The Carbon Isotope Biogeochemistry of ΣCO_2 Production in a Methanogenic Marine Sediment

ABSTRACT

To investigate the relationship between ΣCO_2 $\delta^{13}C$ values and rates of the dominant remineralization processes at the organic-rich field site of Cape Lookout Bight, NC, the isotopic composition of porewater ΣCO_2 was measured on a seasonal basis. The ΣCO_2 $\delta^{13}C$ values varied seasonally in response to changes in rates of sulfate reduction and methanogenesis, the dominant remineralization processes at this site.

A tube incubation experiment was also performed to determine the isotopic signature of the ΣCO_2 produced by sulfate reduction and methanogenesis. The $\delta^{13}C$ of the ΣCO_2 produced in the sulfate reduction zone determined from the tube incubation was -14.3 $\pm$ 1.9, a value enriched in ^{13}C relative to the labile organic fraction. The ^{13}C-enrichment may be caused by low rates of methanogenesis occurring in the sulfate reduction zone. The $\delta^{13}C$ of the ΣCO_2 produced in the methanogenic zone was estimated to be +44 per mil, whereas the co-produced methane was -65 per mil. The fractionation factor for CO_2 reduction was calculated to be 1.055, a value in agreement with previous estimates at this site. The measured concentration and $\delta^{13}C$ of the ΣCO_2 at Cape Lookout was closely reproduced by a diagenetic model using the measured rates of sulfate reduction and ΣCO_2 production, and the isotopic signature of the ΣCO_2 production in the two biogeochemical zones.

INTRODUCTION

The isotopic composition of total dissolved inorganic carbon (ΣCO_2) in sediments is a unique record of diagenetic processes. Presley and Kaplan (1968) used the isotopic signatures of ΣCO_2 to confirm that the downcore increases in ΣCO_2 from nearshore sediments were a result of metabolic activity of organisms in the sediment. Nissenbaum et al. (1972) identified the effects of methane production on the isotopic composition of ΣCO_2. These studies were followed by numerous applications of isotopic measurements of ΣCO_2 to identify the processes that produce and utilize ΣCO_2 in sediments (e.g. LaZerte, 1981; Reeburgh, 1982; McCorkle et al., 1985; Herczeg, 1988; McCorkle and Emerson, 1988).

Closed system box models were first used to try to reproduce the isotopic signatures that were measured in the field (Nissenbaum et al., 1972; Claypool and Kaplan, 1974; Reeburgh, 1982). Applying open system models to stable isotope studies of both freshwater and marine studies allowed for the inclusion of diffusion, bioturbation and sedimentation effects and yielded more information about the processes affecting these profiles such as carbonate dissolution (McCorkle et al., 1985; Alperin, 1988; McNichol et al., 1991), carbon input rates (McCorkle et al., 1985), and methane oxidation (Whelen et al., 1976; Reeburgh, 1982; Herczeg, 1988; Alperin, 1988). To date, diagenetic models of isotopic profiles have required assumptions about the isotopic composition of the ΣCO_2 produced during sulfate reduction and methanogenesis, as well as the isotopic composition of the remineralized organic carbon.

Typical ΣCO_2 $\delta^{13}C$ depth profiles from organic-rich marine sediments exhibit two major trends. The ΣCO_2 initially becomes depleted in ^{13}C relative to overlying seawater ΣCO_2 values (typically near zero per mil) and then, at depth, reverses this trend and becomes ^{13}C-enriched. The initial $^{13}C/^{12}C$ gradient has been attributed to sulfate reduction which produces ΣCO_2 with an isotopic composition similar to the organic matter that has been remineralized (typically -19 to -24 per mil in marine sediments (Fry and Sherr, 1984). Methanogenesis is thought to cause the gradient reversal by utilizing ^{13}C-depleted ΣCO_2 and leaving behind ^{13}C-enriched ΣCO_2 (Nissenbaum et al., 1972). To observe such profiles in typical coastal or deep sea sediments may require analysis of several to 100's of meters of sediment because of the slow rates of remineralization. The sediments of Cape Lookout Bight, North Carolina (Station A-1; Martens, 1976) were chosen for this study because these trends are fully established in the upper 40 cm of sediment due to the extremely high remineralization rates (Chapter 2; Boehme et al., in revision).

The present study attempts to address some of the assumptions applied to isotopic studies of ΣCO_2 and to identify and quantify the controls on the isotopic composition of ΣCO_2 in a methanogenic sediment. Specifically, this study was undertaken to determine quantitatively the processes controlling the isotopic composition of ΣCO_2 in a methane producing marine sediment overlain by a typical sulfate reduction zone. By coupling field and laboratory studies, the parameters that had to be assumed in earlier models could be directly measured in order to estimate the relative importance of the dominant processes controlling the $\delta^{13}C$ composition of

the ΣCO_2. Field measurements of ΣCO_2 $\delta^{13}C$ sedimentary profiles indicated a relationship between rates of the dominant processes and the resultant ΣCO_2 $\delta^{13}C$ profiles. Sediment incubations were used to determine rates of ΣCO_2 production and sulfate reduction as well as the $\delta^{13}C$ of ΣCO_2 produced. From these measurements it was possible to test the hypothesis that the ΣCO_2 $\delta^{13}C$ porewater profiles from anoxic sediments resulted from a balance of ΣCO_2 produced by sulfate reduction and that produced by methanogenesis.

Field Site

Cape Lookout Bight, North Carolina is a partially enclosed marine basin located 110 km southwest of Cape Hatteras. Samples were collected at Station A-1, which has been actively studied for almost 20 years (Martens, 1976; Chanton et al., 1983; Martens and Klump, 1984; and references therein). The dominant remineralization processes within the sediment are sulfate reduction and methanogenesis (Crill and Martens, 1983; 1986; Martens and Klump, 1984). Accumulation rates of 8-12 cm per year for the upper 40 cm of the sediment have been measured at Station A-1 (Chanton et al, 1983; Canuel et al., 1990). Seasonal temperature variations of 20°C drive changes in remineralization rates and fluxes of the diagenetic products. Despite this seasonality, near-steady state conditions occur on yearly time scales. Good agreement between measured and modelled nutrient profiles suggest near steady state conditions may occur on shorter time scales as well (Klump and Martens, 1981; Martens and Klump, 1984; Haddad and Martens, 1990; Chapter 2, Boehme et al., in revision; this study).

METHODS

The collection and analysis of field samples for monthly profiles of ΣCO_2 from Station A-1 have been described previously (Chapter 2; Boehme et al., in revision). A 9.5 cm diameter sediment core was collected at A-1 in June 1991 and sectioned in the lab at 2 cm intervals down to 20 cm to conduct an incubation experiment. Sediment temperature was 25°C, in the field at the time of core collection. Sediments from each depth interval were homogenized and transferred into five 15 ml centrifuge tubes via syringes. The centrifuge tubes from each of the 10 depth intervals were capped and stored in a glove bag and kept under nitrogen at 25°C for the duration of the incubation experiment.

One sample from each of the ten depth intervals was processed immediately (T=0) and then every 40 to 75 hours by centrifugation (3500 rpm for 30 minutes). The extracted porewater was taken up in a syringe, and filtered through a 0.45 μm nylon filter (Micron Separations Inc.). One ml of porewater was injected into a 2.5 ml serum bottle, capped and crimped for analysis of the concentration and $\delta^{13}C$ of the ΣCO_2. The ΣCO_2 samples were maintained frozen until the end of the experiment so that the five samples from each depth could be analyzed on the same day. One to 3 ml of porewater was acidified and stored for Ca^{++} analyses. The remaining porewater was treated with a drop of concentrated $ZnCl_2$ to precipitate sulfide, filtered, and stored refrigerated for sulfate analysis.

Porewater ΣCO_2 samples were analyzed on a GC-TCD adapted with a vacuum line to collect the CO_2 gas for isotopic analysis (Blair and Carter, 1992; Schaff et al.,

1993). The ΣCO_2 serum bottles were injected with 0.2 ml of 1M H_3PO_4 and the resulting CO_2 was swept into the GC column containing Unibeads-1s or 2s silica gel (Alltech) with He as the carrier gas. Water was removed using a Nafion drying tube (Permapure Products). Sample concentrations were determined with a thermal conductivity detector. The CO_2 was trapped in an 1/8 inch o.d. stainless steel trap under liquid nitrogen, and transferred to 6 mm borosilicate glass tubing for storage. Isotopic analyses were done on a modified Finnigan Mat Delta E isotope ratio mass spectrometer (Hayes, 1983). All isotope values are given in the $\delta^{13}C$ notation (See Eqn. 1). The precision and accuracy of the ΣCO_2 concentration measurements determined from standards is 0.5 mM and 1.0 mM respectively, and for ΣCO_2 $\delta^{13}C$ measurements, 0.3 per mil and 0.6 per mil respectively.

Porewater $SO_4^=$ samples were processed by gravimetric analysis of the precipitated barium salt (Chanton, 1985; Chanton et al, 1987). The precision and accuracy of this technique was 0.6 mM and 1.0 mM, respectively.

Porewater Ca^{++} samples were treated as described in Chapter 2. The Ca^{++} measurements were analyzed on a Perkin-Elmer Atomic Absorption spectrometer in the Soil Science Laboratory (NCSU).

RESULTS

The concentration of ΣCO_2 increased and that of sulfate decreased as a function of time in the upper 10 cm during the incubation experiment, with the most rapid changes occurring in the upper 0-2 cm interval (Fig. 3.1). ΣCO_2 $\delta^{13}C$ became progressively depleted in ^{13}C with time for the 0-8 cm intervals, whereas the 8-10 cm

interval became enriched in ^{13}C with time (Fig. 3.1). The rates of ΣCO_2 production and sulfate reduction were determined by linear fits to the concentration data for each of the upper 0-10 cm intervals (r^2 values are given in Table 3.1). The $SO_4^=$ and ΣCO_2 concentrations, and the ΣCO_2 $\delta^{13}C$ values showed little change with time in the samples below 10 cm due to slow rates of remineralization and the relatively short 10-day incubation and therefore are not shown. The Ca^{++} concentrations show little change below the 0-2 cm interval (Fig. 3.5) for the upper 10 cm. The Ca^{++} T=0 profile did not agree well with the porewater collected by squeezer on the same day (Chapter 2; Fig. 2.5). The poor agreement between the squeezer and centrifuged profiles may indicate that one of these techniques causes changes in the porewater concentrations, and therefore this data set was only used to show that there were not large changes in porewater Ca^{++} over the 10 day experiment.

Using the ΣCO_2 concentration and $\delta^{13}C$ values, ^{12}C and ^{13}C production rates were determined. Rate measurements of ΣCO_2 production, and $SO_4^=$ reduction for the upper 10 cm (Fig. 3.2a) as well as $\Sigma^{13}CO_2$ and $\Sigma^{12}CO_2$ production rates calculated using the isotopic signatures of the ΣCO_2 produced (Fig. 3.2b) were fit to an exponential curve of the form:

$$R_z = R_0 \exp^{(-az)} \qquad (10)$$

where

R_z = rate of production or reduction of process (mM/hr),
R_0 = rate at the sediment-water interface (mM/hr),
a = attenuation coefficient (cm^{-1}), and
z = depth into sediment (cm).

Values of R_0 and a were solved iteratively from measured values of Rz with the Sigmaplot curve fitting routine (Jandel Scientific). Curve fitting parameters are given in Table 3.2. The ΣCO_2 concentration and $\delta^{13}C$ values were used to estimate the $\delta^{13}C$ signal of the ΣCO_2 produced for each depth interval using the following equation:

$$\delta_{add} = \frac{C_t\delta_t - C_o\delta_o}{C_{add}} \tag{11}$$

where

C_t = ΣCO_2 concentration at given time
δ_t = $\delta^{13}C$ of ΣCO_2 at given time
C_o = ΣCO_2 concentration at initial time zero for given depth
δ_o = $\delta^{13}C$ of ΣCO_2 at initial time zero for given depth
C_{add} = ΣCO_2 concentration added between C_o and C_t
δ_{add} = $\delta^{13}C$ of ΣCO_2 added between C_o and C_t.

The δ_{add} was solved for by curvefitting the ΣCO_2 concentration and ΣCO_2 $\delta^{13}C$ for each depth interval and the horizontal error bars represent the standard error for each of the values (Fig. 3.2).

The advection-diffusion model (Berner, 1980) was used to convert the measured rates of sulfate reduction, ΣCO_2 production, $\Sigma^{12}CO_2$ production and $\Sigma^{13}CO_2$ production to test whether these rates are representative of <u>in situ</u> conditions. An analytical solution of the advection-diffusion equation

$$\frac{\partial C}{\partial t} = D_s\frac{\partial^2 C}{\partial z^2} - \omega\frac{\partial C}{\partial z} - R \tag{12}$$

was found assuming $\partial C/\partial t = 0$ and the following boundary conditions: as $C_z \dashrightarrow \infty$, $\partial C/\partial z = 0$; for ΣCO_2, C_0 = 2.11 mM, $\delta^{13}C$ = 0.0 per mil; for sulfate, C_0 = 29.6.

Eqn. 12 becomes

$$C_z = C_0 + \frac{R_0}{a^2 D_s + a\omega}(1 - \exp^{-az}), \qquad (13)$$

where

D_s	=	Diffusion coefficient ($D_s = \phi^2 D_0$; $\phi = 0.9$ for upper 20 cm; Chanton et al., 1983; $D_s(HCO_3^-) = 0.0344$ cm/hr; $D_s(SO_4^=) = 0.0312$ cm/hr),
C_z	=	Concentration of species at given depth z
a	=	attenuation coefficient (cm^{-1})
ω	=	sedimentation rate (10.6 cm/yr; Chanton et al., 1983).

Diffusion coefficients for HCO_3^- and $SO_4^=$ were taken from Li and Gregory (1974) and corrected for sediment tortuosity as noted above (Ullman and Aller, 1982). The effects of changing porosity with depth have been shown to alter the results by less than 4% for Cape Lookout sediments and so porosity was assumed to be constant with depth (Klump and Martens, 1989). The comparison of measured and calculated concentrations and the ΣCO_2 $\delta^{13}C$ profiles are shown in Fig. 3.3. The steady state assumption appears to be justified given the agreement between measured and estimated ΣCO_2 profiles determined from the rate measurements (Klump and Martens, 1981;1989; this study). The estimated ΣCO_2 $\delta^{13}C$ profile shown in Fig. 3.3b, agrees with the measured values for the upper 0-12 cm. The two profiles diverge below this depth, because the lower boundary condition forces $\partial C/\partial z$ to 0 at depth. The modelled and measured $SO_4^=$ concentration also agree well, verifying the sulfate reduction rate measurements.

The depth-integrated rates of ΣCO_2 production and sulfate reduction as well as $\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ were determined by integrating Eqn. 11 and multiplying by a

porosity term (ϕ=0.9 cm^3_{pw}/cm^3_{sed}) (Eqn. 12), to compare to previous depth integrated rate measurements:

$$\Sigma R_z = \int_0^{10cm} R_0 \exp^{-az}. \quad (14)$$

The calculated depth integrated rate of sulfate reduction of 21.3 moles-m^{-2}-yr^{-1} is in excellent agreement with a previously measured incubation experiment sulfate reduction rate of 21.0 moles-m^{-2}-yr^{-1} (Klump and Martens 1989) and comparable to previously measured rates of sulfate reduction for June 1978 of 19.3 $\pm$ 3.4 moles-m^{-2}-yr^{-1} and June 1979 of 24.3 $\pm$ 3.0 moles-m^{-2}-yr^{-1}(Martens and Klump, 1984). The depth integrated ΣCO_2 production of 34.6 moles-m^{-2}-yr^{-1} also agrees well with a previous incubation experiment rate of 34.0 moles-m^{-2}-yr^{-1} (Klump and Martens 1989) and is comparable to ΣCO_2 fluxes of 38.9 $\pm$ 0.8 moles-m^{-2}-yr^{-1} measured in June 1978 (Martens and Klump, 1984).

DISCUSSION

The ΣCO_2 $\delta^{13}C$ profiles measured at Cape Lookout are typical of anoxic marine sediments, with a zone of ^{13}C-depletion overlying a zone of ^{13}C enriched ΣCO_2. These profiles are thought to result from sulfate reduction producing ΣCO_2 that is isotopically similar to the organic matter being oxidized in the upper zone and methanogenesis generating ^{13}C-enriched ΣCO_2 at depth. Additionally, ^{13}C-depletions have been attributed to methane oxidation adding ^{13}C-depleted ΣCO_2 in the sulfate reduction zone, particularly near the transition between the sulfate reduction and methane production zones (Reeburgh, 1982; Whiticar and Faber, 1986). In studies

where methane oxidation has been shown to be important, the resultant ΣCO_2 may be distinguishable by the ^{13}C-depleted nature and the concave-up sedimentary methane concentration profiles. At Cape Lookout, methane oxidation is not an obvious control on the ΣCO_2 $\delta^{13}C$ values, and appears to affect only the surficial sediments based on flux experiments and sedimentary CH_4 $\delta^{13}C$ profiles (See Chapter 2, Fig. 2.4; Boehme et al., in revision). The agreement between methane production rates and observed fluxes (Crill and Martens, 1986) is further evidence against significant methane oxidation at this site.

The ΣCO_2 $\delta^{13}C$ profiles from Cape Lookout also exhibit seasonal changes (Chapter 2; Boehme et al., in revision) If sulfate reduction and methanogenesis are controlling the isotopic signature of the ΣCO_2, then the seasonal trends seen in the isotope profiles should correlate to the changing rates of these processes. This can be tested directly using the measured monthly profiles of ΣCO_2 $\delta^{13}C$.

Further, if these processes are occurring in distinct zones, namely sulfate reducing bacteria out-competing the methanogenic consortia down to the depth at which sulfate is depleted, then the isotopic signal of ΣCO_2 being produced should reflect this zonation and should be expressed in the incubation experimental results. The measured isotopic signature for the ΣCO_2 produced in the sediment generally reflects this zonation (Fig. 3.2b).

The use of exponential fits to the $\Sigma^{12}CO_2$ and the $\Sigma^{13}CO_2$ rate measurements results in an estimated $\delta^{13}C$ produced that does not reflect the observed zonation in $\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ production (Fig 3.2b). This is not surprising considering that the

$\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ rate data may not be described by a simple exponential function because these rates result from the addition of two separate processes, sulfate reduction and methanogenesis, with different rates. The exponential fits, despite these problems reproduced the concentration and estimated isotope signatures of the ΣCO_2 measured in the sediment (Fig. 3.3; see discussion below). A comparison of exponential fits to cubic spline fits resulted in little differences in the estimated ΣCO_2 $\delta^{13}C$ profiles. The curve fits were used to determine depth integrated rates for modelling of individual processes. The depth integrated rates determined are consistent with previously measured rates for this site.

Field Studies

Seasonal changes in the ΣCO_2 $\delta^{13}C$ profiles from Cape Lookout (Chapter 2, Fig. 2.2; Boehme et al., in revision) appear to result from the changing rates of sulfate reduction and methanogenesis, the changing depths of sulfate depletion and possibly by changes in methanogenic pathways. This relationship can be seen qualitatively in the steepening concentration gradients and changing porewater ΣCO_2 isotope profiles in Fig. 2.2. In an attempt to quantify this relationship, sulfate concentration gradients and the isotopic gradient for the upper 3 cm of the sediment are compared in Fig. 3.4. Increasing rates generally correlate with increasingly ^{13}C-depleted gradients, however, this comparison does not include the effects of methanogenesis on the isotope profiles. Methane production is altering the isotopic signature of the ΣCO_2 flux. Further, for most of the year methane is produced predominantly via CO_2 reduction, however in July and August, some fraction of the

methane appears to be produced by acetate dissimilation (Crill and Martens, 1986; Blair et al., 1993). The smaller fractionation factor associated with acetate dissimilation may further alter the relationship between the sulfate concentration gradient and isotopic gradient for the months when acetate dissimilation is important.

The comparison shown in Fig. 3.4 suggests a simple correlation, that ΣCO_2 $\delta^{13}C$ values at the sediment surface are dependent on the concentration gradients of sulfate reduction. This may be a useful observation for understanding the range of isotopic signatures that are measured in other field sites, especially environments where methane production is not important. The data imply that if the isotopic signature of the two dominant processes controlling ΣCO_2 concentration in Cape Lookout sediments can be determined, then the overall ΣCO_2 isotopic signature should be predictable based on the changing rates of these two processes.

Incubation Experiment

The incubation experiment was performed to measure directly the isotopic signature of the ΣCO_2 being produced as a function of depth. Variation with depth in the isotopic composition of the ΣCO_2 produced for the upper 10 cm are shown in Fig. 3.2b. For the upper 8 cm, the isotopic signature is relatively constant. The 8-10 cm interval is markedly ^{13}C-enriched with an isotopic signature of $+2 \pm 1.9$ per mil. The two distinct zones are consistent with biogeochemical arguments that have theorized little overlap between the sulfate reduction and methanogenic zones. The isotopic signature of the ΣCO_2 in the upper 8 cm is enriched in ^{13}C relative to the metabolizable organic carbon being at Cape Lookout (-19 per mil; Chapter 2; Boehme

et al., in revision). This may indicate some methane production in the sulfate reduction zone. This hypothesis is consistent with previous measurements of methane production in the sulfate reduction zone (Crill and Martens, 1986). There are other possible causes for this enrichment of the ΣCO_2 that can be considered.

One source of ^{13}C enriched ΣCO_2 to porewaters is the dissolution of calcium carbonate. Porewater concentrations of Ca^{++} were measured during the incubation experiment and suggest some dissolution in the upper 0-4 cm (Fig. 3.5). The previous Ca^{++} measurements for this site also show an increase in the upper two cm indicating dissolution (Chapter 2, Fig. 2.5; Boehme et al., in revision). The increases, however, are not large enough to account for an offset of -5 per mil (-19 per mil organic carbon to the -14 per mil average isotope value for the upper 8 cm) assuming an isotopic composition of the $CaCO_3$ of -0.1 per mil (the average isotopic composition of the particulate inorganic carbon at Cape Lookout). Further, the Ca^{++} profiles measured during the incubation experiment do not indicate dissolution below the 0-2 cm interval over the 10 day incubation experiment (Fig. 3.5). Thus, the isotopic composition of the ΣCO_2 produced should therefore not be significantly affected by calcium carbonate dissolution. This conclusion could be erroneous if dissolution was coupled with precipitation in an exchange reaction. Exchange reactions have been hypothesized to account for differences in modelled and measured ΣCO_2 $\delta^{13}C$ profiles from turbidites (McArthur, 1989) but have typically been ignored because they are considered to be too slow to significantly alter the isotopic composition of rapidly depositing sediments (Nissenbaum et al., 1972). The isotopic

composition of the particulate inorganic carbon is relatively constant with depth (Boehme et al., in revision; Boehme, 1989), however, the possibility of isotopic exchange reactions during dissolution and precipitation of $CaCO_3$ has not been adequately addressed.

Another possible cause of the relatively ^{13}C-enriched ΣCO_2 in the sulfate reduction zone is the preferential remineralization of a ^{13}C-enriched organic carbon fraction during sulfate reduction. A long-term incubation study using sediments from Cape Lookout estimated the $\delta^{13}C$ of the organic matter remineralized during sulfate reduction to be -15.6 per mil (Alperin et al., 1992). If the sulfate reducing bacteria do not significantly fractionate the organic matter during remineralization, then the ΣCO_2 should be similar to the remineralized organic carbon. Preferential remineralization is not consistent with the particulate organic carbon (POC) $\delta^{13}C$ profiles for this site that indicate that the δ value of the organic carbon does not change with depth (Haddad, 1989; Blair and Carter, 1992; Chapter 2; Boehme et al., in revision). If sulfate reduction, the dominant remineralization process in these sediments, is preferentially removing organic carbon with a different isotopic signature than the bulk organic carbon, then this should cause be evident in the POC $\delta^{13}C$ profiles.

If methane production is occurring within the sulfate reduction zone, ^{13}C-enriched ΣCO_2 would be added to the pool of ΣCO_2 produced by sulfate reduction. This is consistent with incubation measurements of methanogenic rates measured by Crill and Martens (1983; 1986) in which low rates of methanogenesis via CO_2

reduction were measured within the sulfate reduction zone using incubation experiments and ^{14}C tracers, especially in the summer months when sulfate is rapidly depleted by 8 to 10 cm. As will be shown later, these rates of sulfate reduction and methanogenesis can be used in conjunction with an estimate of the isotopic signature of the ΣCO_2 produced from these processes to estimate a ΣCO_2 $\delta^{13}C$ value produced in the sulfate reduction zone. Studies have shown that methane production via non-competitive substrates such as methanol and methylamines can also result in methane production (and presumably ΣCO_2 production as well) in salt marsh sediments (King, 1984; King et al., 1985; Oremland et al., 1982; 1993).

The successful use of the rate measurements to estimate ΣCO_2 concentration and $\delta^{13}C$ profiles indicates that a simple two component mixing model can be used to estimate the isotopic signature of the ΣCO_2 produced from an anoxic sediment like Cape Lookout. The mixing model assumes that the isotopic signature of the ΣCO_2 results from the mixing of two sources--oxidative source (sulfate reduction) and methanogenic. This hypothesis can be described mathematically as a mass balance of the processes producing ΣCO_2 in the sediment,

$$R_{tot}\delta_{tot} = R(\Sigma CO_2)_{SR}\delta_{SR} + R(\Sigma CO_2)_M\delta_M \qquad (15)$$

where:

R_{tot}	= ΣCO_2 total production rate (mM/hr),
δ_{tot}	= isotopic signature of ΣCO_2 produced,
$R(\Sigma CO_2)_{SR}$	= ΣCO_2 production from sulfate reduction rate (mM/hr),
δ_{SR}	= isotopic signature of ΣCO_2 from SR
$R(\Sigma CO_2)_M$	= ΣCO_2 production from methanogenesis rate (mM/hr),
δ_M	= isotopic signature of ΣCO_2 from methanogenesis.

The sources of the ^{13}C-enriched ΣCO_2 produced in the sulfate reduction zone cannot be resolved by this study, but the measurement of this isotope signature allows us to model the processes producing these signals in an effort to test some of the hypotheses given here.

The ΣCO_2 produced and its isotopic signature were used to determine the ^{12}C and ^{13}C production rate profiles individually. These profiles were curve fit and extrapolated to estimate the isotopic signature of the ΣCO_2 added to the surface sediments due to sulfate reduction, -19.2 per mil. This value is assumed to be the best guess value for the isotopic composition of the ΣCO_2 produced from sulfate reduction alone (δ_{SR}) because the surface sediments should be the least affected by methanogenesis.

The sulfate reduction rate, R_{SR}, can be used to determine the depth integrated rate of ΣCO_2 produced from sulfate reduction,

$$R(\Sigma CO_2)_{SR}=R_{SR}\tau, \tag{16}$$

where $\tau = 1.78$, the ratio of ΣCO_2 produced to sulfate reduced at the sediment-water interface. The stoichiometric coefficient, τ, is assumed to be constant downcore. This assumption is supported by degradation studies of algae where the calculated composition of the refractory material (calculated as percent proteins, lipids and carbohydrates) was similar to the original composition of the algae (Foree and McCarty 1970) suggesting that no one algal component was preferentially utilized producing a different ratio of ΣCO_2 produced to algae degraded. Previous estimates

of τ for Cape Lookout surface sediments, of 1.7 $\pm$ 0.1 (Alperin et al, 1992) and 1.9 (average of upper 6 cm; Klump and Martens, 1989) supports the τ value used in this study. Factors controlling τ are discussed in further detail in a later section.

The net production of ΣCO_2 during methanogenesis, R_M is calculated by subtracting $R(\Sigma CO_2)_{SR}$ from R_{tot}. The depth integrated δ value of ΣCO_2 produced, δ_{tot} was estimated from the isotopic signature of the depth integrated $\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ production rates, -13.1 per mil. Solving Eqn. 15 for δ_M gives +44.2 per mil for the isotopic composition of the ΣCO_2 produced during methanogenesis. Using the values for ΣCO_2 production from sulfate reduction (-19.2 per mil) and methanogenesis (+44.2 per mil) and the estimated production rates, a ΣCO_2 $\delta^{13}C$ mixing curve was calculated and compared to the measured isotopic signature of the ΣCO_2 produced (Fig. 3.2b). The mixing curve misses the zonation of sedimentary processes, similar to the exponential curve fit results, but the mixing curve is derived from the exponential fits and so the similarity is not unexpected. As noted previously, the estimated concentration from the rate curve fits agrees well with the data. The differences in the curve fits and the measured data are probably not strongly reflected in the estimated isotope profiles because the production rates are low at depth.

Based on mass balance arguments, it should be possible to estimate the isotopic composition of the CH_4 produced in the incubation experiment using the following equation,

$$[R_{tot}+R_{CH_4}]\delta_{remin}=R_{tot}\delta_{tot}+R_{CH_4}\delta_{CH_4} \qquad (17)$$

where

R_{CH4} = rate of methanogenesis (mM/hr),
δ_{CH4} = isotopic signature of methane produced,
δ_{remin} = (-18.9 per mil; Boehme et al., in revision).

To estimate the rate of methane production, the rate of ΣCO_2 produced during methanogenesis can be used in the following stoichiometric relationship:

$$(R_{CH_4}) = \frac{R(\Sigma CO_2)_M}{(\tau - 1)}. \qquad (18)$$

The τ-1 parameter was determined based on oxidation state arguments and will be discussed later. Solving Eqn. 17, the isotopic composition of the methane produced in the sediments is -65.9 per mil. This value is very similar to the measured isotopic signature of methane bubbles collected at the same site in June 1983 and 1984 (-64.3 $\pm$ 0.7; Martens et al., 1986). The mass balance equations accurately estimate the isotopic composition of the methane being produced in this sediment. This is further verification that the mass balance achieved using measured rates of ΣCO_2 production and sulfate reduction adequately represents controlling diagenetic processes.

As noted earlier, the calculated $\delta^{13}C$ for ΣCO_2 produced via sulfate reduction (-19 per mil) and methanogenesis (+44 per mil) can be used to estimate the isotopic signature of the ΣCO_2 produced in the sulfate reduction zone using previously measured rates from Crill and Martens (1983). The rates of sulfate reduction and methane production from the summer months were averaged for the upper 1- 4 cm and 6-11cm (the depths above where sulfate was depleted) and converted to ΣCO_2 production rates using τ and τ-1. The resultant rates were used with the calculated

$\delta^{13}C$ for ΣCO_2 produced via sulfate reduction (-19 per mil) and methanogenesis (+44 per mil) to estimate a $\delta^{13}C$ for ΣCO_2 produced of -18 per mil for the upper 4 cm and -13 per mil for the 6-11cm interval. These calculations suggest that methane production in the sulfate reduction zone can significantly affect the $\delta^{13}C$ of the ΣCO_2 produced and may be responsible for the ^{13}C-enriched ΣCO_2 seen in the sulfate reduction zone.

The determination of the isotopic composition of the methane produced in the sediment allows us to calculate the fractionation factor for the production of methane,

$$\alpha = \frac{\delta^{13}C_{(CO_2)} + 10^3}{\delta^{13}C_{(CH_4)} + 10^3}, \tag{19}$$

where $\delta^{13}C_{(CH4)}$ is -65.9 per mil as calculated above and δCO_2 is the isotopic composition of the CO_2 measured in the methanogenic zone. Using a pH of 6.95 for A-1 sediments (Chanton, 1985; N.E. Blair et al., 1993), the relative contributions of HCO_3^-, CO_2, and $CO_3^=$ can be determined (Stumm and Morgan, 1981). At 8-10 cm, the measured ΣCO_2 $\delta^{13}C$ is -7.0 per mil (Fig. 3.3). To determine the isotopic composition of the CO_2 species, the following equations (Deines et al., 1974; Friedman and O'Neil, 1977; Blair et al., 1993) were solved simultaneously:

$$\delta^{13}C(\Sigma CO_2) = d\delta^{13}C(CO_2) + e\delta^{13}C(HCO_3^-) + f\delta^{13}C(CO_3^-) \tag{20}$$

$$\alpha\left(\frac{HCO_3^-}{CO_2}\right) = \frac{10^3 + \delta^{13}C(HCO_3^-)}{10^3 + \delta^{13}C(CO_2)} \tag{21}$$

$$\delta^{13}C(HCO_3^-) = \delta^{13}C(CO_3^=) \quad (22)$$

The variables d, e, and f represent the fractions of the dissolved ΣCO_2 components. The $\delta^{13}C$ of HCO_3^- and $CO_3^=$ are assumed to be the same (Eqn. 22). The equilibrium fractionation factor for the HCO_3^- and CO_2 equilibrioum is given by:

$$\ln\alpha\left(\frac{HCO_3^-}{CO_2}\right) = \left(\frac{9.552}{T}\right) - 0.0241 \quad (23)$$

Temperature is in Kelvin (Deines et al., 1974) Solving Eqns. 20, 21, 22 and 23 gives a $\delta^{13}C$ value for CO_2 of -14.0 per mil. Substituting this value into Eqn. 19 gives an α for methane production of 1.055. This fractionation factor is remarkably similar to an independent estimate for this site, based on a mechanistic model (1.056 at 25°C; Blair et al., 1993) and is within the range determined in culture studies (1.03 to 1.06; Games et al., 1976; Fuchs et al., 1979; Balabane et al., 1987; Belyaev et al.,1983) and estimated from measured profiles of ΣCO_2 and CH_4 (1.05 to 1.09; Whiticar et al., 1986). The agreement is further evidence to support the model results. This is the first in situ fractionation factor for methane production from CO_2 reduction determined using directly measured rates and the associated isotopic signatures.

Model Sensitivity to Reaction Stoichiometry

The isotopic composition of the ΣCO_2 produced during sulfate reduction and methanogenesis, and the isotopic composition of the methane that were estimated using the mass balance approach are sensitive to the stoichiometries of the sulfate reduction and methane production reactions. The δ_{CH4} is especially sensitive to the

stoichiometry as is seen in Fig. 3.6. These stoichiometries are dependent on the apparent oxidation state of the organic carbon that is remineralized. For the generalized formula $C_xH_yO_z$, the apparent oxidation state of the carbon is defined as

$$OS=\frac{2z-y}{x} for -4\leq OS\leq 4. \tag{24}$$

for the reaction

$$C_xH_yO_z+wSO_4^{=}\rightarrow uCO_2+S^{=}+H_2O, \tag{25}$$

where

$$\tau=\frac{u}{w}=\frac{8}{4-OS}. \tag{26}$$

Similarly for the methanogenic reaction,

$$2C_xH_yO_z\rightarrow uCO_2+vCH_4 \tag{27}$$

the ratio of CO_2 produced to CH_4 produced (u/v) is given by

$$\frac{u}{v}=\frac{4+OS}{4-OS}=\tau-1. \tag{28}$$

The relationship between τ-1, the ratio of CH_4 to ΣCO_2 produced, and oxidation state has been demonstrated in culture experiments (Tarvin and Buswell, 1934; Fig. 3.7).

Solving Eqn. 28 for oxidation state using τ = 1.78 from the model, gives an apparent oxidation state of the organic matter of -0.49. Using the τ determined by

Alperin et al. (1992) of 1.7 ± 0.1 gives an OS values of -0.72 ± 0.28 and using the τ determined by Klump and Martens (1989) of 1.9 gives an OS value of -0.21.

An independent estimate of oxidation state of the remineralized organic matter can be determined from the composition of the remineralized organic matter. At Cape Lookout, 64 ± 17% of the metabolized organic carbon has been identified as carbohydrates, lipids and amino acids, in a ratio of 1.0:1.0:1.9. Using the equation,

$$OS = \frac{(3a+2b+2z-y)}{x} \tag{29}$$

to describe the oxidation state of amino acids with the formula $C_xH_yN_aO_zS_b$ and the amino acid distributions identified at Cape Lookout sediments (Burdige and Martens, 1988), the average oxidation state of the amino acid carbon is estimated to be 0.07. The carbohydrate carbon is assumed to have an average oxidation state of zero. The C_{16} fatty acid was used as a representative lipid for this system (Haddad, 1989) with an oxidation state of -1.75. Given the relative contribution of these fractions to the identified pool, the apparent oxidation state of the identified metabolizable pool is -0.41, in good agreement with the modeled value of -0.49. The OS determined from the organic matter can be applied to Eqns. 26 and 28 to estimate a τ of 1.81, in excellent agreement with the 1.78 determined from this study. McCorkle and Emerson (1988) calculated CO_2/O_2 ratios for a variety of oxic and suboxic sediments. Assuming that oxidation state is the only factor controlling this ratio, their value of -0.54 is consistent with the τ determined for Cape Lookout. Gujer and Zehnder (1983) also noted a relationship between oxidation state, substrate, and CO_2 to CH_4

ratios for anaerobic digesters. Based on their correlations, the substrates that correlate to the oxidation state estimated for Cape Lookout are algae, bacteria, carbohydrates and proteins. The agreement of these various approaches to determining the OS and stoichiometries of sulfate reduction and methanogenesis is further evidence that despite the sensitivity of the model to these parameters (Fig. 3.6), our model is accurately describing the processes and their isotopic signatures in this system.

Implications for Control of Marine CH_4 $\delta^{13}C$

Marine CH_4 $\delta^{13}C$ values exhibit a wide range of values (-110 to -60 per mil; Whiticar et al., 1986). Possible sources of this variation must be due to differences in the relative rates of methanogenic pathways (predominantly CO_2-reduction and acetate dissimilation), differences in fractionation factors, and the isotopic composition of the precursors. The mass balance model presented here indicates that the δCH_4 is also dependent on the relative rates of non-methanogenic oxidative processes (sulfate reduction in this study) and methanogenesis. Further, the model's sensitivity to oxidation state suggests that oxidation state may be important as well.

CONCLUSIONS

The ΣCO_2 $\delta^{13}C$ profiles from Cape Lookout have been shown to be dependent on the rates of the remineralization processes, similar to the results of McCorkle and Emerson (1988). In the methanogenic sediments of Cape Lookout, the ΣCO_2 $\delta^{13}C$ primarily reflects changes in sulfate reduction rate and methanogenesis. The sedimentary profiles are best described by the mixing of ΣCO_2 from two processes: sulfate reduction, producing ΣCO_2 with an isotopic signature of -19.2 per mil, and

methanogenesis, resulting in ΣCO_2 with an isotopic signature of +44.2 per mil. The mass balance calculations generated a reasonable isotope value for methane and a reasonable fractionation factor for methane production. However the calculation is very sensitive to the reaction stoichiometry.

The mass balance approach used suggests that the isotopic signature of both the ΣCO_2 and methane are sensitive to the ratios of ΣCO_2/SR and $\Sigma CO_2/CH_4$ that are ultimately dependent on the oxidation state of the organic matter being remineralized. The oxidation state determined from the incubation experiment agreed with a calculated OS based on the identified fraction of the organic carbon remineralized. We hypothesize that the ratio of sulfate reduction to methanogenesis may be an important contribution to the range of CH_4 isotope values observed in the marine environment. This may ultimately be useful in interpreting ΣCO_2 $\delta^{13}C$ profiles in other environments as well as the isotopic signature of diagenetic carbonates.

Fig. 3.1 ΣCO_2, sulfate and ΣCO_2 $\delta^{13}C$ data for the five depth intervals. The ΣCO_2, $\Sigma^{12}CO_2$, $\Sigma^{13}CO_2$ and sulfate concentrations were fit to a line to determine rates for each depth interval.

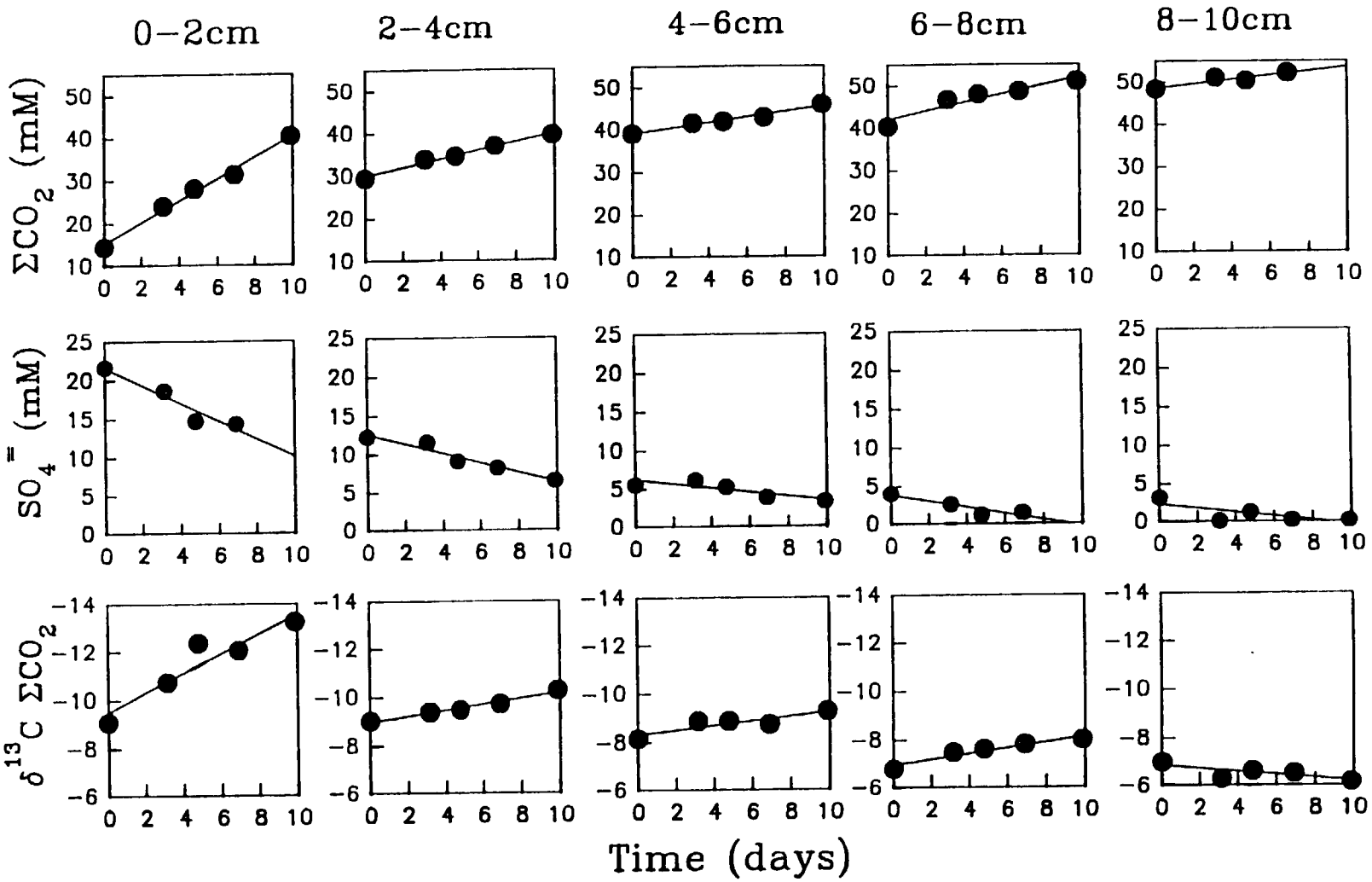
0–2cm
2–4cm
4–6cm
6–8cm
8–10cm
ΣCO2 (mM)
SO4= (mM)
δ13C ΣCO2
Time (days)

Fig. 3.2 (A) Measured rates (symbols) and curvefits to rates (lines) of ΣCO_2 production and sulfate reduction. (B) Isotopic signature of the ΣCO_2 produced in the upper 10 cm (symbols) determined using Eqn. 11. The solid line is the $\delta^{13}C$ of the ratio of the curvefits of the individual $\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ production profiles. The dotted line (.....) is a mixing curve based on the calcualted rates of sulfate reduction and methanogenesis and the associated end member isotopic signatures. The dashed line (- - -) is the ratio of depth-dependent production rates of $\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ solved for using the measured ΣCO_2 concentration and $\delta^{13}C$ profile and the diagenetic equation (Eqn. 12).

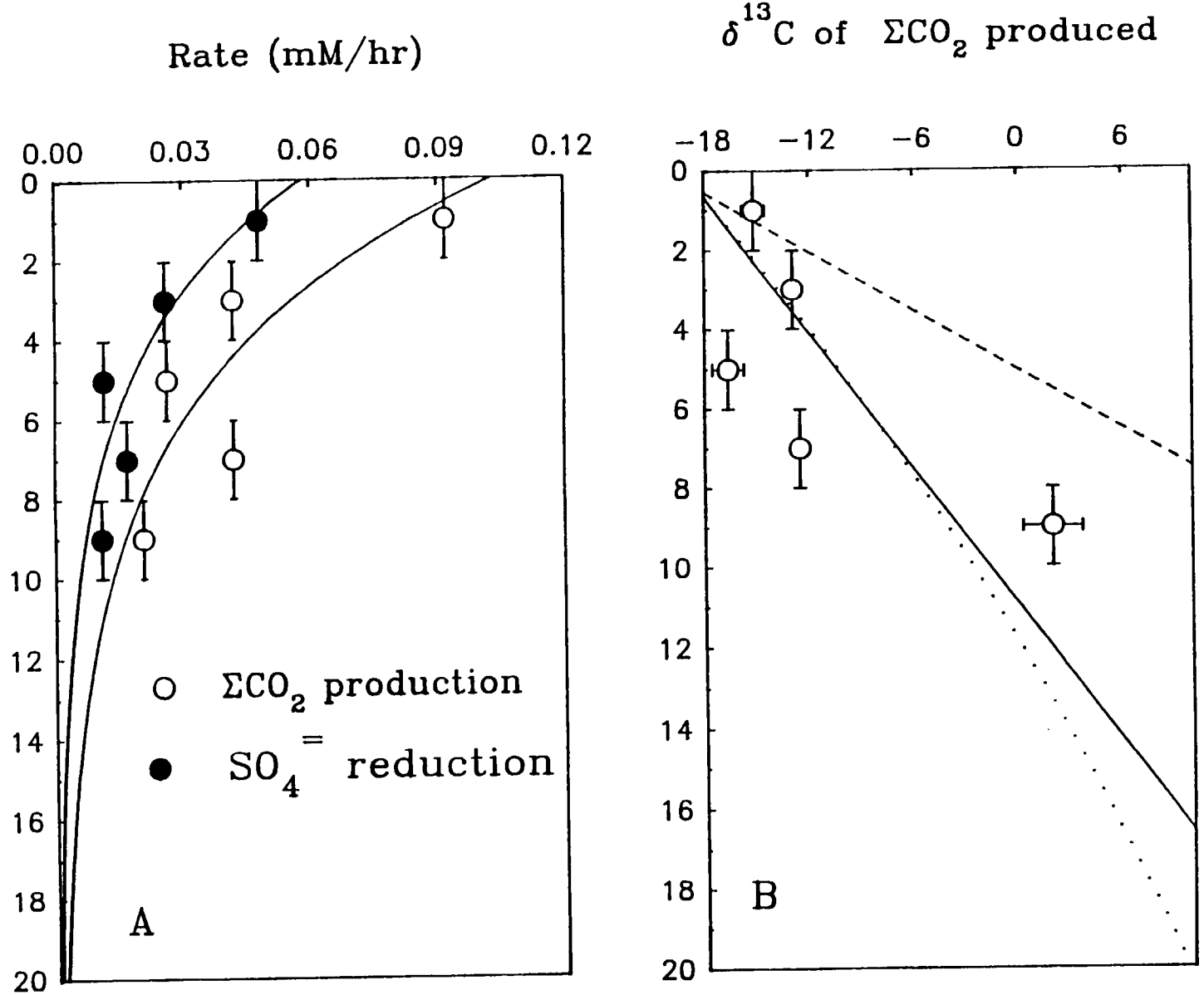
Rate (mM/hr)
0.00
0.03
0.06
0.09
0.12
0
2
4
6
8
10
12
14
16
18
20
ΣCO2 production
SO4= reduction
A
δ13C of ΣCO2 produced
−18
−12
−6
0
6
0
2
4
6
8
10
12
14
16
18
20
B

Fig. 3.3 The measured concentrations of ΣCO_2 and sulfate (symbols) and the model estimates determined using Eqn. 12 and the rate data shown in Fig. 3.2 (solid line) (A). The measured isotopic signature of the ΣCO_2 (symbols) and the modelled estimates determined using Eqn. 12 and the individual rates of $\Sigma^{12}CO_2$ and $\Sigma^{13}CO_2$ production (solid line) (B).

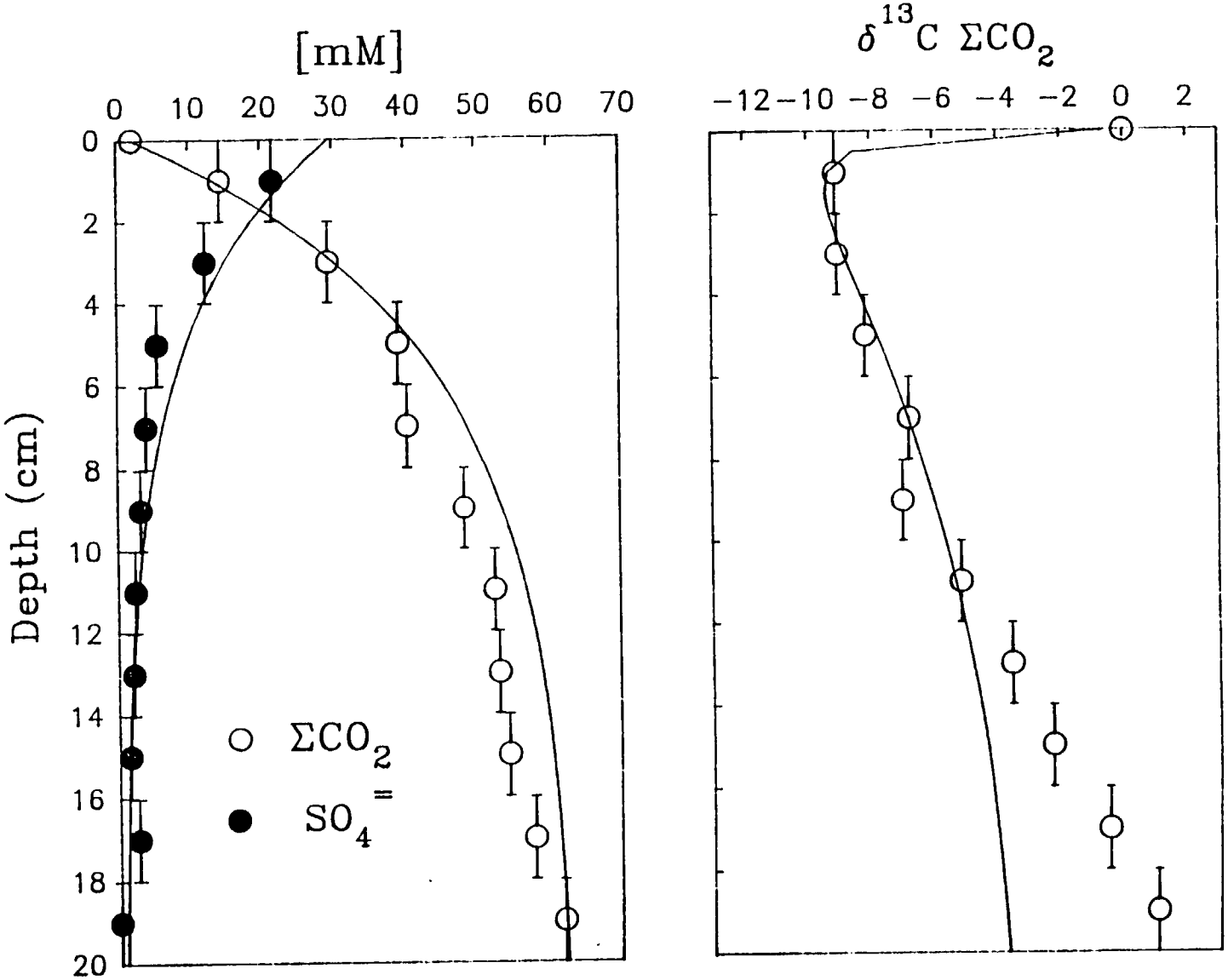
[mM]
0 10 20 30 40 50 60 70
Depth (cm)
0 2 4 6 8 10 12 14 16 18 20
ΣCO2
SO4=
δ13C ΣCO2
−12 −10 −8 −6 −4 −2 0 2

Fig. 3.4 The sulfate gradient versus the ΣCO_2 $\delta^{13}C$ gradient for the upper 3 cm of sediment from porewater profiles collected from 1986 to 1991 at Cape Lookout. The sulfate and ΣCO_2 $\delta^{13}C$ gradients were based on linear fits of concentration over the upper 3 cm (one cm intervals).

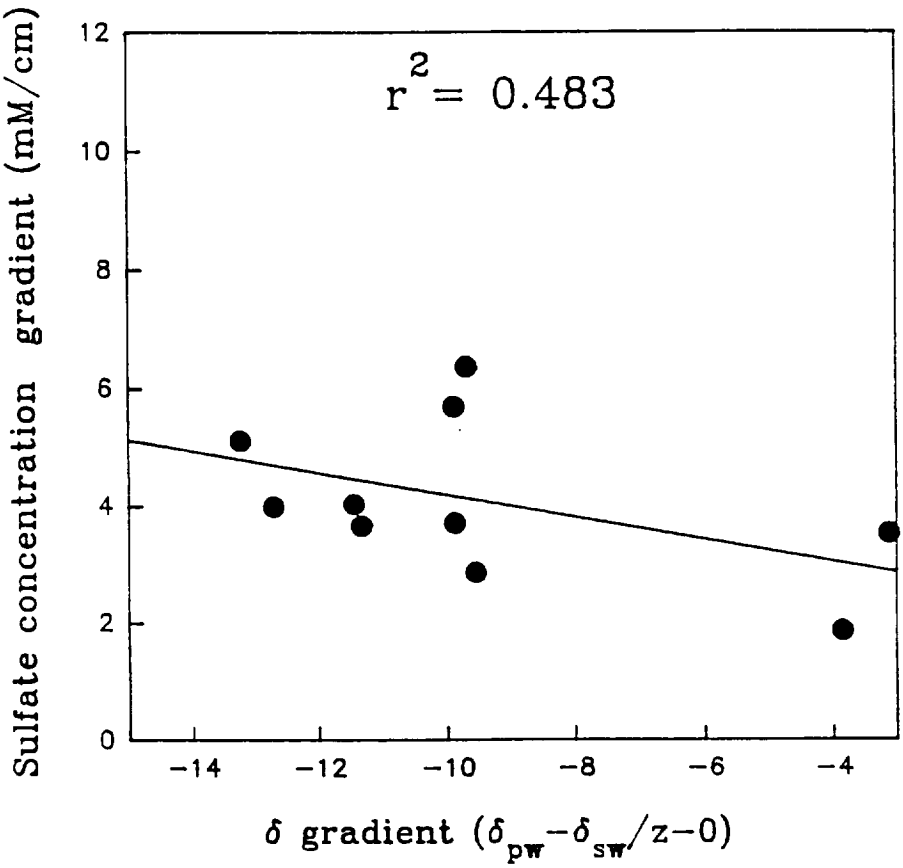
$r^2 = 0.483$
Sulfate concentration gradient (mM/cm)
12
10
8
6
4
2
0
−14
−12
−10
−8
−6
−4
δ gradient ($\delta_{pw}-\delta_{sw}/z-0$)

Fig. 3.5 Porewater Calcium concentration versus time for the upper 10 cm from the incubation experiment.

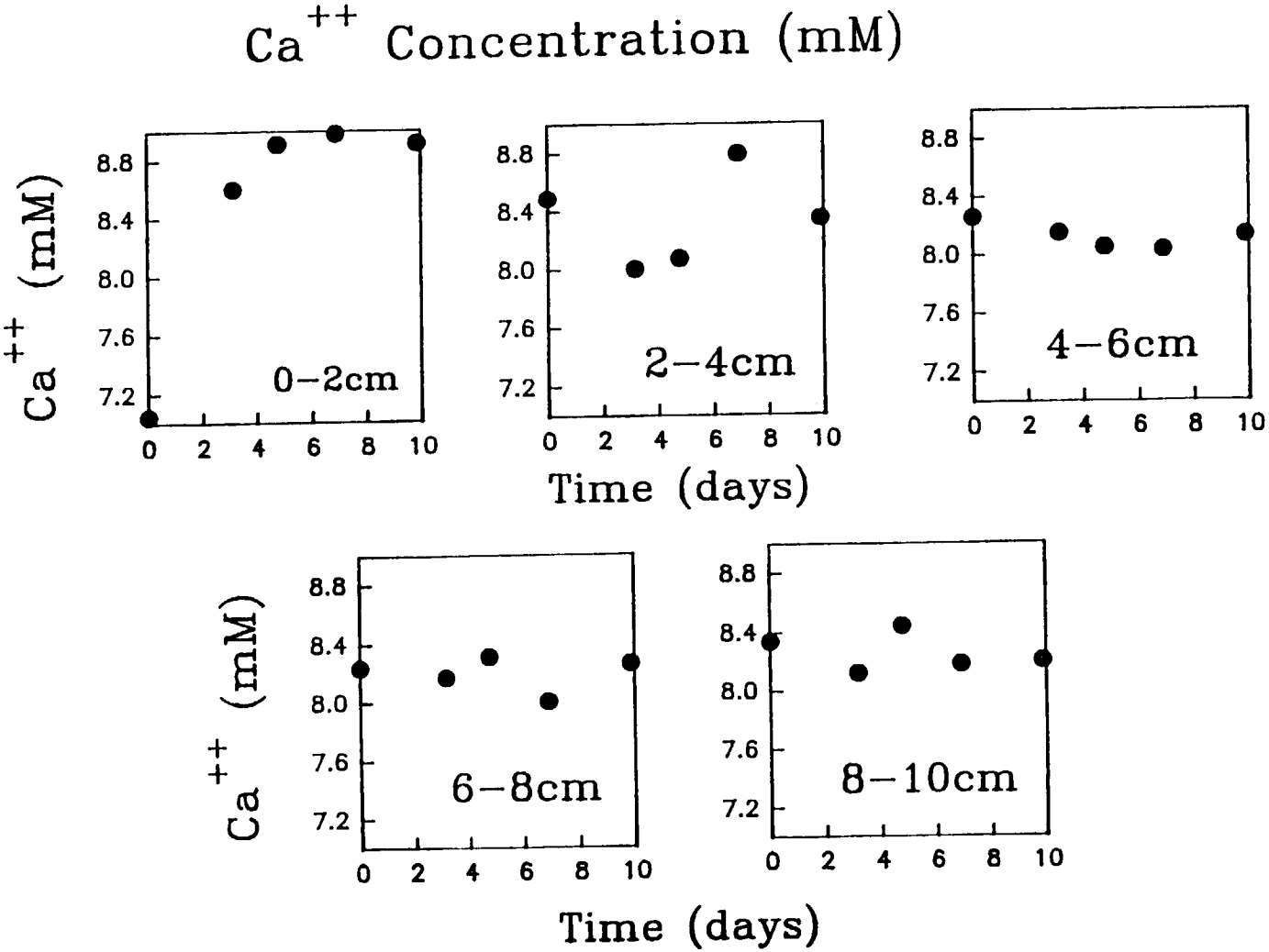
Ca++ Concentration (mM)
Ca++ (mM)
8.8
8.4
8.0
7.6
7.2
0 2 4 6 8 10
0–2cm
2–4cm
4–6cm
Time (days)
6–8cm
8–10cm
Time (days)

Fig. 3.6 The relationship between the oxidation state of the organic carbon, the ratio of ΣCO_2 produced to sulfate reduced (τ) and the modelled isotopic signature of the methane produced.

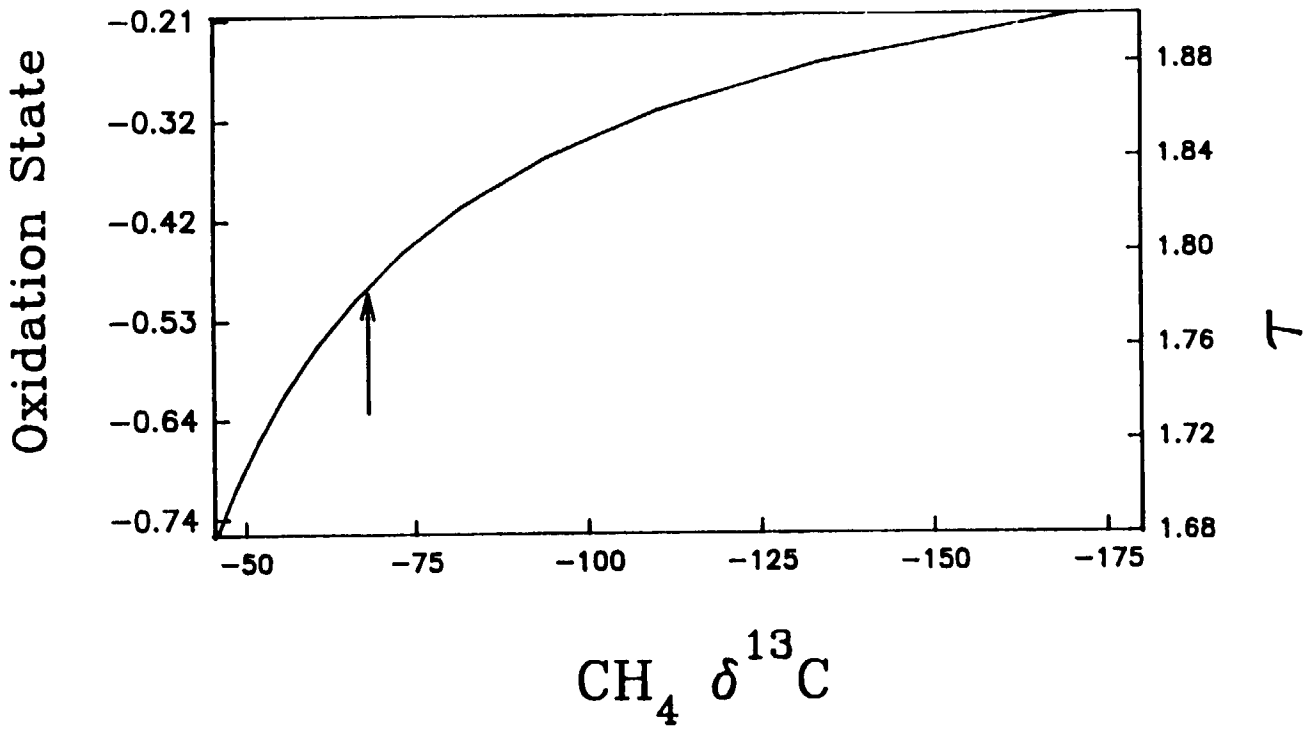
Oxidation State
−0.21
−0.32
−0.42
−0.53
−0.64
−0.74
1.88
1.84
1.80
1.76
1.72
1.68
T
−50
−75
−100
−125
−150
−175
CH_4 $\delta^{13}C$

Fig. 3.7 The relationship of the relative rates of CO_2 and CH_4 production (τ-1) and the apparent oxidation state (OS) of the fermented material. Data is from Tarvin and Buswell (1934). Curve is described by Eqn. 18.

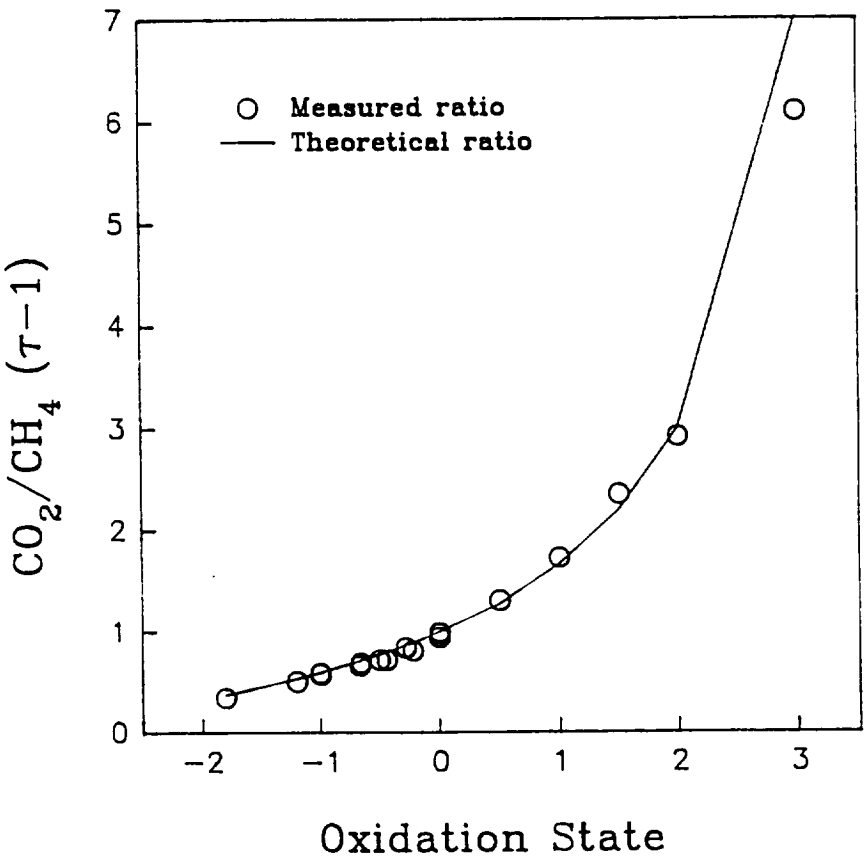
Measured ratio
Theoretical ratio
CO_2/CH_4 $(\tau-1)$
Oxidation State
0
1
2
3
4
5
6
7
−2
−1
0
1
2
3

Table 3.1 Regression coefficients (r^2) for the $SO_4^=$, ΣCO_2, and ΣCO_2 $\delta^{13}C$ incubation data

Depth	$SO_4^=$	ΣCO_2	ΣCO_2 $\delta^{13}C$
0-2 cm	0.92	0.99	0.99
2-4 cm	0.94	0.98	0.98
4-6 cm	0.75	0.96	0.96
6-8 cm	0.85	0.89	0.89
8-10 cm	0.56	0.84	0.84

Table 3.2 Curve fit parameters, estimated depth integrated rates (10 cm), and associated isotopic compositions from tube incubation experiment.

Process	R_0	*a*	*Depth Integrated Rate	$\delta^{13}C$
Total ΣCO_2 Produced	0.103	0.2037	0.439	-13.1
ΣCO_2 Produced from Sulfate Reduction	0.103	0.2341	0.397	-19.2
ΣCO_2 Produced from Methanogenesis	--	--	0.0420	+44.2
Methane Produced	--	--	0.0539	-65.9
Sulfate Reduction	0.058	0.2341	0.223	--

*Units of $mmol*cm^{-2}*hr^{-1}$

A-2

REFERENCES

ALPERIN M.J. (1988) The carbon cycle in an anoxic marine sediment. Ph.D. Dissertation University of Alaska, Fairbanks.

ALPERIN M.J., BLAIR N.E., ALBERT D.B., HOEHLER T.M. and MARTENS C.S. (1992) Factors that control the stable carbon isotopic composition of methane produced in an anoxic marine sediment. *Global Biogeochem. Cycles* 6, 271-291.

BALABANE M., GALIMOV E., HERMANN M. and LETOLLE R. (1987) Hydrogen and carbon isotope fractionation during experimental production of bacterial methane. *Global Biogeochem. Cycles* 2, 279-288.

BELYAEV S.S., WOLKIN R., KENEALY W.R., DENIRO M.J. EPSTEIN S. and ZEIKUS J.G. (1983) Methanogenic bacteria from the Bondyuzhskoe oil field: General characterization and analysis of stable-carbon isotopic fractionation. *Appl. Environ. Microbiol.* 45, 691-697.

BERNER R.A. (1980) Early diagenesis. Princeton University Press.

BLAIR N.E. and CARTER W.D. (1992) The carbon isotope biogeochemistry acetate from a methanogenic marine sediment. *Geochim. Cosmochim. Acta* 56, 1247-1258.

BLAIR N.E., BOEHME S.E., and CARTER W.D. (1993) The carbon isotope biogeochemistry of methane production in anoxic sediments. In *The Biogeochemistry of Global Change: Radiative Trace Gases* (ed. R.S. Oremland) Chapman and Hall.

BOEHME S.E. (1989) Seasonal variation in the production of ΣCO_2 in a methane-producing sediment. M.S. North Carolina State University, Raleigh.

BOEHME S.E., BLAIR N.E., CHANTON J.P. and MARTENS C.S. (in revision) A carbon isotope mass balance for an anoxic marine sediment: Isotopic signatures of diagenesis. Submitted to *Geochim. Cosmochim. Acta.*

BURDIGE D. and MARTENS C.S. (1988) Biogeochemical cycling in an organic-rich coastal marine basin: 10. The role of amino acids in sedimentary carbon and nitrogen cycling. *Geochim. Cosmochim. Acta* 52, 1571-1584.

CANUEL E.A., MARTENS C.S. and BENNINGER L.K. (1990) Seasonal variations in 7Be activity in the sediments of Cape Lookout Bight, North Carolina. *Geochim. Cosmochim. Acta* 54, 237-245.

CHANTON J.P. (1985) Sulfur mass balance and isotopic fractionation in an anoxic marine sediment. Ph.D. Dissertation, University of North Carolina, Chapel Hill.

CHANTON J.P., MARTENS C.S., and KIPPHUT G.W. (1983) Lead-210 sediment geochronology in an organic-rich coastal marine basin. *Geochim. Cosmochim. Acta* **49**, 1791-1804.

CHANTON J.P., MARTENS C.S. and GOLDHABER M.B. (1987) Biogeochemical cycling in an organic-rich coastal marine basin. 7. Sulfur mass balance, oxygen uptake and sulfide retention. *Geochim. Cosmochim. Acta* **51**, 1187-1199.

CLAYPOOL G.E. and KAPLAN I.R. (1974) The origin and distribution of methane in marine sediments. In *Natural Gases in Marine Sediments* (ed. I.R. Kaplan) pp. 99-139. Plenum Press.

CRAIG H. (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* **12**, 133-149.

CRILL P.M. and MARTENS C.S. (1983) Spatial and temporal fluctuations of methane production in anoxic, coastal marine sediments. *Limnol. Oceangr.* **25**, 564-571..

CRILL P.M. and MARTENS C.S. (1986) Methane production from bicarbonate and acetate in an anoxic marine sediment. *Geochim. Cosmochim. Acta* **50**, 2089-2097.

DEINES P., LANGMUIR D., HARMON R.S. (1974) Stable carbon isotope ratios and the existence of a gas phase in the evolution of carbonate groundwaters. *Geochim. Cosmochim. Acta* **38**, 1147-1164.

FOREE E.G. and MCCARTY P.L. (1970) Anaerobic decomposition of algae. *Env. Sci. Technol.* **4**, 842-849.

FRIEDMAN I. and O'NEIL J.R. (1977) Compilation of stable isotope fractionation factors of geochemical interest. U.S. Geological Survey Professional Paper 440-KK.

FRIEDMAN I. and MURATA K.J. (1979) Origin of dolomite in Miocene Monterey Shale and related formations in the Temblor Range, California. *Geochim. Cosmochim. Acta* **43**, 1357-1365.

FRY B. and SHERR E.B. (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. in Mar. Sci.* 27, 13-47.

FUCHS G., THAUER R. ZEIGLER H. and STICHLER W. (1979) Carbon isotope fractionation by *Methanobacterium thermoautotrophicum. Arch. Microbiol.* 120, 135-139.

GAMES L.M., HAYES J.M. and GUNSALUS R.P. (1978) Methane-producing bacteria: natural fractionations of the stable carbon isotopes. *Geochim. Cosmochim. Acta* 42, 1295-1297.2

GUJER W. and ZEHNDER J.B. (1983) Conversion processes in anaerobic digestion. *Water Sci. Technol.* 15, 127-167.

HADDAD R.I. (1989) Sources and reactivity of organic matter accumulating in a rapidly depositing, coastal environment. Ph.D. Dissertation, University of North Carolina, Chapel Hill.

HADDAD R.I. and MARTENS (1987) Biogeochemical cycling in an organic-rich coastal marine basin: 9. Sources and accumulation rates of vascular plant-derived organic material. *Geochim. Cosmochim. Acta* 51, 2991-3001.

HAYES J.M. (1983) Practice and principles of isotope measurements in organic geochemistry. In: *Organic Geochemistry of Contemporaneous and Ancient Sediments* (ed. W.G. Meinschein) Society of Economic Paleontology and Mineralogy. pp 5-1 - 5-31.

HERCZEG A.L. (1988) Early diagenesis of organic matter in lake sediments: A stable carbon isotope study of pore waters. *Chem. Geol.* 72, 199-209.

KING G.M. (1984) Utilization of hydrogen, acetate, and "non-competitive" substrates by methanogenic bacteria in marine sediments. *Geomicrobiol. J.* 3, 275-306.

KING G.M., KLUG M.J. and LOVLEY D.R. (1981) Metabolism of acetate, methanol, and methylated amines in intertidal sediments of Lowes Cove, Maine. *Appl. Environ. Mircrobiol.* 45, 1848-1853.

KLUMP J.V. and MARTENS C.S. (1981) Biogeochemical cycling in an organic-rich coastal marine basin. 2. Nutrient sediment-water exchange processes. *Geochim. Cosmochim. Acta* 45, 101-121.

KLUMP J.V. and MARTENS C.S. (1989) The seasonality of nutrient regeneration in an organic-rich coastal sediment: Kinetic modeling of changing pore-water nutrient and sulfate distributions. *Limnol. Oceanogr.* 34, 559-577.

LAZERTE B.D. (1981) The relationship between total dissolved carbon dioxide and its stable carbon isotope ratio is aquatic sediments. *Geochim. Cosmochim. Acta* **45**, 647-656.

LI Y.H. and GREGORY S. (1974) Diffusion of ions in sea water and in deep-sea sediments. *Geochim Cosmochim. Acta* **38**, 703-714.

MARTENS C.S. (1976) Control of methane sediment-water bubble transport by macroinfaunal irrigation in Cape Lookout Bight, North Carolina. *Science* **192**, 998-1000.

MARTENS C.S. and KLUMP J.V. (1984) Biogeochemical cycling in an organic-rich coastal marine basin. 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. *Geochim. Cosmochim. Acta* **48**, 1987-2004.

MARTENS C.S., BLAIR N.E., GREEN C.D. and DESMARAIS D.J. (1986) Seasonal variations in the stable carbon isotopic signature of biogenic methane in a coastal sediment. *Science* **233**, 1300-1303.

MCARTHUR J.M. (1989) Carbon isotopes in pore water, calcite, and organic carbon from distal turbidites of the madeira Abyssal Plain. *Geochim. Cosmochim. Acta* **53**, 2997-3004.

MCCORKLE D.C. and EMERSON S.R. (1988) The relationship between pore water carbon isotopic composition and bottom water oxygen concentration. *Geochim. Cosmochim. Acta* **52**, 1169-1178.

MCCORKLE D.C., EMERSON S.R. and QUAY P.D. (1985) Stable carbon isotopes in marine porewaters. *Earth Planet. Sci. Let.* **74**, 13-26.

MCNICHOL A.P., DRUFFEL E.R.M. and LEE C. (1991) Carbon cycling in coastal sediments: 2. An investigation of the sources of ΣCO_2 to pore water using carbon isotopes. In *Organic Substances and Sediments in Water* (ed. R.A. Baker) Lewis Publishers, Chelsea, MI.

NISSENBAUM A., BAEDECKER M.J. and KAPLAN I.R. (1972) Studies on dissolved organic matter from interstitial water of a reducing marine fjord. *Adv. in Org. Geochem.* (ed. Braunschweig) Pergammon Press, Oxford, 427-440.

OREMLAND R.S., MARSH L.M. and POLCIN S. (1982) Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. *Nature* **290**, 143-145.

OREMLAND R.S., MILLER L.G., COLBERTSON C.W., ROBINSON S.W., SMITH R.L., LOVLEY D., WHITICAR M.J., KING G.M., KIENE R.P., IVERSEN N. and SARGENT M. (1993) Aspects of the biogeochemistry of methane in Mono Lake and the Mono Basin of California. In *The Biogeochemistry of Global Change: Radiative Trace Gases* (ed. R.S. Oremland) Chapman and Hall.

PRESLEY B.J. and KAPLAN I.R. (1968) Changes in dissolved sulfate, calcium and carbonate from interstitial water of near-shore sediments. *Geochim. Cosmochim. Acta* 32, 1037-1048.

REEBURGH W.S. (1982) A major sink and flux control for methane in marine sediments: Anaerobic consumption. In: The Dynamic Environment of the Ocean Floor. (eds. Fanning K. and Manheim F.T.) Heath, Lexington Mass. pp 203-217.

SCHAFF T., LEVIN L., BLAIR N., DEMASTER D.J., POPE R., and BOEHME S. (1992) Spatial heterogeneity of benthos on the Carolina continental slope: large (110 km)-scale variation. *Mar. Ecol. Prog. Ser.* 88, 143-160.

STUMM W. and MORGAN J.J. (1981) Aquatic Chemistry. John Wiley and Sons, New York.

TARVIN D. and BUSWELL A.M. (1934) The methane fermentation of organic acids and carbohydrates. *J. Amer. Chem. Soc.* 56, 1751-1755.

ULLMAN W.J. and ALLER R.C. (1982) Diffusion coefficients in near-shore marine sediments. *Limnol. oceanogr.* 27, 552-556.

WHELEN T., BERNARD B.B. and BROOKS J.M. (1976) Carbon isotope variations in total carbon dioxide and methane from interstitial waters of nearshore sediments. In *Stable Isotopes in the Earth Sciences* (N.Z. DSIR Bull.) 220, pp.39-47.

WHITICAR M.J. and FABER E. (1986) Methane oxidation in sediment and water column environments--Isotope evidence. *Org. Geochem.* 10, 759-768.

WHITICAR M.J., FABER E. and SCHOELL M. (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation--Isotope evidence. *Geochim. Cosmochim. Acta* 50, 693-709.