic matter. Acetate can be produced by anaerobic bacteria via the degradation of a wide variety of organic compounds, including amino acids (BARKER, 1981; STAMS and HANSEN, 1984), carbohydrates (WINTER and WOLFE, 1980; BHARATI et al., 1980; LAUBE and MARTIN, 1981; LJUNGDAHL and WOOD, 1982; JONES et al., 1984; and many others), and organic acids and alcohols (BRYANT et al., 1977; MCINERNEY et al., 1979; MCINERNEY and BRYANT, 1981; KOCH et al., 1983; EICHLER and SCHINK, 1984; STIEB and SCHINK, 1985, 1986; KREMER et al., 1988). Bacteria capable of producing acetate via CO_2-reduction with H_2 have also been identified (BRAUN et al., 1979, 1981; LEIGH et al., 1981; LJUNGDAHL and WOOD, 1982). The dominant microbial populations responsible for acetate synthesis and the relative importance of the different synthetic pathways in marine sediments are poorly understood.

Acetate is rapidly consumed in sediments by microorganisms linked with terminal oxidative processes such as sulfate reduction and methanogenesis. Sulfate-reducing bacteria appear to be responsible for the oxidation of >95% of the acetate in organic-rich marine sediments (WINFREY and WARD, 1983; SHAW et al., 1984). In sulfate-depleted marine and freshwater sediments, acetate is disproportionated to CH_4 and CO_2 (WARFORD et al., 1979; WINFREY and ZEIKUS, 1979a; SANSONE and MARTENS, 1981, 1982; LOVLEY and KLUG, 1982; CRILL and MARTENS, 1986; SCHUTZ et al., 1989; KUIVILA et al., 1990). Approximately 25–50% of the methane production in organic-rich marine sediments and as much as 60–70% in freshwater sediments results from the dissimilation of acetate (CAPPENBERG and PRINS, 1974; WINFREY and ZEIKUS, 1979a; LOVLEY and KLUG, 1982; CRILL and MARTENS, 1986; KUIVILA et al., 1990). The dissimilation process occurs only in the sulfate-depleted portion of the sediment column because of the competition for acetate between the methanogens and sulfate reducers (SCHONHEIT et al., 1982; LOVLEY and KLUG, 1983a).

In addition to the methanogenic archaebacteria, other microorganisms may be involved in the catabolism of acetate in sulfate-depleted sediments. Van Niel proposed the methane-producing pathway:

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 4H_2 \rightarrow CO_2 + CH_4 + 2H_2O \quad (1)$$

as an alternative process to the direct dissimilation of acetate to methane and CO_2 (BARKER, 1936). An organism capable of the first step of the reaction, the production of CO_2 and H_2 from acetate, has been isolated from a methane-producing thermophilic digester (ZINDER and KOCH, 1984). The microorganism, nicknamed "Reversibacterium," exists in a syntrophic relationship with hydrogen-utilizing methanogens. ^{14}C-tracer studies have indicated that acetate oxidation to CO_2 is an important process in sulfate-depleted marine sediments (WARFORD et al., 1979; SANSONE and MARTENS, 1981, 1982; CRILL and MARTENS, 1986). It has been proposed that an interspecies H_2-transfer consortium between a sulfate reducer and a methanogen may be responsible for pathway (1) in marine sediments (SANSONE and MARTENS, 1982). Alternatively, the acetate oxidation may be mediated by a sulfur-reducing bacterium (WARFORD et al., 1979; WINFREY and ZEIKUS, 1979b) similar to the freshwater *Desulfuromonas acetoxidans* (GEBHARDT et al., 1985).

Isotope effects which might be associated with acetate cycling would have a significant, if not dominant, influence on the isotopic composition of the diagenetic products, ΣCO_2 and CH_4 (LAZERTE, 1981; WHITICAR et al., 1986). For instance, differences in the $\delta^{13}C$ and δD values of methane from marine and freshwater environments have been attributed to differences in the relative importance of the acetate dissimilation and CO_2-reduction pathways (WHITICAR et al., 1986). Similarly, seasonal variations in the isotopic composition of methane from anoxic sediments have been hy-

* *Present address:* Archer Daniels Midland Co., PO Box 10640, Southport, NC 28461, USA.

pothesized to result from changes in acetate cycling processes (MARTENS et al., 1986; BURKE et al., 1988; CHANTON and MARTENS, 1988). Our ability to test those hypotheses, and more generally the development of quantitative models which describe the biogeochemical controls of the carbon isotopic compositions of ΣCO_2 and CH_4, have been limited by the lack of information concerning the isotopic systematics of acetate turnover (GAMES and HAYES, 1976; LAZERTE, 1981).

In an attempt to address some of the issues in question concerning acetate cycling pathways and the associated isotopic fractionations, we have made $\delta^{13}C$ measurements of acetate and its methyl group isolated from the anoxic marine sediments of Cape Lookout Bight, North Carolina. Preliminary measurements suggested that large carbon isotope effects accompany the natural turnover of acetate (BLAIR et al., 1987). The measurements presented in this report provide new information concerning the relative rates and spatial distribution of acetate cycling processes, and the carbon isotope fractionation which occurs during the turnover of acetate.

FIELD SITE

Cape Lookout Bight, North Carolina, is a 1–2 km^2 coastal basin located 115 km SW of Cape Hatteras on the Outer Banks (MARTENS and KLUMP, 1980; MARTENS et al., 1980). Fine-grained sediment with an organic content up to 4% dry weight (MARTENS and KLUMP, 1984), accumulates at a rate of 8–12 cm/yr (CHANTON et al., 1983; CANUEL et al., 1990) at the sampling station, A-1. The organic matter appears to be derived from phytoplankton and seagrass debris (HADDAD and MARTENS, 1987).

The rapid flux of metabolizable organic matter to the seabed results in a high rate of organic carbon remineralization (MARTENS and KLUMP, 1984). Sulfate reduction, occurring in the upper 10 cm of sediment during summer months, and methanogenesis, which occurs in the underlying zone (CRILL and MARTENS, 1983), are the dominant diagenetic processes at this site and, respectively, account for 68 ± 20% and 32 ± 16% of the organic carbon remineralization (MARTENS and KLUMP, 1984). Acetate concentrations over 100 μM have been found in the summer months at the interface between the sulfate-reducing and methane-producing zones (SANSONE and MARTENS, 1982). Approximately 20–30% of the methane is produced via acetate dissimilation (CRILL and MARTENS, 1986).

METHODS

Diver-collected cores were obtained at A-1 with 9.5 cm diameter lucite tubes. Normally, the acetate cores were processed immediately after collection onboard ship. The porewater from cores collected on August 14, 1986, was isolated with a sediment press (REEBURGH, 1967). The porewater samples were frozen immediately after collection and stored at −86°C until analysis. One core from that date was transported to and processed at the Institute of Marine Sciences (Morehead City) in the same manner. Because of our concern for potential artifacts associated with the use of the sediment press and the possibility that the isotopic signature of the acetate may change rapidly after core recovery, samples collected on July 21, 1987, were treated in one of two methods. Each sampling interval was split with one portion immediately centrifuged at 8000 rpm for 10 min. The porewater sample (40 mL) was passed through a Whatman GFA filter, acidified with 5 mL of HPLC grade 85% H_3PO_4 (J. T. Baker) and frozen in polypropylene bottles (Nalgene). The second portion of each sediment interval was rapidly mixed with 50 mL of a 1:1 methanol-H_2O mixture and frozen. The cores were sampled in less than 15 min. after retrieval. The methanol-porewater mixture was isolated later in the laboratory by centrifugation as described above.

The isotopic analysis of porewater acetate followed a modified procedure of BLAIR et al. (1985, 1987). The porewater sample was acidified to pH 1 with concentrated H_3PO_4 and distilled in vacuo cryogenically to produce a volatile acid fraction. The basified (pH > 11) distillate was dried in a Teflon® beaker under N_2 at 135°C. The dried salts were dissolved in 1.0 mL water and 0.9 mL concentrated H_3PO_4. The resulting mixture was distilled in vacuo cryogenically. The distilling pot was maintained at 90–95°C. The resulting solids in the pot were redissolved with 1.0 mL of water and distilled as above. This procedure was repeated a third time with 2.0 mL of water. The volatile acids were concentrated approximately 25-fold with this method.

The volatile acids were separated on a 10 μm RP-8 Lichrosorb column (25 cm × 4.6 mm i.d., Alltech Assoc.) and detected at 210 nm (Lambda-Max Model 481, Waters Chromatog. Div.). The mobile phase was 0.01 M H_2SO_4 maintained at 0.63 mL/min. The acetate was separated from the H_2SO_4 and concentrated by the following drying/distillation steps. The acetate fraction collected from the liquid chromatograph was brought to pH 11 with 20% NaOH and dried, as described above. The salts were dissolved in 50 μL water and 200 μL H_3PO_4, and the resulting solution was distilled as above. The distillate was stored frozen until needed.

The isolated acetate was converted to CO_2 for isotopic analysis with a gas chromatograph–combustion system (MATTHEWS and HAYES, 1978; DES MARAIS, 1978). The Carlo Erba HRGC 5300 Mega series was outfitted with a packed column injector (150°C) and a Superox-FA wide bore capillary column (0.53 mm o.d., 30 m length, Alltech Assoc.). The helium flowrate was 3 mL/min and the temperature was programmed to hold at 80°C for 15 min. and ramped to 110°C at 10°/min. The sample was swept through the combustion furnace (2 mm i.d. quartz tube packed with 80–100 mesh CuO, 780–790°C) with a make-up gas (12 mL/min He). The resulting CO_2 was monitored with a thermal conductivity detector (Gow-Mac Model 40-400) and collected in a ⅛ in. stainless steel multiple loop trap immersed in liquid nitrogen. The CO_2 was then purified cryogenically and stored in a 6 mm o.d. Pyrex breakseal until isotopic analysis. Acetate standards producing >0.3 μmoles CO_2 per 10 μL injection were found to have $\delta^{13}C$ values within 0.4‰ of the accepted values, which were determined by either bomb combustion (BLAIR et al., 1985) or direct gas chromotograph (GC) combustion of large samples. This sample size would typically translate into an original porewater concentration of >30 μM. Smaller standards were depleted in ^{13}C by more than 0.5‰. Accordingly, all $\delta^{13}C$ analyses reported in this paper were from samples > 0.3 μmoles C/injection. The sensitivity of this procedure is comparable to that reported for an alternative method (GELWICKS and HAYES, 1990).

The isotopic analysis of the acetate methyl group was accomplished by the pyrolysis of sodium acetate (OAKWOOD and MILLER, 1950; MEINSCHEIN et al., 1974; BLAIR et al., 1985). A 200:1 mixture of NaOH and acetate (from the last distillation) was dried under N_2 at 135°C in a quartz tube (9 mm i.d. × 20 cm long). The tube was evacuated after drying and heated to 500°C. Methane, which is derived from the methyl group, was quantitatively collected, measured, and injected into the GC combustion system via Toepler pump. The purification of the methane was accomplished on a 2.5 m long × 2 mm i.d. stainless steel column packed with 100–150 mesh Porasil B (Alltech Assoc.) at room temperature. The CO_2 resulting from the combustion of the methane was treated as above for isotopic analysis. As little as 1.6 μmoles of acetate could be analyzed with an accuracy and precision of 0.3‰.

Samples for the $\delta^{13}C$ measurements of the total organic carbon (TOC) fraction were prepared by one of two methods. Samples from 1983 were prepared by a bomb combustion method described previously (BLAIR et al., 1987). Samples from 1986–87 were treated with a modified procedure. Between 0.7 and 1.0 grams of wet sediment were slurried with 1N HCl until bubbling ceased, after which the sample was lypholized. Approximately 20–30 mg of the homogenized sample were combusted in tin boats with a Carlo Erba 1500 CNS

analyzer (UNC-Chapel Hill, Marine Sciences). The resulting CO_2 was collected in a ⅛ in. stainless steel multiple looped trap immersed in liquid nitrogen. The CO_2 was subsequently transferred cryogenically and sealed in a 6 mm o.d. Pyrex tube for later isotopic analysis. The $\delta^{13}C$ values of standards prepared by the CNS analyzer were within 0.3‰ of those prepared by the bomb combustion method referenced above.

Lipid fractions were prepared for isotopic analysis by sonicating 150 mg of freeze-dried sediment with 5 mL of 1:1 methanol-toluene (Burdick and Jackson) for 10 min. The mixture was vortexed for 2 min. The extract-sediment mixture was separated by centrifugation, and the sediment was reextracted as above. The extracts were combined, and the solvent was removed by rotary evaporation. The sample was saponified with a 1:1 aqueous 1 M KOH-methanol solution (J. T. Baker, Burdick and Jackson) at 77°C for 2 h. The KOH pellets had been pretreated by heating at 490°C for 25 min to remove organic contamination. The saponified lipid mixture was extracted three times with previously distilled petroleum ether (40–45°C) to produce the neutral lipid fraction. The KOH mixture was then acidified and reextracted with previously distilled $CHCl_3$ to produce the fatty acid fraction. The volumes of both fractions were reduced by rotary evaporation. The samples were transferred to Ag boats with $CHCl_3$, and the solvent was removed in vacuo. The samples were converted to CO_2 for $\delta^{13}C$ analysis by bomb combustion (BLAIR et al., 1985).

The $\delta^{13}C$ measurements of the CO_2 from the various preparations were analyzed on either a modified Nuclide 6-60 RMS (NASA-Ames Research Center) or one of two Finnigan MAT 251 mass spectrometers (NCSU Stable Isotope Laboratory and the University of Georgia Center for Applied Isotope Studies). A cross-calibration of a CO_2 standard by the three facilities produced results consistent to within 0.25‰. Procedural blanks were collected and used to correct the results of all analyses.

Dissolved sulfate was measured on 5 mL of porewater by the gravimetric analysis of the precipitated barium salt (CHANTON, 1985; CHANTON et al., 1987). Sulfide was removed immediately after the recovery of the porewater sample by the addition of $ZnCl_2$, followed by the filtration of the zinc sulfide precipitate. The accuracy of this procedure is typically ±0.5 mM (CHANTON, 1985).

RESULTS

The $\delta^{13}C$ values of the TOC from Cape Lookout Bight averaged −19.08 ± 0.26‰ (Fig. 1), indicating that the organic matter is predominantly of marine origin (HAINES, 1976; GEARING et al., 1984; HADDAD and MARTENS, 1987). The fatty acid and neutral lipid fractions averaged −22.1 ± .5 and −22.9 ± .3‰, respectively (Fig. 1). Similar ^{13}C-depletions relative to the TOC have been observed in lipid fractions isolated from estuarine sediments (PARKER, 1964), the Eocene Messel shale (HAYES et al., 1987), and a wide variety of biological samples (ABELSON and HOERING, 1961; PARKER, 1964; DEGENS et al., 1968; DENIRO and EPSTEIN, 1977; MONSON and HAYES, 1982a,b). The nearly ubiquitous ^{13}C-depletion of lipids has been attributed to isotope effects associated with the biosynthesis and cycling of the lipid precursor, acetyl CoA (DENIRO and EPSTEIN, 1977; MONSON and HAYES, 1982a,b; BLAIR et al., 1985).

Porewater sulfate and acetate concentrations (Fig. 2) were similar to those observed previously at this site (SANSONE and MARTENS, 1982; CHANTON, 1985; CRILL and MARTENS, 1987). A subsurface maximum in acetate concentration frequently occurs between June and August at the same depth horizon where dissolved sulfate concentrations fall below 1 mM (SANSONE and MARTENS, 1982). The magnitude of the subsurface maximum was highly variable, similar to that seen in other studies at this site using different sediment processing and analytical methods (M. Alperin, per. comm.). The variability between cores is thought to result from spatial heterogeneities in acetate cycling processes. No systematic differences were observed between splits of cores, which were processed by either the immediate centrifugation or methanol-poisoned/centrifugation treatments (Fig. 2C).

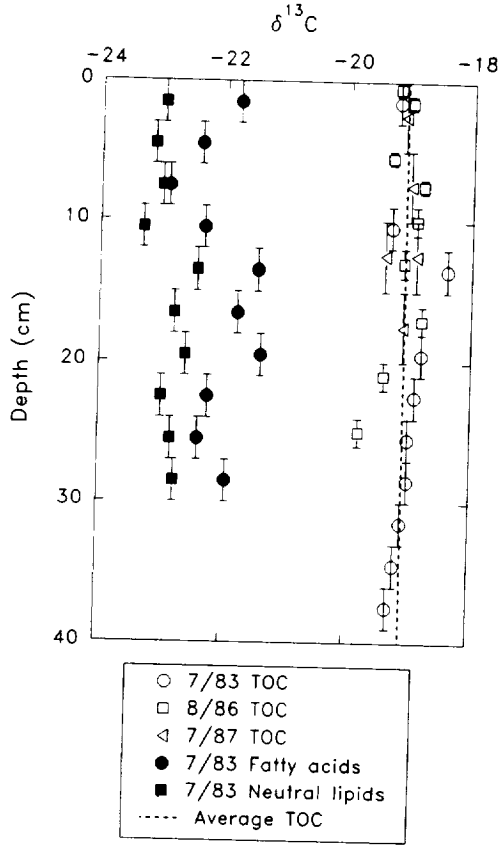

FIG. 1. $\delta^{13}C$ values of total organic carbon and lipid fractions. The TOC samples were collected on July 7, 1983 (○), August 14, 1986 (□), and July 21, 1987 (◁). The fatty acid (●) and neutral lipid fractions (■) were from July 7, 1983. The TOC data is from Blair et al. (1987), Boehme et al. (unpubl. data), and this study.

The $\delta^{13}C$ values of the porewater acetate exhibit excursions of nearly 15‰ with a pronounced subsurface maximum at the base of the sulfate reduction zone (Table 1; Fig. 3). The downcore variation is similar to that observed previously (BLAIR et al., 1987), but the absolute values are 5–10‰ heavier than the earlier data. The differences may represent true temporal variations as the 1983–84 data set was from June to early July. However, it should be noted that the core handling procedures were different in the two studies. In the original investigation, all cores were returned to the laboratory and refrigerated at 4°C until they could be processed (BLAIR et al., 1987). One core was treated similarly in this study. The results from that core (one total and two methyl $\delta^{13}C$ values) were approximately 1‰ lighter than those from a core processed immediately after collection onboard ship. Though the size of the data set prevents a rigorous statistical evaluation of the differences, it appears that the sample processing procedures were not responsible for the large differences between the two studies. Investigations are under way to resolve this issue.

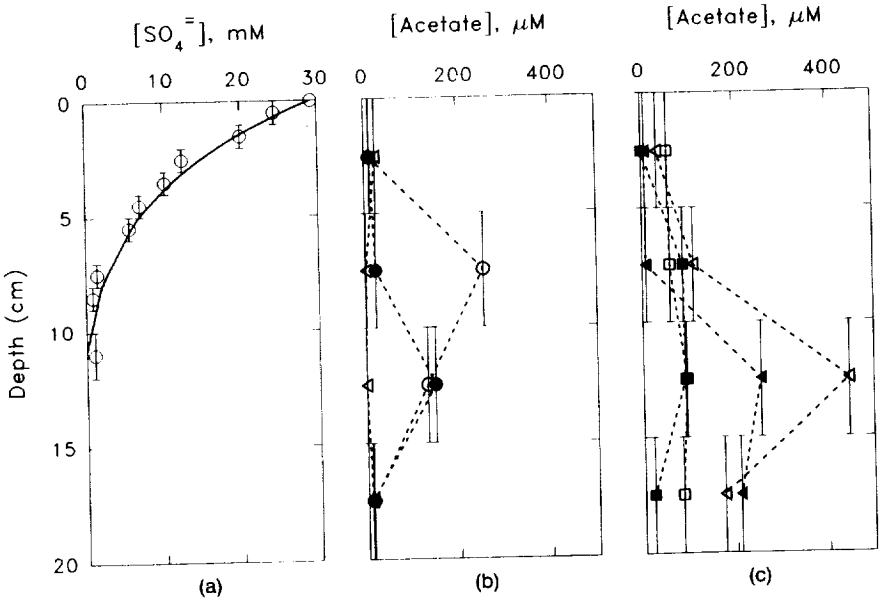

FIG. 2. Porewater sulfate and acetate concentration profiles. (a) Sulfate concentrations on August 14, 1986. (b) Acetate concentrations from triplicate cores collected on August 14, 1986. (c) Acetate concentrations from duplicate cores (triangles and squares) collected on July 21, 1987. Open and filled symbols represent methanol-killed and centrifuged-only samples, respectively (see Methods section for details).

The isotopic variability between cores from the same sampling date (0.2–2‰) was also of concern. No systematic differences were observed between the results of the different sampling procedures; thus, we conclude the noise is due to spatial heterogeneities on the meter scale within the seabed. Accordingly, the results from all treatments on a sampling date were averaged (Table 1; Fig. 3).

The $\delta^{13}C$ values of the acetate methyl group are 2–7‰ lighter than the total molecule (Table 1). The depth profile parallels that of the total acetate (Fig. 3). The $\delta^{13}C$ values of the carboxyl group were determined by a mass balance calculation and are 5–14‰ enriched in ^{13}C relative to the methyl group.

DISCUSSION

In principle, the isotopic composition of sedimentary acetate should be controlled by the (1) isotopic composition of its precursors, (2) isotopic fractionations associated with its synthesis and consumption, and (3) relative rates of all

Table 1: $\delta^{13}C$ values of pore water acetate.

Depth (cm)	CH_3COOH	CH_3-	-COOH[4]
8/14/86			
0-5	-17.6[1]	-21.4(0.7)[2]	-14.4[1]
5-10	-11.4(1.0)[2]	-18.6(0.4)[2]	-4.2(2.4)[2]
10-15	-4.6(0.2)[2]	-10.5(0.4)[2]	1.2(0.1)[2]
15-20	-11.0(0.8)[2]	-13.6(0.8)[2]	-8.4(0.8)[2]
7/21/87			
0-5	-	-24.9(0.8)[2]	-
5-10	-12.0[1]	-18.5(0.2)[2]	-5.3[1]
10-15	-2.8(1.6)[3]	-8.3(0.3)[2]	4.4(1.2)[2]
15-20	-7.2(2.1)[2]	-14.4(0.5)[2]	0.0(3.7)[2]

[1] Single measurement (n=1)
[2] Difference between duplicate core $\delta^{13}C$ values and the mean.
[3] Standard deviation (1σ) for mean of triplicate core values.
[4] Determined by mass balance calculation.

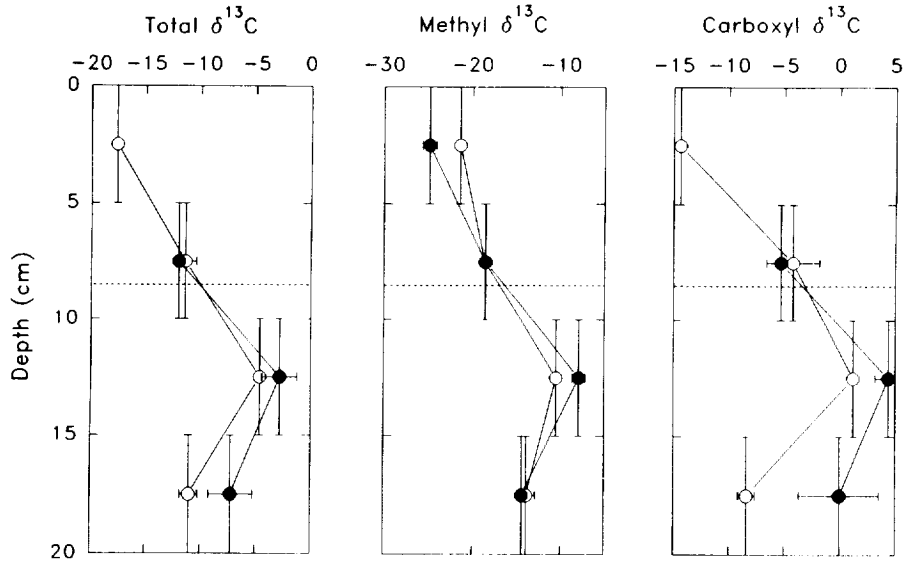

FIG. 3. $\delta^{13}C$ values of porewater acetate. The average $\delta^{13}C$ values of the total acetate, methyl, and carboxyl groups are from August 14, 1986 (○) and July 21, 1987 (●). The horizontal dashed line represents the <1 mM isopleth for dissolved sulfate.

processes which influence its pool size (BLAIR et al., 1985; BLAIR et al., 1987; GELWICKS et al., 1989). The downcore variations in the $\delta^{13}C$ value of acetate from Cape Lookout Bight indicate that changes in one or more of those factors occur as a function of depth within the sediment column. Each of those factors is discussed below.

Carbon Sources

The metabolizable organic carbon, which is the ultimate source of the acetate carbon, is estimated to have approximately the same average $\delta^{13}C$ value as the TOC fraction ($-19.08 \pm .26$) because the $\delta^{13}C$ value of the TOC remains unchanged as a function of depth (Fig. 1), even though 20–30% of the carbon is remineralized (MARTENS and KLUMP, 1984). The $\delta^{13}C$ value reflects a mixture of isotopically distinct sources. The sedimentary organic matter, and by inference, the metabolizable fraction, are derived from a variety of sources including phytoplanktic, microbial, and vascular plant remains (HADDAD and MARTENS, 1987; MARTENS et al., 1992). Visual inspection of cores and lignin analyses have indicated that *Haladule wrightii* and *Zostera marina*, the predominant seagrasses in the area (THAYER et al., 1978), and *Spartina alterniflora* are sources of the vascular plant matter (HADDAD and MARTENS, 1987). These plants typically have $\delta^{13}C$ values of -6 to -13‰ (THAYER et al., 1978; MCMILLAN and SMITH, 1982; STEPHENSON et al., 1984; FRY and SHERR, 1984). This is in contrast to the $\delta^{13}C$ values of coastal plankton, which can range from -20 to -23‰ (HAINES, 1976; GEARING et al., 1984). The relative contributions of the vascular and nonvascular plant sources to the buried organic carbon pool have been estimated to be 17 ± 23% and 83 ± 47%, respectively (HADDAD and MARTENS, 1987). The relative importance of those sources to the metabolizable fraction is unknown. While variations in the relative abundance of those sources within the sediment column could influence the $\delta^{13}C$ depth profile of the acetate, no evidence for significant variations is apparent in either the TOC $\delta^{13}C$ (Fig. 1) or lignin profiles (HADDAD and MARTENS, 1987).

Isotopic heterogeneities which result from differences between compound classes also exist in the metabolizable fraction. Identified amino acid, carbohydrate, and lipid carbon represents 64 ± 17% of the metabolizable pool (BURDIGE and MARTENS, 1988, 1990; HADDAD, 1989; HADDAD and MARTENS, 1990; MARTENS et al., 1992). Lipid fractions are depleted in ^{13}C relative to the TOC fraction (Fig. 1). The $\delta^{13}C$ values of the amino acid and carbohydrate fractions are unknown. Large inter- and intramolecular carbon isotope heterogeneities exist in amino acids produced in a variety of algal and microbial cultures (ABELSON and HOERING, 1961; BLAIR et al., 1985; MACKO et al., 1987), and similar patterns may exist in sediments. The isotopic composition of carbohydrates from different sources is poorly characterized but the bulk carbohydrate pool is typically thought to be similar to that of the total biomass fraction of an organism (DEGENS et al., 1968; BLAIR et al., 1985). However, 3–4‰ differences have been observed between different carbohydrate fractions from marine plankton (DEGENS et al., 1968), and the leaves from the CAM-plant *Bryophyllum daigrmontianum* (DELEENS and GARNIER-DARDART, 1977). The importance of the isotopic heterogeneity within the metabolizable carbon pool is dependent on the extent to which specific organic fractions bypass acetate as an intermediate during diagenesis.

Synthetic Isotope Effects

Little is known about the isotope effects associated with the anaerobic biosynthesis of acetate; however, any synthetic pathway could create a unique isotopic signature in the acetate that it produces. For example, the $\delta^{13}C$ values of the methyl and carboxyl groups of acetate produced aerobically from

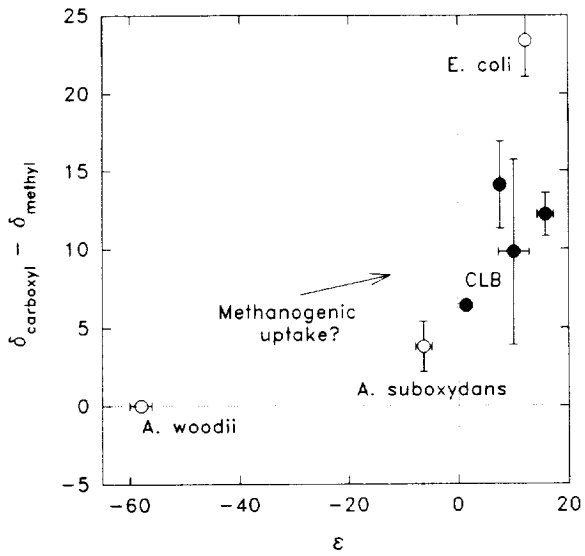

FIG. 4. Isotopic composition of acetate from various biological sources. The parameter, ϵ, is defined by the equation $\epsilon = [(R_{acetate}/R_{substrate}) - 1]10^3$, where R is the $^{13}C/^{12}C$ composition. The substrates for *A. woodii* (GELWICKS et al., 1989), *A. suboxydans* (RINALDI et al., 1974) and *E. coli* (BLAIR et al., 1985) were dissolved inorganic carbon, ethanol, and glucose, respectively. The substrate for the Cape Lookout sediments was assumed to have a $\delta^{13}C$ value of −19.1.

glucose by *Escherichia coli* were approximately 0 and +26‰ relative to the glucose (BLAIR et al., 1985). The large enrichment of ^{13}C in the carboxyl group was proposed to result from the transformation of acetyl phosphate to acetyl-CoA. *Acetobacter suboxydan* expressed a smaller fractionation during the aerobic synthesis of acetate from ethanol where the methyl and carboxyl groups were −9 and 0‰ relative to the corresponding ethanol carbons (RINALDI et al., 1974). The CO_2-reducing anaerobe, *Acetobacter woodii,* produced acetate which was isotopically homogeneous yet depleted in ^{13}C by as much as 57‰ relative to the total carbonate fraction (GELWICKS et al., 1989; PREUß et al., 1989). The isotope effect was believed to be associated with the enzyme carbon monoxide dehydrogenase (GELWICKS et al., 1989). The decarboxylation of lactate by the sulfate reducer, *Desulfovibrio desulfuricans,* produced CO_2 depleted in ^{13}C by 5–13‰ relative to the lactate carboxyl group (KAPLAN and RITTENBERG, 1964). The isotopic composition of the acetate which was produced was not measured; however, the carboxyl group of the acetate could be depleted in ^{13}C if the fractionation occurred during the actual decarboxylation step. A summary of the culture studies is shown in Fig. 4.

Catabolic Processes and Isotope Effects

The potential for isotope effects during the consumption of acetate is equally significant. Several biochemical strategies are used by sulfate- and sulfur-reducing bacteria to oxidize acetate to CO_2 (THAUER et al., 1989). *Desulfobacter postgatei* activates acetate to acetyl CoA via the reaction:

$$\text{Succinyl CoA} + \text{acetate} \rightarrow \text{Succinate} + \text{acetyl CoA} \quad (2)$$

The acetyl CoA is subsequently oxidized to CO_2 via the citric acid cycle (BRANDIS-HEEP et al., 1983; GEBHARDT et al., 1983). *Desulfuromonas acetoxidans,* an anaerobe which grows on acetate and sulfur, utilizes similar pathways (GEBHARDT et al., 1985; THAUER et al., 1989). In contrast, *Desulfotomaculum acetoxidans* and *Desulfobacterium autotrophicum* activate acetate to acetyl CoA by the intermediate formation of acetyl phosphate. The acetyl CoA is oxidized to CO_2 via the carbon monoxide dehydrogenase pathway (SCHAUDER et al., 1986, 1989; SPORMANN and THAUER, 1988, 1989). To our knowledge, the carbon isotopic fractionation associated with the oxidation of acetate by either of those pathways has not been reported.

The methanogenic genera, *Methanosarcina* and *Methanothrix,* are the only known microorganisms capable of dissimilating acetate to CH_4 and CO_2 (THAUER et al., 1989). In both genera, acetate is converted to acetyl CoA, after which the carbon-carbon bond of the acetyl unit is cleaved (GRAHAME and STADTMAN, 1987; THAUER et al., 1989). The methyl group is reduced to methane, and the carbonyl group is oxidized to CO_2 (BUSWELL and SOLLO, 1948; STADTMAN and BARKER, 1949; ZEIKUS, 1983). The fractionation factor (k^{12}/k^{13}) for methane formation from acetate by *Methanosarcina barkerii* is 1.02–1.03 (RISATTI and HAYES, 1983; KRZYCKI et al., 1987). The fractionation factors for acetate dissimilation by other species, including the marine methanogen, *Methanosarcina acetivorans* (SOWERS et al., 1984), have not been reported.

Relative Rates of Acetate Cycling Processes

The relative rates of the acetate cycling processes will have a major influence on the isotopic composition of that compound if any of the processes exhibit a significant isotope effect. The results of ^{14}C-tracer experiments can be used to estimate the relative rates of two processes, the oxidative and dissimilative pathways, by comparing the rates of conversion of the acetate methyl group to CO_2 or CH_4. The fraction of the acetate methyl group, which is converted to methane (f), is defined by the equation:

$$f = r_{CH_4}/(r_{CH_4} + r_{CO_2}), \quad (3)$$

where r_{CH_4} and r_{CO_2} are the rates of the conversion of the acetate methyl group to CH_4 and CO_2, respectively. By inference, f represents the fraction of acetate that is dissimilated directly by methanogens. If uniformly ^{14}C-labelled acetate is used to estimate turnover rate constants, f can be determined with the relationship:

$$f = 2k_{CH_4} + k_{CO_2}), \quad (4)$$

where k_{CH_4} and k_{CO_2} are the rate constants for CH_4 and CO_2 production from the total acetate molecule. It is assumed that the methane is derived solely from the methyl group. Similarly, the respiration index measurement (SANSONE and MARTENS, 1982) can be related to f by:

$$f = 2(1 - \text{RI}), \quad (5)$$

where the respiration index (RI) was determined with U-^{14}C-labelled acetate and is defined as:

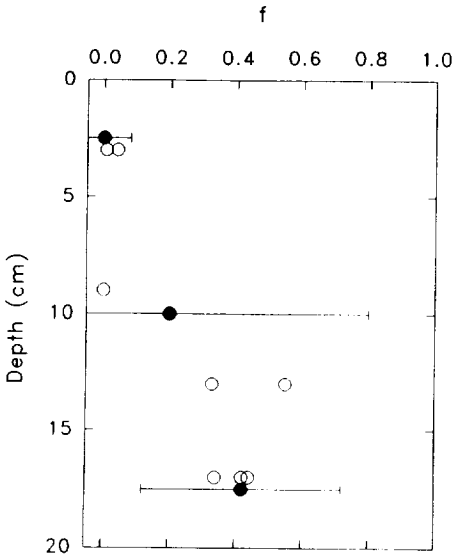

FIG. 5. The relative rate of acetate dissimilation as a function of depth. The parameter, f, was calculated using Eqns. (4) and (5) and ^{14}C-tracer data: ● (SANSONE and MARTENS, 1982); ○ (CRILL and MARTENS, 1986).

$$RI = {}^{14}CO_2 \text{ Production}/({}^{14}CO_2 \text{ Production} + {}^{14}CH_4 \text{ Production}). \quad (6)$$

Data from SANSONE and MARTENS (1982) and CRILL and MARTENS (1986) were used to estimate f for Cape Lookout Bight (Fig. 5). The value of f increases from zero in the sulfate reduction zone to a maximum of 0.55 in the methanogenic zone. In comparison, 70–86% of acetate ($f = 0.70$–0.86) in freshwater sediments is dissimilated directly to CH_4 and CO_2 (CAPPENBERG and PRINS, 1974; WINFREY and ZEIKUS, 1979a; LOVLEY and KLUG, 1982). The fact that f is significantly less than 1.0 indicates that an oxidative process competes with the methanogenic dissimilation of acetate. The similarity in the downcore profiles of f and the acetate $\delta^{13}C$ values suggests that the relative rates of the oxidative and dissimilative processes may be important isotopic controls. This point will be tested with a model which is described in the next section.

The absolute rate constants for acetate turnover, which were reported in the two studies, differ by an order of magnitude or more (SANSONE and MARTENS, 1982; CRILL and MARTENS, 1986). This discrepancy has been attributed to a difference in the incubation times used in the tracer experiments (CRILL and MARTENS, 1986). The consistency of f calculated from the two data sets argues that the relative rates of the acetate cycling processes were insensitive to the differences in methodology.

An Isotopic Model for Porewater Acetate

A simple model is proposed to describe the isotopic composition of acetate in this system. The following equation can be used to describe the isotopic composition of the methyl group, δ_{me}:

$$\delta_{me} = (\delta_{syn} + 10^3)(\alpha_{diss} f + \alpha_{ox}[1 - f]) - 10^3, \quad (7)$$

where δ_{syn} is the isotopic composition of the biosynthesized methyl group before consumption, α_{diss} is the fractionation factor associated with the reduction of the methyl carbon to methane, and α_{ox} is the fractionation factor associated with the oxidation of the methyl group (BLAIR et al., 1985). Steady-state or near-steady-state conditions for the short time frame needed to turn over the acetate pool (15 min to one day, SANSONE and MARTENS, 1982; CRILL and MARTENS, 1986) and first-order kinetics for the uptake of acetate are assumed. The model is simplified by setting $\alpha_{ox} = 1.00$. This is a reasonable first approximation because the $\delta^{13}C$ value of the total acetate molecule in the 0–5 cm interval ($f = 0$) is within 1.5‰ of that estimated for the metabolizable organic carbon fraction. Accordingly, $\delta_{syn} = -23.2 \pm 2.2$‰, which is the average δ_{me} value for the 0–5 cm interval. Average values of f and δ_{me} for the 10–20 cm depth interval, 0.38 ± 0.11 and -11.2 ± 3.0, respectively, were used with the following rearranged expression,

$$\alpha_{diss} = 1 + (\delta_{me} - \delta_{syn})/[f(\delta_{syn} + 10^3)], \quad (8)$$

to estimate α_{diss}. The resulting estimate for α_{diss}, 1.032 ± 0.014, is in good agreement with that observed in acetoclastic cultures (RISATTI and HAYES, 1983; KRZYCKI et al., 1987). An analogous calculation can be done to determine the apparent fractionation factor on the carboxyl group associated with the uptake by methanogens. In that case, α_{diss}(carboxyl) was found to be 1.036 ± 0.019. The agreement between the estimate of α_{diss} from the model and the culture measurements suggests that the rate of the acetoclastic reaction relative to acetate oxidation and the isotopic fractionation associated with methanogenesis are the dominant controls of the downcore variations in δ_{me}. Source effects and the presence of other sinks of acetate, such as biologically unavailable dissolved and adsorbed pools (CHRISTENSEN and BLACKBURN, 1982; SHAW et al., 1984; PARKES et al., 1984; NOVELLI et al., 1988; GIBSON et al., 1989; MICHELSON et al., 1989), would appear to be limited to secondary roles as controlling factors of the isotopic composition of the methyl group.

The 4–6‰ ^{13}C-depletion of the acetate downcore within the 10–20 cm interval indicates that the methanogenic zone cannot be considered spatially homogeneous in terms of microbial processes and may be composed of smaller diagenetic horizons. There is insufficient information to resolve with confidence if the isotopic shift is due to a source or consumptive effect. However, the ^{14}C-tracer studies of CRILL and MARTENS (1986) suggest that the relative rates of acetate oxidation and dissimilation change within the methanogenic zone with the oxidative process becoming progressively more important below the depth of peak methane production. According to our model, such a trend would result in the observed isotopic change.

The isotope model can be used to estimate the $\delta^{13}C$ value of methane produced by acetate dissimilation. Assuming steady-state conditions (HAYES, 1983), the $\delta^{13}C$ of the methane is approximated by:

$$\delta(CH_4/\text{Acet}) = \delta_{syn} - (1 - f)(\alpha_{diss} - 1) * 10^3. \quad (9)$$

Using the average value of f for the 10–20 cm methanogenic zone, $\delta(CH_4/Acet)$ was calculated to be $-43 \pm 10‰$. Approximately 26% of the methane production at this site is provided by acetate; the remainder is generated principally by CO_2-reduction (CRILL and MARTENS, 1986). A mass balance calculation using the average measured $\delta^{13}C$ value of $-59.5 \pm 2.0‰$ for methane produced in July and August at A-1 (MARTENS et al., 1986) indicates that the isotopic signature of the methane produced by CO_2-reduction should be $-65 \pm 5‰$. This result appears to verify the hypothesis that the acetate-dissimilating pathway produces methane which is enriched in ^{13}C relative to that generated by CO_2-reduction (WHITICAR et al., 1986). WHITICAR et al. (1986) hypothesized further that the isotopic differences observed between methane generated in freshwater and marine environments are the result of the distinctive isotopic signatures and the relative importance of the two pathways. Accordingly, freshwater environments, in which acetate dissimilation is the dominant pathway, typically produce methane which is relatively enriched in ^{13}C.

Similarly, the seasonal variations in methane $\delta^{13}C$ and δD values at Cape Lookout have been attributed, in part, to changes in the relative rates of the two methanogenic processes (MARTENS et al., 1986; BURKE et al., 1988). ^{14}C-tracer studies have suggested that the proportion of total methane production from acetate increased from 20% in mid-July 1983 to 29% in late August (CRILL and MARTENS, 1986). The statistical significance of the temporal trend cannot be evaluated; however, the apparent trend toward the greater importance of the acetate dissimilatory pathway in late summer should produce methane enriched in ^{13}C. The observed methane $\delta^{13}C$ values for mid-July and late August are -61.4 ± 1.1 and $-57.7 \pm .3‰$, respectively (MARTENS et al., 1986). This consistency between the model predictions and actual observations not only provides us with some measure of confidence in the general features of the model but also in the relative rates determined with the ^{14}C-tracer experiments. This is an important issue given the current controversial nature of acetate turnover rate measurements (CHRISTENSEN and BLACKBURN, 1982; SHAW et al., 1984; PARKES et al., 1984; NOVELLI et al., 1988; MICHELSON et al., 1989; GIBSON et al., 1989).

Speculations on Microbial Processes in Anoxic Sediments

The calculated $\delta^{13}C$ values of the carboxyl group exhibit a downcore trend nearly parallel to that of the methyl group (Fig. 3) suggesting that they are controlled, in part, by methanogenic activity. Two methanogenic-related processes may influence the carboxyl $\delta^{13}C$ value. The first involves the bond cleavage between the methyl and carbonyl groups of the acetyl intermediate in the acetoclastic reaction sequence. A normal kinetic isotope effect associated with that reaction would explain the methyl group $\delta^{13}C$ values as well. The fact that the isotopic fractionations associated with both the methyl and carboxyl groups are observed in the porewater pool of acetate implies that the steps leading to the isotopically discriminating reactions are reversible. Cell suspensions of *Methanosarcina barkeri* catalyze rapid isotopic exchange between CO_2 and the carboxyl group of acetate (EIKMANNS and THAUER, 1984). Similarly, cell extracts of the same methanogen promoted isotopic exchange between CO_2 and acetyl-CoA, thus providing direct evidence that the carbon-carbon bond cleavage of acetyl-CoA is reversible (FISCHER and THAUER, 1990).

The second process is the isotopic exchange between the carboxyl group and an external CO_2 pool, as demonstrated for *Methanosarcina barkeri*. Thus, the apparent fractionation factor determined for the carboxyl group may not be simply the result of a kinetic isotope effect as treated in our model. The importance of the exchange reaction as an isotopically controlling process will depend on the relative rates of the isotopic exchange and the overall dissimilative process. Other microorganisms, including *Desulfobacterium autotrophicum* (SCHAUDER et al., 1986), *Acetobacterium woodii* (WINTER and WOLFE, 1980), and the syntrophic acetate-oxidizer, "Reversibacterium," (ZINDER and KOCH, 1984) have exhibited similar isotopic exchange capabilities. Carbon monoxide dehydrogenase appears to be the enzyme responsible for the exchange reaction in all of those microorganisms (DIEKERT et al., 1985; THAUER et al., 1989). The extent to which isotopic exchange occurs between acetate and CO_2 in Cape Lookout or other organic-rich marine sediments is unknown but should clearly be investigated.

The similarity of the $\delta^{13}C$ value of the total acetate molecule in the upper 5 cm of sediment with that of the TOC fraction, along with the model results, suggests that the fractionation associated with the uptake by bacteria in the sulfate reduction zone is relatively small. This conclusion, which is admittedly based on a modest data base, merits discussion because of its implications. The intramolecular carbon isotopic difference (ca. 7‰) in the 0–5 cm interval indicates that large isotopic fractionations occur during the biosynthesis of acetate. For an isotope effect of that magnitude not to be reflected in the $\delta^{13}C$ value of the total molecule, a large portion of the metabolizable carbon must be shunted through acetate in the sulfate reduction zone. This would also explain why the isotopic heterogeneity within the metabolizable organic carbon fraction does not manifest itself more obviously. Qualitatively, the isotope data is consistent with previous estimates that 40–60% of the remineralized organic carbon is shunted through acetate in the sulfate reduction zone of coastal sediments (SORENSEN et al., 1981; WINFREY and WARD, 1983). A more quantitative estimate of the flow of carbon through acetate would require substantial information concerning the biosynthetic fractionations.

The similarity of the $\delta^{13}C$ value of the surficial acetate to that of the TOC fraction would appear to preclude the possibility that a significant portion of the acetate could be produced by acetogenic CO_2-reduction because of the large isotope effect associated with that process (GELWICKS et al., 1989; PREUß et al., 1989). Instead, it is likely that the acetate is synthesized via the more direct fermentation of organic species. In contrast, isotope models have suggested that CO_2-reduction is an important source of acetate in freshwater sediments (LAZERTE, 1981; GELWICKS et al., 1989). This con-

tradicts the results of other studies, which indicate that <2% of the acetate in eutrophic lake sediments was produced via CO_2-reduction (LOVLEY and KLUG, 1983b). The apparent contradiction clearly illustrates how little is known concerning acetate cycling and the associated isotope effects in different environments and points out the need for further studies.

SUMMARY AND CONCLUSIONS

The impressive intramolecular carbon isotopic heterogeneity and downcore $\delta^{13}C$ variations exhibited by the dissolved acetate at Cape Lookout Bight are compelling evidence for the presence of large isotope effects associated with some aspect of acetate cycling. The downcore $\delta^{13}C$ profiles are consistent with the hypothesis that both oxidative and dissimilative processes consume acetate in the methanogenic zone of the sediment (WARFORD et al., 1979; SANSONE and MARTENS, 1982). The dominant isotopic fractionation appears to be associated with the methanogenic dissimilation of acetate. A fractionation factor for the conversion of the methyl group of acetate to methane was estimated to be 1.032 $\pm$ 0.014, which is in good agreement with that previously measured in culture (RISATTI and HAYES, 1983; KRZYCKI et al., 1987). The isotopic measurements of the acetate methyl group coupled with estimates of rates of acetate cycling have provided direct evidence in support of the hypothesis that methane produced by the dissimilation of acetate is enriched in ^{13}C relative to that produced by CO_2-reduction (WHITICAR et al., 1986).

The secondary controls of the isotopic composition of acetate are virtually unknown. Experiments employing chemical inhibitors for microbial processes, e.g., $MoO_4^{=}$ for sulfate reduction, and radiotracer rate determinations coupled with the natural abundance $^{13}C/^{12}C$ measurements will provide important information concerning both synthetic and catabolic isotope effects. Culture studies of acetate-producing and -consuming anaerobic microorganisms and their associated isotope effects are needed to establish the isotopic signatures of the different metabolic pathways. If the isotopic signature of the sedimentary acetate can be understood, it would be a sensitive indicator of in situ processes and their relative rates. The isotopic measurements should prove useful in monitoring temporal and spatial changes in the sedimentary microbial ecosystem and the related diagenetic processes. As an example in this study, the $\delta^{13}C$ measurements have indicated that the methanogenic zone should not be viewed as a homogeneous microbial ecosystem but instead appears to be stratified with respect to acetate cycling processes. The exact nature and cause of the stratification is unknown.

Isotopic measurements of diagenetic intermediates such as acetate provide information which is different from, but complementary to, that generated by other methods. In principle, by looking at the natural abundance isotopic composition of a compound from a sediment, we are viewing the result of in situ processes which have not been subjected to the same potential artifacts commonly associated with radiolabel and microbiological methods. Because of that characteristic, the isotopic measurements should serve as unique constraints on models derived from the results of other methodologies.

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