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Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon

W. Brian Jones[1], Luis A. Cifuentes[1,*], James E. Kaldy[2]

[1]Oceanography Department, Texas A&M University, MS 3146, College Station, Texas 77843, USA
[2]Pacific Coastal Ecology Branch, Western Ecology Division, US Environmental Protection Agency, 2111 SE Marine Science Center Dr., Newport, Oregon 97366, USA

ABSTRACT: We measured stable carbon isotope ratios ($\delta^{13}C$) in phospholipid fatty acids (PLFAs) to identify the primary carbon source utilized by sedimentary bacteria in Lower Laguna Madre, Texas, which is a seagrass dominated lagoon. Comparisons were made between 3 differing habitat types consisting of a bare area, a transitional area, and a vegetated area. Using PLFA concentrations, we estimated that bacterial abundance was significantly higher in the vegetated habitat compared with bare or transitional habitats. Seagrass *Thalassia testudinum* above-ground tissues averaged –10.8 ± 0.3‰ and benthic microalgae, based on 20:5Δ3 PLFA, averaged –20.5 ± 0.6‰. The $\delta^{13}C$ of total organic carbon ($TO^{13}C$) from all habitats and depths were within ±2‰ of *T. testudinum* above-ground tissues, suggesting that the majority of sedimentary organic carbon originated from this source. The $\delta^{13}C$ of the ubiquitous 16:0 PLFA indicated more complexity in surface vegetated sediments and at depth (ca. 19 cm) in bare and transitional habitats. In turn, the $\delta^{13}C$ of branched, iso- and anteiso-15:0 (i&a15:0) PLFAs found only in bacteria were within ±3‰ of $TO^{13}C$ in all habitats and at all depths. Our work confirmed coupling between sedimentary bacteria and seagrasses occurs in oligotrophic systems with few allochthonous inputs.

KEY WORDS: Seagrasses · Carbon cycling · Bacteria · Phospholipid fatty acids · Stable carbon isotope ratio

INTRODUCTION

Seagrasses are important primary producers, contributing about 30% of total ecosystem net primary production in the Lower Laguna Madre (Kaldy et al. 2002), with similar contributions to other estuarine systems (Heffernan & Gibson 1983, Moncrieff et al. 1992). Because few animals feed directly on seagrass tissue (Moriarty et al. 1985), the bulk of seagrass biomass is believed to enter the detrital food chain through leaf senescence and release of dissolved organic carbon (DOC) (Moriarty et al. 1985, Ziegler & Benner 1998). Senescent material is then broken down through mechanical and biological processes resulting in particulate detrital material and DOC.

The importance of detrital feeders to the decomposition of seagrass was investigated by a number of researchers (Fenchel & Jorgensen 1977 and references therein). These studies concluded that detrital feeders are incapable of utilizing bulk carbon contained in detrital particles and instead digest the associated microbiota. Therefore, organic carbon produced by macrophytes must enter a bacterial or fungal link before this energy can be utilized by higher organisms (Fenchel & Jorgensen 1977). Bacterial communities are hypothesized to mediate the detrital food chain in estuarine systems (Klung 1980, Ogden 1980) by transferring organic matter to higher trophic levels (Tenore 1977).

Concentrations of phospholipid fatty acids (PLFAs) are used widely in terrestrial environments to study

*Corresponding author. Email: lcifuentes@ocean.tamu.edu

bacterial community structure and estimate bacterial abundance in soils (Zelles et al. 1992, Frostegard & Baath 1996, Klamer & Baath 1998, Olsson et al. 1999). PLFA bacterial biomarkers have advantages over other biomarker compounds (e.g. amino acids, DNA) such as: (1) they are found in all living cells, (2) they have a rapid turnover after cell death, and (3) they are easily extracted from complex matrices (Parkes 1987, Tunlid & White 1992). Additionally, studies have reported that bacterial biomass has an isotopic ratio similar to that of the assimilated substrate (Blair et al. 1985, Coffin et al. 1990, Hullar et al. 1996, Salata 1999). However, lipids in these organisms are depleted in ^{13}C (lighter) relative to total biomass and substrate (Monson & Hayes 1982, Blair et al. 1985, Boschker et al. 1999, Salata 1999). The extent of this depletion is dependent on the carbon source being assimilated (Salata 1999) and the chemical environment during synthesis (i.e. oxic versus anoxic; Teece et al. 1999).

While numerous studies show a correspondence between macrophyte production and benthic bacterial activity (Moriarty & Pollard 1982, Moriarty et al. 1986, Pollard & Moriarty 1991, Minoda & Kimura 1994, Holmer et al. 2001), few unequivocally link bacteria to their primary carbon source. Boschker et al. (1999) used gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) of PLFAs to identify carbon sources assimilated by bacterial communities in 3 Netherlands' salt marshes. In 2 salt marshes containing *Spartina anglica* as the dominant macrophyte, bacterial PLFAs had an average $\delta^{13}C$ that matched the isotopic signature of microalgae rather than macrophytes. A similar result was reported for *Zostera marina* and *Z. noltii* meadows, where these seagrasses were of limited importance as a bacterial carbon source (Boschker et al. 2000). *Scirpus maritimus* was the dominant macrophyte in the only system where bacterial PLFAs matched the isotopic signature of the macrophyte (Boschker et al. 1999). This contrast could be explained by species-specific differences in quantity of DOC released by below-ground macrophyte tissues (Boschker et al. 1999) or the lability of released DOC (Salata 1999). Additionally, a recent study by Holmer et al. (2001) showed a close coupling between seagrass organics and sediment bacterial isotopic compositions. Based on these earlier findings, we hypothesized that isotopic coupling between macrophyte carbon and benthic bacteria will be observed only when the macrophyte is the dominant source of labile carbon.

We examined the above hypothesis in a seagrass meadow of Lower Laguna Madre (LLM), Texas, made up almost exclusively of *Thalassia testudinum* (Quammen & Onue 1993). The local dominance of this species removed the complication of species-specific effects indicated by Boschker et al. (1999) in the Netherlands salt marsh studies. *T. testudinum* autecology has been well documented in the LLM (Herzka & Dunton 1997, 1998, Lee & Dunton 1999a,b, Kaldy & Dunton 2000) and primary production at the study sites was dominated by this species (Kaldy et al. 2002). Furthermore, the potential impact of terrestrial carbon was minimized because LLM is hypersaline, receiving little freshwater input.

MATERIALS AND METHODS

Site description. The Laguna Madre is a coastal lagoon system spanning 200 km between Corpus Christi and Port Isabel, Texas, and is separated from the Gulf of Mexico by a barrier island system. Tidal flats, which are infrequently inundated by wind-driven water movement, separate the lagoon into Upper and Lower Laguna Madre, ULM and LLM respectively. The lagoon is shallow with a mean depth <1 m, but is bisected longitudinally by the deeper Gulf Intracoastal Waterway (GIWW). Circulation patterns in Laguna Madre are primarily wind-driven by prevailing southeast winds and passage of north fronts with minimal astronomical forcing (Brown & Kraus 1997). Predominant current flow in LLM is along the north-south axis of the GIWW (Denison & Henderson 1956, Rusnak 1960, Militello & Kraus 1994). Limited freshwater inflow, the semi-arid nature of the south Texas climate and persistent winds result in hypersaline conditions (Collier & Hegpeth 1950); presently, salinities average around 40‰ (Quammen & Onue 1993).

Sample collection. Sediment samples were obtained from a *Thalassia testudinum* dominated area in Lower Laguna Madre near South Padre Island, Texas. Three different sites were sampled on July 1, 1999, with 6.5 cm (inner diameter; i.d.) core liners pushed by hand approximately 40 cm into the sediment. Each site (hereafter referred to as plots) contained an unvegetated area between 5 and 15 m in diameter and was surrounded by dense growth of *T. testudinum*. Plot 1 was ca. 3 yr old, Plot 2 was ca. 2 yr old, and Plot 3 had been formed ca. 1 yr before sampling (Fig. 1). Plot ages were based on observations made during previous field sampling operations (J. E. Kaldy pers. obs.).

At each plot, duplicate cores were collected in the middle of the bare sediment (no *Thalassia testudinum* present), in the transition zone from bare to vegetated sediment (~1 individual *T. testudinum* plant [ind.] per 10 cm^2), and in the densely vegetated sediment surrounding the bare spot (3 ind. 10 cm^{-2}), making a total of 18 cores. Cores were sectioned immediately and 5 depth intervals from each core were kept for PLFA analyses: 0 to 0.5 cm, 0.5 to 2.5 cm, 4.5 to 6.5 cm, 8.5 to 10.5 cm, 18.5 to 20.5 cm; hereafter denoted as 0, 1, 5, 9

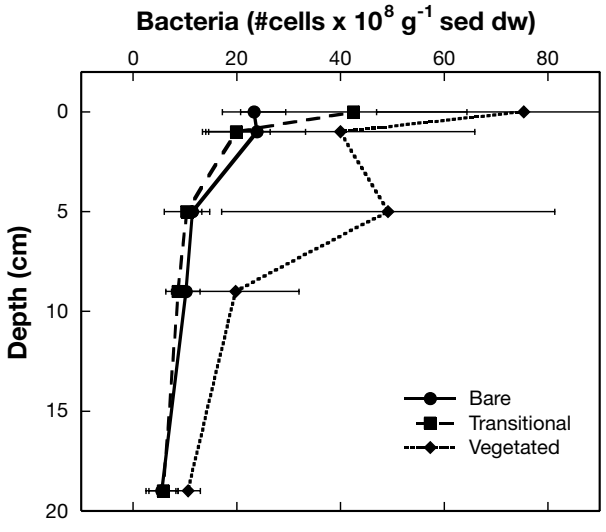

Fig. 1. Bacterial abundance (per g sediment dry wt), based on a conversion factor of 1.7×10^{-17} mol bacterial phospholipid fatty acids (PLFAs) $cell^{-1}$, in the bare, transitional, and vegetated habitats. Values are means ± 1SD

and 19 cm depths, respectively. Each section was wrapped in aluminum foil and frozen until returned to the laboratory, where it was lyophilized and stored at –20°C before extraction of PLFAs.

Sample preparation. Live *Thalassia testudinum* plants present in core tubes were kept for isotopic analysis. Above-ground tissues were separated from the below-ground material. Material was cleaned of epiphytes and washed with 10% HCl to remove inorganic deposits. Both plant fractions were analyzed for bulk carbon isotopic ratios using standard combustion techniques.

Large pieces of shell and organic matter were carefully removed from the lyophilized core sections and the remaining sediment was thoroughly homogenized. Total lipids were extracted with a modified Bligh and Dyer extraction detailed by Cifuentes & Salata (2001). Each core section was extracted in triplicate.

Phospholipid analysis. Fatty acid methyl esters (FAMEs) were prepared from the saponified PLFA fraction with a strong acid methylation (Salata 1999). FAMEs were separated on a 30 m non-polar methylsilicone-bonded phase capillary column (SPB-1, Supelco) with He as the carrier gas at a flow rate of 40 cm s^{-1}. The injector was maintained at 250°C and set in the split mode (1:20) for 15 s. The column was temperature programmed from 100°C (4 min) to 150°C at 5°C min^{-1} and then to 250°C at 4°C min^{-1}. All carbon isotope ratios are expressed in conventional δ notation relative to Pee Dee Beleminite (PDB).

Corrections to measured $\delta^{13}C$. Conversion of free fatty acids to fatty acid methyl esters resulted in the addition of 1 carbon atom to each fatty acid. Therefore, the isotopic ratio of the original free fatty acid ($\delta^{13}C_{FA}$) was calculated with the following mass-balance equation:

$$\delta^{13}C_{FAME} = (x)\,\delta^{13}C_{FA} + (1 - x)\,\delta^{13}C_{MeOH}$$

where $\delta^{13}C_{FAME}$ and $\delta^{13}C_{MeOH}$ 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 fatty acid isotopic ratios presented below were corrected with this equation.

In addition to correcting for methylation, isotopic ratios of fatty acids were corrected for carbon isotope discrimination associated with lipid production. Carbon isotope discrimination in bacterial lipid production has been estimated in a number of studies. Published literature gives discrimination values in bacterial lipid production in the range of –3 to –11‰ relative to bulk biomass (De Niro & Epstein 1977, Monson & Hayes 1982, Blair et al. 1985, Hayes 1993, Boschker et al. 1999, Salata 1999). Fractionation values are dependent on the labile nature of the substrate utilized by the bacteria (Salata 1999). We used a fractionation factor of –5.6‰ based on measurements made in estuarine and salt marsh environments (Boschker et al. 1999, Salata 1999, Boyce et al. 2001).

The column used in these analyses was not capable of separating iso- and anteiso-15:0 fatty acids to baseline; thus, these peak areas and isotope ratios were combined with the isotope balance equation as demonstrated in Boschker et al. 1999.

Estimates of bacterial abundance. Bacterial abundance was estimated in each core section analyzed. Plants and marine algae contain significant amounts of 14:0 and 18:0 fatty acids; however, these fatty acids are not major constituents in the cellular membranes where phospholipids are derived (Harwood & Russell 1984). Instead, cellular membranes of plant and marine algae tend to be comprised of longer-chain fatty acids of 20 carbons or greater, and various carbon length polyunsaturated fatty acids of 3 double bonds or more (Harwood & Russell 1984). As a result, 14:0, i15:0, a15:0, 15:1, 16:1, 17:0, 17:1, 18:0, 18:1, and 18:2 PLFAs were considered to be of bacterial origin. The 16:0 PLFA was excluded from abundance estimates due to its ubiquitous presence in all living cells (Ratledge & Wilkinson 1988).

Abundance estimates were made with peak area calculations for each of the 10 PLFAs considered to be of bacterial origin. Calculations of area vs. concentration were developed with standard curves produced from multiple injections (n = 6) of a quantitative standard (FAME Mix GLC-90, Supelco). Resulting concentrations of these 10 PLFAs were converted to values of

total bacterial PLFA g^{-1} sediment (dry wt). A conversion factor of 1.7×10^{-17} mol bacterial PLFA $cell^{-1}$ was then used to obtain an estimate of the number of bacterial cells g^{-1} sediment (dry wt). This conversion factor is based on pure culture measurements of *Escherichia coli* (White et al. 1977).

Method verification. The extraction procedure and isotope ratio analyses of FAMEs were verified with surrogate and calibration check standards. The surrogate standard, fatty acid 19:0, was used to calculate the efficiency in conversion of fatty acids to FAMEs. All calculated conversion efficiencies averaged 89 ± 6%. The calibration check standard, methyl ester 21:0 ($21:0_{ME}$) (Supelco), was added to the FAMEs after methylation for verification of isotopic calculations and as a check of area to concentration calibrations within each GC/C/IRMS analysis. The $\delta^{13}C$ of $21:0_{ME}$ were within 0.6‰ of standard combustion values in all samples. Area to concentration calibrations were within 1% of expected values based on the standard curve produced.

Statistical methods. For statistical purposes, the 3 sampled plots were treated as replication due to lack of triplicate cores obtained within each habitat and location. Analyses from duplicate cores, triplicate extractions of the same core section, and multiple injections of the same extraction were averaged to 1 value for each core section within each habitat. This process avoided pseudo-replication and resulted in triplicate measurements for each depth interval at each habitat type. The data obtained from this process was analyzed using SPSS v10 with a multivariate ANOVA model (MANOVA, $\alpha = 0.05$). The dependant variables analyzed were the $\delta^{13}C$ of i&a15:0 and 16:0 PLFAs and the $\delta^{13}C$ of total organic carbon ($TO^{13}C$). The main effects were the factors habitat and depth. Normality and homogeneity of variance were tested before ANOVA analyses according to the Kolmogorov-Smirnov test of normality and Levene's test of homogeneity and were found acceptable. Calculated values for number of bacterial cells g^{-1} sediment (dry wt) were divided by 1×10^8 for simplicity. Post-hoc analysis was performed with the Bonferroni correction.

Values of bacterial abundance were treated in the same manner as described above and subjected to a 2-way ANOVA (ANOVA, $\alpha = 0.05$) model using the factors habitat and depth.

RESULTS

Identification

Twelve fatty acids were identified by comparison to retention times of a methyl ester standard (Bacterial Acid Methyl Esters CP Mix, Supelco). These included 14:0, i15:0, a15:0, 15:1, 16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:2, and 20:5Δ3. In turn, the identified fatty acids comprised approximately 96% of total fatty acids observed in the chromatograph based on the sum of peak areas. With the exception of 20:5Δ3, a microalgal biomarker (Ratledge & Wilkinson 1988, Boschker et al. 2000), and 16:0, a ubiquitous fatty acid found in all living organisms (Ratledge & Wilkinson 1988), concentrations of the remaining fatty acids were used to estimate bacterial abundance as described earlier.

Bacterial abundance

Bacterial abundance estimates ranged from 5.6 to 75×10^8 cells g^{-1} sediment (dry wt) (Fig. 1). All 3 habitats had a decrease in bacterial abundance with depth. Bacterial abundance in the bare habitat was 23×10^8 cells g^{-1} sediment (dry wt) at the surface, decreasing to 5.6×10^8 cells g^{-1} sediment (dry wt) in the 19 cm section. Values in the transitional habitat were approximately equal to those seen in the bare habitat, with exception of the surface. Here, bacterial abundance, 43×10^8 cells g^{-1} sediment (dry wt), was approximately twice that found in the bare habitat (Fig. 1). Surface bacterial abundance in all habitats was significantly higher (ANOVA, $p = 0.003$) than deeper sections.

Bacterial abundance in the vegetated habitat was significantly higher (ANOVA, $p = 0.001$) and contained more variation than the bare and transitional habitats (Fig. 1). The surface value was 75×10^8 cells g^{-1} sediment (dry wt), or approximately twice that found in the transitional habitat. A considerable decrease to 40×10^8 cells g^{-1} sediment was observed immediately in the 1 cm section, but was followed by an increase to 50×10^8 cells g^{-1} sediment (dry wt) at 5 cm. Thereafter, bacterial abundance at depth was similar to those measured in the other 2 habitats.

Stable isotope compositions

Plants

Stable carbon isotope ratios ($\delta^{13}C$) of *Thalassia testudinum* above-ground tissues averaged −10.8 ± 0.3‰ (all reported variations represent ±1 SD, n = 6) while below ground tissues averaged −6.3 ± 0.4‰ (n = 6). Similar discrepancies in above- and below-ground tissues have been observed in previous studies of terrestrial and marine plants (Benedict & Scott 1976, Fry & Sherr 1984, Stephenson et al. 1984, Leavitt & Long 1986, Boyce et al. 2001). This discrepancy is hypothesized to be a result of isotopic discrimination in the production of vegetative verses structural components

(Leavitt & Long 1986, Boyce et al. 2001). Detectable concentrations of 20:5Δ3, a proxy for benthic microalgae (Ratledge & Wilkinson 1988, Boschker et al. 2000), were found in all 0 cm sections and in a few of the 1 cm sections. Unfortunately, no estimates of the fractionation due to synthesis of 20:5Δ3 by microalgae are found in the literature. Consequently, we did not correct for isotope discrimination and $\delta^{13}C$ values of 20:5Δ3 averaged –20.5 ± 0.6‰ (n = 16).

Sediments ($TO^{13}C$)

The $TO^{13}C$ for sediments ranged from approximately –13 to –10‰ (Fig. 2A). Depth profiles for bare and transitional habitats were similar, ranging from –11‰ at the surface and becoming lighter with depth. The vegetated habitat profile started lighter at the surface, –12.1‰, and became heavier as it approached 9 cm depth (Fig. 2A), corresponding to the zone where the majority of the *Thalassia testudinum* root system was found. The next depth segment in the vegetated profile (19 cm), well below the root system of *T. testudinum*, shifted to –13.1‰ (Fig. 2A). The $TO^{13}C$ at all depth intervals were within ±2‰ of the $\delta^{13}C$ of *T. testudinum* above-ground tissues.

PLFAs

Corrected $\delta^{13}C$ values of i&a15:0 PLFA from each of the 3 habitats ranged from –13 to –9‰ and displayed considerable variation at each depth interval (Fig. 2B). Isotopic compositions of this biomarker became lighter with depth in all habitats and were within ±3‰ of the $\delta^{13}C$ of *Thalassia testudinum* above-ground tissues. Significant differences were observed between the surface and 9 and 19 cm sections in $\delta^{13}C$ values of i&a15:0 PLFA (MANOVA, p = 0.022 and p = 0.001, respectively) and the surface and 19 cm section in $\delta^{13}C$ of 16:0 PLFA (MANOVA, p = 0.018). The value at the surface (0 cm) of the vegetated habitat, –12.1‰, was lighter compared with bare and transitional habitats, which were approximately –9‰.

Within the bare habitat, surface $\delta^{13}C$ of i&a15:0 and 16:0 PLFAs were both approximately –9‰ (Fig. 2B,C) while the $TO^{13}C$ was –11‰ (Fig. 2A). Below the surface, the $\delta^{13}C$ of i&a15:0 and 16:0 PLFAs, and $TO^{13}C$ exhibited similar values to a depth of 9 cm (Fig. 2). A trend towards ^{13}C-depletion was observed in both PLFA pools with depth relative to $TO^{13}C$. However, the variation of i&a15:0 PLFAs isotope ratios at this depth interval spanned both the $\delta^{13}C$ of 16:0 PLFA and $TO^{13}C$ and no significant differences were observed.

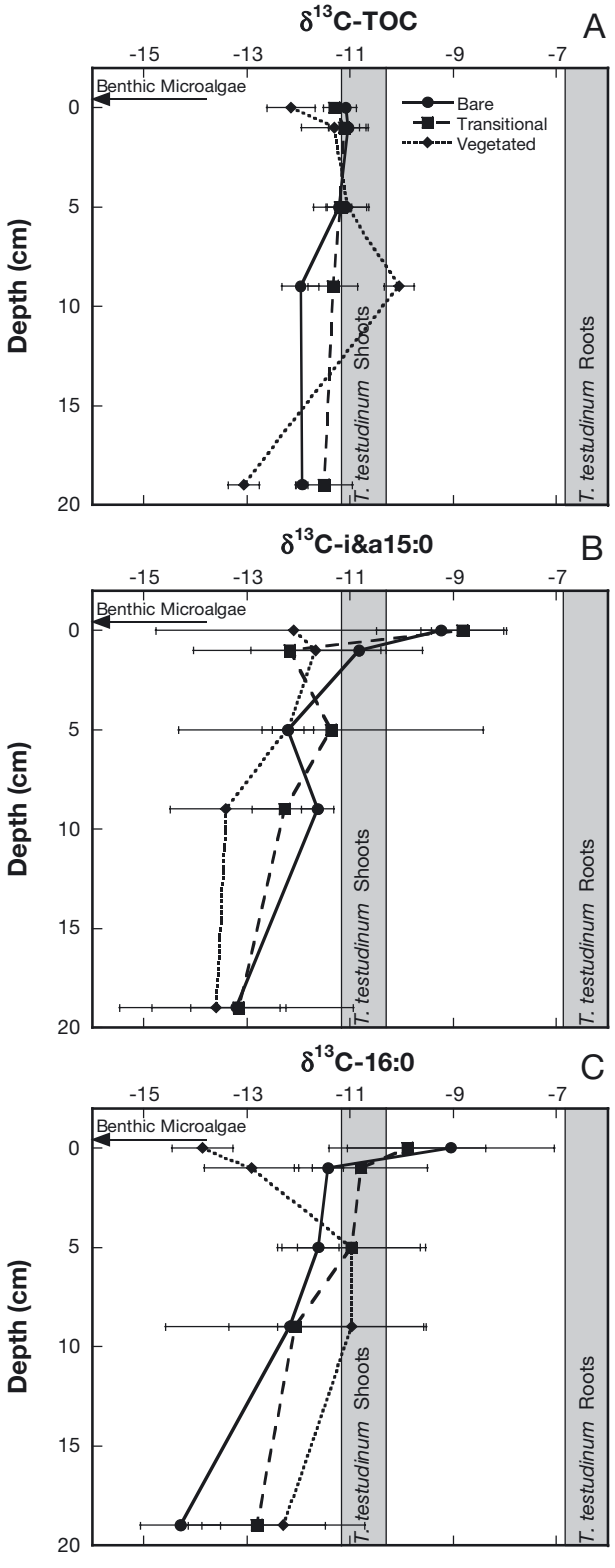

Fig. 2. Stable carbon isotope ratios ($\delta^{13}C$) of (A) total organic carbon ($TO^{13}C$), (B) bacterial biomarker i&a15:0 phospholipid fatty acid (PLFA), and (C) 16:0 PLFA in bare, transitional, and vegetated habitats. Values are means ±1SD. *T. testudinum*: *Thalassia testudinum*

Within the transitional habitat, close agreement between the $\delta^{13}C$ of i&a15:0 and 16:0 PLFAs, and $TO^{13}C$ was observed again. However, the 16:0 PLFA showed a shift of approximately –1‰ from that seen in the same section of the bare habitat (Fig. 2C). The i&a15:0 PLFA at the surface (0 cm) still indicated a ^{13}C-enriched (heavier) carbon source (–9‰); however, the $\delta^{13}C$ of i&a15:0 PLFA from deeper sections appeared similar to that in the bare habitat at the same depth (Fig. 2B). A similar trend toward more negative $\delta^{13}C$ values with depth can be seen again in the 2 PLFA pools while sediment $TO^{13}C$ remained constant at ca. –11‰ throughout the sampled region (Fig. 2).

Profiles of the $\delta^{13}C$ of i&a15:0 and 16:0 PLFAs and $TO^{13}C$ from the vegetated habitat differed from the other 2 habitats. The $TO^{13}C$ started out at approximately –12‰ at the surface and become heavier in the 1, 5 and 9 cm sections, where it reached its highest value of –10.0‰ at 9 cm depth (Fig. 2A). Then, the $TO^{13}C$ value shifted to –13.1‰ in the 19 cm section. The $\delta^{13}C$ of i&a15:0 PLFA was almost identical to the $TO^{13}C$ in the 0, 1 and 19 cm sections (Fig. 2B). However, i&a15:0 PLFA was approximately –1 and –3‰ shifted from $TO^{13}C$ within the 5 and 9 cm sections, respectively. Finally, the 16:0 PLFA pool had a $\delta^{13}C$ of –13.9 at the surface, which increased to ca. –11‰ in both the 5 and 9 cm sections (Fig. 2C). As in all habitats, these values then decreased in the 19 cm section (Figs. 2A to C).

DISCUSSION

Bacterial abundance is generally higher in estuarine sediments compared with those in coastal and deep waters, owing to greater availability of labile organic matter (Cruz-Kaegi 1992, Deming & Baross 1993). In this study, higher bacterial abundance within vegetated sediments in contrast to bare and transitional sediments was consistent with a higher concentration of organic matter in the former (Eldridge & Morse 2000). Although our method for determining bacterial abundance differed from previous studies, estimates (5.6 to 75 $\times 10^8$ cells g^{-1} sediment [dry wt]) were within ranges published in similar environments (Cruz-Kaegi 1992, Deming & Baross 1993, Danovaro et al. 1994). Specifically, Danovaro et al. (1994) reported values in the range of 5 to 322 $\times 10^8$ cells g^{-1} sediment (dry wt) for a *Posidonia oceanica* seagrass bed.

The trend of lower bacterial abundance with increasing sediment depth was in agreement with earlier observations (Cruz-Kaegi 1992, Deming & Baross 1993). The seemingly anomalous data point at the 5 cm section of the vegetated habitat (Fig. 1) corresponded to the region of maximum root biomass for *Thalassia testudinum* in Laguna Madre (Lee & Dunton 2000). Increases in bacterial abundance near the root zone are most likely linked to labile organic matter released from below ground tissues (Eldridge & Morse 2000, Karjalainen et al. 2001).

The $\delta^{13}C$ of sedimentary organic matter ($TO^{13}C$) is highly variable (e.g. Sherr 1982, Cifuentes et al. 1988, Street et al. 1997), ranging from –26 to –13‰ in estuarine sediments (Fry & Sherr 1984, Boutton 1991). For the most part, $TO^{13}C$ are representative of major producers in the environment, whether macrophytes, marine algae or both (Fry & Sherr 1984). In LLM, $TO^{13}C$ were similar to the $\delta^{13}C$ of *Thalassia testudinum* above ground tissues (–10.8‰), suggesting even bare sediments contained organic matter originating from seagrasses. This result contrasts with earlier studies showing a discrepancy between the $\delta^{13}C$ of the dominant seagrass and $TO^{13}C$ (e.g. Simenstad & Wissmar 1985, Fourqurean et al. 1997, Boschker et al. 2000). The lack of a dominant microalgal signal was corroborated by low pigment concentrations measured in bare and vegetated LLM sediments (J. E. Kaldy unpubl. data). As expected, $TO^{13}C$ indicated terrestrial- and phytoplankton-derived organic matter was minimally present in LLM sediments.

The majority of the *Thalassia testudinum* root system is found at the depth zone (Lee & Dunton 2000) where vegetated sediments in LLM were heavier (9 cm; Fig. 2A). *T. testudinum* below-ground tissues ($\delta^{13}C$ = –6.3) were heavier compared with above-ground tissues ($\delta^{13}C$ = –10.8), possibly explaining the isotopic change observed at this depth. Depletion of ^{13}C measured to varying degrees at greater depth (19 cm; Fig. 2A) may signal a population of methanotrophs (Kusel et al. 1999, Sorrell et al. 2002). Although these bacteria most likely did not contribute a large fraction of the sedimentary organic carbon, they could have sufficiently lighter values (Summons et al. 1994, Zyakun 1996) to alter the $TO^{13}C$. This will be discussed further below.

$TO^{13}C$ integrates isotopic signatures of living, recently senesced and older, more refractory, organic matter. Because PLFAs degrade quickly after cell death, detectable concentrations in sediments only represent living and recently senesced cells (Parkes 1987, Tunlid & White 1992). In this study, benthic macrofauna were excluded from PFLA extractions. Therefore, the ubiquitous 16:0 PLFA we detected in sediments had to originate from microheterotrophs (i.e. bacteria, meiofauna) and/or living or newly senesced cells from primary producers (i.e. seagrasses, microalgae). A correspondence between $TO^{13}C$ and $\delta^{13}C$ of 16:0 PLFA implied seagrasses were also the original source of older, more refractory, organic matter in the sediment. In contrast, significant differences

between these isotope ratios indicated either: (1) contribution of living or newly senesced cells with different $\delta^{13}C$ than *T. testudinum* (i.e. below-ground tissues, benthic algae, methanotrophs), or (2) alteration of $TO^{13}C$ following diagenesis (Fig. 3A).

The isotopic data confirmed that above-ground seagrasses were the primary carbon source to LLM sediments. With few exceptions, $TO^{13}C$ and $\delta^{13}C$ of 16:0 PLFA corresponded closely (Fig. 2A) and were similar to that of seagrass above-ground tissues (Fig. 2A,C). Seagrass below-ground tissues were the only identified source of heavier carbon that could account for the more positive $\delta^{13}C$ of 16:0 PLFA recorded in surface transitional and bare sediments. However, it is unlikely that living or recently senesced carbon from seagrass below-ground tissues would be confined to the top 0.5 cm of sediment. Alternatively, extensive recycling of seagrass-derived carbon could lead to isotopic enrichment. Fenton & Ritz (1988) reported positive discrimination (ca. +1‰) in *Heterozostera tasmanica* following 60 d of decomposition. This study, conducted in beakers using filtered (3 µm) seawater as a decomposition medium, may not adequately describe what occurs in sediment. It is possible, therefore, that greater ^{13}C-enrichement occurred in LLM transitional and bare sediments over the longer time span (1 to 3 yr). Because this enrichment was confined to surface sediment, the only depth continuously exposed to oxygen (see Eldridge & Morse 2000), we speculate that this level of isotopic discrimination was enhanced by oxidative degradation.

Previous studies reported benthic microalgae contribute significantly to organic matter in salt marsh sediments (Currin et al. 1995) and seagrass beds (Boschker et al. 2000, Moncreiff & Sullivan 2001). The $\delta^{13}C$ of 16:0 PLFA in 0 and 1 cm sections of the vegetated zone indicated a lighter source of carbon compared with *Thalassia testudinum* (Fig. 2C). In LLM, benthic microalgae had an isotopic composition of –20.5 ± 0.6‰ based on the 20:5Δ3 PLFA proxy

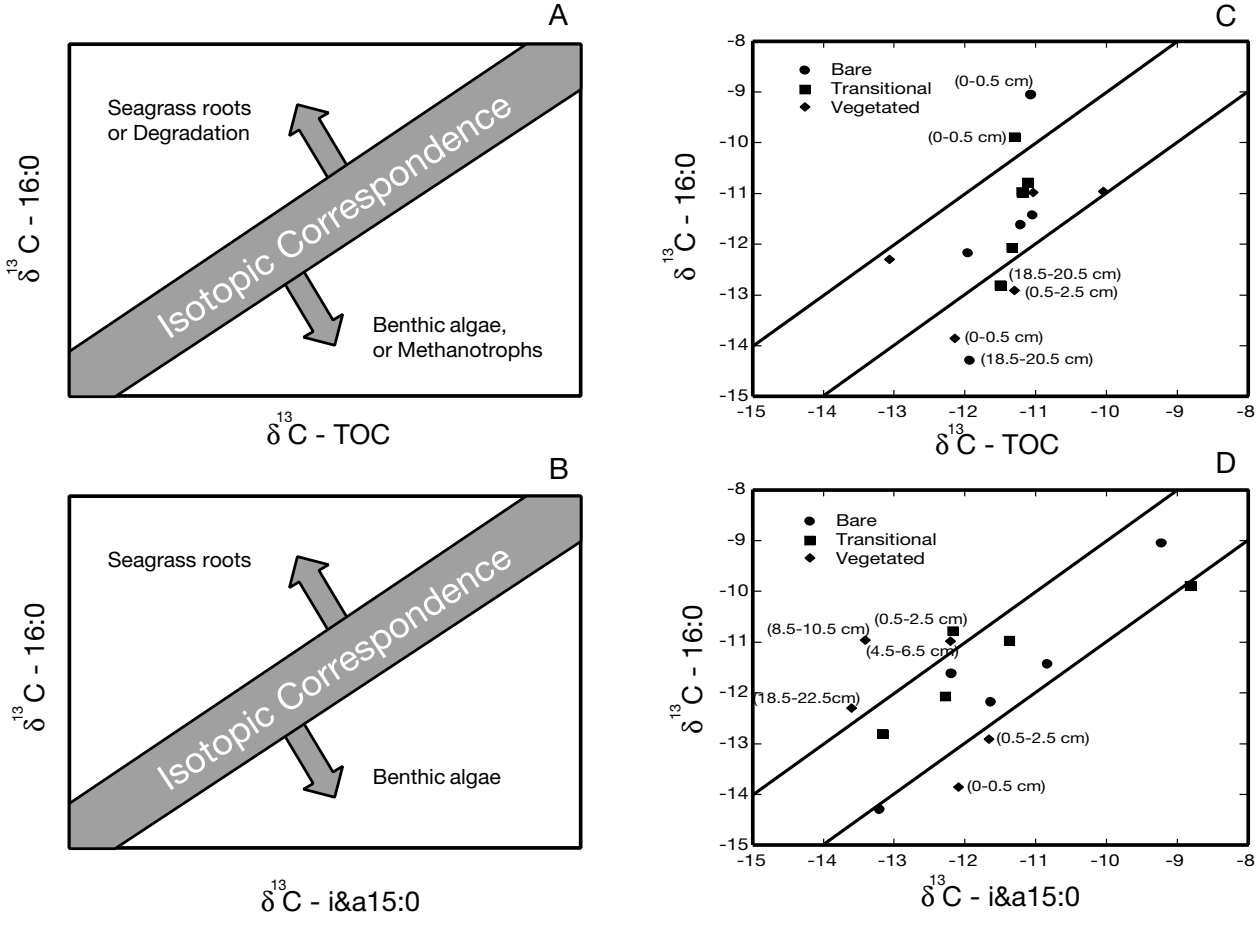

Fig. 3. Diagrams on left indicate sources of carbon which can alter the correspondence between (A) the stable carbon isotope ratio ($\delta^{13}C$) of sedimentary total organic carbon ($TO^{13}C$) and $\delta^{13}C$ of 16:0 phospholipid fatty acid (PLFA) and (B) the $\delta^{13}C$ of i&a15:0 and 16:0 PLFAs. Graphs on right show (C) the $\delta^{13}C$ of sedimentary $TO^{13}C$ versus $\delta^{13}C$ of the 16:0 PLFA and (D) the $\delta^{13}C$ of i&a15:0 PLFA versus the $\delta^{13}C$ of 16:0 PLFA from all 3 habitat types: (●) bare, (■) transitional, and (◆) vegetated. Area between dark lines represents ca. ±1‰ difference around the 1:1 relationship. Values that fall outside of this area are labeled to indicate depth intervals

(Ratledge & Wilkinson 1988, Boschker et al. 2000). This value is likely more heavier, owing to typical isotopic discrimination associated with lipid synthesis (De Niro & Epstein 1977, Monson & Hayes 1982, Blair et al. 1985, Hayes 1993, Boschker et al. 1999, Salata 1999). Using this uncorrected value and a simple isotope-mass balance calculation, we estimated that benthic microalgae contributed ca. 30% of the living or newly senesced carbon. This estimate is high considering the low pigment concentrations measured in these sediments (chl *a*: 0.8 to 8.0 µg pigment g^{-1} sediment, Fucoxanthin: 0.3 to 1.0 µg pigment g^{-1} sediment; J. E. Kaldy unpubl. data). Heavy grazing, however, was implied by the co-occurrence of pigment degradation products (J. E. Kaldy unpubl. data). Therefore, the heavier 16:0 PLFA could have originated from benthic meiofauna, which fed on microalgae (e.g. Montcreiff & Sullivan 2001) as well as seagrass detritus. Alternatively, contributions from epiphytic algae could also explain this difference.

Compared to seagrasses, methanotrophs are highly ^{13}C-depleted (Summons et al. 1994, Zyakun 1996), owing to the isotopic ratio of the substrate (–80 to –60‰ for biogenic methane; see Whiticar 1999). At –70‰, methanotrophs would only have to contribute 5% of the living and recently senesced cells in order to ^{13}C-deplete a seagrass feed consortium (~ –11‰) by 3‰. Although LLM sediments are anoxic at depth (Eldridge & Morse 2000), recent studies have proposed that anaerobic methane oxidation can occur by a consortium of Archaea and sulfate reducing bacteria (Hoehler et al. 1994, Kusel et al. 1999, Boetius et al. 2000, Sorrell et al. 2002). Therefore, we argue that methanotrophs may reach sufficiently high abundance in deep LLM sediments to explain the lighter 16:0 PLFAs at 19 cm depths (Fig. 2C). A similar trend was observed in $TO^{13}C$ of vegetated sediments (Fig. 2A). If methanotrophs were responsible for this ^{13}C-depletion in total sedimentary carbon, these organisms would have to contribute not just 5% of the living and recently senesced cells, but 5% of the total carbon. It is not known whether methanotrophs can accumulate such biomass.

The i&a15:0 PLFAs are found only in bacteria (Parkes 1987, Tunlid & White 1992), and thus were used as the biomarker for bacterial carbon isotopic compositions (see Boschker et al. 1998, 1999, 2000). Therefore, correspondence between the $\delta^{13}C$ of 16:0 and i&a15:0 PLFAs suggested that either bacteria were the predominant source of living or recently senesced cells or there was close coupling between bacteria and the major source of carbon in the sediments (i.e. seagrasses, benthic algae). Isotopic differences between these PLFA pools implied a significant contribution of living or newly senesced carbon of non-bacterial origin with a different isotope ratio (Fig. 3D). Alternatively, discrimination during lipid synthesis varies among aerobic and anaerobic environments (Teece et al. 1999) and could explain contrasting $\delta^{13}C$ of 16:0 and i&a15:0 PLFAs. Although data for this mechanism is limited, the data available indicate small differences compared with other mechanisms such as methanotropy. Thus, we conclude that this mechanism is a limited factor on isotopic differences seen.

For the majority of LLM sediments we studied, bacteria were the principal source of living and/or recently senesced carbon. With the exception of the vegetated site, the $\delta^{13}C$ of 16:0 and i&a15:0 PLFAs were reasonably correlated (Fig. 3D). The vegetated sediment showed ^{13}C-enrichment in 16:0 PLFA compared with i&a15:0 PLFA at 9 cm (Fig. 3D), where the root zone is concentrated (Lee & Dunton 2000). At this depth, *Thalassia testudinum* below-ground tissues contributed to the extracted PLFA pool, but did not appear to be dominantly assimilated by the bacteria. Instead, we hypothesize that seagrass below-ground tissues release soluble carbohydrates produced in the above-ground tissues which are preferentially assimilated by the bacteria. Although the below-ground tissues contain a significant amount of carbon, much of this carbon is contained in structural components of the root system and is more refractory by nature (Boyce et al. 2001 and references therein). Due to the difficulty in processing this carbon source by the bacteria, the below-ground biomass is assimilated at a slower rate and only minimally affects the isotopic composition of the bacterial community. This hypothesis would explain the continued presence of the below-ground tissues in the older bare plots; however, more research is required to adequately characterize this mechanism. Similarly, isotopic difference between 16:0 and i&a15:0 PLFAs in surface, vegetated sediments (Fig. 3B) indicated the presence, but not assimilation, of benthic algae or epiphyte carbon. This was corroborated by the close coupling between the $\delta^{13}C$ of i&a15:0 PLFA and $TO^{13}C$ (Fig. 3A,B), verifying that bacteria were consuming bulk organic matter, which was isotopically similar to *T. testudinum* above-ground tissues.

To emphasize the coupling between *Thalassia testudinum* and bacteria throughout LLM sediments, weighted averages, based on bacterial abundance, were calculated for the $\delta^{13}C$ of i&a15:0 from all 3 habitats. These were clearly more similar to the isotopic composition of *T. testudinum* than those of other known sources (e.g. microalgae, methane) within the system. Our conclusion contrasts with studies reporting that benthic algae contribute organic matter in macrophyte dominated environments (Currin et al. 1995, Boschker et al. 1999, 2000, Moncreiff & Sullivan 2001). However, a recent study by Holmer et al. (2001)

supports our conclusion of close coupling in seagrass organics and sediment bacterial communities.

The close isotopic coupling between *Thalassia testudinum* and bacteria in LLM was not expected in the bare site, where we predicted that other sources of carbon would become important over time, particularly the older plot (ca. 3 yr). The isotopic data, however, demonstrated that there was sufficient *T. testudinum* derived carbon at this site to continue supporting microbial production.

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