The Carbon Isotope Biogeochemistry of $\Sigma$CO$_2$ Production in a Methanogenic Marine Sediment

ABSTRACT

To investigate the relationship between $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$ was measured on a seasonal basis. The $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$ produced by sulfate reduction and methanogenesis. The $\delta^{13}$C of the $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ at Cape Lookout was closely reproduced by a diagenetic model using the measured rates of sulfate reduction and $\Sigma$CO$_2$ production, and the isotopic signature of the CO$_2$ production in the two biogeochemical zones.
INTRODUCTION

The isotopic composition of total dissolved inorganic carbon (ΣCO₂) in sediments is a unique record of diagenetic processes. Presley and Kaplan (1968) used the isotopic signatures of ΣCO₂ to confirm that the downcore increases in ΣCO₂ 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 ΣCO₂. These studies were followed by numerous applications of isotopic measurements of ΣCO₂ to identify the processes that produce and utilize ΣCO₂ 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 ΣCO₂ produced during sulfate reduction and methanogenesis, as well as the isotopic composition of the remineralized organic carbon.
**Typical ΣCO₂ δ¹³C depth profiles from organic-rich marine sediments exhibit two major trends. **The ΣCO₂ initially becomes depleted in ¹³C relative to overlying seawater ΣCO₂ values (typically near zero per mil) and then, at depth, reverses this trend and becomes ¹³C-enriched. The initial ¹³C/¹²C gradient has been attributed to sulfate reduction which produces ΣCO₂ 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 ¹³C-depleted ΣCO₂ and leaving behind ¹³C-enriched ΣCO₂ (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 ΣCO₂ and to identify and quantify the controls on the isotopic composition of ΣCO₂ in a methanogenic sediment. Specifically, this study was undertaken to determine quantitatively the processes controlling the isotopic composition of ΣCO₂ 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 δ¹³C composition of
the ΣCO₂. Field measurements of ΣCO₂ δ¹³C sedimentary profiles indicated a relationship between rates of the dominant processes and the resultant ΣCO₂ δ¹³C profiles. Sediment incubations were used to determine rates of ΣCO₂ production and sulfate reduction as well as the δ¹³C of ΣCO₂ produced. From these measurements it was possible to test the hypothesis that the ΣCO₂ δ¹³C porewater profiles from anoxic sediments resulted from a balance of ΣCO₂ 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 $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$. The $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ 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.,
The \( \Sigma \text{CO}_2 \) serum bottles were injected with 0.2 ml of 1M \( \text{H}_3\text{PO}_4 \) and the resulting \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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 \( \delta^{13} \text{C} \) notation (See Eqn. 1). The precision and accuracy of the \( \Sigma \text{CO}_2 \) concentration measurements determined from standards is 0.5 mM and 1.0 mM respectively, and for \( \Sigma \text{CO}_2 \ \delta^{13} \text{C} \) measurements, 0.3 per mil and 0.6 per mil respectively.

Porewater \( \text{SO}_4^{2-} \) 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 \( \text{Ca}^{++} \) samples were treated as described in Chapter 2. The \( \text{Ca}^{++} \) measurements were analyzed on a Perkin-Elmer Atomic Absorption spectrometer in the Soil Science Laboratory (NCSU).

RESULTS

The concentration of \( \Sigma \text{CO}_2 \) 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). \( \Sigma \text{CO}_2 \ \delta^{13} \text{C} \) became progressively depleted in \( ^{13} \text{C} \) 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 $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ concentrations, and the $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$ concentration and $\delta^{13}$C values, $^{12}$C and $^{13}$C production rates were determined. Rate measurements of $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ produced (Fig. 3.2b) were fit to an exponential curve of the form:

$$ R_z = R_0 \exp(-az) $$

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 $R_z$ with the
Sigmaplot curve fitting routine (Jandel Scientific). Curve fitting parameters are given
in Table 3.2. The $\Sigma CO_2$ concentration and $\delta^{13}C$ values were used to estimate the
$\delta^{13}C$ signal of the $\Sigma CO_2$ produced for each depth interval using the following
equation:

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

(11)

where

- $C_t$ = $\Sigma CO_2$ concentration at given time
- $\delta_t$ = $\delta^{13}C$ of $\Sigma CO_2$ at given time
- $C_o$ = $\Sigma CO_2$ concentration at initial time zero for given depth
- $\delta_o$ = $\delta^{13}C$ of $\Sigma CO_2$ at initial time zero for given depth
- $C_{add}$ = $\Sigma CO_2$ concentration added between $C_o$ and $C_t$
- $\delta_{add}$ = $\delta^{13}C$ of $\Sigma CO_2$ added between $C_o$ and $C_t$.

The $\delta_{add}$ was solved for by curvefitting the $\Sigma CO_2$ concentration and $\Sigma CO_2$ $\delta^{13}C$ 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, $\Sigma CO_2$ production, $\Sigma^{12}CO_2$ production and $\Sigma^{13}CO_2$
production to test whether these rates are representative of in situ conditions. An
analytical solution of the advection-diffusion equation

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

(12)

was found assuming $\partial C/\partial t = 0$ and the following boundary conditions: as $C_z \rightarrow \infty$,
$\partial C/\partial z = 0$; for $\Sigma CO_2$, $C_0 = 2.11$ mM, $\delta^{13}C = 0.0$ per mil; for sulfate, $C_0 = 29.6$. 
Eqn. 12 becomes

\[ C_z = C_0 + \frac{R_0}{a^2 D_s + a \omega} (1 - \exp^{-a \omega}), \]  

(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; \( D_s(SO_4^{2-}) = 0.0312 \) cm/hr),
- \( C_z \) = Concentration of species at given depth \( z \)
- \( a \) = attenuation coefficient (cm\(^{-1}\))
- \( \omega \) = sedimentation rate (10.6 cm/yr; Chanton et al., 1983).

Diffusion coefficients for \( HCO_3^- \) and \( SO_4^{2-} \) 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 \( \Sigma CO_2 \) \( \delta^{13}C \) profiles are shown in Fig. 3.3. The steady state assumption appears to be justified given the agreement between measured and estimated \( \Sigma CO_2 \) profiles determined from the rate measurements (Klump and Martens, 1981; 1989; this study). The estimated \( \Sigma CO_2 \) \( \delta^{13}C \) 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 \( SO_4^{2-} \) concentration also agree well, verifying the sulfate reduction rate measurements.

The depth-integrated rates of \( \Sigma CO_2 \) 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 \((\phi=0.9 \text{ cm}^3_{pw}/\text{cm}^3_{sed})\) \((\text{Eqn. 12})\), to compare to previous depth integrated rate measurements:

\[
\Sigma R_z = \int_0^{10cm} R_0 \exp^{-az}. \tag{14}
\]

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\(^{-1}\) \((\text{Klump and Martens 1989})\) 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\(^{-1}\) \((\text{Martens and Klump, 1984})\). The depth integrated \(\Sigma\text{CO}_2\) 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\(^{-1}\) \((\text{Klump and Martens 1989})\) and is comparable to \(\Sigma\text{CO}_2\) fluxes of 38.9 \pm 0.8 moles-m\(^{-2}\)-yr\(^{-1}\) measured in June 1978 \((\text{Martens and Klump, 1984})\).

**DISCUSSION**

The \(\Sigma\text{CO}_2\) \(\delta^{13}\text{C}\) profiles measured at Cape Lookout are typical of anoxic marine sediments, with a zone of \(^{13}\text{C}\)-depletion overlying a zone of \(^{13}\text{C}\) enriched \(\Sigma\text{CO}_2\). These profiles are thought to result from sulfate reduction producing \(\Sigma\text{CO}_2\) that is isotopically similar to the organic matter being oxidized in the upper zone and methanogenesis generating \(^{13}\text{C}\)-enriched \(\Sigma\text{CO}_2\) at depth. Additionally, \(^{13}\text{C}\)-depletions have been attributed to methane oxidation adding \(^{13}\text{C}\)-depleted \(\Sigma\text{CO}_2\) in the sulfate reduction zone, particularly near the transition between the sulfate reduction and methane production zones \((\text{Reeburgh, 1982; Whiticar and Faber, 1986})\). In studies
where methane oxidation has been shown to be important, the resultant $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$, 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 $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$ being produced should
reflect this zonation and should be expressed in the incubation experimental results.
The measured isotopic signature for the $\Sigma$CO$_2$ 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
\( \Sigma^{12}\text{CO}_2 \) and \( \Sigma^{13}\text{CO}_2 \) 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 \( \Sigma\text{CO}_2 \) 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 \( \Sigma\text{CO}_2 \delta^{13}\text{C} \) 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 \( \Sigma\text{CO}_2 \delta^{13}\text{C} \) 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 \( \Sigma\text{CO}_2 \) 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 \(^{13}\text{C}-\)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 \( \Sigma\text{CO}_2 \) flux. Further, for most of the year methane is produced predominantly via \( \text{CO}_2 \) 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 $\Sigma CO_2$ $\delta^{13}C$ 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 $\Sigma CO_2$ concentration in Cape Lookout sediments can be determined, then the overall $\Sigma CO_2$ 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 $\Sigma CO_2$ being produced as a function of depth. Variation with depth in the isotopic composition of the $\Sigma CO_2$ 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 $\Sigma CO_2$ 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 ΣCO₂ that can be considered.

One source of ¹³C enriched ΣCO₂ 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 CaCO₃ 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 ΣCO₂ 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 ΣCO₂ δ¹³C 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 $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ 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 $\delta$ 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 $\Sigma$CO$_2$ would be added to the pool of $\Sigma$CO$_2$ 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 $^{14}\text{C}$ 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 $\Sigma\text{CO}_2$ produced from these processes to estimate a $\Sigma\text{CO}_2$ $\delta^{13}\text{C}$ 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 $\Sigma\text{CO}_2$ 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 $\Sigma\text{CO}_2$ concentration and $\delta^{13}\text{C}$ profiles indicates that a simple two component mixing model can be used to estimate the isotopic signature of the $\Sigma\text{CO}_2$ produced from an anoxic sediment like Cape Lookout. The mixing model assumes that the isotopic signature of the $\Sigma\text{CO}_2$ 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 $\Sigma\text{CO}_2$ in the sediment,

$$R_{\text{tot}}\delta_{\text{tot}} = R(\Sigma\text{CO}_2)_{\text{SR}}\delta_{\text{SR}} + R(\Sigma\text{CO}_2)_{\text{M}}\delta_{\text{M}}$$  \hspace{1cm} (15)

where:

- $R_{\text{tot}}$ = $\Sigma\text{CO}_2$ total production rate (mM/hr),
- $\delta_{\text{tot}}$ = isotopic signature of $\Sigma\text{CO}_2$ produced,
- $R(\Sigma\text{CO}_2)_{\text{SR}}$ = $\Sigma\text{CO}_2$ production from sulfate reduction rate (mM/hr),
- $\delta_{\text{SR}}$ = isotopic signature of $\Sigma\text{CO}_2$ from SR
- $R(\Sigma\text{CO}_2)_{\text{M}}$ = $\Sigma\text{CO}_2$ production from methanogenesis rate (mM/hr),
- $\delta_{\text{M}}$ = isotopic signature of $\Sigma\text{CO}_2$ from methanogenesis.
The sources of the $^{13}$C-enriched $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ 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 $\Sigma$CO$_2$ produced from sulfate reduction alone ($\delta_{SR}$) 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 $\Sigma$CO$_2$ produced from sulfate reduction,

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

(16)

where $\tau = 1.78$, the ratio of $\Sigma$CO$_2$ produced to sulfate reduced at the sediment-water interface. The stoichiometric coefficient, $\tau$, 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 (Forsee and McCarty 1970) suggesting that no one algal component was preferentially utilized producing a different ratio of $\Sigma$CO$_2$ produced to algae degraded. Previous estimates
of \( \tau \) for Cape Lookout surface sediments, of 1.7 ± 0.1 (Alperin et al, 1992) and 1.9 (average of upper 6 cm; Klump and Martens, 1989) supports the \( \tau \) value used in this study. Factors controlling \( \tau \) are discussed in further detail in a later section.

The net production of \( \Sigma \text{CO}_2 \) during methanogenesis, \( R_M \) is calculated by subtracting \( R(\Sigma \text{CO}_2)_{SR} \) from \( R_{tot} \). The depth integrated \( \delta \) value of \( \Sigma \text{CO}_2 \) produced, \( \delta_{tot} \) was estimated from the isotopic signature of the depth integrated \( \Sigma^{12}\text{CO}_2 \) and \( \Sigma^{13}\text{CO}_2 \) production rates, -13.1 per mil. Solving Eqn. 15 for \( \delta_M \) gives +44.2 per mil for the isotopic composition of the \( \Sigma \text{CO}_2 \) produced during methanogenesis. Using the values for \( \Sigma \text{CO}_2 \) production from sulfate reduction (-19.2 per mil) and methanogenesis (+44.2 per mil) and the estimated production rates, a \( \Sigma \text{CO}_2 \) \( \delta^{13} \text{C} \) mixing curve was calculated and compared to the measured isotopic signature of the \( \Sigma \text{CO}_2 \) 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 \( \text{CH}_4 \) produced in the incubation experiment using the following equation,

\[
[R_{tot} + R_{\text{CH}_4}] \delta_{\text{remia}} = R_{tot} \delta_{tot} + R_{\text{CH}_4} \delta_{\text{CH}_4}
\]  

(17)
where

\[ \frac{R_{\text{CH}_4}}{\delta_{\text{CH}_4}} = \text{rate of methanogenesis (mM/hr),} \]
\[ \delta_{\text{CH}_4} = \text{isotopic signature of methane produced,} \]
\[ \delta_{\text{rem}in} = (-18.9 \text{ per mil; Boehme et al., in revision).} \]

To estimate the rate of methane production, the rate of \( \Sigma \text{CO}_2 \) produced during methanogenesis can be used in the following stoichiometric relationship:

\[ (R_{\text{CH}_4}) = \frac{R(\Sigma \text{CO}_2)_M}{(\tau - 1)}. \]  (18)

The \( \tau - 1 \) 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 ± 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 \( \Sigma \text{CO}_2 \) production and sulfate reduction adequately represents controlling diagenetic processes.

As noted earlier, the calculated \( \delta^{13}\text{C} \) for \( \Sigma \text{CO}_2 \) produced via sulfate reduction (-19 per mil) and methanogenesis (+44 per mil) can be used to estimate the isotopic signature of the \( \Sigma \text{CO}_2 \) 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 6-11 cm (the depths above where sulfate was depleted) and converted to \( \Sigma \text{CO}_2 \) production rates using \( \tau \) and \( \tau - 1 \). The resultant rates were used with the calculated
$\delta^{13}C$ for $\Sigma CO_2$ produced via sulfate reduction (-19 per mil) and methanogenesis (+44 per mil) to estimate a $\delta^{13}C$ for $\Sigma CO_2$ produced of -18 per mil for the upper 4 cm and -13 per mil for the 6-11 cm interval. These calculations suggest that methane production in the sulfate reduction zone can significantly affect the $\delta^{13}C$ of the $\Sigma CO_2$ produced and may be responsible for the $^{13}C$-enriched $\Sigma CO_2$ 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,

$$\alpha = \frac{C_{(CO_2)}^{13} \times 10^3}{C_{(CH_4)}^{13} \times 10^3},$$

(19)

where $\delta^{13}C_{(CH_4)}$ is -65.9 per mil as calculated above and $\delta CO_2$ 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 $CO_3^{2-}$ can be determined (Stumm and Morgan, 1981). At 8-10 cm, the measured $\Sigma CO_2 \delta^{13}C$ 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:

$$\delta^{13}C(\Sigma CO_2) = d\delta^{13}C(CO_2) + e\delta^{13}C(HCO_3^-) + f\delta^{13}C(CO_3^{2-})$$

(20)

$$\alpha(HCO_3^-/CO_2) = \frac{10^3 \times \delta^{13}C(HCO_3^-)}{10^3 \times \delta^{13}C(CO_2)}$$

(21)
The variables d, e, and f represent the fractions of the dissolved \( \Sigma CO_2 \) components.

The \( \delta^{13}C \) of \( HCO_3^\cdot \) and \( CO_3^= \) are assumed to be the same (Eqn. 22). The equilibrium fractionation factor for the \( HCO_3^\cdot \) and \( CO_2 \) equilibrioum is given by:

\[
\ln \alpha \left( \frac{HCO_3^\cdot}{CO_2} \right) = \left( \frac{9.552}{T} \right) - 0.0241
\]  

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 \( \alpha \) 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 \( \Sigma CO_2 \) 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 \( \Sigma CO_2 \) 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 \( \delta_{CH_4} \) 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

$$OS=\frac{2z-y}{x} \text{ for } -4 \leq OS \leq 4.$$  \hspace{1cm} (24)

for the reaction

$$C_xH_yO_z + wSO_4^- \rightarrow uCO_2 + S^- + H_2O,$$  \hspace{1cm} (25)

where

$$\tau = \frac{u}{w} = \frac{8}{4-OS}. $$  \hspace{1cm} (26)

Similarly for the methanogenic reaction,

$$2C_xH_yO_z \rightarrow uCO_2 + vCH_4,$$  \hspace{1cm} (27)

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

$$\frac{u}{v} = \frac{4+OS}{4-OS} = \tau - 1.$$  \hspace{1cm} (28)

The relationship between $\tau-1$, the ratio of $CH_4$ to $\Sigma CO_2$ produced, and oxidation state has been demonstrated in culture experiments (Tarvin and Buswell, 1934; Fig. 3.7).

Solving Eqn. 28 for oxidation state using $\tau = 1.78$ from the model, gives an apparent oxidation state of the organic matter of -0.49. Using the $\tau$ determined by
Alperin et al. (1992) of 1.7 ± 0.1 gives an OS values of -0.72 ± 0.28 and using the
\( \tau \) 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,

\[
OS = \frac{3a + 2b + 2z - y}{x}
\]  

(29)
to describe the oxidation state of amino acids with the formula C\(_x\)H\(_y\)N\(_z\)O\(_2\)S\(_b\) 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 \( \tau \) 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 \( \tau \) determined for Cape Lookout. Gujer and Zehnder
(1983) also noted a relationship between oxidation state, substrate, and CO\(_2\) to CH\(_4\)

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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 $\delta$CH$_4$ 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 $\Sigma$CO$_2$ $\delta^{13}$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 $\Sigma$CO$_2$ $\delta^{13}$C primarily reflects changes in sulfate reduction rate and methanogenesis. The sedimentary profiles are best described by the mixing of $\Sigma$CO$_2$ from two processes: sulfate reduction, producing $\Sigma$CO$_2$ with an isotopic signature of -19.2 per mil, and
methanogenesis, resulting in $\Sigma CO_2$ 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 $\Sigma CO_2$ and methane are sensitive to the ratios of $\Sigma CO_2/\text{SR}$ 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 $\Sigma CO_2 \delta^{13}C$ profiles in other environments as well as the isotopic signature of diagenetic carbonates.
Fig. 3.1  \( \Sigma \text{CO}_2 \), sulfate and \( \Sigma \text{CO}_2 \ \delta^{13}\text{C} \) data for the five depth intervals. The \( \Sigma \text{CO}_2 \), \( \Sigma^{12}\text{CO}_2 \), \( \Sigma^{13}\text{CO}_2 \) and sulfate concentrations were fit to a line to determine rates for each depth interval.
Fig. 3.2  (A) Measured rates (symbols) and curvefits to rates (lines) of $\Sigma CO_2$ production and sulfate reduction. (B) Isotopic signature of the $\Sigma CO_2$ 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 calculated 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 $\Sigma CO_2$ concentration and $\delta^{13}C$ profile and the diagenetic equation (Eqn. 12).
The measured concentrations of $\Sigma CO_2$ 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 $\Sigma CO_2$ (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).
The diagram shows the change in δ¹³C compared to the sum of CO₂ over depth (cm) in the sediment. The upper graph represents δ¹³C vs. ΣCO₂, while the lower graph shows SO₄²⁻ vs. ΣCO₂. The data points are plotted with error bars to indicate variability.
Fig. 3.4 The sulfate gradient versus the $\Sigma CO_2 \delta^{13}C$ gradient for the upper 3 cm of sediment from porewater profiles collected from 1986 to 1991 at Cape Lookout. The sulfate and $\Sigma CO_2 \delta^{13}C$ gradients were based on linear fits of concentration over the upper 3 cm (one cm intervals).
\[ r^2 = 0.483 \]
Fig. 3.5 Porewater Calcium concentration versus time for the upper 10 cm from the incubation experiment.
Fig. 3.6  The relationship between the oxidation state of the organic carbon, the ratio of $\Sigma CO_2$ produced to sulfate reduced ($\tau$) and the modelled isotopic signature of the methane produced.
Oxidation State

$\text{CH}_4$ $\delta^{13}\text{C}$
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.
The graph shows the measured ratio of $\text{CO}_2/\text{CH}_4$ versus oxidation state. The measured ratio is represented by open circles, while the theoretical ratio is represented by a solid line. The y-axis represents $\text{CO}_2/\text{CH}_4 \times (\tau - 1)$, and the x-axis represents the oxidation state.
Table 3.1  Regression coefficients ($r^2$) for the SO$_4$$^-$, ΣCO$_2$, and ΣCO$_2$ δ$^{13}$C incubation data

<table>
<thead>
<tr>
<th>Depth</th>
<th>SO$_4$$^-$</th>
<th>ΣCO$_2$</th>
<th>ΣCO$_2$ δ$^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 cm</td>
<td>0.92</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>2-4 cm</td>
<td>0.94</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>4-6 cm</td>
<td>0.75</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>6-8 cm</td>
<td>0.85</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>8-10 cm</td>
<td>0.56</td>
<td>0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Table 3.2  Curve fit parameters, estimated depth integrated rates (10 cm), and associated isotopic compositions from tube incubation experiment.

<table>
<thead>
<tr>
<th>Process</th>
<th>$R_0$</th>
<th>$a$</th>
<th>*Depth Integrated Rate</th>
<th>$\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total $\Sigma$CO$_2$ Produced</td>
<td>0.103</td>
<td>0.2037</td>
<td>0.439</td>
<td>-13.1</td>
</tr>
<tr>
<td>$\Sigma$CO$_2$ Produced from Sulfate Reduction</td>
<td>0.103</td>
<td>0.2341</td>
<td>0.397</td>
<td>-19.2</td>
</tr>
<tr>
<td>$\Sigma$CO$_2$ Produced from Methanogenesis</td>
<td>--</td>
<td>--</td>
<td>0.0420</td>
<td>+44.2</td>
</tr>
<tr>
<td>Methane Produced</td>
<td>--</td>
<td>--</td>
<td>0.0539</td>
<td>-65.9</td>
</tr>
<tr>
<td>Sulfate Reduction</td>
<td>0.058</td>
<td>0.2341</td>
<td>0.223</td>
<td>--</td>
</tr>
</tbody>
</table>

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


BOEHME S.E. (1989) Seasonal variation in the production of $\Sigma CO_2$ in a methane-producing sediment. M.S. North Carolina State University, Raleigh.


