

MICROBIAL CARBON SOURCES ON THE SHELF AND SLOPE OF
THE NORTHWESTERN GULF OF MEXICO

A Thesis

by

CARLTON DAVID RAUSCHENBERG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Oceanography

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Approved by:

Chair of Committee, Luis Cifuentes
Committee Members, Dan Thornton
David Zuberer
Head of Department, Wilf Gardner

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ABSTRACT

Microbial Carbon Sources on the Shelf and Slope of the
Northwestern Gulf of Mexico. (August 2005)
Carlton David Rauschenberg, B.S., DePaul University
Chair of Advisory Committee: Dr. Luis A. Cifuentes

Over the past five years, gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) analyses of phospholipid fatty acids (PLFAs) has been increasingly used to link organic matter (OM) sources with sedimentary bacteria. This technique has been applied across diverse estuarine and coastal sediments, including lower Laguna Madre, TX, an oligotrophic, coastal lagoon dominated by a single OM source, seagrasses; shelf stations, a eutrophic coastal region receiving multiple sources of OM, hypoxic regions that occur seasonally and deep slope and abyssal plain sediments of the Gulf of Mexico (GOM). Previous reports using the Laguna Madre data as examples, have been used to make comparisons of PLFA 16:0 and PLFA 15:0 isotope ratios and PLFA 16:0 and total organic carbon isotope ratios. Deviations from the 1:1 line in the former indicate living or recently senescent sources of organic matter are not predominantly bacterial. Deviations from the 1:1 line in the latter indicate living or recently senescent sources of organic matter differ isotopically from detrital or older OM in sediments. Prior to the work of Goni et al. (1998), carbon isotope ratios of OM in GOM sediments were interpreted as marine in origin. Based on a series of geochemical measurements, Goni et al. suggested that GOM sediments are largely composed of terrestrial organic carbon (OC_{terr}). Furthermore, They went on to show that shelf and slope sediments were primarily C_3 and C_4 respectively. I report on the preferential utilization of autochthonous OM by sedimentary bacteria at the sediment surface and the shift to recalcitrant, terrestrially derived OM with depth.

DEDICATION

I am dedicating this piece of work
To those I love the most
First of all, the Man up stairs
My family, friends, and dogs
Without them, none of this would have been possible

And of course to the love of my life
Sara Elizabeth Keach
Her never-ending patience and strong will
Propelled me through this task

Thank you

ACKNOWLEDGMENTS

I thank my committee chair Luis (El Jefe) Cifuentes and my committee members David Zuberer and Dan Thornton. A special thanks goes to Brian Jones who taught me everything that I needed to know in order to get this thing done. If he had not been around, then I would be up a creek without a paddle. Thanks man.

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INTRODUCTION

Continental shelves and slopes account for less than 20% of all the oceans in the world (Walsh et al. 1981; Smith and MacKenzie 1987), but are the primary sinks of organic carbon (OC) in marine sediments (Bernier 1989). The high concentration of OC along these margins is maintained by the export of dissolved organic carbon (DOC) and particulate organic carbon (POC) from rivers (Hedges et al. 1997). Each year rivers carry 0.4 Gt of carbon to the ocean in the forms of DOC (0.25 Gt) and POC (0.15 Gt) (Schlesinger and Melack 1981). Current research suggests that the majority of marine derived OC is utilized within the water column before reaching the sediment (Hedges et al. 1997; Gordon et al. 2001). It has also been suggested that a large majority of terrestrially derived OC is exported from coastal margins to the shelf/slope and deep ocean (Bianchi et al. 1997; Goni et al. 1998). Both terrestrial organic carbon (OC_{terr}) and marine organic carbon (OC_{mar}) are likely sources of OC in the abyssal ocean. There is some debate, however, as to how much OC_{terr} reaches the deeper regions of the ocean (Hedges and Parker 1976; Bianchi et al. 1997; Goni et al. 1998).

By isolating lignin-phenol biomarkers that are indicative of terrestrial origin, such as vanillyl and syringyl, Hedges and Parker (1976) showed an exponential decrease in OC_{terr} with increasing water depth along the shelf and slope. Similar to Hedges and Parker (1976), Goni et al. (1998) made stable carbon isotope measurements of individual lignin phenols and showed that OC_{terr} close to the Mississippi River drainage basin had C_3 terrestrial signatures whereas outer shelf and slope regions had a distinctive C_4 signature. Gordon et al. (2001) came to a similar conclusion in their study on the inner Louisiana shelf. They reported that more OC_{terr} exported by the Atchafalaya River is remineralized on the shelf than is contributed by autochthonous activity. In addition, more autochthonous OC is remineralized in the water column as opposed to OC_{terr} that undergoes degradation in sediments.

This thesis follows the style of Marine Ecology Progress Series.

Finally, Bauer and Druffel (1998) conducted an investigation concerning the relative concentrations of OC_{terr} present in the Equatorial North Pacific and Western North Atlantic. Utilizing radiocarbon isotopic dating to determine age ($\Delta^{14}C$) and stable carbon isotopic techniques to determine the origin ($\delta^{13}C$), they were able to show that input of OC from margins to the open ocean is greater than surface production OC to deep open oceans, and recent, surface-produced OC is degraded much faster allowing the refractory margin-derived OC to contribute more to the deep open ocean. Two important points can be taken from the research described above: (1) OC produced by marine plankton, in the form of POC and DOC, is efficiently recycled within the water column and water/sediment interface (see Wakeham and Lee 1993), and (2) OC_{terr} is not as labile as OC_{mar} resulting in higher concentrations available for export to the deep ocean.

Keil et al. (1994) suggested that a strong relationship exists between mineral surface area and organic matter (OM) preservation and that this process may be the largest controlling factor of OM preservation. The binding of OM to sediment surfaces gives rise to a physical transport mechanism capable of OC transport. It was also shown that components of OC_{terr} associate with distinct sediment types (Keil et al. 1998). They demonstrated along the Washington Margin that shelf sediments, which were coarse silts and sands, contained more lignin compounds than the fine silts and clays of the slope, which were high in n-alkanes, indicating compound-specific binding to source specific sediment types. Another important mechanism controlling the export of OC from coastal surface waters to shelf/slope sediments is sinking particulate organic matter (POM) (Minor et al. 2003). POM is the product of primary productivity, incorporating fecal pellets, amino acids, lipids, cell walls, carbohydrates, proteins as well as terrestrial derived molecules such as lignins and cellulose (Hedges et al. 1997). Minor et al. (2003) expanded on the work by Keil et al. (1994) with a study analyzing the changes of organic compounds in POM with depth. They suggested that in addition to sorptive binding of OC to mineral grains, the biological community is able to break down and utilize the compounds contained within POM. Minor et al. (2003) suggest that this is another fundamental process that controls the amount and type of OC to sediments.

Stable carbon isotope ($\delta^{13}\text{C}$) analysis has many applications and has been widely used over the past 50 years. Due to isotopic discrimination in the different photosynthetic pathways utilized, C_3 and C_4 plants have unique stable carbon isotope signatures: -28‰ to -25‰ , and -10‰ to -12‰ respectively (Fry and Sherr 1984). Temperate marine phytoplankton, on the other hand, has values of 22‰ to -19‰ (Fry and Sherr 1984). These differences in isotopic signature, in addition to the small fractionation of organic carbon (1‰) that occurs during the transfer of carbon from one trophic level to another, make it possible for carbon to be traced through food webs (Peterson and Fry 1987; Rundel et al. 1989). For example, Parker (1964) conducted an investigation in a shallow marine estuary near Port Aransas, TX. He compared the sedimentary stable carbon isotopic signature of the total organic carbon (TO^{13}C) with all biological members of the marine community to determine whether carbon isotopes of individual species would reflect food chain patterns. With one primary source of carbon present in the system, Parker was able to utilize $\delta^{13}\text{C}$ values of organisms and roughly trace the pathways of carbon in the system. He identified the major end-member of carbon to the system and illustrated its complexity. Haines (1976) conducted similar research in a Georgia salt marsh and concluded that either: 1) a substantial amount of estuarine carbon is derived from phytoplankton and C_3 plant material or 2) the carbon from the dominant macrophyte is fractionated to a form that is different from the live plant. Stable carbon isotopes also present a practical way to characterize the health of a polluted system. For instance, Sackett (1986) describes a study in which large amounts of sludge were discarded off the coast of New York and the extent of contamination was under investigation. Stable carbon isotopes were sampled along a transect through the polluted dumpsite. Unpolluted sediments in the area were -22‰ and the polluted site was -26‰ , indicating the severity of contamination and providing researchers with a general idea of pollutant composition. These initial investigations laid the foundation for stable carbon isotope research in environmental studies.

The development of compound-specific isotopic analysis (CSIA) has made microbial biomarker isolation and analysis more practical. Conventional combustion

techniques for $\delta^{13}\text{C}$ analysis of microbial biomarkers were difficult and time consuming resulting in limited research concerning utilization of carbon by bacterial communities (Coffin et al. 1990). The ability to identify and trace specific compounds within OC has made it possible to undergo more thorough investigations of carbon cycling by identifying specific molecules and determining their $\delta^{13}\text{C}$ values (e. g., Goni et al. 1998; Cifuentes and Salata 2001; Jones et al. 2003). For example, Abrajano et al. (1994) used CSIA to demonstrate that fatty acids of marine mytilids from the Gulf of Mexico and Newfoundland have similar carbon isotopic signatures as their carbon source. The available carbon source in the GOM was CH_4 (-52‰) whereas it was CO_2 (-29‰) in Newfoundland waters. A novel use of CSIA is in the field of bioremediation. A study conducted by Lollar et al. (1999) examined the usefulness of CSIA in evaluating the extent of bioremediation occurring within chlorinated-hydrocarbon polluted systems. A consortium of facultative anaerobic bacteria cultured from a contamination site was used in laboratory tests to observe the degradation of trichloroethylene (TCE) and toluene. CSIA was used to analyze $\delta^{13}\text{C}$ of these compounds throughout the degradation process. Lollar et al. (1999) report that the large fractionation associated with degradation of TCE by a bacterial assemblage may provide a useful method for evaluating the process of bioremediation.

Recent research has utilized CSIA technology in conjunction with microbial phospholipid fatty acids (PLFAs) to better understand the role of microbial populations in the environment (ie. Boschker et al. 1999; Cifuentes and Salata 2001; Jones et al. 2003; Pancost and Sinninghe 2003). The use of microbial PLFAs as biomarkers for bacterial communities has many advantages over other biomarkers: (1) PLFAs are membrane constituents found in all living cells, (2) they are extremely labile after cell death, and (3) are easily extracted from sediments (Tunlid and White 1992). Teece et al. (1999) used *Shewanella putrefaciens*, a gram-negative facultative marine bacterium to demonstrate the isotopic fractionation of fatty acids under oxic and anoxic conditions. He concluded that anaerobically derived fatty acids were significantly more depleted in ^{13}C ($\delta^{13}\text{C} = -37.8\text{‰}$) than aerobically derived fatty acids ($\delta^{13}\text{C} = -28.8\text{‰}$). Boschker et

al. (1999) utilized PLFAs and CSIA to study the source of carbon for bacterial communities in three distinct salt-marsh ecosystems. They used specific PLFAs as biomarkers to determine the carbon isotopic composition of the native bacterial communities present. Boschker was able to isolate methyl-branched $i14:0$ and $i15:0$, which are indicative of bacteria, and determine where the bacterial carbon source was originating. Boschker et al. (1999) report that in a *Spartina* dominated salt-marsh, the dominant macrophyte was not the prevailing source of organic carbon for bacteria; the dominant source was determined to be of algal origin instead. Jones et al. (2003) conducted a recent investigation examining the relationship between sediment bacterial communities and the seagrass *Thalassia testudinum* in Lower Laguna Madre, TX. Using methyl-branched PLFA $i15:0$ as a proxy for benthic bacteria, he showed that a close relationship is present between the dominant carbon sources (*T. testudinum*) and the benthic bacterial community ($\delta^{13}\text{C}$ of above ground tissue = -10.8, surface bacteria = -12.1). The findings of Jones et al. (2003) on the relationship between dominant seagrasses and carbon sources for bacterial communities differ from previous investigations conducted by Boschker et al. (1999) indicating that carbon flow in salt-marsh communities is more complex than first assumed. Pancost and Sinninghe (2003) successfully utilized CSIA to examine carbon cycling in a peat deposit. By determining the $\delta^{13}\text{C}$ of lipids specific to individual bacterial and archaeal populations, they identified that the carbon flow in the peat deposit began with higher plants and traveled to aerobic bacteria, heterotrophic methanogens, and then to autotrophic methanogens.

The research described above clearly illustrates how $\delta^{13}\text{C}$ of fatty acids, in particular PLFAs, can be used to identify sources of OC to benthic bacterial communities. The Gulf of Mexico (GOM) provides a unique setting to study organic matter sources to benthic bacterial communities owing to significant inputs of terrestrial organic carbon (OC_{terr}) from the Mississippi River and Atchafalaya River (Bianchi et al. 1997; Goni et al. 1998; Gordon et al. 2001). If, as suggested by recent work, more OC_{terr} is ultimately deposited and buried in offshore sediments of the GOM, then the specific terrestrial isotopic signature should be present in the bacterial PLFAs. I proposed to test

this hypothesis by comparing stable carbon isotopes of bacterial PLFAs in the Dead Zone region and along a shelf/slope transect of the Northwestern GOM.

Experimental Design

To test the hypothesis stated above, a variety of sampling locations were selected. The criteria used to determine these locations were bottom depth, environmental conditions such as oxygen concentrations and productivity, and influences from riverine sources. The shelf stations are shallow with a suspected higher influence of OC_{terr} being supplied to the benthic community. The Dead Zone or hypoxic zone is defined as <1.4 ml/L of dissolved oxygen in the water (Justic et al. 1993). The hypoxic station is slightly deeper (Table 1) than that of the shelf stations but with a significantly lower oxygen concentration. The hypoxic station should also have a higher OC_{terr} signature due to influences by the Mississippi and Atchafalaya Rivers. The Mississippi Trough (MT) stations are significantly deeper (Table 1) than the other stations. The sampling locations within the study area were selected to illustrate differences in bulk carbon isotopic ratios and bacterial communities. To illustrate the differences in utilization of individual carbon sources by benthic bacteria in differing ecosystems, a comparison was made between data collected from GOM samples and results published by Jones et al. (2003). Sedimentary $TO^{13}C$ was used in conjunction with PLFA to determine: (1) if bacteria make up a significant portion of total living biomass, and (2) to determine whether or not total living organisms are utilizing the bulk $TO^{13}C$ or a specific fraction. In addition to the use of carbon isotopic signatures, C/N ratios and %OC values were used to help elucidate the organic carbon usage by bacterial assemblages. Table 1 below lists the sampling station locations and the depths. Their locations in the GOM are shown in Figure 1.

Table 1. Sampling station locations and depths.

Station	Latitude	Longitude	Water depth (meters)
MT-1	28 32.4352	89 49.7257	481m
MT-2	28 27.0646	89 40.3563	678m
MT-3	28 13.2246	89 29.7679	986m
Hypoxic Zone 1	28 59.5500	92 38.5546	28m
Shelf 1	29 38.4074	93 38.6871	9m
Shelf 2	29 50.9454	92 38.2907	20m
Laguna Madre	26 04.0482	97 25.1205	3m
Bush Hill	27 37.1052	91 42.9825	500m

MATERIALS AND METHODS

Site Description

The Northwestern Gulf of Mexico is different from other U.S. continental margins in that it is significantly influenced by terrestrial inputs from the Mississippi and Atchafalaya Rivers (Bianchi et al. 1997). The Mississippi River drains 40% of the continental U.S. (Milliman and Meade 1983), which is composed of C₃ plants in the northern region and C₄ plants near coastal areas (Teeri and Stowe 1976). Due to large amounts of OC_{terr} and nutrients exported by these rivers, an area within the Mississippi River Plume becomes hypoxic between the months of April through October (Justic et al. 1993). This occurs from the increased nutrient load exported by the rivers (130.3 million kg-atoms N/year), which increases primary production (Justic et al. 1993). Justic et al. (1993) suggest that the mechanism for transporting organic matter (OM) to the benthos is not sinking phytoplankton but fecal pellets from a well developed zooplankton community. The resulting hypoxic region within the GOM will provide a unique environment for certain benthic bacterial communities to thrive.

Outside the Dead Zone, shelf and slope waters are classified as oligotrophic due to diminishing anticyclonic warm-core eddies from the Caribbean Sea (Biggs 1992). Cyclonic cold-core eddies, scattered between warm-core eddies, replenish nutrient supplies through upwelling (Biggs 1992). Phytoplankton communities are impacted by these events and populations vary over space and time (Biggs 1992). Lambert et al. (1999) conducted a study on phytoplankton diversity and distribution and found that diatoms and cyanobacteria dominate the shelf waters while prymnesiophytes dominate slope waters.

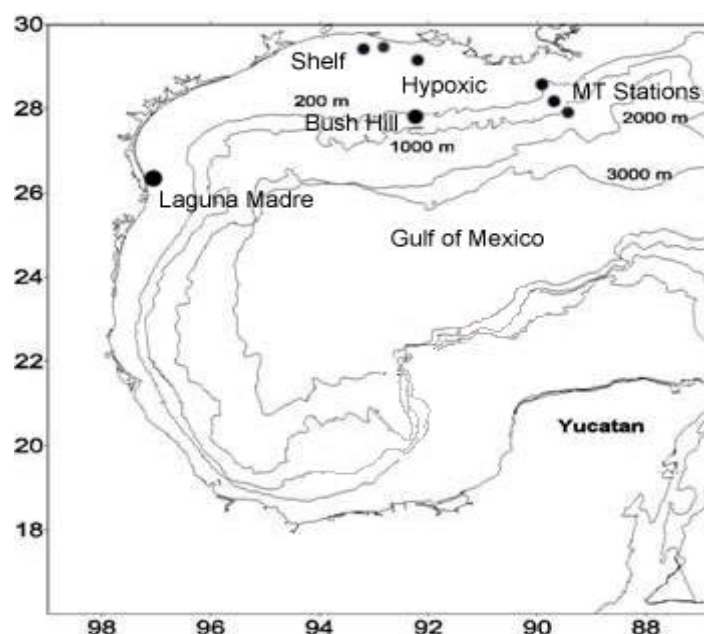


Fig. 1. Sampling locations in the Gulf of Mexico. Mississippi Trough (MT) Stations, Shelf Stations and Hypoxic Stations sampled in present study. Laguna Madre Station sampled by Jones et al (2003). Bush Hill Station sampled by Cifuentes and Salata (2001).

Sample Collection

Sediment samples were collected with a box corer from one site within the hypoxic area, two sites in coastal waters, and three sites on the shelf/slope region of GOM during the summer of 2002 on the R/V Gyre (Figure 1). One box core was taken at each site. Core liners with a 6.5 cm inner diameter (i.d.) were pushed by hand to sub-sample the box core. The depth of sediment collected was determined by the box core recovery. The depth of sediment collected in the core liner was approximately 30 cm. Two sub-samples were collected per site, and a total of 20 sub-samples were collected. Cores were capped at the top and bottom and immediately frozen. In the laboratory, cores were sectioned at 3-cm intervals, wrapped in aluminum foil, lyophilized, and stored at -20°C prior to lipid extraction. Phospholipid fatty acids (PLFAs) were extracted (see below) from each sediment section. The $\delta^{13}\text{C}$ of bulk organic carbon

(TO¹³C) was also measured with standard sealed tube combustion (Cifuentes et al. 1988). In addition to collection of sediments, sargassum samples were collected from the inner shelf of the Gulf of Mexico. A net was used for the collection of these samples taken off the coast of Galveston the following summer during a brief sampling trip.

Sample Preparation

The method used below was described previously by Cifuentes and Salata (2001) and Jones et al (2003). Slight variations were made to the procedure to accommodate sampling location. Before samples were thoroughly homogenized, pieces of shell were removed from each core section. Total lipids were extracted from each core section in a 250 ml Erlenmeyer flask using a modified Bligh and Dyer procedure described by White et al. (1979). This method requires the combination of 30 g of sediment, approximately 500 ng nonadecanoic acid (surrogate standard 19:0), and extraction solution consisting of milliQ-H₂O:methanol:chloroform (0.8:2:1). The sediment was added to the extraction mixture using 1 g sediment:1 ml chloroform. The flask was shaken for 24 hrs. The sediment and solution are then transferred to centrifuge tubes and centrifuged for 5 min. at 2000 rpm. After centrifugation the supernatant was decanted into a 250 ml separatory funnel. MilliQ-H₂O:chloroform was added to the separatory funnel for a final solvent volume ratio of 0.9:1:1. The separatory funnel was shaken vigorously to induce phase separation. When the upper aqueous phase was no longer cloudy the lower organic phase was collected into a 250 ml round-bottom flask. The solvent was removed using a rotary evaporator under N₂. The remaining dark lipid extract was transferred to a 50 ml screw-cap test tube. The round-bottom flask was washed with chloroform, transferring residual lipid extract to the test tube. The excess chloroform in the test tube was removed under a stream of N₂ and the total lipid fraction stored at -20°C.

The lipid extract was then resuspended using a minimal amount of chloroform. The extract was loaded onto a silicic acid column (Supelclean LC-Si, Supelco, Bellefonte, PA.) for separation of lipid classes. Neutral lipids, glycolipids, and phospholipids were separated with equal amounts of chloroform, acetone, and methanol. The phospholipid

fraction was collected in a separate test tube. The other lipid classes were stored at -20°C for further investigation.

Fatty acid methyl esters (FAMES) were prepared from the phospholipid fraction with strong acid methylation (Salata 1999). The FAMES are prepared in 1 ml of hexane containing an internal standard of eicosanoic methyl ester (21:0 ME_I). The sample was analyzed using GC/C/IRMS.

FAMES were separated on a 30-m slightly polar methylsilicone bonded-phase capillary column (SPB-5) using He as the carrier gas with a flow rate of 14 cm³s. The injection temperature was maintained at 250°C and the split set to 1:10. The temperature of the column was programmed from 100°C (4 min.) to 150°C at $5^{\circ}\text{C min}^{-1}$ and then to 250°C at $4^{\circ}\text{C min}^{-1}$. The compounds separated on the GC were converted to CO₂ by means of a combustion unit utilizing an oxidized Ni/Pt/Cu catalyst maintained at 950°C . The CO₂ was passed into a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS) for stable isotopic analysis. All carbon isotopic ratios are expressed in conventional δ notation relative to Pee Dee Belemnite (PDB) by the following equation:

$$\delta^{13}\text{C}_{\text{PDB}} (\text{‰}) = [(\text{R}_{\text{sample}}/\text{R}_{\text{standard}})-1] \times 1000$$

Where R_{sample} and $\text{R}_{\text{standard}}$ are the $^{13}\text{C}/^{12}\text{C}$ isotopic ratios of the sample and the standard respectively (Craig, 1957). The $\delta^{13}\text{C}$ isotopic ratios were determined by comparison to a working standard of CO₂ (99.996%, $\delta^{13}\text{C}_{\text{PDB}} = -11.66$) introduced in triplicate at the beginning of each run.

When the free fatty acid is converted to a fatty acid methyl ester there is an addition of one carbon atom to each fatty acid. This additional carbon alters the isotopic ratio and can be accounted for using the following mass balance equation:

$$\delta^{13}\text{C}_{\text{FAME}} = (x) \text{d}^{13}\text{C}_{\text{FA}} + (1-x) \text{d}^{13}\text{C}_{\text{MeOH}}$$

where $\delta^{13}\text{C}_{\text{FAME}}$ and $\delta^{13}\text{C}_{\text{MeOH}}$ are the measured isotopic ratios of the fatty acid methyl ester and the methanol used in the strong acid methylation, respectively, and x is the fractional carbon contribution of the free fatty acid to the ester (Abrajano et al. 1994). All values presented in this investigation will be corrected using this equation.

A sub sample from each core section was prepared for sedimentary total organic carbon analysis (TO^{13}C). Samples were acidified with 10% HCl to remove carbonates present in the sample. Approximately 10 mg of sediment were sealed in a quartz tube containing cupric oxide and combusted. The CO_2 created by the combusted sediment was transferred cryogenically and analyzed on the MAT 252.

A portion of the acidified sub sample was used to measure elemental C:N. 60 mg of the acidified sample was weighed into a silver capsule and combusted at 1000°C on a Costech Elemental Analyzer. The combusted sediment first flows through a quartz tube filled with chromium oxide and cobaltic oxide to remove halogenated compounds. The remaining gas was reduced in another quartz tube filled with reduced copper wire. A gas chromatography column separated the gases and a thermal conductivity detector measured the concentrations.

The sargassum samples were washed with 10% HCl to insure the removal of carbonates present on the sample. The samples were then freeze dried and homogenized. Approximately 5 g of sample was weighed into a silver capsule and combusted using the Costech Elemental Analyzer to determine TO^{13}C and C:N.

Bacterial Abundance

In order to make measurements of bacteria, a biomarker that provides an unambiguous link to bacteria is required. Phospholipid fatty acids (PLFA) make up a significant portion of the lipids present within the cellular membrane. Iso and anteiso 15:0 are specific types of PLFA's that are only found in bacteria. 16:0 PLFA is another lipid that is found in all living of recently senescent organisms. These two compounds were used as biomarkers in this study. The relative bacterial abundance was determined

for each core section collected in the present study. In order to make the calculation, the following equation was employed:

$$\text{Relative Bacterial Abundance} = \frac{i\&a\ 15:0}{16:0}$$

Where $i\&a\ 15:0$ represent the peak area of the bacterial biomarker divided by the ubiquitous $16:0$. The values used for the bacterial biomarker $i\&a\ 15:0$ were the peak areas obtained during the isotopic analysis of the individual core sections. The reason for this method was not to determine exact bacterial counts (Jones et al. 2003) but to understand how the microbial community interacts with the organic carbon present. Mancuso et al (1990) utilized a similar technique consisting of dividing the sum of the bacterial biomarkers $i\&a\ 15:0$ by the ubiquitous $16:0$. This revealed the proportion of bacterial biomass in the sample. An increase in the ratio revealed an increase in the relative abundance of bacterial signatures present. Numerous studies have been conducted on bacterial concentrations in environmentally different locations on the ocean floor (Rowe and Deming 1985), thus influencing this investigation to focus on the relative concentrations at each sediment interval. By comparing the peak area of the bacterial biomarker $i\&a\ 15:0$, values from each sampling station can be compared to determine relative increases and decreases in bacterial abundance.

In order to determine relative biological abundance of living or recently senescent bacteria, a modification of the formula utilized by Mancuso et al. (1990) was used:

$$\frac{i\&a\ 15:0 + 16:0}{\text{grams of sediment}}$$

Where $i\&a\ 15:0$ represents the peak area of the bacterial biomarker, $16:0$ represents the peak area of the ubiquitous biomarker produced by all living organisms, and grams of sediment is the weight of sediment from each depth interval used to extract the biomarkers. Combining the two biomarkers allows for a comparison of biological abundance throughout a depth profile of each core.

Statistical Design

At each sampling station, two push-cores (n=2) were taken for each box-core retrieved. The data obtained from each core section were averaged for each station. This data are represented graphically in the following sections. Due to the large amount of samples, two replicate push-cores were taken but triplicate injections were not made for isotopic analysis. To avoid artifacts in statistical results, an independent one-way ANOVA test was used to determine whether individual sampling stations were different. The dependent variables used were $\delta^{13}\text{C}$ values of the total organic carbon (TO^{13}C), and the bacterial biomarkers i&a 15:0 and 16:0. To determine significant differences for individual data points with depth in the sediment layer, a paired t-test was used.

RESULTS

 TO^{13}C

All sampling locations showed consistent down-core TO^{13}C values. Mississippi Trough (MT) stations had carbon isotopic signatures ranging from -22.1 to -20.3 ‰ (Figure 2). The hypoxic station also maintained a consistent down-core TO^{13}C trend (Figure 3) with values from -21.1 ‰ at the sediment surface and -21.5 ‰ at the bottom. The Shelf stations varied slightly at the sediment surface with values of -21.2 and -21.4 ‰. Both shelf stations became lighter down-core.

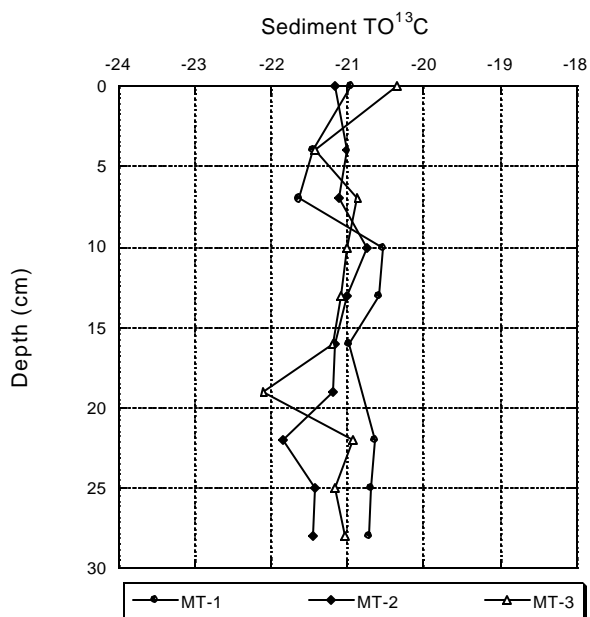


Fig. 2. Sediment TO^{13}C values from the Mississippi Trough (MT) Stations. Data points represent averaged values from 2 replicate cores.

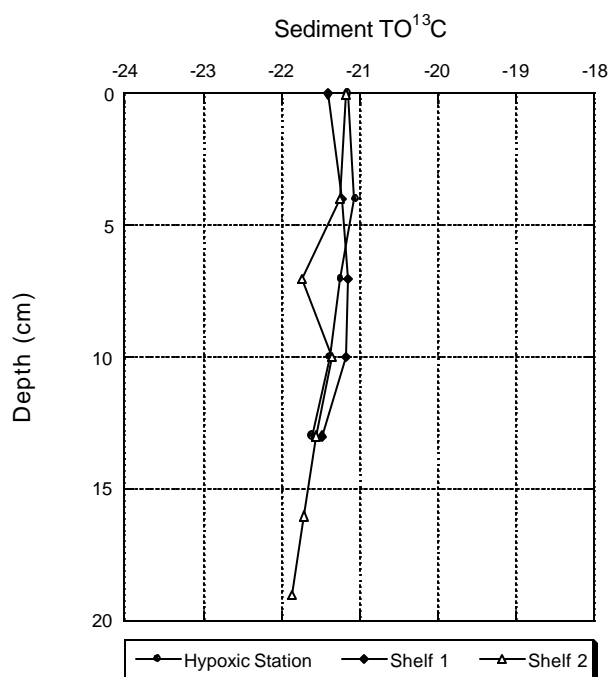


Fig. 3. Sediment TO¹³C values from the Hypoxic Station and Shelf Stations. Data points represent averaged values from 2 replicate cores.

PLFA

Results from PLFA analysis are illustrated in Figures 4-6. Within the MT stations, the bacterial biomarker i&a 15:0 maintained large variations from sediment surface to core bottom. Station MT-1 had a value of -17‰ on the sediment surface and decreased to -19.7‰ at the bottom of the core. It is worth mentioning that at 25cm, i&a 15:0 was in a 1:1 isotopic correspondence with TO¹³C (Figure 4). Similar patterns were found at stations MT-2 and MT-3. There was a large difference between TO¹³C and i&a 15:0 at the sediment surface whereas isotopic correspondence occurred at the bottom of the core.

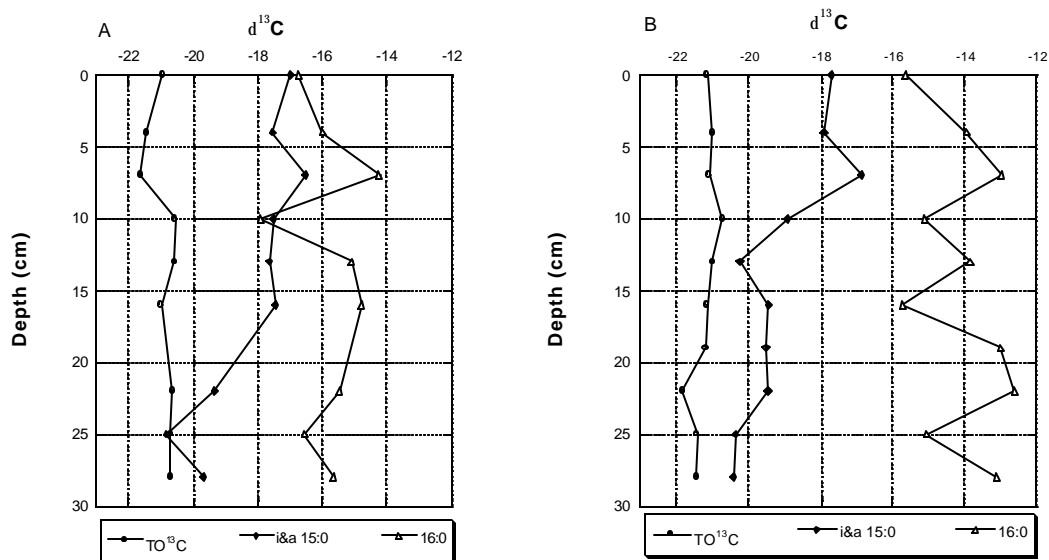


Fig. 4. Down-core $d^{13}C$ values of the bacterial biomarker i&a 15:0 and 16:0 for MT-1 (A) and MT-2 (B). Data points represent averaged values from 2 replicate cores.

Such contrast can then be compared with the hypoxic station at which i&a 15:0 was distinctively separate from TO¹³C at the sediment surface with values of -17.6‰ and -21.1‰ respectively (Figure 5). At the bottom of the core the difference in carbon isotopic values between the two were still maintained.

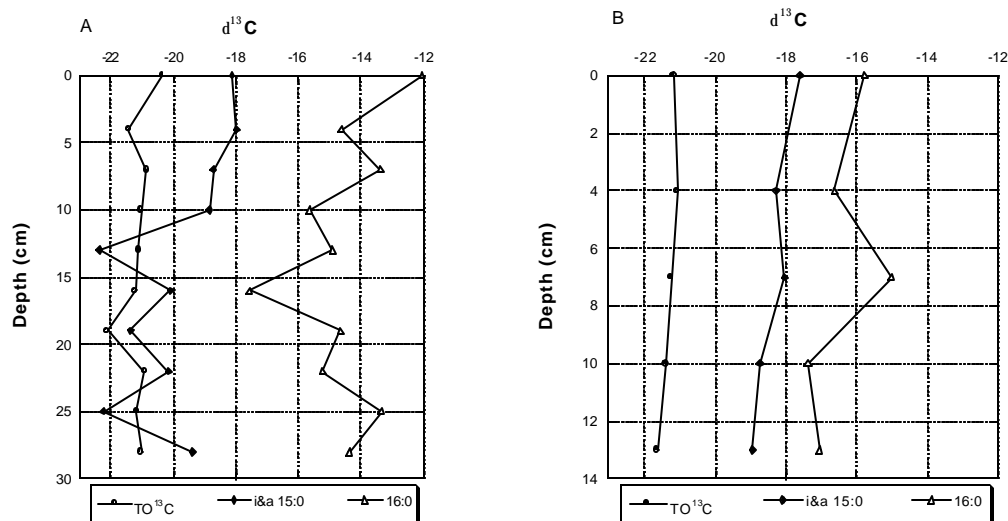


Fig. 5. Down-core $d^{13}C$ values of the bacterial biomarker $i&a\ 15:0$ and $16:0$ for MT-3 (A) and Hypoxic Station (B). Data points represent averaged values from 2 replicate cores.

The shelf stations (Figure 6) had $d^{13}C$ values of the bacterial biomarker $i&a\ 15:0$ that were distinctly different from the $TO^{13}C$ in down-core samples. Shelf station 1 began with an $i&a\ 15:0$ value of -17.1‰ at the sediment surface and bottom of the core with some variation, mainly below 7cm core depth. Shelf station 2 had a value of -18.1‰ at the sediment surface and -20.5‰ at the bottom.

Phospholipid fatty acid (PLFA) $16:0$, indicative of living or recently senescent organisms, followed a trend that was maintained throughout the MT stations. PLFA $16:0$ differed slightly from $i&a\ 15:0$ at the sediment surface in station MT-1 with a value of -16.8‰ . At the bottom of the core PLFA $16:0$ became more positive with a value of -15.6‰ . The difference in PLFA $16:0$ from sediment surface to bottom became larger with each consecutive MT station i.e., with increasing depth of the water column. (Figures 4-5). The difference between PLFA $16:0$ and $i&a\ 15:0$ for the hypoxic station was relatively uniform when compared to the MT stations. There were no significant

differences between PLFA 16:0 and i&a 15:0 from the sediment surface to bottom. The two biomarkers maintained an isotopic separation of approximately 2‰ with PLFA 16:0 being isotopically heavier.

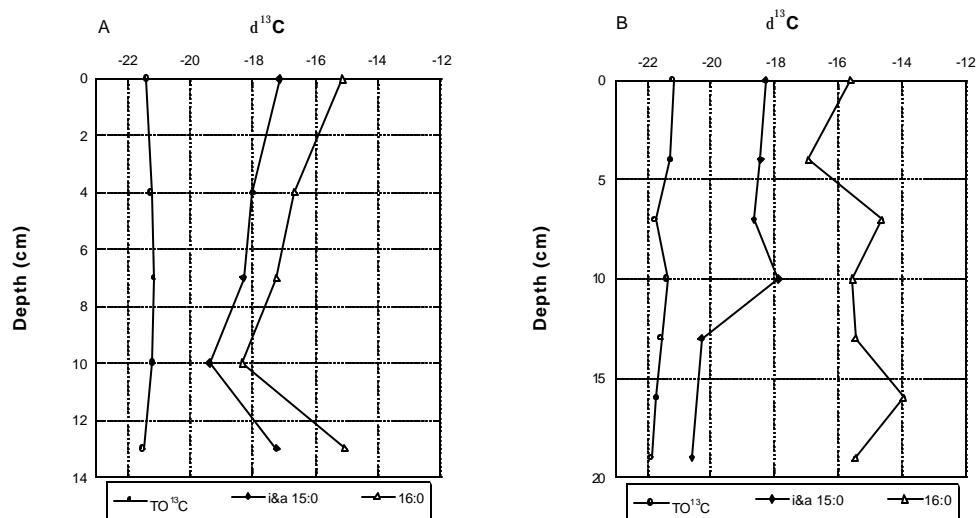


Fig. 6. Down-core $d^{13}C$ values of the bacterial biomarker i&a 15:0 and 16:0 for Shelf Stations 1 & 2 (A and B). Data points represent averaged values from 2 replicate cores.

At Shelf station 1, 16:0 had the same value of $-15.1‰$ at the sediment surface and bottom of the core. This trend was the same as i&a 15:0, maintaining a $+2‰$ separation. Shelf station 2 also had the same value of $-15.8‰$ at the sediment surface and bottom of the core with some variation in between. Station 2 showed a slight trend toward the isotopic value maintained by the $TO^{13}C$ at the bottom of the core.

Percent Organic Carbon in Sediments

Down-core concentrations of % Organic Carbon (% OC) ranged from 1.01% to 1.18% for sediment surface samples at the MT stations. The highest %OC was observed at station MT-1. Values for core-bottom samples were consistently less than sediment surface samples. Minimum values of % OC at MT stations ranged from 0.65 to 0.9 %OC. Sediment samples at the hypoxic station had 0.48% OC at the surface and 0.43%

OC at the bottom of the core. The two shelf stations displayed a decrease in %OC with increasing depth. Both the hypoxic and shelf stations had less % organic carbon at the sediment surface and bottom than the MT stations (Figure 7).

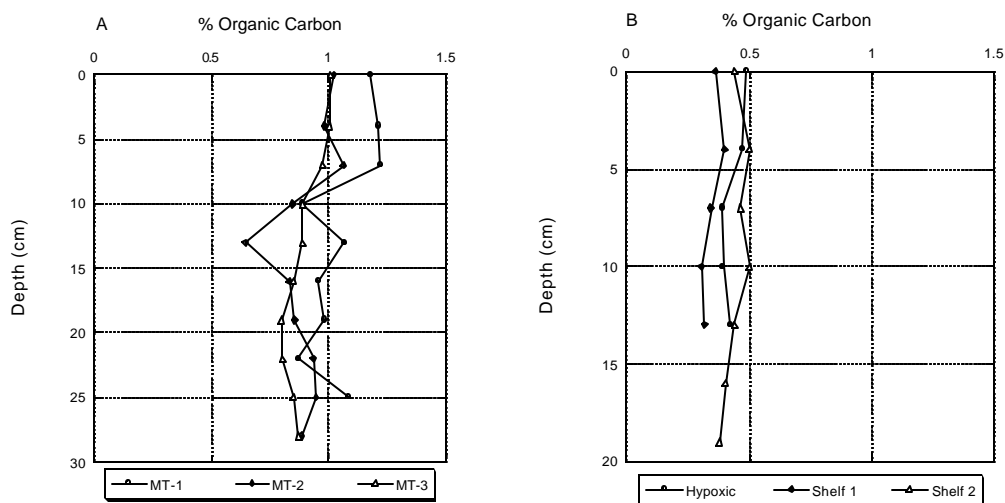


Fig. 7. Relationship of % organic carbon and depth in cores for MT (A), Hypoxic, and Shelf Stations (B). Data points represent averaged values from 2 replicate cores.

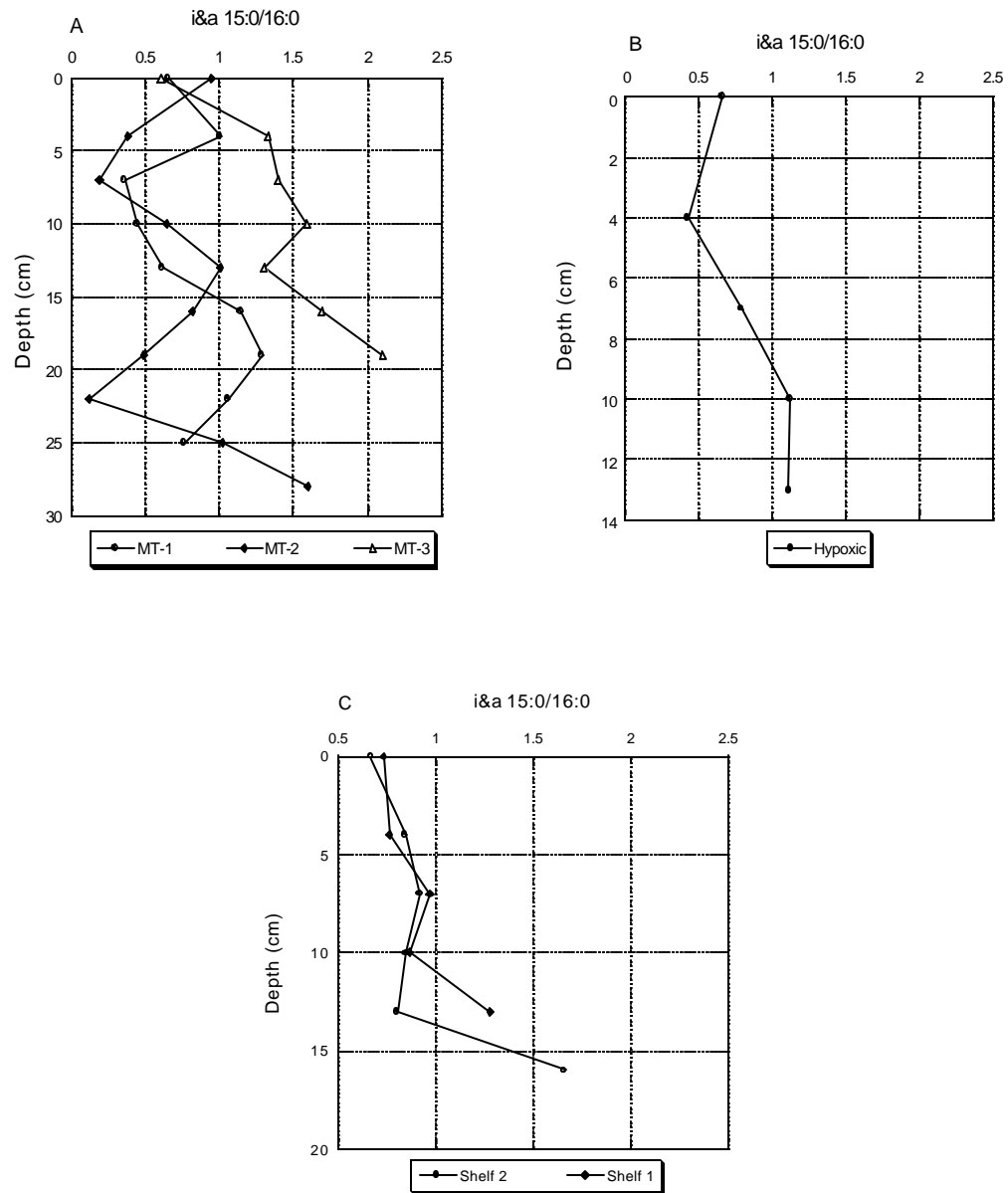


Fig. 8. Relative abundance of bacterial communities in MT (A), Hypoxic (B), and Shelf (C) Stations. Data points represent averaged values from 2 replicate cores.

Relative Bacterial Abundance

The proportion of bacteria within sediment cores was determined by dividing the peak area of the bacterial biomarker i&a 15:0 by the ubiquitous 16:0. If the ratio increases, a larger proportion of bacteria is present. A decrease in the ratio indicates a lesser abundance of bacteria in the total biomass. A similar trend was observed at all sampling locations. The ratio increases with depth indicating that bacteria are becoming more prevalent among the total biomass represented by 16:0 (Figure 8). All stations show similar sediment surface values between 0.6 and 1.0. All stations increase to approximately 1.0 to 1.5. Station MT-3 displayed the largest increase down-core with a value of 2.1.

The total proportion of living organisms in the sediment core samples (Figure 9) was determined by combining the peak area of the bacterial biomarker i&a 15:0 with the ubiquitous 16:0. This takes into consideration bacterial and non-bacterial influences. The value is then divided by the total amount of sediment used to extract the biomarkers. A similar trend in all samples was the decrease in combined biological signals with core depth. The MT stations displayed more variability in biological signals for down-core samples than the other stations. The hypoxic and shelf stations displayed a rapid decrease with sediment depth. The hypoxic station contained the highest levels of biological signals with a value of 43.0 at the sediment surface.

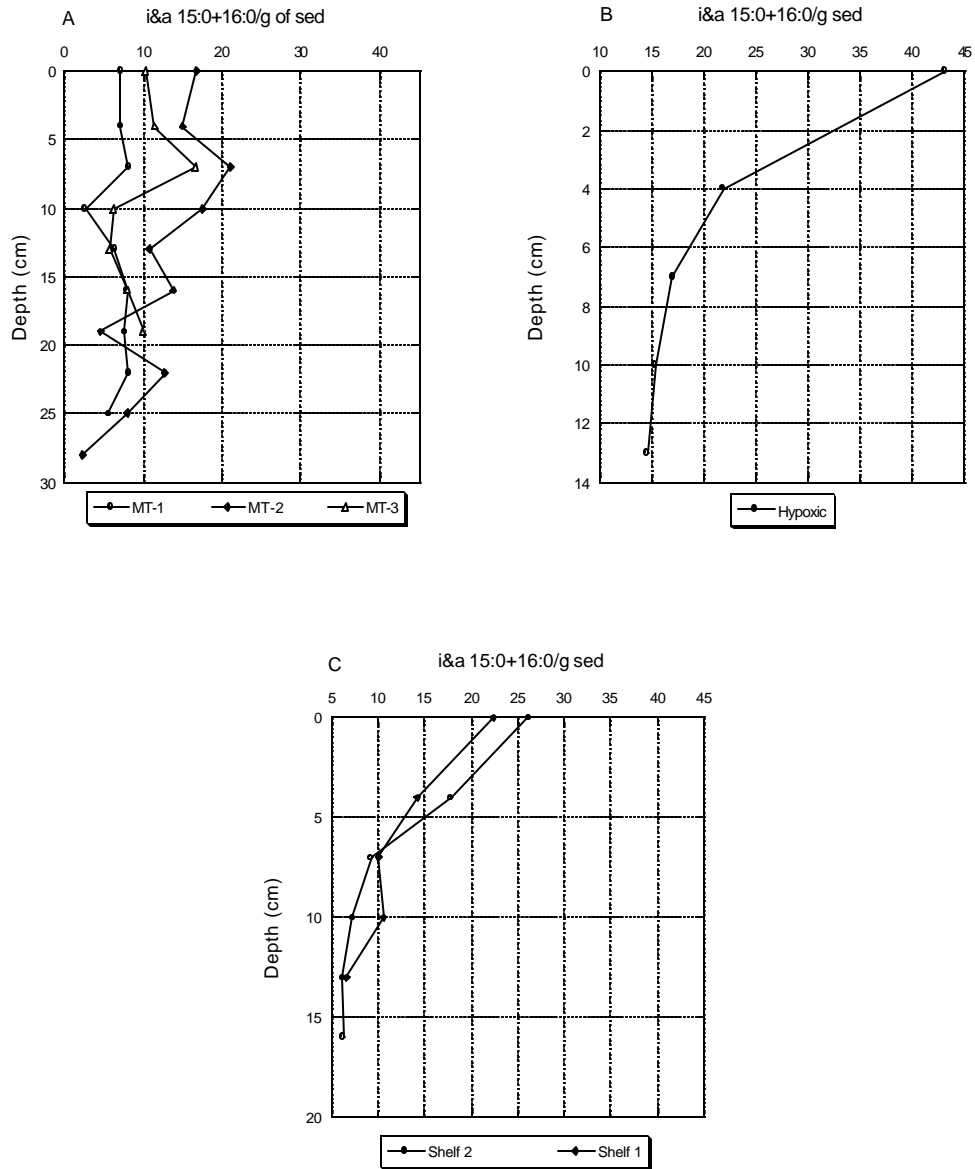


Fig. 9. Relative abundance of all living organisms in sediments for MT (A), Hypoxic (B), and Shelf (C) Stations. Data points represent averaged values from 2 replicate cores.

C:N

The C/N values for the MT stations ranged from 9 to 11 at the sediment surface and 10 to 12.5 for core-bottoms. There was a general increase in C/N values down-core (Figure 10). Values for the hypoxic station began at 9.5 at the sediment surface and steadily increased to 11 at the core-bottom (Figure 10). Both shelf stations had a C/N value of 10.2 at the sediment surface and increased with depth.

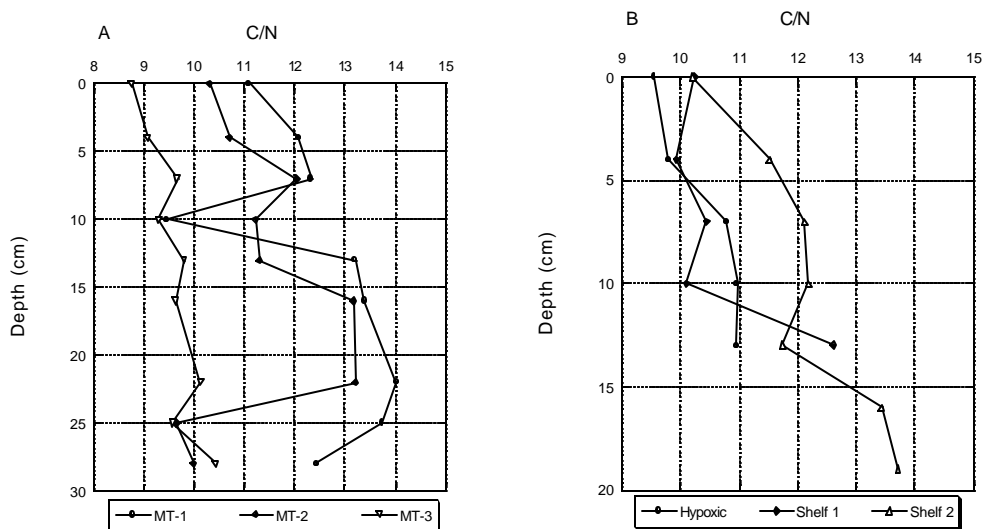


Fig. 10. Down-core C/N ratios for sediments from the MT (A), Hypoxic, and Shelf (B) Stations. Data points represent averaged values from 2 replicate cores.

DISCUSSION

Spatial Variations in Sediment Surface TO¹³C

Although sampling locations in the present study varied significantly with respect to water depth (shelf to slope) and environmental conditions (hypoxic to oxic), TO¹³C did not vary substantially among locations (Figure 11). The mean TO¹³C value measured in this study, about $-21 \pm 1\%$, was well within the range reported by Goni et al. (1998; -21.7 to -19.7%) in the same region. Similar values found at all sampling locations indicate a common type of organic matter (OM) and the presence of recalcitrant OM in the sediment. These TO¹³C values could be interpreted as a signature dominated by autochthonous OM but Goni et al. (1998) argued this would be incorrect. They suggest a predominantly terrestrial signal consisting of C₃ and C₄ vegetation originating from the Mississippi River drainage basin. To help substantiate their argument Goni et al. (1998) conducted radiocarbon dating on the TOC of surface sediment and found ages ranging from 2580 years before present (ybp) for inner-shelf samples to 6770 ybp for deep-slope samples, indicating terrestrial in origin. Autochthonous detritus in surficial sediments (0-2 cm) has been shown to vary in age from 3 ybp in nearshore environments to 400 ybp offshore (Lin 1990). Goni et al. (1998) concluded that the TOC present in the GOM is terrestrial in origin and highly reworked.

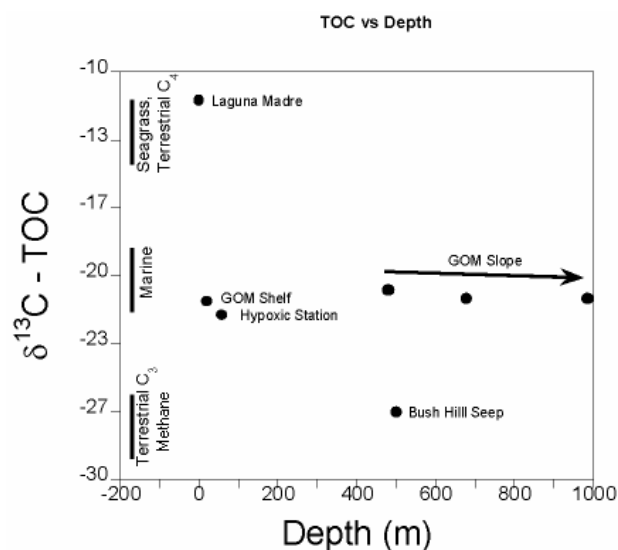


Fig. 11. Sediment surface TO^{13}C values from the present study as well as from previous investigations by Jones *et al.* (2003) and Cifuentes and Salata (2001).

Variations in $\delta^{13}\text{C}$ of Sediment Surface PLFA

The bacterial biomarker i&a 15:0 and ubiquitous 16:0 can be used in conjunction with TO^{13}C to help elucidate the cycling of OM in benthic communities. These biomarkers are closely linked to the organic carbon pool due to the utilization of OM or a specific fraction therein. This allows for the determination OM being taken up by these organisms.

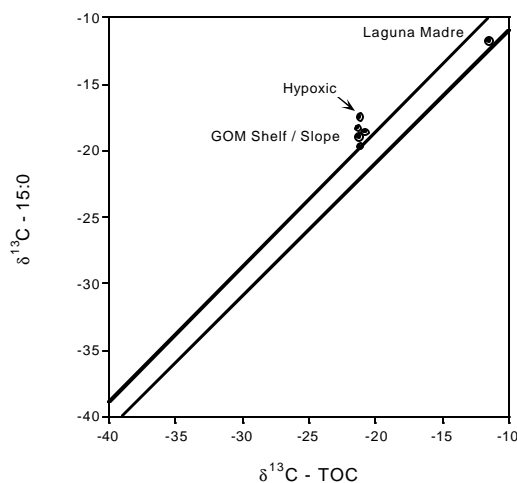


Fig. 12. Sediment surface $\delta^{13}\text{C}$ values of i&a 15:0 from the present study and Laguna Madre. The two solid lines indicate isotopic correspondence with TO^{13}C .

Isolated environments favor the unique determination of carbon sources for bacterial communities. The sources of carbon fueling benthic communities in Laguna Madre (Jones et al. 2003) and Bush Hill (Cifuentes and Salata 2001) have significantly different TO^{13}C values. The seagrass station is dominated by the species *Thalassia testudinum* with a reported above ground tissue $\delta^{13}\text{C}$ value of -10.8‰ and a TO^{13}C sediment surface value of -11.5‰ (Jones et al. 2003). This demonstrates that the primary source of OM for this area originates from the seagrass. Considering this distinction, stations sampled in the GOM showed uniformity in sediment surface $\delta^{13}\text{C}$ values for i&a 15:0 (Figure 12). When the bacterial biomarker i&a 15:0 falls within the limits of isotopic correspondence; i.e. $\delta^{13}\text{C}$ of i&a 15:0 = TO^{13}C , the bacterial community present in that location is utilizing the majority of TO^{13}C present. If the values are not in isotopic correspondence with TO^{13}C then the bacterial community is utilizing another source of organic carbon. It can also be the case that instead of using a completely isolated source of organic carbon, the bacterial community is utilizing a specific fraction of the TO^{13}C available, perhaps a more labile fraction. The Laguna

Madre station is the only sediment surface sample that is in isotopic correspondence with TO^{13}C (Jones et al. 2003). The bacteria, represented by i&a 15:0, are utilizing the dominant source of carbon (Jones et al. 2003). When comparing the GOM stations, all are similar in value (Figure 12). The recalcitrant nature of GOM sediments combined with a small fraction of labile autochthonous OC explain the lack of isotopic correspondence between bacterial assemblages and TOC. These differences can be attributed to the uniquely productive location created in part, by nutrients supplied from the Mississippi River. In addition to high rates of productivity, the Mississippi River is introducing a complex mixture of terrestrial OM to sediments (Goni et al. 1998).

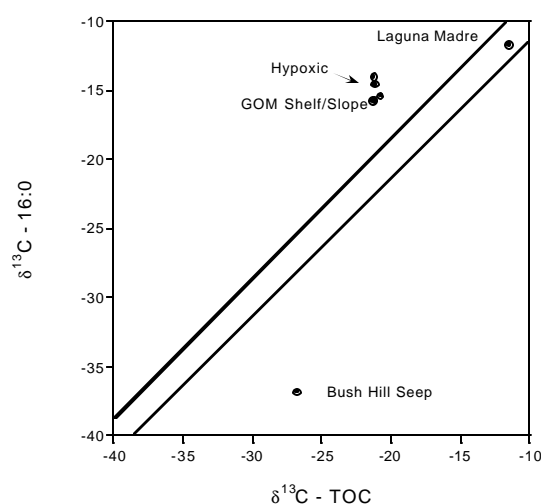


Fig. 13. Sediment surface $\delta^{13}\text{C}$ values of 16:0 from the present study, Laguna Madre, and Bush Hill. The two solid lines indicate isotopic correspondence with TO^{13}C .

An important aspect of this study is to understand the relationship between living carbon and TO^{13}C in coastal and deep ocean environments. Sediment surface values for the biomarker 16:0, representative of all living organisms, are shown in Figure 13. Failure to reach isotopic correspondence between $\delta^{13}\text{C}$ of 16:0 and TO^{13}C indicates that living or recently senescent organisms did not utilize a significant portion of the TO^{13}C .

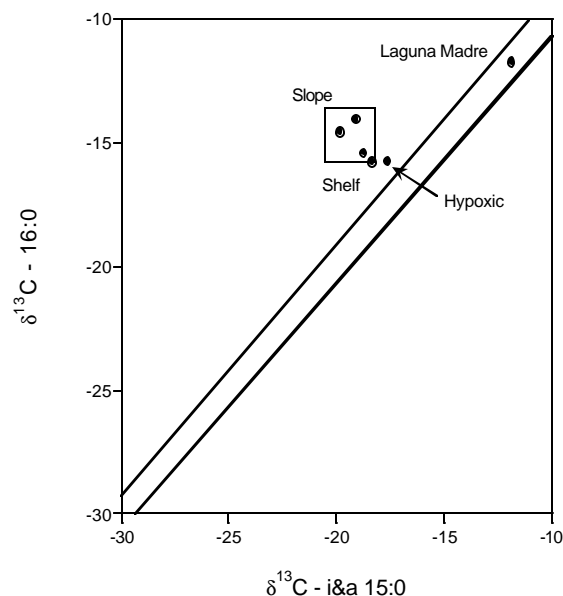


Fig. 14. Sediment surface $\delta^{13}\text{C}$ values of i&a 15:0 and 16:0 from the present study and Laguna Madre. The two solid lines indicate isotopic correspondence with TO^{13}C .

By making a comparison between Figures 12 and 13, it becomes apparent that the bacterial biomarker i&a 15:0 is closer to isotopic correspondence than 16:0. This indicates that there is a specific and sufficient supply of organic matter that is providing isotopically different carbon to the organisms represented by 16:0. By comparing i&a 15:0 with the ubiquitous 16:0 (Figure 14), it becomes evident that bacterial assemblages are not in isotopic correspondence with living carbon. The Bush Hill station is a thermogenic methane seep that creates a unique environment with chemosynthetic communities and bacterial mats (Chen et al. 2004). The Bush Hill station is not in isotopic correspondence with TO^{13}C . Owing to high concentrations of methane, bacteria are able to utilize methane as a source of carbon, thus generating a specific isotopic signature in the organic carbon present in the sediments (Kotelnikova 2002). Organisms represented by 16:0 are receiving the majority of their carbon from the methane expelled from the seep (Cifuentes and Salata 2001). In contrast, the $\delta^{13}\text{C}$ of 16:0 at the Laguna

Madre station is in isotopic correspondence with TO^{13}C (Jones et al. 2003). This is expected due to the isolated source of organic carbon present in this environment. Jones et al. (2003) suggested the isotopic correspondence between 16:0 and TO^{13}C found in Laguna Madre is occurring because the dominant seagrass is the primary contributor of 16:0.

Down-core d^{13}C Variations in Sediment

Owing to the recalcitrant nature of the sedimentary TOC, hypoxic and shelf stations remained consistent in TO^{13}C values with depth (Figures 2-3). This is mirrored by bacterial biomarker i&a 15:0 data illustrated in Figures 4-6. The bacterial assemblages present in all surface sediments have a distinctly different d^{13}C signature relative to the bulk sedimentary organic carbon. This indicates that bacteria are selectively incorporating a more labile portion of TO^{13}C . The slope stations, however, illustrate a different pattern. The variability displayed in down-core measurements of i&a 15:0 and 16:0 at the Mississippi Trough stations (MT-1 through MT-3) suggest different fractions of OM are being utilized. At the MT-1 station, the bacterial biomarker i&a 15:0 displays a trend of becoming more negative with depth (Figure 4). Comparison of i&a 15:0 and TO^{13}C indicate that bacterial communities are utilizing a specific component of TOC at the water-sediment surface interface. As the depth increases the d^{13}C of i&a 15:0 gradually becomes more negative until it reaches isotopic correspondence with TO^{13}C at the bottom of the core. The correspondence between i&a 15:0 and TO^{13}C illustrates the change in OM being used by the bacterial community from the top of the core to the bottom. This general trend persists with MT-2 and MT-3 stations demonstrating the change from one source of OM at the top of the core and eventually utilizing the recalcitrant TOC at the bottom of the core. (Figures 4 and 5). Jones et al. (2003) found similar processes occurring in the Laguna Madre seagrass site. He found that at certain depths where root biomass is highest, bacterial i&a 15:0 varies significantly from TO^{13}C . Jones et al. (2003) attributed this deviation to the release of soluble carbohydrates that were being incorporated by bacteria.

The research by Goni et al. (1998) showing that TOC in the GOM is highly recalcitrant OM derived from terrestrial sources raises the question of what the bacteria are consuming is raised. It was shown that a large percentage (>50%) of the sedimentary TOC from the inner shelf and slope regions of the GOM contained lignin phenols indicative of C₃ and C₄ vegetation (Goni et al. 1998). Furthermore, Goni et al. (1998) demonstrated that lignin phenols representative of C₃ and C₄ plants had d¹³C values of -26.3 to -17.5‰ respectively. Given the d¹³C values of i&a 15:0 (-16.9 to -18.0‰), it is possible that the bacterial assemblages are consuming a more labile fraction of the sedimentary TOC that originated from terrestrial sources. Another more likely explanation is the bacterial assemblages are consuming a newer more labile OC that is autochthonous in origin.

The more ubiquitous 16:0 was near isotopic correspondence with i&a 15:0 at the sediment surface for station MT-1 and at 10cm. The correspondence at the sediment surface can be explained by the benthic microbial loop. This complex cycle uses and recycles OM, distributing it to differing trophic levels (Pace et al. 1984). Jones et al. (2003) found that an isotopic correspondence between i&a 15:0 and 16:0 is representative of the bacterial community constituting the majority of living or recently senescent biomass. The correspondence at 10cm is not representative of this feature but possibly from predation of the bacterial assemblage by benthic fauna. Benthic fauna such as meiofauna and protozoans have been shown to graze heavily on bacteria (Sun et al. 2002). A decrease occurs in bacterial abundance at 10 cm, supporting the likelihood of predation.

A common trend present at all MT stations was the gradual departure from isotopic correspondence at the sediment surface between i&a 15:0 and 16:0 (Fig. 4-5). As the water depth at each slope station increased, the bacterial biomarker i&a 15:0 became more negative and the ubiquitous 16:0 more positive. This departure at the sediment surface is two-fold. The MT-1 station shows that the bacterial community in addition to other higher trophic level organism are engaging in the benthic microbial loop and cycling the same OM source at the sediment surface. The MT-2 and MT-3

stations show a departure from this relationship due to a change in the source of organic matter. A decrease in C/N ratios throughout the slope stations suggest a change in OM (Figure 5). This is expected due to the location of the sampling sites. MT-1 is the closest to land, which explains why MT-1 would have more influence from terrestrial organic matter sources. MT-3 being the most distal sampling location explains why it receives a larger fraction of autochthonous OM. All MT sites display an increase in C/N down-core which is likely due to the loss of nitrogen (N) from diagenetic activity (Patience et al. 1990). This corresponds well with the relative decrease in % organic carbon (%OC) with depth at all MT stations.

Samples collected at the hypoxic site in the Gulf of Mexico (GOM) show a different trend. Similar to the Mississippi Trough stations, the $TO^{13}C$ remains constant with depth (Fig 5). The bacterial biomarker i&a 15:0 was found to be less variable with depth in contrast to the MT stations. A major difference occurred at the hypoxic station where isotopic correspondence is never reached between i&a 15:0 and $TO^{13}C$. This indicates a constant and sufficient supply of OM, other than bulk TOC, that is being utilized by the benthic bacterial community. The more ubiquitous 16:0 becomes slightly more negative with depth. Isotopic correspondence was never reached between i&a 15:0 and 16:0 in the hypoxic location. This indicates that the bacteria represented by i&a 15:0 did not contribute significantly to the formation of 16:0, thus, the organisms represented by 16:0 are utilizing a specific fraction of the TOC as are the bacteria (Figure 5).

There are several likely reasons for these differences. Van Moody et al. (2002) conducted a study in the Equatorial Tropical North Pacific (ETNP) to determine how suboxic and anoxic conditions effect the degradation of sinking particulate organic carbon (POC) on its way to sediments. They concluded that more POC reaches sediments and is less degraded in suboxic water columns. Since the hypoxic station sampled in this investigation maintains similar characteristics as the previously mentioned study, it can be assumed that a larger amount of OC reaches the sediment. It can also be assumed that the OC reaching the sediment is less degraded and contains more labile OM. The high concentrations of sedimenting POC with a significant input of

OC_{terr} supplied by the Mississippi River (Goni et al. 1998) provide bacterial communities a steady source of labile OC. Sun et al. (2002) demonstrated that organic matter is degraded at a slower rate in hypoxic conditions. This means that the increase in OM reaching the sediment is going to last longer, allowing the benthic community to selectively utilize the OC during seasonal hypoxic events. Sun et al. (2002) also showed that grazing on bacteria is significantly reduced in hypoxic regions. This corresponds well with the greater abundance of bacteria detected at this station (Figure 8). This is likely due to the high rate of OM remineralization (Justic et al. 1993).

In contrast to the previous observation, the %OC is lower at the hypoxic station than the MT stations (Fig. 13-15). This is in disagreement with studies by (Sun et al. 2002) showing that hypoxic environments decrease OM degradation. It is likely that the temporal component related to the hypoxic region in the GOM is dynamic and fluctuates significantly. This would allow an increase in bacterial abundance due to a loss of grazing (Sun et al. 2002) as well as rapid OM degradation. The C/N signals observed in the hypoxic station are similar to those observed at the MT-3 station. This seasonally hypoxic region was located just off the coast of Louisiana at the time sampling occurred. Based on the location, it was expected that the surficial sediment sample would have a more terrestrial C/N signature, similar to that of station MT-1. Instead, it had a more autochthonous signature. This signature is probably related to the extremely high productivity rates that occur in this region due to high nutrient concentrations being transported by the Mississippi and Atchafalaya Rivers. The high primary productivity leads to an increase in sedimenting phytodetritus. Since the location is hypoxic this causes more phytodetritus to reach the sediment surface (Sun et al. 2002), thus influencing the C/N with a more marine signature (9.5) Tiessen et al. (1984) showed C/N values of soils from the Mississippi River drainage basin to range from 10-13. This indicates that the hypoxic station is being influenced with OM from both the OC_{terr} supplied by the Mississippi River and autochthonous sources.

The shelf stations display a trend similar to the hypoxic station but different from MT stations in that the i&a 15:0 values remain relatively consistent down-core

(Figure 6). The ubiquitous 16:0 and i&a 15:0 also maintain the same isotopic differences. This leads to the assumption that the benthic organism(s) responsible for creating the $\delta^{13}\text{C}$ value for 16:0 are linked to the bacterial assemblages present. This is reinforced by the nearly identical isotopic differences in i&a 15:0 and 16:0 with depth in shelf station 1 (Figure 6). It has been shown that benthic macro and mesofauna (Sun et al. 2002) are closely linked to OM degradation, transport, and bacterial grazing. One possibility is that the benthic fauna are selectively utilizing a specific labile portion of the bulk OC. The waste and/or partially degraded fraction is then made available to the bacterial community.

Shelf stations also have a C/N ratio indicative of OC_{terr} input (10.3). Due to the close proximity to coastal regions, this is expected. Similar to the hypoxic station, the bacterial abundance is high, relative to the MT stations (9). In addition, the %OC is low relative to the MT stations (0.36). The C/N ratios down-core were different than those observed at the other GOM stations in that they remained relatively constant. The similarity of C/N ratios down-core is likely due to the preservation of carbon (Patience et al. 1990). The low %OC and high bacterial abundance indicate a high rate of OM degradation.

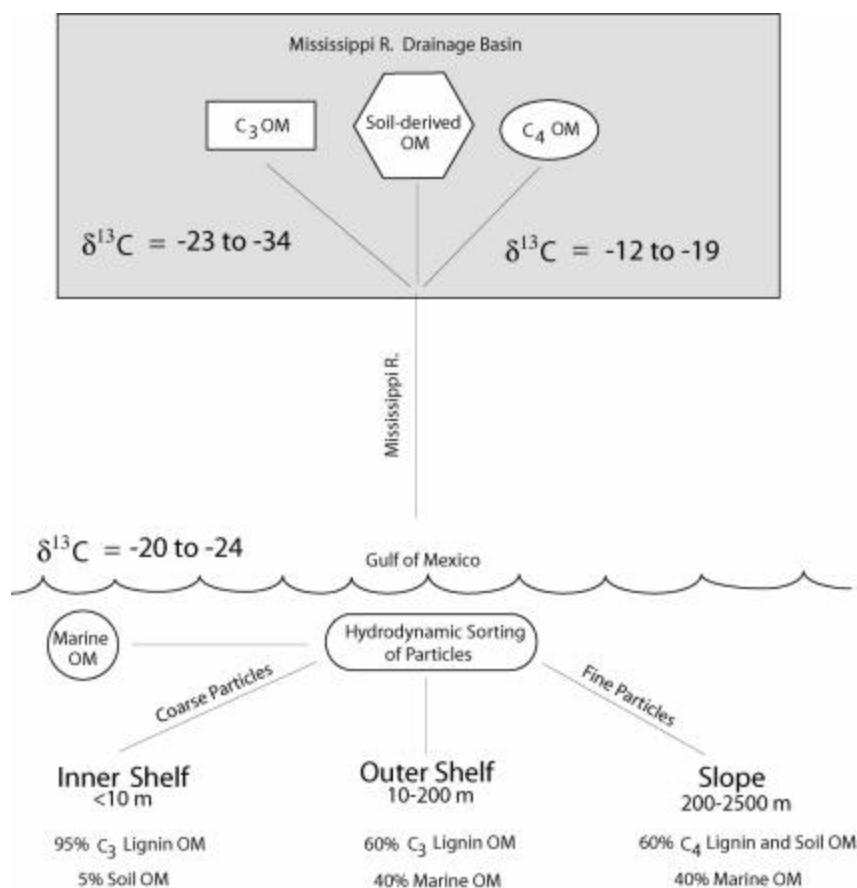


Fig. 15. Flow diagram of OM constituents and fractions as described by Goni *et al.* (1998) and Gordon and Goni (2004).

The fate of OM transported into the GOM from the Mississippi River is an essential component in understanding the utilization of OM by bacteria in GOM sediments. Are bacterial communities utilizing a specific fraction of terrestrially derived OM? Gordon and Goni (2004) showed that a marine signature is identical to a heterogeneous OC_{terr} signature composed of both C₃ and C₄ OM. It is likely that marine OM is contributing the specific fraction of OM to bacterial communities at outer shelf and slope locations based on estimates from Goni *et al.* (1998) and Gordon and Goni

(2004). This becomes apparent due to the homogeneous bacterial biomarker signal obtained at all locations. This is inferred from the similarity in isotopic signatures observed in the bacterial biomarker i&a 15:0 ($-17.5 \pm 0.5\%$) at all sampling locations. I propose that an autochthonous source of OC is reaching the sediment surface interface. Its descent through the water column has slightly altered the carbon isotope ratio causing it to be more ^{13}C enriched. Gordon and Goni (2004) have shown that marine OC is significantly more labile. Bacterial assemblages preferentially consume this more labile autochthonous source of OC before utilizing the recalcitrant OC_{terr} . Given that terrestrial OM is highly degraded and recalcitrant, it seems an unlikely source for the more positive, labile fraction observed in bacterial assemblages. Goni et al. (1998) demonstrated that a significant portion of GOM sediments were old, indicating a terrestrial origin. It is possible that a unique fraction was overlooked when measuring bulk sediments. Perhaps dating specific compounds should be employed in upcoming investigations.

Another question arises in regard to the depth at which the ubiquitous 16:0 is found in sediment samples. How does this biomarker, indicative of all living or recently senescent organisms, achieve such depth in deep ocean sediments? Hughes et al. (2005) collected box cores in deep north-east Atlantic sediment and found burrow cavities exceeding 26 cm in depth. He suspects that the burrows were created by echiuran worms. Considering the depths at which the worms can burrow, Hughes et al. (2005) determined that these megafauna are capable of sequestering phytodetrital material deep within borrows. Similar studies have shown that two species of ghost shrimp (Thalassinidea) have been found to burrow up to 30 cm in depth in Gulf of Mexico sediments (Stanzel and Finelli 2004). These studies indicate that it is quite realistic to obtain a biological signal for viable organisms at depths of 30 cm in GOM sediments.

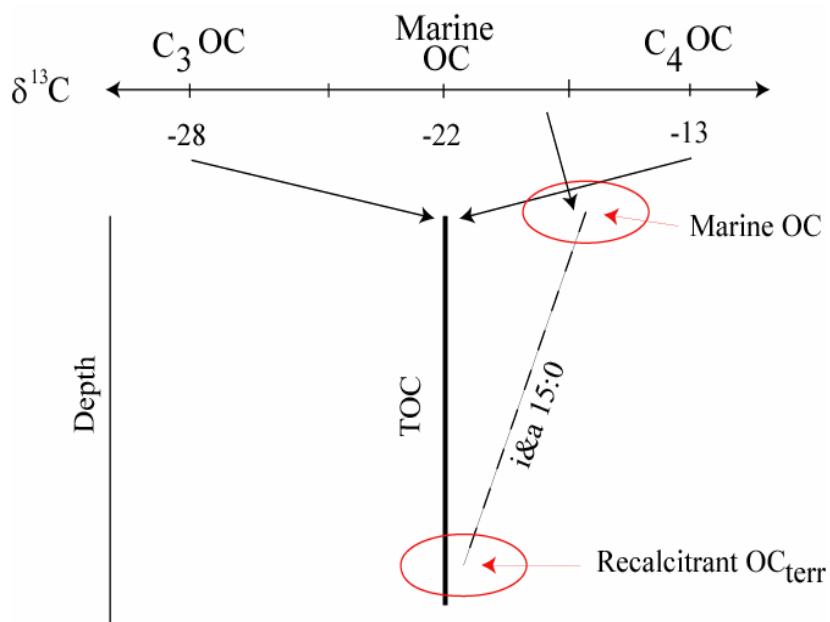


Fig. 16. Illustration of origin for TOC and labile source of OC for bacterial assemblages.

CONCLUSION

It is imperative to understand the role that coastal oceans play in the preservation of carbon. In order to understand the functioning of the coastal ocean, we must determine where the carbon originated and how it is being incorporated into the food web. The data shown above illustrates that bacterial communities in the Gulf of Mexico are utilizing a specific, more labile fraction of the bulk sedimentary organic carbon at the sediment surface interface. The inner shelf stations and hypoxic station are different from slope stations (MT stations) in that bacteria do not revert to the TOC down-core at depths that were sampled. This is due to the high sedimentation rates present in shelf regions. It is suspected that deeper cores would show that bacteria utilize bulk TOC at greater depths once the more labile marine OC is gone. The slope stations appear to use up this labile fraction of the TOC at a shallower depth, thus resorting to the more recalcitrant bulk TOC. This agrees with the findings of Gordon and Goni (2004). The authors found that the sedimentation rate for inner and outer shelf stations in the GOM are much higher than slope stations. Therefore, it seems likely that bacterial assemblages at similar depths in the GOM are mineralizing the labile fraction of TOC slower in slope environments rather than shelf stations (Figure 16).

LITERATURE CITED

- Abrajano TA, Murphy DE, Fang J, Comet P, Brooks JM (1994) $^{13}\text{C}/^{12}\text{C}$ ratios in individual fatty acids of marine mytilids with and without bacterial symbionts. *Organic Geochemistry* 21:611-617.
- Bauer JE, Druffel ERM (1998) Ocean margins as a significant source of organic matter to the deep open ocean. *Nature* 392:482-485.
- Berner RA (1989) Biochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over Phanerozoic time. *Palaeogeography Palaeoclimatology Palaeoecology* 75:97-122.
- Bianchi TS, Lambert CD, Santschi PH, Guo L (1997) Sources and transport of land-derived particulate and dissolved organic matter in the Gulf of Mexico (Texas shelf/slope): The use of lignin-phenols and loliolides as biomarkers. *Organic Geochemistry* 27:65-78.
- Biggs DC (1992) Nutrients, plankton, and productivity in a warm-core ring in the Western Gulf of Mexico. *Journal of Geophysical Research* 97:2143-2154.
- Boschker HTS, de Brouwer JFC, Cappenberg TE (1999) The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. *Limnology and Oceanography* 44(2):309-319.
- Chen DF, Cathles LM, Roberts HH (2004) The geochemical signatures of variable gas venting at gas hydrate sites. *Marine Petroleum Geology* 21:317-326.
- Cifuentes LA, Sharp JH, Fogel ML (1988) Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Limnol Oceanogr* 33(5):1102-1115.
- Cifuentes LA, Salata GG (2001) Significance of carbon isotope discrimination between bulk carbon extracted phospholipid fatty acids in selected terrestrial and marine environments. *Organic Geochemistry* 32:613-621.
- Coffin RB, Velinsky DJ, Devereux R, Price WA, Cifuentes LA (1990) Stable carbon isotope analysis of nucleic acids to trace sources of dissolved substances used by bacteria. *Applied and Environmental Microbiology* 56(7):2012-2020.

- Craig H (1957) Isotopic standards for carbon and oxygen and the correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta* 12:133-149.
- Fry B, Sherr EB (1984) $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions to Marine Science* 27:13-47.
- Goni MA, Ruttenger KC, Eglinton TI (1998) A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochimica et Cosmochimica Acta* 62(18):3055-3075.
- Gordon ES, Goni MA, Roberts QN, Kineke GC, Allison MA (2001) Organic matter distribution and accumulation on the inner Louisiana shelf west of the Atchafalaya River. *Continental Shelf Research* 21(16-17):1691-1721.
- Gordon ES, Goni MA (2004) Controls on the distribution and accumulation of terrigenous organic matter in sediments from the Mississippi and Atchafalaya River margin. *Marine Chemistry* 92:331-352.
- Haines EB (1976) Stable carbon isotope ratios in the biota, soils and tidal waters of a Georgia salt marsh. *Estuarine and Coastal Marine Science* 4:609-616.
- Hedges JJ, Parker PL (1976) Land-derived organic matter in the surface sediments from the Gulf of Mexico. *Geochimica et Cosmochimica Acta* 40:1019-1029.
- Hedges JJ, Keil RG, Benner R (1997) What happens to terrestrial organic matter in the ocean? *Organic Geochemistry* 27(5-6):195-212.
- Hughes DJ, Brown L, Cook GT, Cowie G, Gage JD, Good E, Kennedy H, MacKenzie AB, Papadimitriou S, Shimmiel GB, Thomson J, Williams M (2005) The effects of megafaunal burrows on radiotracer profiles and organic composition in deep-sea sediments: Preliminary results from two sites in the bathyal north-east Atlantic. *Deep-Sea Research I* 52:1-13.
- Jones WB, Cifuentes LA, Kaldy JE (2003) Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon. *Marine Ecology Progress Series* 255:15-25.
- Justic D, Rabalais NN, Turner RE, Wiseman WJ (1993) Seasonal coupling between riverborne nutrients, net productivity and hypoxia. *Marine Pollution Bulletin* 26(4):184-189.

- Keil RG, Montlucon DB, Prahl FG, Hedges JI (1994) Sorptive preservation of labile organic matter in marine sediments. *Nature* 370:549-552.
- Keil RG, Tsamakis E, Giddings JC, Hedges JI (1998) Biochemical distributions (amino acids, neutral sugars, and lignin phenols) among size classes of modern marine sediments from the Washington Coast. *Geochimica et Cosmochimica Acta* 62(8):1347-1364.
- Kotelnikova S (2002) Microbial production and oxidation of methane in deep subsurface. *Earth-Science Reviews* 58:367-395.
- Lambert CD, Bianchi TS, Santschi PH (1999) Cross-shelf changes in phytoplankton community composition in the gulf of Mexico (Texas shelf/slope): The use of plant pigments as biomarkers. *Continental Shelf Research* 19:1-21.
- Lin S (1990) Environmental controls on sulfate reduction and iron sulfide mineral formation. PhD dissertation, Texas A&M University, College Station, TX
- Lollar BS, Slater GF, Ahad J, Sleep B, Spivack J, Brennan M, MacKenzie P (1999) Contrasting carbon isotope fractionation during biodegradation of trichloroethylene and toluene: Implications for intrinsic bioremediation. *Organic Geochemistry* 30:813-820.
- Mancuso CA, Franzmann PD, Burton HR, Nichols PD (1990) Microbial community structure and biomass estimates of a methanogenic Antarctic lake ecosystem as determined by phospholipid analyses. *Microbial Ecology* 19:73-95.
- Milliman JD, Meade RH (1983) World-wide delivery of river sediment to the oceans. *Journal of Geology* 91:1-21.
- Minor EC, Wakeham SG, Lee C (2003) Changes in the molecular-level characteristics of sinking marine particles with water column depth. *Geochimica et Cosmochimica Acta* 67(22):4277-4288.
- Pace ML, Glasser JE, Pomeroy LR (1984) A simulation analysis of continental shelf food webs. *Marine Biology* 82:47-63.
- Pancost RD, Sinninghe D (2003) Carbon isotopic compositions of prokaryotic lipids as tracers of carbon cycling in diverse settings. *Chemical Geology* 195(1-4):17-28.
- Parker PL (1964) The biogeochemistry of stable isotopes of carbon in a marine bay. *Geochimica et Cosmochimica Acta* 28:1155-1164.

- Patience RL, Clayton CJ, Kearsley AT, Rowland SJ, Bishop AN, Rees AWG, Bibby KG, Hopper AC (1990) An integrated biochemical, geochemical and Sedimentological study of organic diagenesis in sediments from Ocean Drilling Program Leg 112, College Station TX, Part B (E. Suess, R. von Huene, et al, eds) 135-153.
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecological Systems* 18:293-320.
- Rowe GT, Deming JW (1985) The role of bacteria in the turnover of organic carbon in deep-sea sediments. *Journal of Marine Research* 43:925-950.
- Rundel PW, Ehleringer JR, Nagy KA (1989) *Stable isotopes in ecological research*. Springer, New York.
- Sackett W (1986) Uses of stable carbon isotope compositions of organic carbon in sedimentological studies on tropical marine systems. *The Science of the Total Environment* 58:139-149.
- Salata GG (1999) *Stable carbon isotopes as tracers of microbial degradation*. PhD dissertation, Texas A&M University, College Station, TX
- Schlesinger WH, Melack JM (1981) Transport of organic carbon in the world's rivers. *Tellus* 33:172-187.
- Smith S, MacKenzie F (1987) The ocean as a net heterotrophic system: Implications for the carbon biogeochemical cycle. *Global Biogeochemistry Cycles* 1:187-198.
- Stanzel C, Finelli C (2004) The effects of temperature and salinity on ventilation behavior of two species of ghost shrimp (Thalassinidea) from the northern Gulf of Mexico: A laboratory study. *Journal of Experimental Marine Biology and Ecology* 312 (1):19-41.
- Sun MY, Aller RC, Lee C, Wakeham SG (2002) Effects of oxygen and redox oscillation on degradation of cell-associated lipids in surficial marine sediments. *Geochimica et Cosmochimica Acta* 66(11):2003-2012.
- Teece MA, Fogel ML, Dollhopf ME, Nalson KH (1999) Isotopic fractionation associated with biosynthesis of fatty acids by a marine bacterium under oxic and anoxic conditions. *Organic Geochemistry* 30:1571-1579.
- Teeri JA, Stowe LG (1976) Climatic patterns and the distribution of C₄ grasses in North America. *Oecologia* 23:1-12.

- Tiessen H, Stewart JWB, Hunt HW (1984) Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions. *Plant and Soil* 76:287-295.
- Tunlid A, White DC (1992) Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. *Soil Biology and Biochemistry* 7:229-262.
- Van Moody BAS, Keil RG, Devol AH (2002) Impact of suboxia on sinking particulate organic carbon: Enhanced carbon flux and preferential degradation of amino acids via denitrification. *Geochimica et Cosmochimica Acta* 66(3):457-465.
- Wakeham SG, Lee C (1993) Production, transport, and alteration of particulate organic matter in the marine water column. In: *Organic Geochemistry*, Engel MH, and Macko SA, (eds.) pp. 145-169. Plenum, New York.
- Walsh J, Rowe G, Iverson R, McRoy C (1981) Biological export of shelf carbon is a sink of the global CO₂ cycle. *Nature* 292:196-201.
- White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40:51-62.

VITA

Carlton David Rauschenberg was born to Robert David Rauschenberg and Martha Kathrine Brittain on July 24, 1978 in Dallas, Texas. Carlton attended First Baptist Academy until the 10th grade when he made the transfer to Booker T. Washington High School for the Performing and Visual Arts. There he studied the stand-up and electric basses and performed in numerous musical groups. Carlton attended DePaul University in Chicago, Illinois and graduated in 2002 with a double major in biology and environmental science and a minor in chemistry. During his tenure at DePaul, Carlton worked with exotic big cats and conducted several investigations concerning the utilization of nutrients in a remnant tallgrass prairie. He then moved back to Texas and began his master's degree in oceanography at Texas A&M University during the fall of 2002. During this time Carlton was able to participate in several research cruises in the Gulf of Mexico and off the western coast of Africa. He graduated from A&M during the summer of 2005. He has received a position as research associate for Bigelow Laboratory for Ocean Research and is moving to Boothbay Harbor Maine at the end of June.

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