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Utilization of DOC from seagrass rhizomes by sediment bacteria: ^{13}C-tracer experiments and modeling

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

[1]Department of Oceanography, Texas A&M University, MS 3146, College Station, Texas 77843, USA
[2]US EPA—Western Ecology Division, Pacific Coastal Ecology Branch, 2111 SE Marine Science Center Drive, Newport, Oregon 97365, USA

ABSTRACT: Seagrasses are widely recognized as contributing to net ecosystem primary production and to supporting heterotrophy in estuarine systems. We investigated the linkage between seagrass (*Thalassia testudinum*) rhizosphere carbon exudation and sediment bacteria. In microcosms, we simulated summer conditions and enriched the water column DIC (dissolved inorganic carbon) pool with ^{13}C, then followed the tracer into the sediment porewater DOC (dissolved organic carbon) and the bacterial biomarkers (phospholipid fatty acids, PLFAs). Subsequently, we developed an inverse analysis of the seagrass microcosm system and calculated the flux of carbon between biological and geochemical compartments. After 18 d, the bacterial pool was enriched by about Δ +4‰, while the DOC pool was enriched by about Δ +50 to +60‰. We estimate that about 15 to 30% of gross primary production was exuded from the root/rhizome and that this accounts for about 41 to 61% of the carbon required by sediment bacteria. Mineralization of detrital seagrass leaf material or refractory seagrass DOC rhizodeposition may account for the other 38 to 59% of the bacterial carbon demand. We suggest that the sediment bacteria in the seagrass rhizosphere rapidly utilize labile DOC and, consequently, build up ^{13}C label in the refractory DOC pool. Our results conclusively show a direct linkage between seagrass carbon exudation and sediment biogeochemical processes.

KEY WORDS: *Thalassia testudinum* · Stable isotope · DOC · Tracer · Phospholipid fatty acid · Rhizodeposition · Carbon flux

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INTRODUCTION

Bacterially mediated processes are a driving factor in sediment nutrient (carbon and nitrogen) cycling. One of the principal themes of estuarine ecology has been the identification of carbon sources contributing to the autotrophic–heterotrophic balance in estuarine ecosystems (Kaldy et al. 2005). There are a number of autochthonous and allochthonous sources that can contribute to carbon cycling in sediments. For example, in permeable sediments advective transport of particles, including microalgal cells, can influence biogeochemical cycling (Huettel & Rusch 2000, Rusch & Huettel 2000, Rusch et al. 2001, Precht & Huettel 2003). At the scale of whole estuaries, seagrasses can contribute about 30% of total net ecosystem primary production, with various macro- and microalgal components making up the remainder (Moncreiff et al. 1992, Moncreiff & Sullivan 2001, Kaldy et al. 2002). On a more limited spatial scale (e.g. patches), seagrass production has been hypothesized to support the microbial loop in both the water column (Velimirov 1986, Velimirov & Walenta-Simon 1993, Ziegler & Benner 1998, 1999) and the sediments (Fenchel 1977, Holmer et al. 2001). The release of root exudates by plants is termed 'rhizodeposition' and is of ecological significance because it represents a loss of fixed carbon and it fuels the sediment microflora (Nguyen 2003). Recent

*Address for correspondence: Newport.
Email: kaldy.jim@epa.gov

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work has shown that photosynthetically produced O_2 contributes to sediment biogeochemical processes (e.g. Eldridge & Morse 2000, Lee & Dunton 2000, Burdige & Zimmerman 2002). We suggest that rhizodeposition also plays an important ecological role in estuarine sediment carbon cycling.

The conceptual relationship between seagrass and sediment bacteria is that a proportion of the carbon allocated to the below-ground tissues (i.e. roots and rhizomes) is exuded as dissolved organic carbon (DOC) into the sediment porewater. Several studies have attempted to examine the mechanistic relationships between sediment-associated bacteria and macrophytes. The few studies available for temperate estuarine plants (marsh plants and seagrasses) found little evidence for coupling of macrophytes and sediment bacterial production (Boschker et al. 1999, 2000). These pioneering studies were relatively short-term experiments and did not consider seasonality or a wide range of species. However, both Holmer et al. (2001) and Jones et al. (2003), working in tropical/sub-tropical systems, have shown direct isotopic evidence that bacteria utilize seagrass-derived carbon as a metabolic substrate. It remains unclear if the carbon source is detrital seagrass (e.g. dead leaves, roots, and rhizomes) or if it is exuded DOC. We hypothesize that the exuded labile DOC pool is preferentially incorporated into bacterial biomass.

Seagrass research has frequently used micro- and mesocosm experiments to test specific hypotheses that require experimental manipulations of the chemical environment (Burkholder et al. 1992, Durako & Sackett 1993, Short et al. 1995, Taylor et al. 1995). Short-term microcosm experiments permit the quasi-realistic culture of seagrass communities, thus representing a dynamic ecosystem (see Lalli 1990 for review). Lepoint et al. (2004) recently suggested that isotopic enrichment experiments will be useful in elucidating organic matter fluxes in seagrass beds. Here, we use a stable isotope tracer experiment conducted in seagrass microcosms to test the hypothesis that seagrass-derived DOC supports sediment bacterial production. Until recently, determining the isotope ratio of bacteria was difficult. However, advances in stable isotope analyses of compound-specific natural abundances have made the use of bacterial biomarkers (e.g. phospholipid fatty acids, PLFAs) more routine. Additionally, recent work has verified that bacterial biomarkers rapidly take on the isotopic signature of the substrate metabolized (Cifuentes & Salata 2001). Unfortunately, few studies have taken the manipulative isotope tracer studies and combined them with modeling (Van den Meersche et al. 2004).

Our objectives were 2-fold: to determine the importance of seagrass-derived (*Thalassia testudinum*) DOC to sediment microbial communities and to develop a model to quantify these dynamics. To achieve our objectives, we conducted a ^{13}C stable isotope tracer experiment in seagrass microcosms. We labeled the dissolved inorganic carbon (DIC) in the water column with ^{13}C-DIC and traced the isotope into the porewater DOC and ultimately into the bacteria. We also developed models of seagrass carbon allocation (inverse analysis) and the stable isotope ratio of geochemical pools.

MATERIALS AND METHODS

Experimental design and microcosm set-up. The experiment was a 2×2 factorial, with replication, habitat, and enrichment as the experimental factors. The 2 habitat types were bare and seagrass-vegetated sediments, and the 2 enrichments were ^{13}C-DIC amended and no ^{13}C-DIC; these treatments will be referred to as 'spike' and 'no-spike', respectively, throughout the paper. Each factor was run in duplicate for a total of 8 microcosms. Each microcosm consisted of a 20 l plastic container (28.5 cm diameter, 36 cm height), with an intact sediment plug (27 cm diameter, 10 to 15 cm thick), maintained at a light level of 279 ± 47 µmol photons m^{-2} s^{-1} (saturating irradiance 220 and 290 µM quanta m^{-2} s^{-1}; Herzka & Dunton 1997, Kaldy & Dunton 1999) over a 24 h light period; leaves were trimmed to minimize self-shading. Seagrass plugs were obtained from a monotypic stand of *Thalassia testudinum* in Lower Laguna Madre (LLM), Texas, in July 1999. Microcosm chambers were returned to the laboratory within 10 h of collection, and treatment placement was randomized. Microcosm samples were collected near the site of previous seagrass studies (Herzka & Dunton 1997, Kaldy 1997, Kaldy & Dunton 1999, 2000, Eldridge & Morse 2000). Temperature and salinity conditions in the microcosms were similar to field conditions and were maintained throughout the course of the experiment. LLM water was collected at the same time for use in the microcosms. To characterize field conditions, all microcosms were sampled for sediment porewater DOC concentration and isotope ratio and for bacterial biomarker isotope ratio within 24 h of arriving at the laboratory. Deionized water was used to balance evaporative losses. Microcosm temperature ranged between about 27 and 30°C, and salinity, between 35 and 42 psu, comparable to summer field conditions (Quammen & Onuf 1993, Kaldy & Dunton 2000). Mechanical mixing of the microcosms prevented stratification. All microcosms were allowed to acclimate to laboratory conditions for 10 d prior to the initiation of enrichment experiments. The manipulative experiment was initiated on 10 July 1999, with final sampling conducted on 28 July 1999.

Plant measurements. On Day 4 of the enrichment experiment, all plants in the vegetated treatments were marked to assess leaf growth over the remaining 14 d of the experiment. Leaf marking was conducted as described by Kaldy & Dunton (2000). Care was taken to avoid isotopic cross contamination. At the end of the experiment, all seagrass material was retained, sorted into above- and below-ground components, dried, and weighed. Direct isotopic analysis of seagrass tissue samples was not conducted. Microphytobenthic biomass was assessed at the beginning of the experiment by sampling the top 2 mm of sediment, extracting with 100% acetone, and analyzing the supernatant for pigments with high performance liquid chromatography (HPLC). Water column chlorophyll was sampled at the end of the experiment; 100 ml was filtered through clean GFF and extracted as above.

Stable isotope enrichment experiments. After an initial acclimation period (10 d), the experiment was initiated with a complete water change; no-spike treatments (n = 4) received 12 l filtered (0.1 µm) LLM water, and spike treatments received 12 l filtered LLM water amended with ^{13}C-DIC. Enrichment consisted of 0.856 g ^{13}C bicarbonate (Isotec) added to 70 l of 0.1 µm filtered LLM water to give an enrichment of about +5100‰ ^{13}C-DIC.

Isotope measurements. DIC concentration and δ^{13}C-DIC were measured at the beginning of the experiment using the methods of Salata et al. (2000). Briefly, DIC samples were collected in clean 60 ml Quorpak bottles, fixed with mercuric chloride, sealed with Teflon-lined caps, and refrigerated. For analysis, a water aliquot was introduced to an evacuated vacutainer and acidified with phosphoric acid; pressure was then equalized with helium gas. Samples were allowed to equilibrate, and head space gas was analyzed by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) using a FinniganMat 252. Using standard curves we calculated both the δ^{13}C-DIC and the DIC concentration (Salata et al. 2000).

Bulk and sipper samples were collected for analysis of sediment porewater DOC concentration and isotope ratio. Bulk porewater DOC was sampled at the end of the experiment. Bulk samples were collected by centrifuging a sediment core (2.7 cm i.d. by 10 cm), filtering the supernatant (combusted 0.45 µm GFF) into acid-washed and combusted scintillation vials, and frozen on dry ice. Samples were stored frozen until analysis. A time series of porewater samples from 3 and 8 cm sediment depths was obtained using porewater sippers. Sipper samplers were a modification of the design by Zimmerman et al. (1978). Porewater collected from sippers was filtered and stored as described above. The time series of δ^{13}C-DOC porewater samples collected from the sippers was statistically evaluated using non-parametric, 1-way repeated-measures ANOVA on ranks using Sigma-Stat 3.1. Because of heterogeneity of variance and non-normality that could not be corrected with transformations, non-parametric analyses were conducted. Tukey's test was used to examine differences between groups, and all tests were performed with $\alpha = 0.05$. DOC concentration was measured by high temperature combustion of acidified samples using a Shimadzu 5000 TOC analyzer with a platinum catalyst. Prior to analysis for each batch of samples, a standard curve was determined, and a standard was run between every 10th sample.

Isotopic analysis of porewater DOC was accomplished by coupling the Shimadzu 5000 TOC analyzer to the GC/C/IRMS (Kaldy et al. 2005). Briefly, a water sample was combusted at 720°C in a column packed with crushed quartz glass. After passing through the chlorine and moisture scrubbers and the infrared detector, CO_2 in the Shimadzu effluent stream was retained in Sample Loop 1 with silica gel crystals at −70°C. After collection, the valve configuration was altered, allowing transfer from the Shimadzu instrument to the GC, and Sample Loop 1 was heated to about 200°C. The CO_2 released from Sample Loop 1 was retained in Sample Loop 2 and packed with Porapak (Porapak U, Alltech Associates) at −180°C (liquid nitrogen). After complete transfer of CO_2 to Sample Loop 2, the GC run was initiated and the CO_2 was introduced to the GC/C/IRMS by heating Sample Loop 2 to 100°C. The detection limit for this method was about 2.5 mg C l^{-1}. Prior to sample analysis, a series of standards were run to determine a correction factor (typically <3‰); all samples were analyzed in duplicate.

PLFAs were used as a biomarker for live and recently senesced marine bacteria (Cifuentes & Salata 2001, Jones et al. 2003). The isotope ratio of the iso-anteiso-15:0 PLFA (i&a 15:0) was examined to determine bacterial dependence on seagrass-derived DOC, while a suite of 10 PLFAs was used to quantify bacterial abundance. Detailed PLFA analytical methods were presented by Jones et al. (2003). The field PLFA sample collected before the initiation of the isotope enrichment experiment was a composite of sediments from all 4 replicate tanks (seagrass and bare, respectively). During the final sampling , duplicate sediment samples (~12 g dry wt sediment) obtained with cores from the center of the tanks were extracted for total lipids using a modified Bligh–Dyer extraction. Fatty acid methyl esters (FAMEs) were prepared from the phospholipid fraction using a strong acid methylation. FAMEs were analyzed by GC/C/IRMS; recovery was 89 ± 6% (Jones et al. 2003). Corrections for methylation, verification methods, and bacterial biomass calculations were as detailed by Jones et al. (2003). Because of het-

erogeneity of variance that could not be corrected with transformation, a Mann-Whitney rank sum test was used to assess differences in the δ^{13}C-PLFA between the seagrass spike and no-spike treatments.

Modeling. The conceptual basis for the allocation of carbon is that DIC is fixed by photosynthesis in the leaves and then transported through the plant, released to the environment, and ultimately incorporated into bacterial biomass. An inverse model is used to estimate the fluxes associated with the seagrass plant, and a separate tracer model uses the results of the allocation model to predict how the ^{13}C-tracer moves from the enriched DIC in the water column, through the plant, and into the sediments and sediment bacteria.

Inverse analysis: The allocation model for *Thalassia testudinum* is based on microcosm data collected during the present study and some literature values. The model uses inverse analysis to estimate the flow of carbon produced by photosynthesis to either the synthesis of plant structural components or to the maintenance of plant physiological processes. Vezina & Pahlow (2003) characterize inverse methods as merging observations with mechanistic models in order to recover unobserved features of natural systems. This type of model has been used extensively in food web research (Vezina & Platt 1988, Jackson & Eldridge 1992); here, we apply the same concepts of material flow to predict the flux of C between plant compartments. The inverse analysis is a mass-balance model that assumes the seagrass–bacterial system is composed of a finite set of compartments; material flows entering a compartment balance those leaving the compartment. Furthermore, we assume that this is a closed system, with no atmospheric exchange. The model is solved as the minimization of the sum of the squares of the flows among plant and sediment compartments ($\|\boldsymbol{A}x - \boldsymbol{b}\|$), with the additional requirement that the solution be consistent with the constraints ($\boldsymbol{G}x \geq \boldsymbol{h}$). $\boldsymbol{A}$ is a matrix of equations describing the flow of carbon (Table 1), $\boldsymbol{b}$ is a vector of 0-values for the mass-balance equations (Table 1), $\boldsymbol{G}$ is a set of inequalities that delimit ranges of data used in the model's physiological information, and $\boldsymbol{h}$ specifies the bounds that the inequality must satisfy (Vezina et al. 2000) (Table 2). Detailed solution methods for inverse analysis are described in the literature (Vezina & Platt 1988, Vezina & Pace 1994, Vezina et al. 1997).

Table 2. Inverse analysis constraint relationships. Additional constraints not shown require all C flows to be >0 (mn: minimum constraints; mx: maximum constraints). Units are mmol C m^{-2} d^{-1}; symbols as in Table 1. 1: Herzka & Dunton (1997); 2: Kaldy & Dunton (1999); 3: Wetzel & Penhale (1979); 4: Kaldy (1997); 5: Vezina & Platt (1988); 6: Eldridge & Sieracki (1993); 7: present study

Description	Constraints in $\boldsymbol{G}x \geq \boldsymbol{h}$, where $\boldsymbol{h}$ specifies the bounds the inequality must satisfy	Source
Gross primary production mn	$F_{di\rightarrow lf} \geq 692$	1
Gross primary production mx	$F_{di\rightarrow lf} \leq 898$	1
Leaf respiration mn	$F_{lf\rightarrow di} \geq 54$	2
Leaf respiration mx	$F_{lf\rightarrow di} \leq 201$	2
Short shoot respiration mn	$F_{ss\rightarrow di} \geq 56$	2
Short shoot respiration mx	$F_{ss\rightarrow di} \leq 93$	2
Root/Rhizome respiration mn	$F_{rr\rightarrow di} \geq 385$	2
Root/Rhizome respiration mx	$F_{rr\rightarrow di} \leq 637$	2
Leaf loss + growth mn	$F_{lf\rightarrow de} + F_{lf\rightarrow gr} \geq 105$	2
Leaf DOC loss	$F_{lf\rightarrow dc} - 0.01F_{di\rightarrow lf} > 0$	1
Root/Rhizome DOC loss	$F_{rr\rightarrow dc} - 0.12F_{di\rightarrow lf} \geq 0$	3
Leaf loss + growth mx	$F_{lf\rightarrow de} + F_{lf\rightarrow gr} \leq 272$	4
Bacteria respiration	$F_{ba\rightarrow di} \geq 1.3F_{ba} - C\ e^{0.0693T}$	5, 6, 7
Bacteria net production efficiency	$0.9F_{dc\rightarrow ba} - F_{ba\rightarrow di} \geq 0$	5

Table 1. Inverse analysis model equations. Symbols representing import/export terms are detritus (de), sediment organic carbon (oc), and *Thalassia* growth (gr). Carbon flows used in the equations are denoted as $F_{i\rightarrow j}$ where i is the donor compartment and j is the recipient compartment. DIC: dissolved inorganic carbon; DOC: dissolved organic carbon

Compartment	Equations in matrix $\boldsymbol{A}x = \boldsymbol{b}$, where $\boldsymbol{b}$ is a vector of 0-values
Leaf (lf)	$F_{di\rightarrow lf} - F_{lf\rightarrow di} - F_{lf\rightarrow ss} - F_{lf\rightarrow dc} - F_{lf\rightarrow de} - F_{lf\rightarrow gr} = 0$
Short shoot (ss)	$F_{lf\rightarrow ss} - F_{ss\rightarrow di} - F_{ss\rightarrow rr} - F_{ss\rightarrow dc} - F_{ss\rightarrow de} - F_{ss\rightarrow gr} = 0$
Root/Rhizome (rr)	$F_{ss\rightarrow rr} - F_{rr\rightarrow di} - F_{rr\rightarrow dc} - F_{rr\rightarrow de} - F_{rr\rightarrow gr} = 0$
Bacteria (ba)	$F_{dc\rightarrow ba} - F_{ba\rightarrow di} - F_{ba\rightarrow de} = 0$
DIC (di)	$F_{lf\rightarrow di} + F_{ss\rightarrow di} + F_{rr\rightarrow di} + F_{ba\rightarrow di} - F_{di\rightarrow lf} = 0$
DOC (dc)	$F_{ss\rightarrow dc} + F_{rr\rightarrow dc} + F_{oc\rightarrow dc} - F_{dc\rightarrow ba} = 0$

The plant compartments for the analysis were leaves, short shoot (also known as the vertical rhizome), and root/rhizome; the chemical compartments were DOC and DIC. The only input flux to photosynthesis was DIC, while the export fluxes were detritus, DIC, and DOC. Plant growth measured during the experiment was incorporated into this steady state analysis as an export flux, since compartment size does not change in steady state analysis. We model a range of seagrass rhizodeposition rates (5 to 30% of gross primary production) in order to examine how rhizodeposition can influence the isotope ratio of the DOC and bacterial PLFAs.

Allocation model sensitivity analysis: We tested the effect of data variability on the carbon

allocation analysis by increasing and decreasing the value of each datum individually by 20%. The sensitivity of the model to these changes was determined by calculating 2 response measures (Eldridge et al. 2005). The first response measure is the ratio of system respiration to gross primary production (C_m):

$$C_m = \frac{R_{seagrass} + R_{bacteria}}{GPP_{seagrass}} \quad (1)$$

where $R_{seagrass}$ and $R_{bacteria}$ represent the respiration for all seagrass and bacterial pools and $GPP_{seagrass}$ is gross primary production. Respiration was a consistently available output variable for seagrass and bacteria, and is a measure of carbon utilization by an organism or plant organ (Eldridge & Jackson 1993). The second response measure is based on the root-mean-squared difference (RMSD), also referred to as the standard deviation (Zar 1984, his Eq. 4.11, p. 31), between output from the standard model results and the model results with 1 parameter changed at a time. The RMSD response variable provides an integrated measure of how model output changes in response to a change in a single input parameter.

We use a sensitivity index $S(A,p)$ developed by Fasham et al. (1990) to relate a change in an input parameter to a change in the response measure, as calculated by the model subject to the new parameter:

$$S(A,p) = \left(\frac{A(p) - A_s}{A_s}\right)\left(\frac{p - p_s}{p_s}\right)^{-1} \times 100 \quad (2)$$

where p_s and p are the standard and changed parameter values and A_s and $A(p)$ are the standard and changed response measure (C_m and RMSD). We calculate S as the percentage change in the output of the response measure for each change made in a model input parameter. If S is 0, then A does not change with the parameter. If S is 100%, a 20% increase in the parameter caused a 20% increase in A. For $S > 100$%, the change in A is disproportionately large relative to the change in the input parameter (Fasham et al. 1990, Eldridge & Jackson 1993).

Tracer model: A simulated ^{13}C-tracer model was developed to reconcile the microcosm experimental results ($\delta^{13}C$ measurements) with our inverse model results (flux estimates). The predicted flows from the inverse analysis were used to parameterize a C-tracer model that was developed by Jackson & Eldridge (1992) and revised for ^{13}C stable isotopes. Detailed description of the tracer model is provided in Appendix 1. Briefly, a simulated ^{13}C-tracer was added to the DIC compartment of the analysis in the same proportion as in the experimental treatment and then followed through the plant and into the geochemical and bacterial compartments of the sediment over 20 d.

RESULTS

Seagrass characteristics

The average biomass in the seagrass spike treatments was roughly twice the average biomass in the seagrass no-spike treatments; highlighting the heterogeneous aerial distribution of seagrasses (in this case, *Thalassia testudinum*). However, the biomass allocation was similar in all microcosms, with 85 to 87% of the total biomass in the below-ground (rhizome + root) fraction (Table 3). In general, seagrass characteristics (e.g. biomass, shoot density, and growth rates; Table 3) in the microcosms were within the range documented for this population (Kaldy & Dunton 2000). During the experiment, the bare treatments developed algal blooms with 19 ± 10 and 15 ± 16 µg chl *a* l^{-1} (mean ± SD, n = 2) in the water column of the spike and no-spike treatments, respectively, seagrass treatments did not exhibit blooms with <2.5 µg chl *a* l^{-1} in the water column. Sediment chlorophyll levels measured before the experiment ranged between 0.039 ± 0.045 and 0.043 ± 0.042 µg chl *a* cm^{-2} for the seagrass and bare treatments, respectively.

Table 3. Seagrass and sediment bacteria characteristics measured at the end of the microcosm experiment system. Values represent means (±SD, n = 2), except for bacterial estimates. GS: grass spike; BS: bare spike; GNS: grass no spike; BNS: bare no spike; sed: sediment

	Leaf biomass (g dry wt m^{-2})	Below-ground biomass (g dry wt m^{-2})	Shoot density (shoots m^{-2})	Seagrass growth (g dry wt m^{-2} d^{-1})	Bacterial density[a] (10^{10} cells g^{-1})	Bacterial biomass[b] (mg C g^{-1} sed.)
GS	138.7 ± 35.8	837.9 ± 96.7	1511 ± 135	2.26 ± 1.0	4.62	1.98
BS	–	–	–	–	2.25	0.97
GNS	66.9 ± 13.8	478.1 ± 5.9	899 ± 12	1.52 ± 0.4	0.30	0.13
BNS	–	–	–	–	0.63	0.27

[a]Estimate based on phospholipid fatty acid abundance (Jones et al. 2003)
[b]Biomass estimate based on 0.043 pg C $cell^{-1}$ (Bratbak 1985)

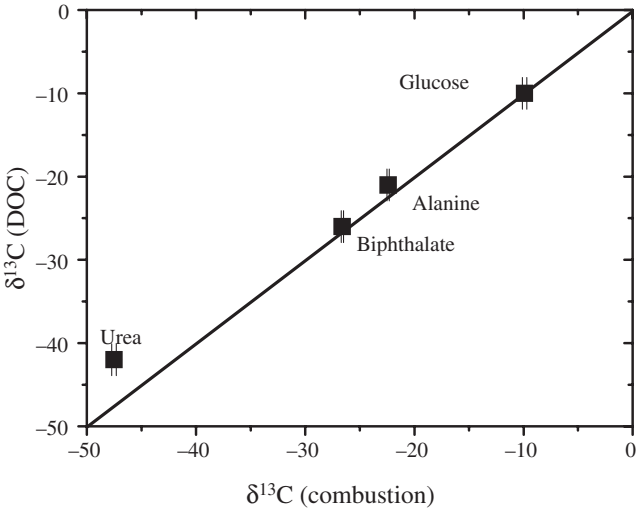

Fig. 1. Comparison of traditional combustion and the coupled TOC/GC/C/IRMS (total organic carbon/gas chromatography/combustion/isotope ratio mass spectrometer) analytical method for various laboratory standards (mean ± SD, n = 5)

Stable isotope enrichment

We calculated that the water column DIC pool was enriched to +5100‰ by the addition of ^{13}C bicarbonate. Analysis of water samples diluted 1:10 with Milli-Q water (–12.6 ± 0.5‰) confirmed that the DIC pool was enriched to +5110 ± 32‰.

To verify that the new δ^{13}C-DOC analytical method worked, we compared the results of our TOC/GC/C/IRMS method with those obtained from traditional combustion methods (i.e. sealed tube with cupric oxide) for a series of standards (Fig. 1). Of the 4 standards used, only urea exhibited a deviation from the 1:1 line. Additionally, for each standard, we ran a series of concentrations and determined the minimum detection limit to be about 2.5 mg C l^{-1}, equivalent to 0.21 mM (data not shown). Sediment porewater typically has DOC concentrations above this detection limit (Burdige 2002).

The size of the DOC pools was fairly consistent throughout the experiment, indicating that there were no unexplained sources or sinks of DOC in the microcosm sediments and that the addition of carbon isotope label did not stimulate productivity (i.e. not carbon limited). The concentration of DOC in the sediment porewater ranged between 5 and 70 mg l^{-1} (Fig. 2). Field values for the seagrass treatments were higher than those from the bare treatments; however, by Day 3 those differences were gone. For all treatments and at both sediment depths, the lowest [DOC] measured was on the last sampling date (Fig. 2). In all of the no-spike microcosms, the δ^{13}C-DOC time series and final measurements were similar to the field values, suggesting that there was no isotopic contamination and that there were no real artifacts associated with the microcosm incubations (Fig. 3). Additionally, there was no significant difference in the time series of δ^{13}C-DOC between the bare and seagrass no-spike treatments (χ^2 = 7.32, df = 3, p = 0.062). In contrast, the δ^{13}C-DOC of the spike microcosms exhibited several interesting characteristics (Fig. 3). At both sediment depths, the δ^{13}C of porewater DOC from the spike seagrass treatment increased (i.e. more positive) during the experiment from field values of about –10‰ to about +50‰, resulting in enrichment factors of Δ +40 to +60‰ (Fig. 3). In general, the δ^{13}C-DOC time series from the seagrass spike treatment was significantly enriched relative to the δ^{13}C-DOC of the bare spike treatment (χ^2 = 23.5, df = 7, p = 0.001). Multiple comparisons tests indicate that samples from seagrass spike Replicate B at 3 cm depth were significantly enriched relative to samples

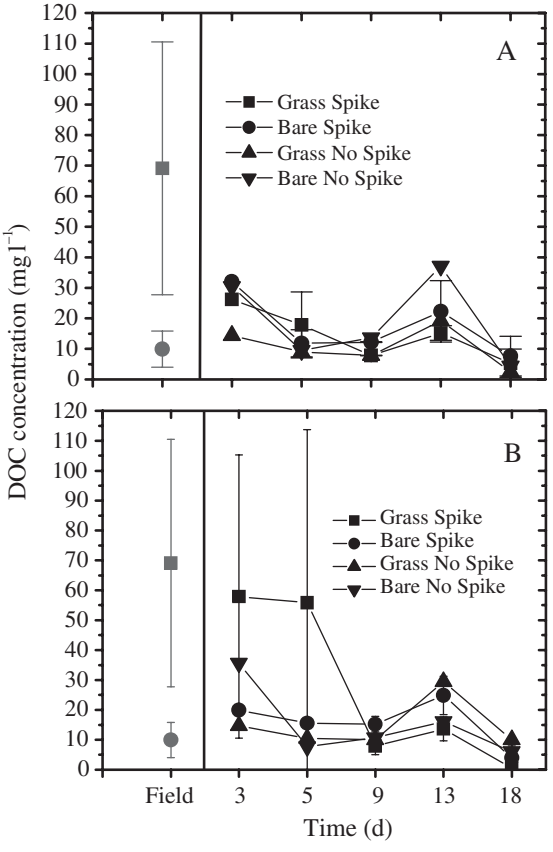

Fig. 2. Time series of sediment porewater dissolved organic carbon (DOC) concentration (mean ± SD, n = 2) from sipper samples at (A) 3 cm sediment depth and (B) 8 cm sediment depth. Also shown for comparison is the average field concentration (n = 4) from bulk sediment analysis (see 'Materials and methods'). Final bulk DOC concentrations were not measured, since the entire sample was required for isotopic analysis

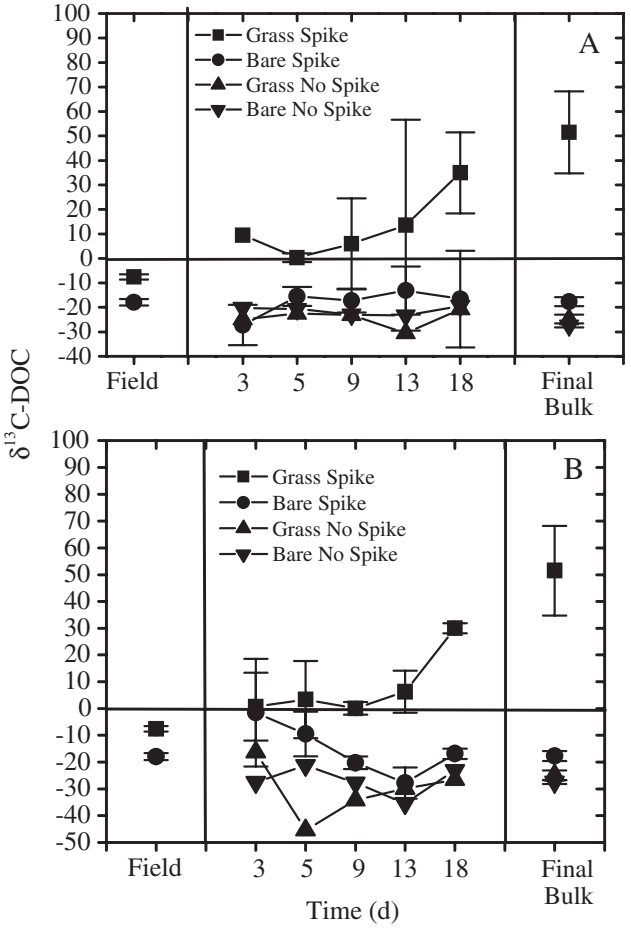

Fig. 3. Time series of δ^{13}C-DOC (mean ± SD, n = 2) from sipper samples at (A) 3 cm sediment depth and (B) 8 cm sediment depth. Also shown for comparison are average field and final δ^{13}C-DOC from pooled bulk porewater samples (n = 4)

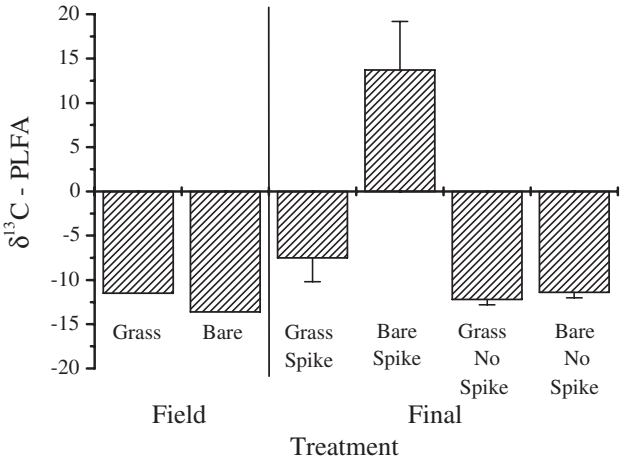

Fig. 4. Sediment-associated bacterial δ^{13}C-PFLA (phospholipid fatty acid mean ± SD, n = 2) at the beginning (field) and end (final) of the experiment

from bare spike Replicate B at 3 and 8 cm sediment depth ($p < 0.05$). In both the seagrass and bare spike treatments, there was no significant difference in the δ^{13}C-DOC between the 3 and 8 cm sipper sampling depths (seagrass $\chi^2 = 5.1$, df = 3, p = 0.19; bare $\chi^2 = 5.88$, df = 3, p = 0.118). As expected, samples from 3 cm sediment depth in the no-spike and bare spike treatments did not exhibit increased isotope ratios (Fig. 3A). The δ^{13}C-DOC of the bare spike treatment at 8 cm sediment depth on Day 3 is about 0‰, representing an unrealistically large enrichment that did not persist (Fig. 3B). This may be an artifact of the sipper sampler leaking and overlying water inadvertently being sampled, since the sipper at 3 cm depth did not exhibit this enrichment. Additionally, the 8 cm depth seagrass no-spike treatment exhibited δ^{13}C-DOC as low as −45‰ (Fig. 3B).

The PLFA biomarker (i&a 15:0) for bacteria was enriched in the spike treatments relative to the no-spike treatments (Fig. 4). The no-spike microcosms exhibited no evidence of isotopic enrichment, and bacterial PLFA was similar to the field samples. The mean i&a 15:0 PLFA from the seagrass spike was −7.5 ± 2.7‰, while that from the bare spike was +13 ± 5.5‰, resulting in enrichment factors of about Δ +4 and +30‰, respectively (Fig. 4). Mann-Whitney rank sum tests indicated that the median δ^{13}C-PLFA of samples from the seagrass spike treatment were significantly enriched relative to the δ^{13}C-PLFA of samples from the seagrass no-spike treatment ($t = 26$, n = 4, p = 0.029). Additionally, the bacteria in the seagrass spike Microcosm A appear to be more enriched than in the seagrass spike Microcosm B, which is probably related to the amount of biomass in the replicate tanks (Table 4). The PLFA biomarker (20:5ω3) for microalgae was detected in most samples; however, the peaks were not large enough to accurately assess the isotope ratio.

Modeling

Inverse analysis simulations were run with 5 to 30% of GPP allocated to rhizodeposition (Table 5). Subsequently, we ran tracer simulations using the output from the inverse analysis for each rhizodeposition rate and compared the tracer output to the empirical data (Fig. 5). Based on the comparison of simulated tracer output to empirical data we chose a range of rhizodeposition rates (Table 5) consistent with the data. Because tracer simulations ran using the inverse analysis flux rates (Table 5) from the 15 to 30% rhizodeposition simulations encompass the empirical data (Fig. 5), we averaged the flux rates from the 15, 20 and 30% rhizodeposition simulations to estimate carbon allocation (Fig. 6).

Table 4. Isotope ratios of the iso- anteiso-15:0 phospholipid fatty acid bacterial biomarker (δ^{13}C-PLFA), the total seagrass biomass in the seagrass microcosm tanks, and the isotope ratio of bulk DOC measured at the end of the experiment

Isotope treatment	Microcosm	Core	δ^{13}C-PLFA (‰)	Seagrass biomass (g dry wt m^{-2})	Bulk δ^{13}C-DOC final (‰) ±SD
Spike	A	1	−5.53	1085.7	+65 ± 0.5
	A	2	−4.85		
	B	1	−10.62	882.9	+52 ± 4
	B	2	−8.81		
No spike	A	1	−12.85	550.7	−22 ± 2
	A	2	−12.23		
	B	1	−11.49	539.4	−26 ± 2
	B	2	−12.04		

The sensitivity analysis tests the effect that each model input variable has on model performance. As expected, the allocation model (inverse analysis) was most sensitive to changes in metabolism associated with large pools; for example, gross primary production, rhizome/root respiration, DOC losses, and bacterial net production efficiency (Table 6). The sensitivity index (S) for the metabolic parameter $S(C_m, p)$ ranged between −12.9 and 18.2%, while the S(RMSD, p) ranged between −6.9 and 7.7% (Table 6). The S(RMSD, p) generally mirrored trends shown by the $S(C_m, p)$ sensitivity parameter.

Tracer simulations exhibit expected patterns of ^{13}C incorporation to the plant tissues (Fig. 5A). The δ^{13}C-DIC decreased throughout the period of simulation as a function of uptake and utilization in photosynthesis. There was a corresponding increase in the isotope ratio of the plant compartments, with much of the tracer being incorporated initially into the leaves and subsequently distributed throughout the plant (Fig. 5A). The ^{13}C-tracer simulation predicts that detectable isotope changes should occur in <24 h, even in the below-ground compartments. Note that the DIC pool is about 30% smaller than the leaf carbon pool (Table A1 in Appendix 1); thus, the draw-down of the DIC was expected. The tracer model simulations using rhizodeposition rates equivalent to 15–30% of GPP reproduced the measured isotope ratios of the sediment porewater DOC pool and the PLFA bacterial biomarker (Fig. 5B). The simulated bacterial pool exhibited a slight enrichment Δ^{13}C-PLFA (Fig. 5B) that was slightly greater than the measured Δ^{13}C-PLFA of +4‰ (Fig. 4). The calculated isotope ratio of the DOC pool also compared well with measured values, since the predicted isotope ratios encompass the empirical data (Fig. 5B).

Using the combined inverse and tracer analysis estimates, we can quantify the amount of seagrass-derived DOC released to the environment. The models suggest that the plants fixed 781 ± 80 mmol C m^{-2} d^{-1} and that about 78% (~614 ± 79 mmol C m^{-2} d^{-1}) was allocated to below-ground tissues (Fig. 6). Leaf DOC losses were

Table 5. Carbon flux rates (mmol C m^{-2} d^{-1}) calculated using inverse analysis. Rhizodeposition rates expressed as a percentage of gross primary production (GPP). [a]Sub-components of equations in Table 1 with same subscripts given here in the form: compartment (C) i to (TO) compartment j

Text description of flux	Equation description flux[a]	Percent of GPP 5%	10%	15%	20%	30%
Production to leaf	CdiTOlf	692.39	692.39	714.53	759.75	869.86
Leaf to respiration	ClfTOdi	104.73	70.11	54.09	54.09	54.09
Short shoot to respiration	CssTOdi	56.11	56.11	56.11	56.11	56.11
Root/rhiz. to respiration	CrrTOdi	384.80	384.80	384.80	384.80	384.80
Leaf to short shoot	ClfTOss	475.53	510.15	548.09	592.86	701.86
Short shoot to root/rhiz.	CssTOrr	419.42	454.04	491.98	536.75	645.76
Leaf to DOC	ClfTOdc	6.92	6.92	7.15	7.60	8.70
Root/rhiz. to DOC	CrrTOdc	34.62	69.24	107.18	151.95	260.96
Leaf to detritus	ClfTOde	52.61	52.61	52.61	52.61	52.61
Short shoot to detritus	CssTOde	0	0	0	0	0
Root/rhiz. to detritus	CrrTOde	0	0	0	0	0
Sed. organic C to DOC	CocTOdc	128.44	132.29	136.75	142.23	155.56
Bacteria to detritus	CbaTOde	16.31	20.15	24.39	29.42	41.65
Bacteria to respiration	CbaTOdi	146.75	181.37	219.54	264.76	374.87
DOC to bacteria	CdcTOba	163.06	201.53	243.93	294.18	416.52
Leaf to growth	ClfTOgr	52.61	52.61	52.61	52.61	52.61
Short shoot to growth	CssTOgr	0	0	0	0	0
Root/rhiz. to growth	CrrTOgr	0	0	0	0	0

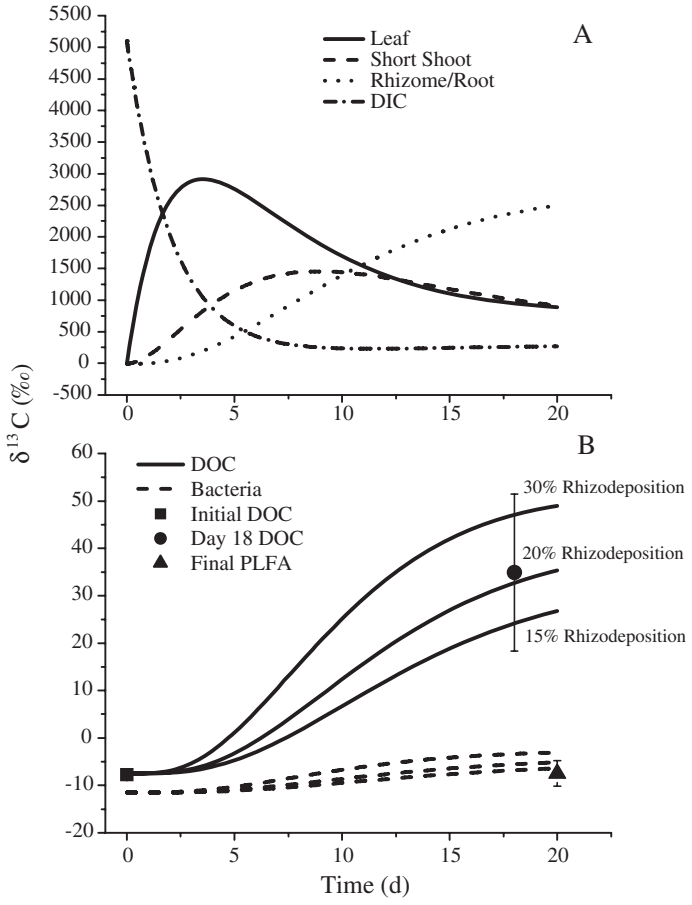

Fig. 5. Isotopic tracer simulations. (A) Predicted $\delta^{13}C$ (‰) for the individual plant compartments (leaves, short shoots, and rhizome/roots), as well as the dissolved inorganic carbon (DIC) pool for the 20% rhizodeposition simulation only. (B) Predicted $\delta^{13}C$ (‰) for the sediment porewater dissolved organic carbon (DOC) pool and bacteria for the 15, 20, and 30% rhizodeposition of gross primary production. Also shown for comparison are measured values of field $\delta^{13}C$-DOC, Day 18 $\delta^{13}C$-DOC and final phospholipid fatty acid (PLFA) isotope values. Error bar is SD

set at 1% of GPP, while root/rhizome DOC losses were estimated to range between 15 and 30% of the gross primary production (Fig. 6). Rhizodeposited DOC accounted for 41 to 61% of bacterial C demand, while decomposition of sediment organic material (detritus) accounted for 38 to 59% of the sediment bacterial carbon demand. Additionally, the flux of DOC from the sediment organic pool and the root/rhizome complex were of similar magnitude (Fig. 6).

DISCUSSION

We conclusively show that in a sub-tropical *Thalassia testudinum* microcosm system, sediment bacteria utilize both DOC exuded from the root/rhizome tissues (rhizodeposition) and sediment organic material derived from detrital *T. testudinum* leaf material (Jones et al. 2003). Our results suggest that about 15 to 30% of the seagrass gross primary production was exuded (rhizodeposited) as bio-available DOC through the below-ground tissues (Fig. 6). These are the highest rhizodeposition rates estimated for seagrasses (Table 7), but are comparable to rates for terrestrial angiosperms (17%; Nguyen 2003).

Boschker et al. (2000) working with temperate *Zostera* species found little evidence of coupling between the seagrass and sediment microbial communities. However, there were important biological and methodological differences between the studies. The *Zostera* spp. enrichments were short-term (24 h) field experiments, compared to our 18 d microcosm enrichments. An additional difference is that our microcosms were under 24 h illumination, which could have resulted in the plants allocating more carbon to rhizodeposition than in the natural environment. However, it is unlikely that the photoperiod in the experiments significantly altered carbon allocation. Independent field studies in Laguna Madre suggest that under natural conditions *Thalassia testudinum* releases so much organic carbon via the below-ground tissues that it diffuses from the sediments and fuels water column

Table 6. Allocation model sensitivity (S). Model was run at parameter values $p = \pm (0.2 \times p_s)$ of a standard case for both C_m (the ratio of system respiration to gross primary production) and RMSD (the root-mean-squared difference). The min. and max. headings represent the sensitivity parameters when an input variable was decreased (min.) or increased (max.) relative to the standard case. The mn and mx indexes denote lower and upper bounds, respectively, for a constraint in the model. GPP: gross primary production; mn: minimum; mx: maximum; resp.: respiration; SS: short shoot; R/R: rhizome/root; Bact.: bacteria; BNPE: bacterial net production efficiency

Parameter	$S(C_m, p)$		S(RMSD, p)	
	Min.	Max.	Min.	Max.
GPP mn	1.4	10.3	−0.6	7.7
GPP mx	0.0	0.0	0.0	0.0
Leaf resp. mn	0.0	0.0	0.0	0.0
Leaf resp. mx	−0.2	0.0	−0.1	0.0
SS resp. mn	0.0	0.0	0.0	0.8
SS resp. mx	−0.3	0.0	−0.8	0.0
R/R resp. mn	0.0	0.0	0.0	6.5
R/R resp. mx	−9.1	0.0	−6.9	0.0
Leaf loss + growth mn	0.0	18.2	0.0	1.4
Leaf loss + growth mx	0.5	0.0	−1.4	0.0
Leaf DOC loss	16.1	0.0	−1.3	0.0
R/R DOC loss	0.0	1.2	0.0	1.4
Bact. resp.	0.0	0.0	0.0	0.0
BNPE mn	0.0	−12.9	0.0	1.1
BNPE mx	−7.2	0.0	−0.6	0.0

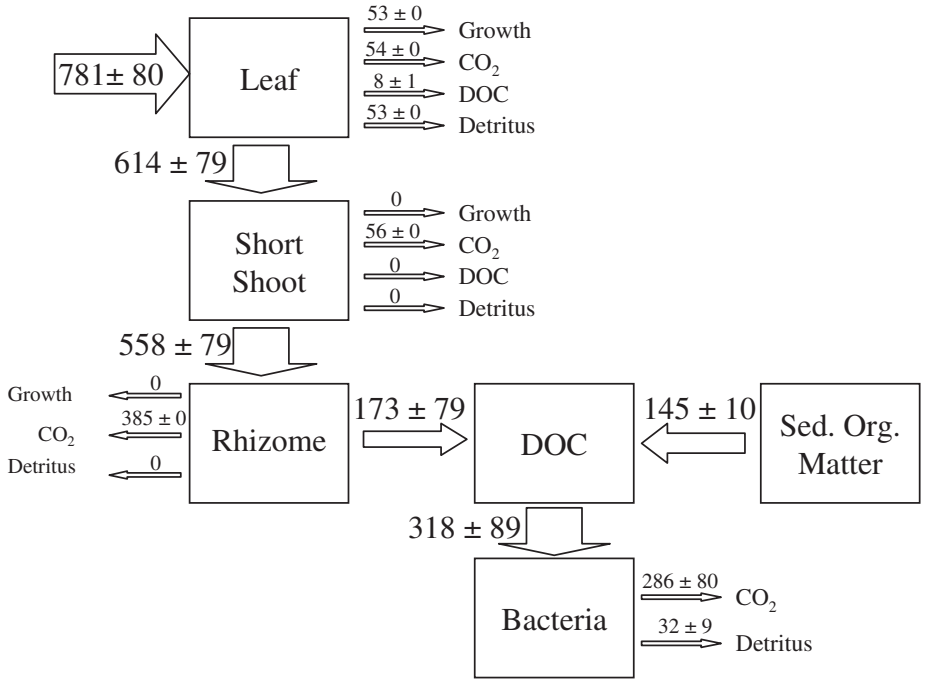

Fig. 6. Box model representation of the carbon allocation model results. All values reported are mean ± SD in mmol C m^{-2} d^{-1}. Short-shoot DOC exudation, rhizome growth, and detritus were assumed to be 0 over the 18 d simulation period

heterotrophy (Ziegler & Benner 1998, 1999). These high rhizodeposition rates may be characteristic of this *T. testudinum* population. Nutrient-limited seagrasses may have reduced growth and photosynthetic production (Agawin et al. 1996), resulting in less carbon fixation and leaf elongation than nutrient-replete populations. If our experimental culture conditions were severely nutrient limited, this could result in reduced rhizodeposition. Previous studies have shown that Laguna Madre is an oligotrophic system with water-column nutrient concentrations typically <3 µM (Ziegler & Benner 1998, 1999, Kaldy & Dunton 2000, Lee & Dunton 2000) and that *T. testudinum* growth is nitrogen-limited in this system (Lee & Dunton 1999). Leaf growth rates measured in the microcosms (Table 3) were comparable to field measurements (Kaldy & Dunton 2000), suggesting that there was enough N available in the microcosms to support leaf growth rates characteristic of the field population. Our laboratory results suggest that rhizodeposition rates for *T. testudinum* may be higher than previously believed. However, as with any study, extrapolation of laboratory results to the field should be exercised with caution (Herzka & Dunton 1997).

Our δ^{13}C-DOC data indicate that measurable enrichment of the DOC pool occurs in <3 d (Fig. 3), and model results suggest that enriched isotope ratios of plant tissue should be detectable within 24 h of exposure. Experiments with *Zostera marina* confirm the rapid enrichment of seagrass below-ground tissue (J. E. Kaldy unpubl. data). Additionally, the architecture and biomass allocation differences between *Z. marina* and *Thalassia testudinum* probably influence seagrass–microbial interactions. *T. testudinum* has a large biomass (500 to 1000 g dry wt m^{-2}) and allocates 85 to 87% of its biomass to below-ground tissues (Table 3) (see also Kaldy & Dunton 2000), while *Z. marina* generally allocates about 20 to 50% to below-ground tissues (Thayer et al. 1984, J. E. Kaldy unpubl. data). The greater isotopic

Table 7. Comparison of literature values for DOC excretion by seagrass. Values expressed as a percentage of gross primary production

Seagrass	Leaves (%)	Root/Rhizome (%)	Source
Thalassia testudinum	1	15–30	Present study
		~1	Wetzel & Penhale (1979)
	1.3		Brylinsky (1977)
T. hemprichii		5.4	Holmer et al. (2001)
Halodule wrightii	1	6–17	Moriarty et al. (1986)
Posidonia oceanica	1.9		Velmirov (1986)
Cymodocea rotundata		7.0	Holmer et al. (2001)
Zostera marina	2		Kirchman et al. (1984)

enrichment of bacteria in the seagrass spike Microcosm A was probably a result of the high *T. testudinum* biomass (Table 4), since larger biomass implies more allocation to below-ground tissues. Consequently, seagrass–microbial coupling appears to be more important in sub-tropical *T. testudinum* systems, and may be related, in part, to plant architecture or biomass partitioning. However, additional studies will be necessary to critically evaluate this hypothesis.

There are a number of organic carbon sources that can contribute to carbon cycling within seagrass beds. Recent work has shown that advective transport of bacteria and microalgae contribute to carbon cycling in permeable sediments (Huettel & Rusch 2000, Rusch & Huettel 2000, Rusch et al. 2001, Precht & Huettel 2003). However, the potential for advective transport of microalgal material into Laguna Madre sediments is limited by detrital leaf material, which forms a barrier and effectively seals off the sediments (J. E. Kaldy pers. obs.). Although the porosity of sediments from the *Thalassia testudinum* bed in Laguna Madre were similar to Huettel's 'permeable sands', the grain size characteristics were different. The sediments were poorly sorted and contained about 30% silt/clay (Dunton et al. 2003), which reduces the permeability. Additionally, stable isotope biomarker studies in LLM found no significant contribution of microalgal carbon in *T testudinum* sediments (Jones et al. 2003).

Several other isotope studies have suggested that there may be a strong linkage between seagrass and sediment microbes (Holmer et al. 2001, Jones et al. 2003); however, these studies could not discriminate bacterial consumption of exuded DOC from detrital seagrass carbon. Our work clearly shows that isotopically enriched seagrass-derived carbon was actively incorporated into bacterial biomass. Low replication of the experimental units (2 microcosms treatment^{-1}) limited our statistical analysis to simple non-parametric comparisons of means and 1-way repeated-measures analysis of ranks. Even though there was a large degree of spatial and temporal heterogeneity and low replication, we still obtained statistically significant differences between treatments.

Most seagrass–sediment interaction research has focused on the importance of photosynthetically derived O_2 in rhizosphere geochemistry (e.g. Pedersen et al. 1998, Eldridge & Morse 2000, Lee & Dunton 2000, Burdige & Zimmerman 2002, Greve et al. 2003, Hebert & Morse 2003). Although O_2 dynamics are important to geochemical processes such as sulfide oxidation at the shoot scale, seagrass–sediment microbial carbon dynamics also impact estuarine scale processes such as ecosystem net heterotrophy (Velmirov 1986, Velmirov & Walenta-Simon 1993, Ziegler & Benner 1998, 1999, Barron et al. 2004, Bouillon et al. 2004). Recent work suggests that DOC diffusing from sediment colonized by *Thalassia testudinum* supported water column heterotrophy (Ziegler & Benner 1998, 1999). The coupling of rhizodeposition and water column heterotrophy further complicates the issues associated with determining the relative importance of allochthonous versus autochthonous production in estuaries, since autochthonous production is usually assumed to be algal (Kaldy et al. 2005). The coupling of seagrass production and water column processes underscores the need for advanced analytical methods such as the δ^{13}C-DOC method to examine the contribution of carbon sources determining estuarine trophic status. In general, seagrass rhizodeposition appears to influence both water column and sediment biogeochemistry in at least some sub-tropical seagrass-dominated systems.

Enrichment experiment

The δ^{13}C-DOC and PLFA results lead to an apparent paradox. In the seagrass microcosms, where the plant acts as a conduit supplying fixed carbon to the sediments, the porewater DOC pool was enriched (Δ +60‰), yet the bacterial PLFA biomarker was only slightly enriched (Δ +4‰). In comparison, in the bare microcosms, the porewater DOC was not enriched, while the bacterial PLFA biomarker was enriched (Δ +30‰). If vascular plants are a conduit to the sediments, why were the bacteria in the bare sediments so enriched? We believe that 'carry-over' caused by core sampling methods can explain the apparent data anomalies. Both replicates of the bare spike developed algal blooms, which probably contributed large amounts of highly enriched DOC and particulate matter to the surface sediments; no blooms were observed in the seagrass tanks. Sediment samples for PLFAs were integrated from the surface to about 10 cm sediment depth. Consequently, bacteria at the sediment surface could have been highly enriched (e.g. maybe >500‰) and even a small amount of highly enriched surface bacteria could easily account for the observed values (Fig. 4). Additionally, the final bulk sediment porewater δ^{13}C-DOC samples, also obtained with cores, were about 10 to 20‰ more positive than the sipper samples obtained on Day 18 of the time series. This additional enrichment was likely a result of highly enriched DOC from overlying water collected with the core. It is possible that the enrichment was caused by microalgae, but this is unlikely since the quantity of microalgal PLFA biomarker (20:5ω3 PLFA) was so small that the isotope ratio could not be measured. Sampling methods are the only reasonable explanation, since there is no other transport mechanism (including diffusion) that can account for these dis-

crepancies. Future studies should examine the vertical distribution of isotope tracer in the sediments and isotope ratios of metabolic by-products (e.g. CO_2).

In the enriched seagrass microcosms we expected to find highly labeled sediment bacteria; however, our data indicate that the porewater DOC pool was more enriched than the bacteria. Our modeling results, which incorporate typical bacterial physiology, also exhibited only a slight enrichment. Although the bacterial biomarker was not highly enriched, the δ^{13}C-PLFA in the seagrass spike treatments was significantly ($p < 0.05$) enriched relative to seagrass no-spike treatments. The slight enrichment of the δ^{13}C-PLFA biomarker can be explained based on pool sizes (e.g. isotope dilution) and DOC reactivity. The bacterial biomass in the seagrass microcosm sediments was roughly twice that in the bare sediments (Table 3). A field study in LLM found that bacterial abundance in vegetated sediments was 3-fold greater than in adjacent unvegetated sediments (Jones 2001). Consequently, the bacteria would have to turn-over a large unenriched C pool (e.g. unenriched detrital seagrass material, unenriched bacterial C, unenriched labile DOC) before the rhizodeposited ^{13}C-signal would be detectable in the bacteria. Effectively, the ^{13}C-signal was probably diluted by the presence of a large unenriched C pool. This hypothesis is supported by our modeling efforts (Fig. 5B), which indicate that, depending on the rate of rhizodeposition, it takes 5 to 10 d before there is a noticeable change in the isotope ratio of the bacteria. Additionally, bacterial degradation changes the chemical composition and bioreactivity of DOC, which results in the build up of refractory DOC pools (Amon et al. 2001). Exudates from terrestrial plants tend to be low molecular weight compounds like simple sugars and organic acids (Baudon et al. 2003). It is likely that seagrasses also rhizodeposit simple sugars and organic acids, since simple sugars are the dominant carbohydrate storage compounds (Touchette & Burkholder 2000). Thus, bioreactive isotopically enriched DOC may have been rapidly degraded, leaving behind a highly enriched refractory DOC pool (e.g. enriched refractory by-products or 'leftovers').

Our carbon stable isotope enrichment experiment clearly illustrates the pathway of carbon from the water column into the sediment porewater DOC and, ultimately, into the i&a 15:0 PLFA bacterial biomarker. This experiment also suggests that there were other interesting biogeochemical processes occurring in the sediments. Our data from the seagrass no-spike microcosms suggest that at times during the experiment the sediments were anoxic and that methanogenic/methanotrophic bacteria may have been important. For example, the dramatic decrease in the isotope ratio of DOC (–45‰) in the seagrass no-spike treatment is consistent with reports in the literature of biogenic methane ranging between –80 and –60‰ (Summons et al. 1994). Field samples from the same geographic region also suggest that methanotrophic bacteria may contribute to ^{13}C-depletion in vegetated sediments (Jones et al. 2003). Clearly, the interactions between seagrasses and anoxic geochemical cycling will require additional research.

Modeling

Inverse analysis has been used to examine the flux of materials between compartments in general (Bennett 1992, Parker 1994) and, particularly, for marine food webs (Vezina & Platt 1988, Jackson & Eldridge 1992, Vezina & Pace 1994, Vezina et al. 1997, 2000). We applied the inverse analysis framework to an experimental seagrass system in order to quantify the linkage between seagrasses and microbial communities. The strength of inverse analysis is that it can predict unmeasured flow of materials between compartments in a manner that is consistent with empirical constraints. To our knowledge, this is the first application of inverse analysis to a estuarine plant/sediment/bacteria system. Using a series of relatively easily measured parameters (e.g. above- and below-ground biomass, leaf growth rates) and several assumptions based on published literature, we estimated the allocation of carbon resources to various plant (leaf, rhizome, root) and environmental (porewater, water column) compartments. We found that modeled rhizodeposition rates of 15 to 30% GPP were consistent with empirical DOC and bacterial PLFA isotope data from our experiment. Lepoint et al. (2004) suggest that bacterial biomarkers and stable isotopes represent a new horizon for understanding organic fluxes in seagrass ecosystems. In the present study, we showed that coupling these powerful empirical tools with models can provide valuable insight into ecological processes, such as determining the contribution of multiple carbon sources (rhizodeposited DOC versus detrital DOC) supporting sediment bacteria. Integrative studies using biomarkers, isotopic tracers, and models are important for studying the linkages between macrophytes and bacteria (Lepoint et al. 2004, Van den Meersche et al. 2004).

Although models, coupled with experimental data, can be useful, they are not a panacea. Because models are a simplification they are subject to limitations. It is not possible to measure everything, and, as a result, there is a need to estimate some parameters based on literature values, which causes uncertainty. Identifying the most sensitive model parameters gives an indication of how robust the model simulations are and helps

to focus future research. The metabolic sensitivity parameter $S(C_m, p)$ examines only the response to catabolic and anabolic carbon inputs, whereas the generalized sensitivity parameter S(RMSD, p) is an integration of all flows and, hence, shows a smaller response to single parameter changes than $S(C_m, p)$ (Table 6). S values of 100% indicate that a 20% change in an input parameter causes a 20% change in the model output. Our sensitivity analysis found that all S values were <20%, indicating that the allocation model is very robust to variations in the input parameters. As expected, the input variables that had the largest influence on the model output were the metabolic rates associated with large carbon pools, such as gross primary production, rhizome/root respiration, leaf loss, DOC loss, and bacterial net production efficiency.

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Appendix 1. Tracer model

This stable isotope tracer model is a modification of the Jackson & Eldridge (1992) tracer analysis. To develop the tracer analysis we first create a vector ***mass*** in which the elements $i = 1 \rightarrow 8$ represent the biomass of each compartment used in the allocation model (Table A1) and an extra compartment for growth and detritus (Compartment 8 in Table A1). The results of the inverse analysis are placed in a vector ***flows***, and corresponding vectors ***to*** and ***from*** are created to represent the source and recipient compartment from the allocation model. The elements of ***to*** and ***from*** are the indexes from the vector ***mass***. The vectors ***flow***, ***to***, and ***from*** are all of length n = number of allocation model flows. A new matrix ***RATE*** is developed from the vectors ***mass***, ***flow***, ***to***, and ***from*** such that:

$$RATE = \sum_{i=1}^{n} \left[\frac{flow_i}{mass_{from_i}} \right]_{to_i, from_i}$$

RATE is an 8 × 8 matrix that includes growth and detritus in the last row and column. We sum the first 7 rows of ***RATE*** to retrieve the kinetic rate for each compartment i for which there is a corresponding biomass:

$$r_i = \sum_{j=1}^{8} RATE_{i,j}$$

and then we eliminate the last row and column of ***RATE*** so that only the compartments of interest are left. The initial conditions for the model all consist of 0-values, except for the DIC compartment in which a 1 is placed; this is the compartment in which the tracer is initially added. The amount of tracer (Y) in each compartment at each time point (T) is solved with a matrix equation:

$$\frac{dY}{dt} = RATE \cdot Y - Y \cdot r^T$$

Table A1. Parameters used in the tracer model, carbon flow description as in Table 5. Compartment 8 is for release of the tracer to non-carbon cycling pools. NR: not required; DIC: dissolved inorganic carbon; DOC: dissolved organic carbon

	Compartment	Concentration (mmol C m^{-2})	$\delta^{13}C$
1	Leaf (lf)	4185	–10.8
2	Short shoot (ss)	3195	–6.3
3	Root/Rhizome (rr)	12557	–6.3
4	Bacteria (ba)[a]	64	–11.5
5	DIC (di)	1617	–1.6
6	DOC (dc)[b]	967	–7.5
7	Sediment organic mater (oc)[c]	198750	–13.0
8	Export/import (i.e. detritus, growth)	0	NR

[a]Bacterial carbon calculated assuming 4.62×10^{10} cells g^{-1} sediment (Table 3) and 0.043 pg C $cell^{-1}$ (Bratbak 1985)
[b]Sediment DOC concentration assumed porosity of 0.7 with 1 SD
[c]Sediment organic matter assumed 2% organic mater with a porosity of 0.7 and a 15 cm sediment layer in the mesocosm

The system of differential equations is solved using an ODE solver from Matlab (Mathworks; www.mathworks.com). The tracer results are now scaled between 0 and 1. To express the results in δ notation, the output vector ***y*** is multiplied by the initial ^{13}C-enrichment ($\delta^{13}C = 5100$) and the natural abundance $\delta^{13}C$ of each compartment is added to the appropriate column (j) of Y:

$$\delta Y = 5100 Y + \delta^{13} C_j Y_j$$

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