

## Dissolved organic carbon production and consumption in anoxic marine sediments: A pulsed-tracer experiment

Carol A. Hee,<sup>1</sup> Tamara K. Pease,<sup>2</sup> Marc J. Alperin, and Christopher S. Martens

Department of Marine Sciences, University of North Carolina at Chapel Hill, 12-7 Venable Hall, Chapel Hill, North Carolina 27599-3300

### Abstract

The degradation of phytoplankton and seagrass organic carbon (OC) in sulfate-reducing (SR) and methane-producing (MP) sediments was tracked by measuring concentrations of particulate OC (POC), hydrolysis products (HP), fermentation products (FP), and inorganic end products (EP). This experiment used the novel approach of amending sediment with organic substrates having  $\delta^{13}\text{C}$  values unique from that of the OC pool originally present in the sediment. As a result, we could monitor changes in the dynamic dissolved OC pool and gain insight into processes that control the fate of OC. Rates of hydrolysis, fermentation, and terminal metabolism were greater in the phytoplankton-amended treatments than in the seagrass-amended treatments during the period of active decomposition. At the end of the incubation, concentrations of HP and FP in amended treatments were not significantly different from those in the controls. An analysis of the  $\delta^{13}\text{C}$  values of HP from the amended treatments indicated that the addition of fresh organic matter stimulated the decomposition of carbon present in the sediment at the time of collection. Hydrolysis of this carbon accounted for  $\geq 50\%$  of the total carbon hydrolyzed in the sediment amended with seagrass.

The factors that control whether marine sedimentary organic matter is ultimately remineralized or preserved are not well understood (Henrichs 1992; Canfield 1994; Hedges and Keil 1995). However, recent work has demonstrated that burial efficiency in continental margin sediments is inversely correlated to oxygen exposure time (Hartnett et al. 1998; Hedges et al. 1999). This finding suggests that organic matter preservation is promoted by conditions that are unique to anaerobic environments. An important distinction between metabolism in anaerobic versus aerobic sediments is that, under reducing conditions, organic matter is degraded by a multistep process involving a consortium of bacteria (Fenchel and Finlay 1995).

A simple conceptual model of organic matter degradation in anoxic sediments is shown in Fig. 1. In this model, reactive components of the particulate OC (POC) pool are remineralized by stepwise degradation beginning with hydrolysis (H) to form dissolved OC (DOC). In sulfate-reducing (SR) and methane-producing (MP) environments, the products of H undergo fermentation (F) and are transformed to volatile fatty acids such as acetate. Finally, F products (FP) are converted to  $\Sigma\text{CO}_2$  and  $\text{CH}_4$  by sulfate reduction and methanogenic bacteria in a process known as terminal metabolism (TM; Fenchel and Finlay 1995).

The simple, linear degradation model may be complicated

by processes that produce recalcitrant organic substrates or promote the degradation of less reactive compounds. For example, the introduction of fresh organic matter may stimulate the degradation of unreactive or “relic” POC and/or DOC via a mechanism known as cometabolism or priming. Furthermore, some of the compounds produced by H may be resistant to further degradation due to their inherent stability or as a result of abiotic reactions that protect the molecule from enzymatic attack. These processes can be thought of as adjustable valves (Fig. 1a–c) that interact with other factors to determine the fate of organic matter.

The details of anaerobic, organic matter degradation can only be understood by tracing the carbon as it flows from the POC pool through intermediate DOC pools and finally to end products (EP). To accomplish this, we conducted pulsed-tracer experiments by adding isotopically distinct reactive OC to relatively old sediment that contained only relic POC and DOC. We were interested specifically in determining the timescale of recalcitrant DOC formation and assessing whether the decomposition of recalcitrant POC or DOC is stimulated by the input of fresh, reactive organic matter. To evaluate the influence of organic matter source and terminal metabolic community on these processes, we conducted two experiments. Phytoplankton and seagrass were added to sediment that contained relic POC and DOC, and SR and MP treatments were established.

### Methods

*Study site*—Cape Lookout Bight (CLB) was chosen as our study site because it is well studied, and the lifetime of reactive sedimentary organic matter ( $\sim 1$  yr; Martens et al. 1992) is amenable to laboratory investigations. CLB is a semi-enclosed marine basin located approximately 105 km southwest of Cape Hatteras at the southern end of the Outer Banks barrier island chain in North Carolina. This site is

<sup>1</sup> Corresponding author (chee@email.unc.edu).

<sup>2</sup> Present address: Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, Georgia 31411.

### Acknowledgments

Thanks are extended to Howard Mendlovitz, who assisted with the stable carbon isotope analyses; Dan Albert, who analyzed the organic acid samples and participated in several helpful discussions about this experiment; and two anonymous reviewers, who provided insightful comments. This research was supported by NSF grants OCE 9217570 and OCE 9633456 and an EPA STAR fellowship to C.A.H.

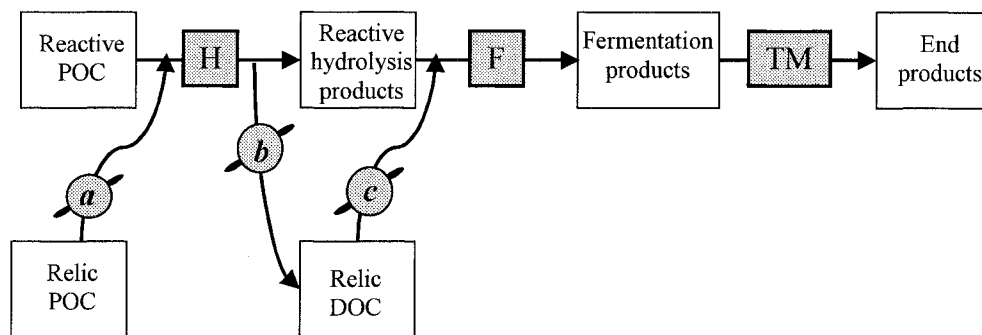


Fig. 1. Pathways of carbon flow during organic matter degradation in anoxic marine sediments. The shaded boxes represent hydrolysis (H), fermentation (F), and terminal metabolism (TM). The valves (a–c) represent additional processes that may influence the fate of organic matter.

notable for its extremely high rates of sedimentation ( $10.3 \pm 1.7 \text{ cm yr}^{-1}$ ; Chanton and Martens 1983) and organic matter deposition ( $165 \pm 20 \text{ mol C m}^{-2} \text{ yr}^{-1}$ ; Martens et al. 1992).

The large input of OC and rapid remineralization at the sediment surface leads to the depletion of oxygen within millimeters of the sediment–water interface. Consequently, organic matter degradation is dominated by anaerobic bacteria. Sulfate reduction is the dominant mode of TM until sulfate is depleted (within 10 cm of the sediment surface during the summer and 25 cm during the winter; Klump and Martens 1987). Thereafter, methanogens control TM. Measurements of inorganic carbon (IC) fluxes, coupled with rate measurements of sulfate reduction and methanogenesis, suggest that  $68 \pm 20\%$  of the integrated OC remineralization occurs via sulfate reduction, and  $32 \pm 16\%$  is associated with methanogenesis (Martens and Klump 1984). Thus,  $\text{NO}_3^-$ , Mn(IV), and Fe(III) reduction and aerobic metabolism are minor pathways of organic matter remineralization at this site.

Sedimentary POC concentrations decrease from a maximum of  $\sim 4\text{--}5\%$  at the surface to  $\sim 3\%$  at 80 cm, with 98% of remineralization occurring within the upper 30 cm (Martens and Klump 1984; Klump and Martens 1987). POC deposited to CLB sediment is derived from multiple sources, including phytoplankton and seagrass. Seasonal variations in the  $\delta^{13}\text{C}$  of OC in surface sediments (0–0.5 cm) suggest temporal changes in the dominant source of OC. From September to February, the  $\delta^{13}\text{C}$  of sedimentary POC decreases from  $-18.2$  to  $-20.3\text{‰}$  (Canuel and Martens 1993). This shift reflects enhanced deposition of algal carbon with a mean  $\delta^{13}\text{C}$  signature of  $-22.0 \pm 1.6\text{‰}$  (Haines 1976). From March to September, the  $\delta^{13}\text{C}$  of sedimentary POC shifts toward heavier values ( $-18.1\text{‰}$ ; Canuel and Martens 1993). This shift coincides with the summertime senescence and transport to CLB of seagrass, which has a  $\delta^{13}\text{C}$  of  $-8$  to  $-13\text{‰}$  (Thayer et al. 1978). Additionally, microscopic analysis of CLB sediment reveals abundant diatom tests and fragments of seagrass blades, and biomarker studies indicate the presence of algal and seagrass carbon (Haddad and Martens 1987; Canuel and Martens 1993).

*Experimental design*—Sediment for this experiment was collected in October 1996 using a gravity corer. To minimize

interference from the degradation of the native organic matter, only sediment containing organic matter buried below the zone of remineralization ( $>50 \text{ cm}$ ; Martens and Klump 1984) was retained. Approximately 10 liters of sediment was pooled and covered to prevent desiccation. In the laboratory, the sediment was subjected to vigorous stirring, which presumably released methane contained in gas bubbles. The porosity of the sediment was increased from 0.80 to 0.88 via the addition of a degassed 34% NaCl solution to match the mean porosity of the upper 30 cm of CLB sediment (Klump and Martens 1987) and to ease mixing and subsampling. Six 2-liter Erlenmeyer flasks were filled with  $\sim 1.5$  liters of homogenized sediment. For three of the flasks, the solution used to adjust porosity also contained  $\text{Na}_2\text{SO}_4$  so that initial sulfate concentrations were  $\sim 30 \text{ mM}$ , sufficient to promote a SR microbial community (hereafter referred to as the SR treatment). In the remaining three flasks, sulfate concentrations were  $<0.05 \text{ mM}$ , and MP bacteria were assumed to be the dominant terminal metabolizers (the MP treatment). The presence of sulfate does not exclude MP bacteria from contributing to the observed remineralization in the SR treatments. Measurements of methane concentrations in SR treatments reveal that methanogenesis accounted for up to  $\sim 30$  and 4% of the observed remineralization in the SR treatments in the phytoplankton and seagrass addition experiments (Pease 2000).

A pair of flasks (one from each treatment) received no carbon addition (“control”), a second pair received an addition of 0.50 moles phytoplankton carbon (“+phytoplankton”), and a third pair received an addition of 0.52 moles seagrass carbon (“+seagrass”). The phytoplankton was a paste-like mixture of fresh algae consisting primarily of diatoms (Coast Seafoods). Healthy *Zostera marina* blades were harvested from a seagrass bed near CLB. Blades without perceptible epiphytes were rinsed with 34% saline solution and were sliced into 2-mm pieces to facilitate an even distribution of seagrass throughout the flasks. These carbon additions increased the POC content from 2.9 to  $\sim 4.3\%$ , an OC content comparable to CLB surface sediment (Martens and Klump 1984).

Following the additions, all flasks were flushed with  $\text{N}_2$  and plugged with stoppers fitted with ports and stopcocks to permit subsampling of whole sediment and headspace gases.

Flasks were shaken vigorously, wrapped in aluminum foil to prevent light exposure, and incubated in the dark at  $\sim 23^{\circ}\text{C}$  (average summer sediment temperature at CLB; Kipphut and Martens 1982). A total of 14 subsamples were taken on a logarithmic timescale during the 176-d experiment, a period that encompasses the residence time of reactive OC in CLB sediment.

At each sampling time, a known aliquot of headspace gas was withdrawn using a glass syringe. This gas was transferred to a combusted 10-ml serum vial by displacing distilled water as the vial was held upside down and submerged. The vial was quickly sealed with a lightly greased stopper and crimp-seal cap and was stored inverted in a freezer until analysis. Flasks that contained pressure in excess of the volume needed for analysis were depressurized by measuring and releasing excess gas using a glass syringe.

After gas-phase sampling, the flasks were shaken and inverted, and 45 ml of whole sediment was withdrawn using a 60-ml catheter-tip syringe. An equal volume of  $\text{N}_2$  was added to replace the withdrawn sediment. Five milliliters of whole sediment was dispensed into a precombusted glass scintillation vial and lyophilized for total OC (TOC) analysis. Thirty milliliters of whole sediment was placed in a combusted 50-ml Corex screw-top centrifuge tube that had been flushed with  $\text{N}_2$ , and pore water was separated by centrifuging for 20 min at  $5,700 \times g$  (5,000 rpm). After centrifugation, pore water was withdrawn using a 20-ml plastic syringe. Pore water was then filtered through a prerinsed  $0.45\text{-}\mu\text{m}$  Millipore polyvinylidene fluoride syringe filter. After discarding  $\sim 0.5$  ml, aliquots of filtered pore water were dispensed into combusted glass vials and stored frozen until analysis. Blanks for pore-water and gas-phase analyses were prepared using filtered ( $0.45\text{-}\mu\text{m}$ -Millipore) samples of 34‰ saline solution at every sampling period.

*Analytical procedures*—To monitor organic matter transformations during the degradation experiment, we measured concentrations of four active carbon pools: POC, H products (HP), FP, and EP. The subdivision of active carbon pools in this manner is based on the microbial decomposition processes that operate in highly reducing, anoxic sediment (Fig. 1). Concentrations of the active pools are presented in terms of millimoles carbon per liter of whole sediment ( $\text{mM}_{\text{ws}}$ ) to facilitate interpool comparisons.

*POC*—The POC pool is operationally defined as the OC content of the sediment fraction greater than  $45\text{-}\mu\text{m}$  and may contain discrete organic particles as well as carbon that is associated with the mineral matrix (Mayer 1993; Keil et al. 1994; Hedges and Keil 1995). Typically, POC is measured as %OC of freeze-dried and vapor-acidified whole sediment (Hedges and Stern 1984). Because this measurement includes the contribution of carbon originally dissolved, %OC actually provides an estimate of TOC. In most natural samples, the contribution of DOC to TOC is negligible (e.g., DOC accounts for  $<0.5\%$  of TOC in CLB sediments; Alperin et al. 1994). However, during this experiment, DOC concentrations reached maximum values of  $\sim 150\text{ mM}_{\text{ws}}$ , accounting for nearly 20% of TOC; and, early in the experiment, volatile fatty acids accounted for  $\geq 90\%$  of DOC. This

complicated determination of POC from TOC because an unknown fraction of volatile fatty acids is lost during the vapor acidification process. Consequently, POC concentrations could not be determined with confidence from %OC analyses alone.

Because the degradation experiment was conducted in sealed flasks, the total inventory of carbon remained constant if the fraction removed during subsampling is taken into account. Thus, the concentration of POC at any time during the incubation ( $[\text{POC}]_t$ ) could be determined by difference using the TOC initially present in each flask ( $[\text{TOC}]_0$ ) and the concentration of carbon in each of the three measured carbon pools at each time

$$[\text{POC}]_t = [\text{TOC}]_0 - [\text{HP}]_t - [\text{FP}]_t - [\text{EP}]_t$$

The value for  $[\text{TOC}]_0$  in the +phytoplankton and +seagrass experiments was calculated as the concentration of  $\text{POC}_0$  in the controls plus the OC added as phytoplankton or seagrass at the start of the incubation. The uncertainty in  $[\text{POC}]_t$  is  $\pm 2\%$ , propagating the maximum possible error associated with each of the contributing pools. Concentration changes in particulate IC (PIC) were not measured and were assumed to be negligible during the experiment. Carbonate dissolution most likely did not occur due to the increases in alkalinity and dissolved IC (DIC) that accompany sulfate reduction and methanogenesis. If carbonate precipitation occurred, estimates of remineralization efficiency based on EP concentrations should be considered lower limits. (Methods for measuring concentrations of HP, FP, and EP and estimating their uncertainty are described below.)

The mass-balance approach to estimating POC was validated by correcting measurements of TOC for the contribution of DOC. We assumed HP were conserved during vapor acidification and subtracted [HP] from [TOC] for each time. As previously stated, some FP are acid volatile and may be lost during vapor acidification. To estimate this loss, we compared total carbon present in acidified and nonacidified subsamples of the solid phase. The contribution of  $\text{CaCO}_3$  to nonacidified samples was determined as the difference in total carbon in acidified and nonacidified control samples that had very low concentrations of acid-volatile DOC. The difference in total carbon between acidified and nonacidified samples attributable to the loss of acid-volatile DOC averaged 60% of the total FP concentration. Using 60% as a correction factor for volatile fatty acid loss, we approximated the contribution of DOC to TOC to arrive at an estimate of [POC]. Despite uncertainties inherent in both methods of calculation, POC concentrations agreed within  $\leq 10\%$  for the +phytoplankton experiment. Due to difficulties homogenizing and subsampling sediment in the seagrass-amended flasks, only the mass-balance approach to estimating [POC] could be applied to the +seagrass experiment.

*HP*—The HP pool contains compounds such as proteins, peptides, long-chain fatty acids, and other nonvolatile biomolecules that are produced during the first step of POC degradation. We assume that these molecules are small enough to pass through a  $0.45\text{-}\mu\text{m}$  filter (equivalent to a molecular weight of  $<10^8$  Daltons; Thurman 1985) and that

volatile fatty acids, CO<sub>2</sub>, and CH<sub>4</sub> are not direct products of extracellular H. HP were measured by dehydrating acidified pore water (to drive off acid-volatile OC and IC) and oxidizing the OC retained with the inorganic salts by sealed tube combustion (STC; Alperin and Martens 1993). Subsamples (1.0 ml) of pore water were pipetted into combusted (5.5 h, 850°C) double-walled quartz test tubes (25.5 cm in length) and acidified with 0.2 ml 50% (v/v) H<sub>3</sub>PO<sub>4</sub>. The “double-walled” technique of Minagawa et al. (1984) was used to avoid explosion of the closed quartz tube caused by high temperature reactions between SiO<sub>2</sub>, H<sub>3</sub>PO<sub>4</sub>, and acidic sea salts. The test tubes were capped with combusted (8 h, 520°C) Pyrex caps that contained a 22-cm length (4-mm O.D.) of Pyrex tubing that served as a conduit for purified air. A Whatman TOC gas generator purified ambient air by removing particles, OC and IC, and water. A manifold containing a series of flow meters split the generator’s outflow into five Teflon tubes that passed through a vent into an oven. Five sample tubes in a rack were placed in this oven, attached to the tubes supplying clean air, and heated to 80°C. The test tube rack was tilted 70° to promote faster evaporation by increasing the pore-water surface area. Water, IC, and acid-volatile OC were removed from the samples by the constant flow (150 ml min<sup>-1</sup>) of purified air across the water surface. After 24 h, the samples were removed from the oven, the Pyrex caps and drying tubes were withdrawn, and combusted (5.5 h, 850°C) quartz ampoules with 0.15 g CuO (a source of oxygen; Buchanan and Corcoran 1959) and 0.2 g WO<sub>3</sub> (a source of an acidic flux; Kissa 1957) were added. The tubes were attached to a vacuum manifold, evacuated, and sealed using a hydrogen and oxygen flame. The dried organics were then combusted at 850°C for 5.5 h. Resultant CO<sub>2</sub> was manometrically measured and sealed in Pyrex ampoules for isotopic analysis (described below).

Accuracy of the STC technique was assessed by using glucose standards in 35‰ NaCl and by comparing STC measurements of DOC in a pore-water sample from CLB with very low concentrations of acid-volatile OC to measurements of the same sample using high-temperature catalytic oxidation (HTCO; Alperin and Martens 1993). The average recovery of glucose standards across a range of concentrations (1–50 mM) was 99.7 ± 1.1% (*n* = 12). The concentration of HP in the CLB pore-water sample as determined by the STC method, 4.2 ± 0.2 mM (*n* = 5), agreed with that determined by HTCO, 3.9 ± 0.1 mM (*n* = 3). Analytical precision for the STC method is 1–2%, as determined by replicate analyses of samples across the range of concentrations measured in this experiment.

**FP**—Fermentative bacteria produce acetate and other volatile organic acids and alcohols that are removed from pore water during the drying phase of the STC method. Therefore, the concentration of FP could be calculated as the difference between concentrations of total DOC (TDOC) and HP. Concentrations of TDOC were measured by HTCO after 300-μl aliquots of pore water were diluted with 3 ml of 0.2% (v/v) HCl and purged of IC using a stream of carbon-free air flowing for 12 min at 30 ml/min. The analytical precision of the TDOC measurement, expressed as the coefficient of variation of triplicate injections of a sample, is generally

<2%. However, total uncertainty in TDOC is on the order of 7–10% (based on 11 analyses of duplicate pore-water aliquots). This higher uncertainty is probably related to particles that appeared after frozen pore-water samples were thawed. Propagating the error associated with measurement of HP and TDOC resulted in an average relative error of ±10% for the FP concentrations.

Concentrations of FP determined by difference were verified by comparison with measurements of low-molecular-weight organic acids. Aliquots of pore-water samples were diluted 250-, 20-, or 2-fold to adjust sample concentrations to the optimal range for analysis. Samples were derivatized in acid-cleaned, precombusted (450°C, 3–4 h), 4-ml borosilicate glass vials with Teflon-lined caps and analyzed via high-performance liquid chromatography (HPLC) of their 2-nitrophenylhydrazide (NPH) derivatives (Albert and Martens 1997). Organic acid concentrations compared well with concentrations of FP that were determined by difference: the organic acids measured by the NPH method (acetate, formate, propionate, and *n*- and *iso*-valerate) accounted for 88% of FP on average. The unaccounted 12% can be explained by the presence of acid-volatile organics other than the six organic acids measured by the NPH method.

**EP**—The EP of anaerobic metabolism include DIC and CH<sub>4</sub>. Since the experimental flasks contained both pore water and gas phases, dissolved and gaseous concentrations were taken into account. To measure DIC, 2 ml of 1 M H<sub>3</sub>PO<sub>4</sub> saturated with CuSO<sub>4</sub> (to scavenge excess hydrogen sulfide) was added to 0.5–1.0-ml pore-water samples stored in sealed serum vials. The resultant CO<sub>2</sub> (g) was removed by vacuum distillation, purified, and measured manometrically. Precision and accuracies of this method are 0.4 and 0.1 mM, respectively (based on analyses of bicarbonate standards with a wide range of concentrations, *n* > 75; Boehme et al. 1996).

Headspace gases, CO<sub>2</sub> (g) and CH<sub>4</sub> (g), were separated on a Porapak Q (0.32 μm i.d., 25 m, film thickness of 10 μm, Hewlett Packard) column using a Hewlett Packard 5980 gas chromatograph with helium (12 psi) as a carrier gas as described in Pease (2001). The separated gases were transferred to a Finnigan-MAT 252 magnetic sector isotope ratio-monitoring mass spectrometer controlled by Isodat software (V5.2-0009). Gas concentrations were calculated from the integrated areas reported by the mass spectrometer relative to authentic and prepared gas standards (Scott Specialty Gases, Scotty Analyzed<sup>®</sup>). The concentration of CH<sub>4</sub> (aq) was assumed to have remained at saturation and constant throughout the experiment and was therefore not included in the calculation of total EP. The relative error of the CO<sub>2</sub> (g) and CH<sub>4</sub> (g) measurements, determined by duplicate analyses of 28 samples, was between 0.1 and 26%, averaging 7 ± 6%. Concentrations of DIC, CO<sub>2</sub> (g) and CH<sub>4</sub> (g) were summed to give the concentration of total EP, and the error in each of these measurements was propagated to arrive at a total relative error of 2–13%.

**Stable isotope analyses**—As another means of monitoring carbon transformations, the δ<sup>13</sup>C values of the phytoplankton, seagrass, and HP were measured. Small samples of phy-

toplankton and seagrass were freeze dried, pulverized, vapor acidified with 12 M HCl (to remove calcium carbonate), and subjected to combustion and quantification using a Carlo Erba CNS 1500 elemental analyzer (Hedges and Stern 1984). The resultant CO<sub>2</sub> was passed to a Finnigan MAT 252 stable isotope mass spectrometer for determination of  $\delta^{13}\text{C}$ . The error associated with the measurement of  $\delta^{13}\text{C}$  of the phytoplankton and seagrass as determined by duplicate analyses was  $\pm 0.2$  and  $\pm 0.02\%$ , respectively. The  $\delta^{13}\text{C}$  values of HP were determined by subjecting CO<sub>2</sub> collected during the STC method (described above) to stable carbon isotope analysis on the same mass spectrometer. Isotopic analyses of dissolved glucose standards ( $n = 12$ ) across a range of concentrations (1–50 mM) gave a standard deviation of  $\pm 0.16\%$ , and triplicate analysis of a sample from this experiment resulted in a standard deviation of  $\pm 0.07\%$ .

## Results and discussion

**Concentration data**—Concentrations of POC, HP, FP, and EP in sediments amended with phytoplankton and seagrass are presented in Fig. 2. Linear regressions of the POC, HP, FP, and EP data for the SR and MP control treatments indicate that, for each subpool, the slope with its associated standard deviation was not statistically different from zero, indicating that the degradation of relic CLB OC was not detectable in the controls.

**POC**—In amended treatments, POC concentrations decreased from initial highs to near constant values by day 60 (Fig. 2a,b). POC concentrations in the SR and MP treatments paralleled each other and differed only in the absolute amount of carbon present at the start and end of the experiment. At the end of the incubation, concentrations of POC in both the +phytoplankton and +seagrass experiments had not returned to control values: approximately 30% of the added phytoplankton remained in the POC pool, and >85% of the added seagrass remained as POC. The disparity in the total amount of carbon hydrolyzed can be attributed to compositional differences between the carbon sources. Phytoplankton contains a greater percentage of protein, which degrades rapidly (Henrichs and Doyle 1986) compared to seagrasses, which contain more structural components that are resistant to degradation (Godschalk and Wetzel 1978; Pellikaan 1984; Enriquez et al. 1991).

Other experiments in which POC was added to anoxic sediment have shown that a large fraction of added carbon was not degraded over the duration of the experiment. For example, Holmer (1996) added fresh phytoplankton detritus to surface (0–2 cm) and deep (10 cm) intertidal sediment from Odense Fjord and found that 81% of the added carbon remained at the end of a 30-d experiment. Anderson (1996) found that ~40% of the carbon added as <sup>14</sup>C-labeled diatoms to samples of intertidal sediment from Saanich Inlet was unreactive after 80 d. In contrast to these earlier studies, the duration of the experiments presented here exceeded the residence time of reactive OC in CLB sediment; thus, our incubation experiments captured the period critical to organic matter degradation at CLB.

**HP**—HP concentrations in the +phytoplankton experiment (Fig. 2c) exhibited maximum values at the first sampling time after the addition of phytoplankton carbon, most likely due to high concentrations of soluble organic compounds in the algal paste. Concentrations of HP decreased from initial maximum values (~10 times control values) to background levels (3.5 mM<sub>ws</sub>) by day 60 without any distinguishable differences between the SR and MP treatments. Concentrations of HP in the +seagrass treatments (Fig. 2d) at  $t = 0$  were comparable to the controls. However, concentrations increased rapidly in the first few days and reached their highest values (~2 times control values) at about day 5, after which time HP concentrations decreased until reaching background levels. Similar to the +phytoplankton experiment, concentrations of HP in the SR and MP treatments paralleled each other throughout the +seagrass experiment and did not indicate net DOC production.

At the end of the incubation period, net DOC production was not evident in either experiment: concentrations in samples from days 90, 121, and 176 were not statistically different from the controls ( $t$ -test,  $P < 0.05$ ). Thus, processes responsible for the net production of refractory DOC must operate on longer timescales.

In other experiments during which carbon was added to anoxic sediment, net DOC accumulation was extremely low relative to  $\Sigma\text{CO}_2$  production, similar to the results reported here. Holmer (1996) noted transient net DOC production during the course of an incubation of surface and deep sediment amended with detritus and relatively little net DOC production relative to  $\Sigma\text{CO}_2$  production at the end of a 56-d incubation. DOC turnover rates were estimated to be 4–11% d<sup>-1</sup>. Kristensen et al. (1995) reported low rates of DOC release to water overlying sediment samples that were amended with aged diatoms and concluded that anaerobic TM was highly efficient at degrading DOC.

**FP**—FP (Fig. 2e,f) were negligible (<30  $\mu\text{M}$ ) in the controls. With the introduction of fresh phytoplankton, FP concentrations increased from zero to maximum values at around day 11. At this point, FP accounted for 15 and 12% of the total carbon present in the SR and MP +phytoplankton treatments, respectively. The degradation of the fresh seagrass also caused the pooling of FP. By day 7, FP concentrations were ~8 mM<sub>ws</sub>—significantly higher than the background levels—but only ~5% of the peak measured in the +phytoplankton experiment. In both experiments, FP concentrations decreased to background levels earlier in the SR treatments than in the MP treatments. However, by the end of the experiment, concentrations of FP had returned to levels below detection in all treatments. Thus, all DOC characterized as FP was degraded within the first 60 d of the incubations.

**EP**—Similar to each of the other pools, the control treatments did not exhibit a consistent change in the concentration of EP throughout the course of the incubations. This reinforces the assumption that the carbon present in the sediment collected from below 50 cm at CLB is unreactive on annual timescales, even if a fresh oxidant (e.g., sulfate) is added. Additionally, it is notable that increasing the porosity

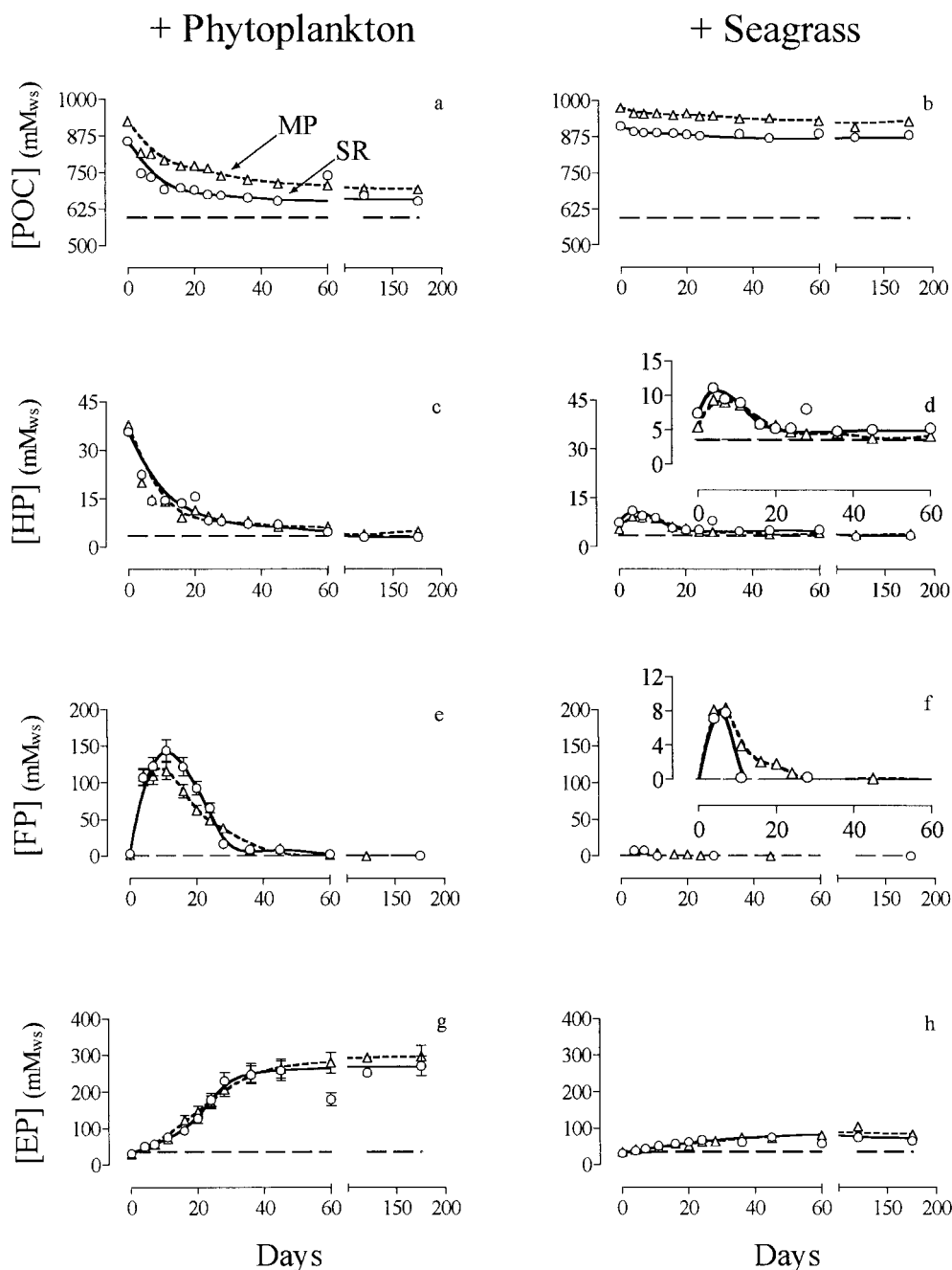


Fig. 2. Concentration changes in the four active carbon pools [(a,b) particulate organic carbon, (c,d) hydrolysis products, (e,f) fermentation products, and (g,h) inorganic end products] for the controls, sulfate-reducing (SR), and methane-producing (MP) treatments. Error bars represent the calculated standard deviation and in some cases are smaller than the symbols. Lines are cubic splines fit to smooth the data. Inserts show expanded concentration scales. Average values for the controls are represented by a dashed line, the thickness of which indicates one standard deviation.

and homogenizing the sediment did not promote the desorption of OC, which subsequently was remineralized.

In the treatments that received an addition of fresh carbon, the remineralization of OC was detectable as an increase in the concentration of DIC,  $\text{CO}_2$  (g), and  $\text{CH}_4$  (g) (Fig. 2g,h). EP concentrations in both the +phytoplankton and +seagrass experiments increased until reaching asymptotic values

at day 60. The amount of carbon remineralized in the +phytoplankton experiment was ~70% of the added carbon, whereas the carbon converted to EP in the +seagrass totaled an amount equivalent to only ~15% of carbon added as seagrass.  $\text{CH}_4$  (g) accounted for 30 and 4% of the total EP accumulated in the SR +phytoplankton and SR +seagrass treatments, respectively, indicating the presence of an active

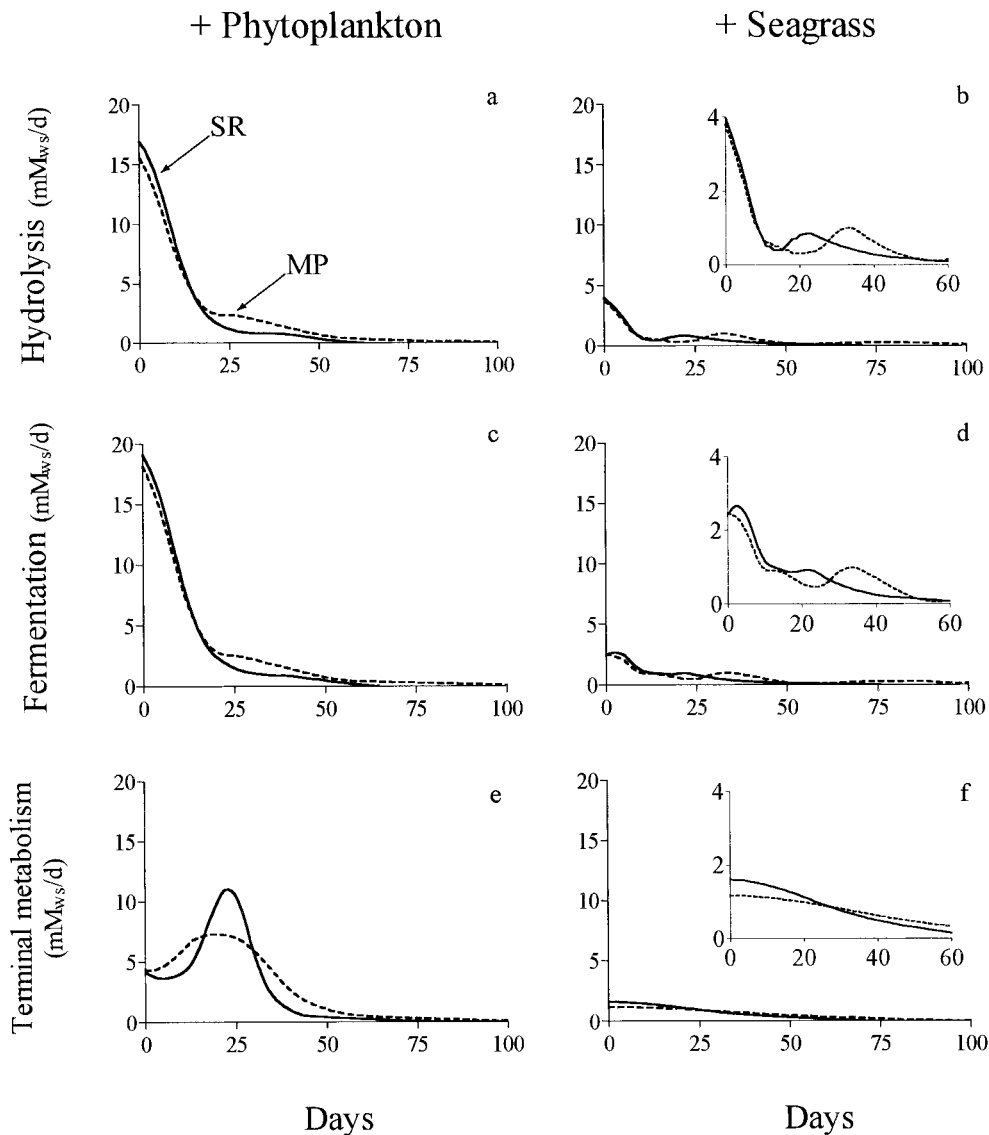


Fig. 3. Rates of transformation between carbon pools in sulfate-reducing (SR) and methane-producing (MP) treatments for phytoplankton- and seagrass-amended incubations determined by fitting cubic splines to the concentration data and taking the derivative of this continuous function.

methanogenic community within the SR treatments throughout the incubation (Pease 2000). At the end of the incubation, there was not a significant difference between the SR and MP treatments in the amount of EP accumulated in either the +phytoplankton or +seagrass experiment.

*OC degradation rates*—Rates of H, F, and TM at any time during the incubation experiment can be calculated from slopes of the concentration versus time data (see Fig. 1).

$$H = -\frac{d[\text{POC}]}{dt}, \quad F = H - \frac{d[\text{HP}]}{dt}, \quad \text{TM} = \frac{d[\text{EP}]}{dt}$$

First derivatives were evaluated by fitting the concentration data with cubic spline functions (DeBoor 1978). Cubic splines are well suited for derivative evaluation because they have the flexibility to reproduce the features of nearly any

data set while minimizing the artificial oscillations that plague polynomial functions (Ahlberg et al. 1967). Cubic splines also have the capability of filtering noisy data (Craven and Wahba 1979). Random errors in the concentration measurements can give rise to large oscillations in the derivatives, especially during periods when concentrations are changing slowly. Fortunately, oscillations in the second derivative induced by random errors occur with a wavelength exactly twice the sampling interval. By selecting the minimum degree of smoothing sufficient to eliminate the short wavelength ( $2\Delta t$ ) oscillations in the second derivative, the data can be filtered with minimal impact on the primary signal (equivalent to a low-pass filter).

*Hydrolysis*—The H of OC commenced immediately after the addition of phytoplankton and seagrass (Fig. 3a,b) with-

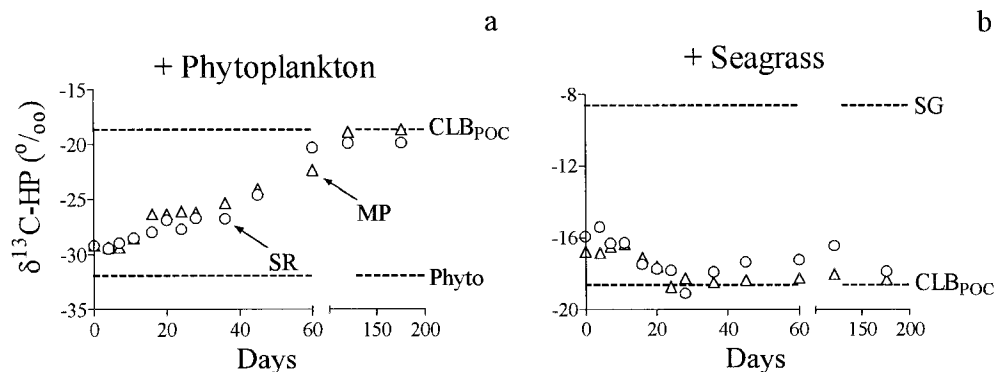


Fig. 4.  $\delta^{13}\text{C}$  of hydrolysis products (HP) in sulfate-reducing (SR) and methane-producing (MP) treatments for (a) phytoplankton- and (b) seagrass-amended incubations. The standard deviation is smaller than the symbol size. The  $\delta^{13}\text{C}$  values of phytoplankton ( $-31.9\text{‰}$ ), seagrass ( $-8.6\text{‰}$ ), and relic Cape Lookout Bight POC are indicated by dashed lines.

out requiring an observable time for microorganisms to initiate enzyme production or to increase biomass. This implies that there is a viable population of organisms capable of H in CLB sediment at depths below the zone of active remineralization and that their metabolism is limited (at least in part) by a lack of available reactive OC. While microorganisms may have been introduced to the incubation jars with the phytoplankton and seagrass, we assume that these organisms were obligate aerobes and did not contribute significantly to the observed carbon metabolism.

In both the +phytoplankton and +seagrass experiments, H rates were at maximum values at  $t = 0$ . The maximum rates of H observed in the phytoplankton-amended treatments ( $\sim 16 \text{ mM}_{\text{ws}} \text{ d}^{-1}$ ) were approximately four times greater than the maximum H rates observed in the +seagrass experiment, demonstrating that reactive constituents of the phytoplankton degrade much faster than those in seagrass. In both experiments, H rates initially decreased rapidly but declined at a slower rate after  $\sim 20$  d, conforming to the multi-G model of organic matter decomposition (Westrich and Berner 1984). Centered on days 22 and 33 in the SR and MP +seagrass treatments, there is a slight bump in the H rates. This reflects the fact that increases in [EP] did not precisely coincide with decreases in [FP]. Carbon leaving the FP pool but not entering the EP pool must be transferred to the POC pool. Subsequent degradation of this carbon thus appears as secondary maxima in the H rate data. In all treatments, H ceased by day 60 (Fig. 3a,b), even though much of the added carbon remained as POC (Fig. 2a,b).

**Fermentation**—Rates of F (Fig. 3c,d) also exhibited maximum values at the start of the experiment. High rates of F during the earliest days of the experiments indicate the presence of a viable population of fermentative organisms below the zone of active diagenesis at CLB. F rates in the SR and MP treatments were not significantly different from each other; however, F in the +phytoplankton experiment was approximately six times as great as in the +seagrass experiment. After small secondary maxima as observed in the H rate data, F rates decreased to zero by day 60 when FP and HP concentrations equaled control values.

**TM**—TM was detectable immediately. In the +phytoplankton experiment, rates of TM in the SR treatment exceeded that in the MP treatment between days 17 and 29; at all other times, rates of TM were greater in the MP treatment. In the +seagrass experiment, rates of TM initially were slightly higher in the SR compared to the MP treatment. However, with time, rates of TM in the +seagrass SR treatment slowed to lower rates than in the MP +seagrass treatment. Higher maximum values in the SR treatments compared to the MP treatments (Fig. 3e,f) caused FP concentrations to decrease more rapidly in the SR treatment compared to the MP treatment in both the +phytoplankton and +seagrass experiments (Fig. 2e,f). Interestingly, TM continued for a longer period of time at a low rate in the MP treatments, causing rate differences to have a negligible effect on the amount of carbon degraded in the MP compared to the SR treatments. Thus, the terminal metabolic community was not the primary determinant of remineralization efficiency.

The slower response by the methanogens is due to their slower doubling time, a consequence of the lower energy yield of methanogenesis compared to sulfate reduction (Fenchel and Blackburn 1979). It has been hypothesized that slower FP consumption by methanogens decreases the thermodynamic favorability of F, causing organic matter decomposition in methanogenic sediments to be less efficient than that in SR sediments (Dolfing 1988). However, in these experiments, lower rates of TM in MP treatments compared to SR treatments did not precipitate a negative feedback on decomposition efficiency.

**Stable isotope data**—The  $\delta^{13}\text{C}$  values of HP ( $\delta^{13}\text{C}\text{-HP}$ ) are shown in Fig. 4a,b. During the 176-d incubation,  $\delta^{13}\text{C}\text{-HP}$  remained constant in the controls ( $-18.7 \pm 0.4\text{‰}$ ,  $n = 28$ ) and did not differ significantly from the  $\delta^{13}\text{C}\text{-POC}$  in un-amended CLB sediment ( $-18.4 \pm 0.3\text{‰}$ ,  $n = 28$ ). In the +phytoplankton experiment,  $\delta^{13}\text{C}\text{-HP}$  at  $t = 0$  ( $-29.2\text{‰}$ ) reflects the contribution of the freshly added phytoplankton. As the experiment progressed,  $\delta^{13}\text{C}$  values continuously became enriched and approached background values after 90 d. In the +seagrass experiment,  $\delta^{13}\text{C}\text{-HP}$  values were ini-

tially enriched by approximately 4‰, a difference large enough to indicate the contribution of fresh seagrass carbon but not as great a difference as noted in the +phytoplankton experiment. Throughout the course of the incubation,  $\delta^{13}\text{C}$ -HP values in the seagrass-amended treatments became increasingly depleted until reaching control values at about day 30. At days 40, 60, and 90, the  $\delta^{13}\text{C}$ -HP of SR and MP samples from the +seagrass treatment became slightly enriched again. At the final time point,  $t = 176$ , the  $\delta^{13}\text{C}$ -HP in both treatments in the +seagrass experiment remained slightly enriched relative to the controls, an indication that some seagrass-derived DOC persisted in the HP pool. Throughout the duration of both experiments, the  $\delta^{13}\text{C}$  values of HP in SR and MP treatments were not consistently different from each other.

**DOC dynamics**—In conjunction with the concentration and rate data, the stable isotope data can be used to test various models of carbon flow during the organic matter decomposition process (Fig. 1). With the definition of  $\delta^{13}\text{C}$ ,

$$\delta^{13}\text{C} = \left( \frac{^{13}\text{C}}{^{12}\text{C}} \div R - 1 \right) \times 1,000$$

where  $R = 0.0112372$ , the ratios  $^{12}\text{C}/\text{C}$  and  $^{13}\text{C}/\text{C}$  can be determined.

$$\frac{^{12}\text{C}}{\text{C}} = \left[ 1 + R \times \left( \frac{\delta^{13}\text{C}}{1,000} + 1 \right) \right]^{-1}, \quad \frac{^{13}\text{C}}{\text{C}} = 1 - \frac{^{12}\text{C}}{\text{C}}$$

Consequently, the fraction of  $^{12}\text{C}$  and  $^{13}\text{C}$  in the phytoplankton or seagrass (Phyto/SG) and relic POC and DOC pools each can be determined, giving rise to 12 subpools. For example, the POC pool is divided as follows:

$$\text{PO}^{12}\text{C}_{\text{Phyto/SG}} = \text{POC}_{\text{Phyto/SG}} \times \left( \frac{^{12}\text{C}}{\text{C}} \right)_{\text{Phyto/SG}}$$

$$\text{PO}^{13}\text{C}_{\text{Phyto/SG}} = \text{POC}_{\text{Phyto/SG}} \times \left( \frac{^{13}\text{C}}{\text{C}} \right)_{\text{Phyto/SG}}$$

$$\text{PO}^{12}\text{C}_{\text{Relic}} = \text{POC}_{\text{Relic}} \times \left( \frac{^{12}\text{C}}{\text{C}} \right)_{\text{Relic}}$$

$$\text{PO}^{13}\text{C}_{\text{Relic}} = \text{POC}_{\text{Relic}} \times \left( \frac{^{13}\text{C}}{\text{C}} \right)_{\text{Relic}}$$

In the simplest version of the model (Model 1), degradation of the freshly added carbon is assumed to proceed linearly, with carbon transformed from POC to HP to FP to EP without the complicating influence of interaction with the relic CLB OC. Additionally, it is assumed that the processes of H and F do not discriminate between  $^{13}\text{C}$  and  $^{12}\text{C}$ . This scenario is equivalent to closing the a, b, and c valves in Fig. 1. Thus, changes in the carbon subpools can be specified as follows:

$$\frac{d\text{PO}^{12}\text{C}_{\text{Phyto/SG}}}{dt} = -H \times \text{PO}^{12}\text{C}_{\text{Phyto/SG}} \quad (1)$$

$$\frac{d\text{PO}^{13}\text{C}_{\text{Phyto/SG}}}{dt} = -H \times \text{PO}^{13}\text{C}_{\text{Phyto/SG}} \quad (2)$$

$$\frac{d\text{PO}^{12}\text{C}_{\text{Relic}}}{dt} = \frac{d\text{PO}^{13}\text{C}_{\text{Relic}}}{dt} = 0 \quad (3)$$

$$\frac{d\text{HP}^{12}\text{C}_{\text{Reactive}}}{dt} = H \times \text{PO}^{12}\text{C}_{\text{Phyto/SG}} - F \times \text{HP}^{12}\text{C}_{\text{Reactive}} \quad (4)$$

$$\frac{d\text{HP}^{13}\text{C}_{\text{Reactive}}}{dt} = H \times \text{PO}^{13}\text{C}_{\text{Phyto/SG}} - F \times \text{HP}^{13}\text{C}_{\text{Reactive}} \quad (5)$$

$$\frac{d\text{DO}^{12}\text{C}_{\text{Relic}}}{dt} = \frac{d\text{DO}^{13}\text{C}_{\text{Relic}}}{dt} = 0 \quad (6)$$

These differential equations were solved numerically using a fourth-order Runge–Kutta method with the following initial conditions:

$$[\text{POC}_{\text{Phyto/SG}}]_0 = [\text{POC}]_{\text{added as phytoplankton or seagrass}}$$

$$[\text{POC}_{\text{Relic}}]_0 = [\text{POC}]_{\text{average control}}$$

$$[\text{HP}_{\text{Reactive}}]_0 = [\text{HP}]_0 - [\text{HP}]_{\text{average control}}$$

$$[\text{DOC}_{\text{Relic}}]_0 = [\text{HP}]_{\text{average control}}$$

$$\delta^{13}\text{C}\text{-HP}_{\text{Reactive}} = -31.9\text{‰} (+\text{phytoplankton experiment}) \\ \text{or } -8.6\text{‰} (+\text{seagrass experiment})$$

$$\delta^{13}\text{C}\text{-DOC}_{\text{Relic}} = -18.6\text{‰}.$$

$\delta^{13}\text{C}$ -HP values are then calculated as follows:

$$\delta^{13}\text{C}\text{-HP} = \left( \frac{\text{HP}^{13}\text{C}}{\text{HP}^{12}\text{C}} \div R - 1 \right) \times 1,000$$

The results of Model 1 are presented as a solid line in Fig. 5a–d. This approach closely approximates the observed  $\delta^{13}\text{C}$  values in the +phytoplankton experiment (Fig. 5a,b), indicating that reactive OC flows as if through a pipeline from POC to inorganic EP. However, this simple model clearly fails in the +seagrass experiment (Fig. 5c,d): the predicted  $\delta^{13}\text{C}$  values are significantly enriched compared to the observed values. At  $t = 0$ , the measured  $\delta^{13}\text{C}$  is depleted compared to the predicted value by 2.5‰ in the SR treatment and by 1.7‰ in the MP treatment. An offset is also observable in the +phytoplankton experiment at  $t = 0$ : the measured values are  $\sim 1.4\text{‰}$  heavier than those predicted by Model 1. Regardless, in the case of the +phytoplankton experiment, the systematic, incubation-long offset conspicuous in the +seagrass experiment is not evident.

The failure of Model 1 to fit the data in the +seagrass experiment indicates the occurrence of a process not included in Model 1. Thus, Models 2 and 3 introduce additional layers of complexity. In Model 2a, Valve b controlling exchange between HP and unreactive DOC is opened, and Valves a and c are kept closed. Mathematically, this is equivalent to multiplying the total H rate by a fraction  $b$ . When  $b = 0$ , the valve is shut, and Model 2 simplifies to Model 1. As the value of  $b$  approaches 1, an increasingly greater

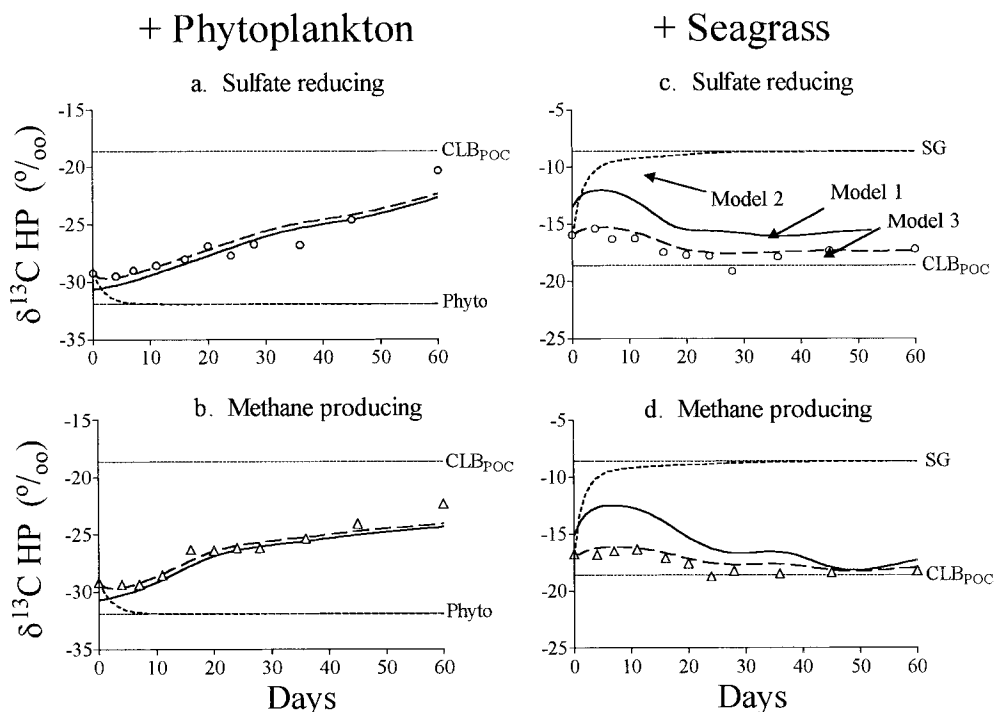


Fig. 5.  $\delta^{13}\text{C}$  of hydrolysis products (HP) in sulfate-reducing (SR) and methane-producing (MP) treatments for phytoplankton- and seagrass-amended incubations with model-derived lines. Best fits are provided by Model 1 for the +phytoplankton experiment and Model 3 for the +seagrass experiment with  $P = 0.5$  (SR) and  $0.6$  (MP). For the +phytoplankton experiment, the good fit of Model 1 is slightly improved by Model 3 with  $P = 0.05$ . See text for full description of models.

fraction of the total H rate creates unreactive DOC. Accordingly, Eqs. 4–6 become the following:

$$\frac{d\text{HP-}^{12}\text{C}_{\text{Reactive}}}{dt} = H \times (b - 1) \times \text{PO}^{12}\text{C}_{\text{Phyto/SG}} - F \times \text{HP}^{12}\text{C}_{\text{Reactive}}$$

$$\frac{d\text{HP-}^{13}\text{C}_{\text{Reactive}}}{dt} = H \times (b - 1) \times \text{PO}^{13}\text{C}_{\text{Phyto/SG}} - F \times \text{HP}^{13}\text{C}_{\text{Reactive}}$$

$$\frac{d\text{DO}^{12}\text{C}_{\text{Relic}}}{dt} = H \times b \times \text{PO}^{12}\text{C}_{\text{Phyto/SG}}$$

$$\frac{d\text{DO}^{13}\text{C}_{\text{Relic}}}{dt} = H \times b \times \text{PO}^{13}\text{C}_{\text{Phyto/SG}}$$

The initial conditions used in this model are the same as in Model 1.

Model 2a places upper limits on the amount of refractory DOC that could have been produced from the H of the freshly added carbon. When  $b > 0.01$ , concentrations of reactive HP become negative. By integrating the total H rate, it can be shown that 1% of the total carbon hydrolyzed is  $\sim 2 \text{ mM}_{\text{ws}}$ , a quantity that would have been detectable as elevated HP concentrations in amended treatments at the end of the incubation. However, as previously noted, the concentration data indicate that the average amount of carbon measured in amended treatments between  $t = 90$  and  $176 \text{ d}$  is not sig-

nificantly different from the average of the controls ( $t$ -test,  $P < 0.05$ ).

Allowing the formation of new reactive HP to stimulate the decomposition of otherwise recalcitrant DOC (i.e., opening Valve c), does not improve the fit of Model 2 to the data or prevent HP concentrations from becoming negative. Under this scenario, HP- $\delta^{13}\text{C}$  values reach the  $\delta^{13}\text{C}$  of the added carbon by day 9 in the +phytoplankton experiment and by day 35 in the +seagrass experiment (Model 2b; Fig. 5). Thus, a net production of unreactive DOC did not occur during this experiment, and relic CLB DOC can be rightfully termed “refractory,” although its source and mechanism of formation remain undetermined.

The last model, Model 3, tests the hypothesis that the addition of fresh substrate stimulates the decomposition of relic POC. This corresponds to opening Valve a in Fig. 1. In this case, the total H rate is multiplied by a fraction,  $a$ , which indicates the fraction of H that degrades relic CLB POC compared to degrading freshly added phytoplankton or seagrass POC. When  $a = 0$ , the valve is shut, and Model 3 simplifies to Model 1. As the value of  $a$  approaches 1, an increasingly greater fraction of the total H rate degrades relic POC. Accordingly, the equations describing changes in carbon subpools are modified as follows:

$$\frac{d\text{PO}^{12}\text{C}_{\text{Phyto/SG}}}{dt} = -H \times (1 - a) \times \text{PO}^{12}\text{C}_{\text{Phyto/SG}}$$

$$\begin{aligned} \frac{d\text{PO}^{13}\text{C}_{\text{Phyto/SG}}}{dt} &= -H \times (1 - a) \times \text{PO}^{13}\text{C}_{\text{Phyto/SG}} \\ \frac{d\text{PO}^{12}\text{C}_{\text{Relic}}}{dt} &= -H \times a \times \text{PO}^{12}\text{C}_{\text{Relic}} \\ \frac{d\text{PO}^{13}\text{C}_{\text{Relic}}}{dt} &= -H \times a \times \text{PO}^{13}\text{C}_{\text{Relic}} \\ \frac{d\text{HP}^{12}\text{C}_{\text{Reactive}}}{dt} &= -\frac{d\text{PO}^{12}\text{C}_{\text{Phyto/SG}}}{dt} - \frac{d\text{PO}^{12}\text{C}_{\text{Relic}}}{dt} \\ &\quad - F \times \text{HP}^{12}\text{C}_{\text{Reactive}} \\ \frac{d\text{HP}^{13}\text{C}_{\text{Reactive}}}{dt} &= -\frac{d\text{PO}^{13}\text{C}_{\text{Phyto/SG}}}{dt} - \frac{d\text{PO}^{13}\text{C}_{\text{Relic}}}{dt} \\ &\quad - F \times \text{HP}^{13}\text{C}_{\text{Reactive}} \\ \frac{d\text{DO}^{12}\text{C}_{\text{Relic}}}{dt} &= \frac{d\text{DO}^{13}\text{C}_{\text{Relic}}}{dt} = 0 \end{aligned}$$

Model 3 differs significantly from the other two models because it provides a mechanism through which carbon with a  $\delta^{13}\text{C}$  different from phytoplankton or seagrass can enter the HP pool. Thus, the  $\delta^{13}\text{C}$  of reactive HP must be calculated from the  $\delta^{13}\text{C}$  of phytoplankton/seagrass, the  $\delta^{13}\text{C}$  of relic POC, and the fraction of HP derived from each of these pools.

$$\begin{aligned} \delta^{13}\text{C}\text{-HP} &= \{(\delta^{13}\text{C}\text{-POC}_{\text{Relic}} \times [\text{HP}]_{\text{Relic POC}}) \\ &\quad + (\delta^{13}\text{C}\text{-POC}_{\text{Phyto/SG}} \times [\text{HP}]_{\text{Phyto/SG POC}}) \\ &\quad + (\delta^{13}\text{C}\text{-DOC}_{\text{Relic}} \times [\text{DOC}]_{\text{Relic}})\} \\ &\quad \div \{[\text{HP}]_{\text{Relic POC}} + [\text{HP}]_{\text{Phyto/SG POC}} + [\text{DOC}]_{\text{Relic}}\} \end{aligned}$$

where  $\text{HP}_{\text{Relic POC}}$  is the concentration of HP derived from  $\text{POC}_{\text{Relic}}$ , and  $\text{HP}_{\text{Phyto POC}}$  is the concentration of HP derived from  $\text{POC}_{\text{Phyto}}$ .

The results of Model 3 are plotted as dashed lines in Fig. 5a–d. Best fits for the +seagrass experiment are obtained when  $a = 0.5$  for the SR treatment and  $a = 0.6$  for the MP treatment. In other words, 50–60% of the total POC hydrolyzed in the +seagrass experiment was relic POC—an amount equivalent to  $\sim 4\%$  of CLB  $\text{POC}_{\text{initial}}$ . The degradation of relic POC was unexpected since, in unamended treatments, CLB POC was unreactive and since diagenesis is not detectable at the depth from which this sediment was collected. Similar to the results noted for Model 2b, opening Valve c to allow  $\text{HP}_{\text{Relic}}$  to enter the degradation pathway causes  $\text{HP}\text{-}\delta^{13}\text{C}$  to reach  $\delta^{13}\text{C}$  of the added carbon by day 10 in the +phytoplankton experiment and by day 35 in the +seagrass experiment.

The success of Model 3 in approximating the  $\delta^{13}\text{C}\text{-HP}$  data for the +seagrass SR and MP treatments indicates that the addition of fresh OC stimulated the degradation of carbon that was diagenetically stable in situ. This phenomenon is referred to as “cometabolism” (Schink 1988; Canfield 1994) or, more accurately, “priming” (Aller 1994). It is also notable that while Model 1 closely approximates the  $\delta^{13}\text{C}\text{-HP}$  data for the +phytoplankton SR and MP treatments, the introduction of  $a = 0.05$  improves the fit to the data during

the first 30 d of the experiment. A value of  $a = 0.05$  implies that  $\sim 2\%$  of  $\text{POC}_{\text{CLB,initial}}$  was hydrolyzed in the +phytoplankton experiment, an amount similar to that noted in the +seagrass experiment.

Although the mechanisms by which priming occurs remain undetermined, several possible explanations exist. The addition of fresh organic matter may have stimulated H by supplying a missing micronutrient or by promoting the release of nonspecific hydrolytic enzymes. Alternatively, if the DOC produced via the H of the fresh carbon had a high affinity for sorption sites, it may have replaced and liberated into the DOC pool relic carbon previously adsorbed in the particulate phase. The finding that the degradation of relic carbon was promoted by the addition of fresh organic matter parallels observations that the addition of growth substrates to systems containing persistent toxic organics can promote bioremediation of the recalcitrant pollutants (Aitken 1998). Additionally, priming induced by the introduction of fresh OC to depth by burrowing organisms may partially explain the observation that bioturbated sediments often exhibit greater organic matter decomposition efficiencies than non-bioturbated sediments (Kristensen and Blackburn 1987; Canfield and Van Cappellen 1992; Aller 1994). This lends additional credence to the hypothesis that the presence of oxygen leads to more extensive degradation (Hulthe et al. 1998; Hedges et al. 1999), because oxygen is required by the benthic fauna responsible for bioturbation.

An alternative explanation for the observed trends in the isotopic values of the HP is that the microbial community preferentially hydrolyzed a subpool of POC that had an isotopic signature distinct from the bulk POC. While fractionation cannot be discounted with absolute certainty, it seems unlikely to account for the observed signals. The available compound-specific isotope data indicate that proteins and carbohydrates are isotopically similar or slightly heavier than bulk POC and that lignin is slightly lighter than bulk POC (Macko et al. 1993). For the +seagrass treatments, a simple isotope mass-balance calculation, following Model 1’s assumption that CLB POC was unreactive, indicates that the carbon hydrolyzed was as much as 5% lighter than bulk seagrass—a difference far greater than the expected range of variation within a single organism or cell.

In conclusion, deciphering the processes involved in organic matter degradation in anoxic sediments remains a complex task. By following the transformations of naturally labeled phytoplankton and seagrass carbon as it was transformed from POC to inorganic EP, we observed that the addition of fresh substrates stimulated the decomposition of relic POC. Thus, all POC buried below the zone of active remineralization cannot be considered intrinsically refractory. This may partially explain the correlation between higher remineralization efficiency and high levels of bioturbation. In oxic regions with significant communities of infauna, bioturbation may introduce fresh organic matter to depth and promote the degradation of less reactive OC via priming.

Net DOC production was not observed; thus, the processes responsible for the formation of refractory DOC must operate on timescales longer than observed here. Additionally, new production of reactive DOC did not stimulate decomposition of relic DOC, indicating the true recalcitrance

of DOC buried beneath the zone of diagenesis at CLB. Finally, although there was a significant difference in the amount of phytoplankton degraded compared to seagrass, the terminal metabolic community (SR or MP) was not a primary determinant of remineralization efficiency. The only noteworthy contrast between SR and MP treatments was a divergence in rates of TM during the first 60 d of the incubation.

## References

- AHLBERG, J., E. NILSON, AND J. WALSH. 1967. The theory of splines and their applications. Academic.
- AITKEN, M. D. 1998. Mechanisms of organic pollutant transformation and degradation by microorganisms, p. 333–383. *In* S. K. Sikdar and R. I. Irvine [eds.], *Bioremediation: Principles and practice—fundamentals and applications*. Technomic.
- ALBERT, D. B., AND C. S. MARTENS. 1997. Determination of low-molecular-weight organic acid concentrations in seawater and pore-water samples via HPLC. *Mar. Chem.* **56**: 27–37.
- ALLER, R. C. 1994. Bioturbation and remineralization of sedimentary organic matter: Effects of redox oscillation. *Chem. Geol.* **114**: 331–345.
- ALPERIN, M. J., D. B. ALBERT, AND C. S. MARTENS. 1994. Seasonal variations in production and consumption of dissolved organic carbon in an organic-rich coastal sediment. *Geochim. Cosmochim. Acta* **58**: 4909–4930.
- , AND C. S. MARTENS. 1993. Dissolved organic carbon in marine pore waters: A comparison of three oxidation methods. *Mar. Chem.* **41**: 135–143.
- ANDERSON, F. O. 1996. Fate of organic carbon added as diatom cells to oxic and anoxic marine sediment microcosms. *Mar. Ecol. Prog. Ser.* **134**: 225–233.
- BOEHME, S. E., N. E. BLAIR, J. P. CHANTON, AND C. S. MARTENS. 1996. A mass balance of  $^{13}\text{C}$  and  $^{12}\text{C}$  in an organic-rich methane-producing marine sediment. *Geochim. Cosmochim. Acta* **60**: 3835–3848.
- BUCHANAN, D. L., AND B. J. CORCORAN. 1959. Sealed tube combustions for the determination of carbon-14 and total carbon. *Anal. Chem.* **31**: 1635–1638.
- CANFIELD, D. E. 1994. Factors influencing organic carbon preservation in marine sediments. *Chem. Geol.* **144**: 315–329.
- , AND P. VAN CAPPELLEN. 1992. How bioturbation may enhance the degradation rates of refractory sedimentary organics. *Geol. Soc. Am. Abstr. Prog.* **24**: A22.
- CANUEL, E. A., AND C. S. MARTENS. 1993. Seasonal variations in the sources and alteration of organic matter associated with recently deposited sediments. *Geochim. Cosmochim. Acta* **20**: 563–577.
- CHANTON, J. P., AND C. S. MARTENS. 1983. Lead-210 sediment geochronology in a changing coastal environment. *Geochim. Cosmochim. Acta* **47**: 1791–1804.
- CRAVEN, P., AND G. WAHBA. 1979. Smoothing noisy data with spline functions. *Numerische Mathematic* **31**: 377–403.
- DEBOOR, C. 1978. *A practical guide to splines*. Springer.
- DOLFING, J. 1988. Acetogenesis, p. 417–468. *In* A. J. B. Zehnder [ed.], *Biology of anaerobic microorganisms*. Wiley.
- ENRIQUEZ, S., C. M. DUARTE, AND K. SAND-JENSEN. 1991. Patterns in decomposition rates among photosynthetic organisms: The importance of detritus C:N:P content. *Oecologia* **94**: 457–471.
- FENCHEL, T., AND T. H. BLACKBURN. 1979. *Bacteria and mineral cycling*. Academic.
- , AND B. J. FINLAY. 1995. *Ecology and evolution in anoxic worlds*. Oxford.
- GODSCHALK, G. L., AND R. G. WETZEL. 1978. Decomposition of aquatic angiosperms c. *Zostera marina* L. and a conceptual model of decomposition. *Aquat. Bot.* **5**: 329–354.
- HADDAD, R. I., AND C. S. MARTENS. 1987. Biogeochemical cycling in an organic-rich coastal marine basin. 9. Sources and accumulation rates of vascular plant-derived organic matter. *Geochim. Cosmochim. Acta* **51**: 2991–3001.
- HAINES, E. B. 1976. Stable carbon isotope ratios in the biota, soils, and tidal water of a Georgia salt marsh. *Estuarine Coastal Mar. Sci.* **4**: 607–616.
- HARTNETT, H. E., R. G. KEIL, J. I. HEDGES, AND A. H. DEVOL. 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. *Nature* **391**: 572–574.
- HEDGES, J. I., F. S. HU, A. H. DEVOL, H. E. HARTNETT, E. TSA-MAKIS, AND R. G. KEIL. 1999. Sedimentary organic matter preservation: A test for selective degradation under oxic conditions. *Am. J. Sci.* **299**: 529–555.
- , AND R. G. KEIL. 1995. Sedimentary organic matter preservation: An assessment and speculative synthesis. *Mar. Chem.* **49**: 81–115.
- , AND J. H. STERN. 1984. Carbon and nitrogen determinations in carbonate-containing solids. *Limnol. Oceanogr.* **29**: 657–663.
- HENRICHS, S. M. 1992. Early diagenesis of organic matter in marine sediments: Progress and perplexity. *Mar. Chem.* **39**: 119–149.
- , AND A. P. DOYLE. 1986. Decomposition of  $^{14}\text{C}$ -labeled organic substances in marine sediments. *Limnol. Oceanogr.* **31**: 765–778.
- HOLMER, M. 1996. Composition and fate of dissolved organic carbon derived from phytoplankton detritus in coastal marine sediments. *Mar. Ecol. Prog. Ser.* **141**: 217–228.
- HULTHE, G., S. HULTH, AND P. O. J. HALL. 1998. Effect of oxygen on degradation rate of refractory and labile organic matter in continental margin sediments. *Geochim. Cosmochim. Acta* **62**: 1319–1328.
- KEIL, R. G., D. B. MONTLUCCON, F. G. PRAHL, AND J. I. HEDGES. 1994. Sorptive preservation of labile organic matter in marine sediments. *Nature* **370**: 549–552.
- KIPPUR, G. W., AND C. S. MARTENS. 1982. Biogeochemical cycling in an organic-rich coastal marine basin: 3. Dissolved gas transport in methane-saturated sediments. *Geochim. Cosmochim. Acta* **46**: 2049–2060.
- KISSA, E. 1957. The microdetermination of carbon and hydrogen in compounds containing alkali and alkaline earth metals. *Microchem. J.* **1**: 203–207.
- KLUMP, J. V., AND C. S. MARTENS. 1987. Biogeochemical cycling in an organic-rich coastal marine basin. 5. Sedimentary nitrogen and phosphorus budgets based upon kinetic models, mass balances, and the stoichiometry of nutrient regeneration. *Geochim. Cosmochim. Acta* **51**: 1161–1173.
- KRISTENSEN, E., S. I. AHMED, AND A. H. DEVOL. 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? *Limnol. Oceanogr.* **40**: 1430–1437.
- , AND T. H. BLACKBURN. 1987. The fate of organic carbon and nitrogen in experimental marine systems: Influence of bioturbation and anoxia. *J. Mar. Res.* **45**: 231–257.
- MACKO, S. A., M. H. ENGEL, AND P. L. PARKER. 1993. Early diagenesis of organic matter in sediments: Assessment of mechanisms and preservation by the use of isotopic molecular approaches, p. 211–224. *In* M. H. Engel and S. A. Macko [eds.], *Organic geochemistry*. Plenum.
- MARTENS, C. S., R. I. HADDAD, AND J. P. CHANTON. 1992. Organic matter accumulation, remineralization, and burial in an anoxic sediment, p. 82–98. *In* J. Whelan and J. W. Farrington [eds.],

- Organic matter productivity, accumulation, and preservation in recent and ancient sediments. Columbia Univ. Press.
- , AND J. V. KLUMP. 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.
- MAYER, L. M. 1993. Organic matter at the sediment–water interface. *In* M. H. Engel and S. A. Macko [eds.], *Organic geochemistry*. Plenum.
- MINAGAWA, M., D. A. WINTER, AND R. F. VACARRO. 1984. Comparison of Kjeldahl and combustion methods for measurement of nitrogen isotope ratios in organic matter. *Anal. Chem.* **56**: 1859–1861.
- PEASE, T. K. 2000. Seagrass and phytoplankton deposition and remineralization in coastal lagoonal sediments. Ph.D. dissertation, Univ. of North Carolina-Chapel Hill.
- PELLIKAAN, G. C. 1984. Laboratory experiments on eelgrass (*Zostera marina* L.) decomposition. *Neth. J. Sea Res.* **18**: 360–383.
- SCHINK, B. 1988. Principles and limits of anaerobic degradation: Environmental and technological aspects, p. 771–846. *In* A. B. J. Zehnder [ed.], *Biology of anaerobic microorganisms*. Wiley.
- THAYER, G. W., P. L. PARKER, M. W. LACROIX, AND B. FRY. 1978. The stable carbon isotope ratio of some components of an eelgrass, *Zostera marina*, bed. *Oecologia* **35**: 1–12.
- THURMAN, E. M. 1985. *Organic geochemistry of natural waters*. Kluwer.
- WESTRICH, J. T., AND R. A. BERNER. 1984. The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. *Limnol. Oceanogr.* **29**: 236–249.

*Received: 15 February 2001*

*Accepted: 17 July 2001*

*Amended: 7 August 2001*