Microbial Utilization of Estuarine Dissolved Organic Carbon: a Stable Isotope Tracer Approach Tested by Mass Balance

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The natural stable isotope values of different plants have been used to trace the fate of organic carbon that enters estuarine ecosystems. Experiments were designed to determine the magnitude of $\delta^{13}\text{C}$ changes of dissolved organic carbon (DOC) derived from tidal marsh vegetation that occurred during bacterial decomposition. Bacteria were grown on DOC leached from estuarine *Spartina alterniflora* and *Typhus angustifolia* plants. In four experiments, 25 to 80% of the initial carbon (2.6 to 9.1 mM organic C) was converted to bacterial biomass and CO_2 . Mass balance calculations showed good recovery of total C and ^{13}C at the end of these experiments ($100\% \pm 14\%$ total C; $\pm 1\%$ $\delta^{13}\text{C}$). The $\delta^{13}\text{C}$ values of DOC, bacterial biomass, and respired CO_2 changed only slightly in the four experiments by average values of -0.6 , $+1.4$, and $+0.5\%$, respectively. These changes are small relative to the range of $\delta^{13}\text{C}$ values represented by different organic carbon sources to estuaries. Thus, microbial $\delta^{13}\text{C}$ values determined in the field helped to identify the source of the carbon assimilated by bacteria.

The composition of dissolved organic carbon (DOC) varies along estuarine salinity gradients as the result of water masses from freshwater and saltwater sources mixing, in situ production by phytoplankton and macrophytes, lateral inputs from surrounding marshes, and microbiological processing. Analyses of $\delta^{13}\text{C}$ -DOC and $\delta^{13}\text{C}$ -dissolved inorganic carbon in the Parker River estuary ($\delta^{13}\text{C}$ -DIC) (7, 21) have revealed that carbon distributions along the seaward course of an estuary are dynamic and not simply predicted by the mixing of DOC in the freshwater and seawater end members. Inputs of DOC from algal blooms and the *Typhus angustifolia* and *Spartina alterniflora* marshes that surround the estuary (7, 21) produce significant departures from the DOC distribution expected from conservative mixing of river and ocean water. Elevated bacterial production can result from marsh DOC inputs (13, 24).

The carbon which supports microbial production is a small fraction of the total DOC pool (14). These sources of available DOC can vary in importance seasonally or over a tidal cycle. Since the bulk DOC pool is made up of several sources of carbon with various degrees of microbial lability, the component that supports microbial production is hard to identify. However, the stable isotope value of estuarine bacteria reflects the isotopic composition of that portion of the bulk DOC pool that is available for microbial growth (5, 16). Therefore, stable isotope analysis of bacteria may provide evidence for sources of carbon which support microbial production in ecosystems.

We performed a series of experimental tests of the feasibility of using $\delta^{13}\text{C}$ values to trace the origins of carbon used to support bacterial productivity in estuaries. Four experiments were conducted with DOC leached from *T. angustifolia* and *S. alterniflora* to determine how these sources of estuarine DOC were processed by bacteria and whether the resultant CO_2 (DIC), bacterial biomass, and residual DOC pools displayed

significant ^{13}C fractionation versus the initial DOC pool. The mass and stable isotope ratio of the initial DOC, respired CO_2 , bacterial biomass, and residual DOC were measured. Isotopic variation was quantified by using mass balance. The objective was to determine whether microbial biomass and CO_2 were isotopically similar to the substrate used for growth. If so, field measurements of the isotopic composition of microbes could be a valuable tool to assess the source of carbon that supports secondary production.

MATERIALS AND METHODS

Experimental design. Experiments were designed to assess whether the photosynthetic mode (C_3 or C_4 ; *S. alterniflora* or *T. angustifolia*) or plant senescence could affect bacterial use and isotopic fractionation of DOC. The carbon concentrations and $\delta^{13}\text{C}$ values of DOC, respired CO_2 , and bacterial biomass were independently measured to compute a complete carbon and isotope mass balance. Experiments 1 and 2 compared the bacterial decompositions of *T. angustifolia* (C_3) and *S. alterniflora* (C_4) plants. Experiments 3 and 4 were designed to compare the bacterial decomposition of *T. angustifolia* plants in different states of senescence.

Sample preparation. During July 1990, fresh green *S. alterniflora* and *T. angustifolia* leaves (experiments 1 and 2) were collected from marshes next to the Parker River in Massachusetts. During July 1991, fresh green and senescent *T. angustifolia* leaves (experiments 3 and 4) were collected from marshes in Woods Hole, Mass. Samples were cut into 2.0-cm pieces. Fifty grams of green vegetation (experiments 1, 2, and 3) and 100 g of senescent *T. angustifolia* (experiment 4) were macerated in a blender with the inorganic salt solution described below. Large particles were removed from the leachate with a coarse mesh net (cheesecloth), and the leachate was filtered through a 0.22- μm -pore-size cartridge filter (Whatman Polycap 75).

Salt solution. Leaves were extracted in an inorganic salt solution (pH 7.8) since natural estuarine waters have high DIC concentrations that would obscure the stable isotope values of respired DIC. The salt solution consisted of distilled deionized water with final concentrations of 10 mM phosphate buffer, 20.0 mM Na_2SO_4 , and 1.0 mM KNO_3 . For the first set of experiments (experiments 1 and 2), sodium chloride was added to 15‰. Experiments 3 and 4, with the freshwater marsh plant *T. angustifolia*, were conducted without NaCl. To remove labile carbon in the stock solution, an inoculum of estuarine sediment was added to the medium for 24 h before each experiment was started. The solution was subsequently filtered through a 0.2- μm -pore-size filter to remove the initial inoculum and any cells that had grown on the labile DOC in the medium.

Experimental procedure. Plant extracts were added to prefiltered mineral salts solution. The dilute extracts were inoculated with 0.6 μm of filtered Parker River water to a final concentration of 2% (vol/vol). In experiments 1 and 2, dilute *S.*

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alterniflora and *T. angustifolia* extracts were distributed into two 10-liter carboys and 60-ml gastight serum bottles. Samples were incubated in the dark for 48 h at 25°C. $\delta^{13}\text{C}$ -DOC, DOC, bacterial biomass, and bacterial counts were measured in the 10-liter containers, while DIC plus $\delta^{13}\text{C}$ -DIC were assayed from parallel samples in serum bottles. In experiments 3 and 4, 500-ml aliquots of senescent and green *T. angustifolia* extracts were added to three replicate 2-liter glass jars. $\delta^{13}\text{C}$ -DOC, DOC, bacterial biomass, and bacterial counts were measured. Parallel bottles for analysis of DIC plus $\delta^{13}\text{C}$ -DIC were incubated in 250-ml bottles with 125 ml of inoculated leachate. Samples were incubated in the dark at 25°C for 36 h. Control treatments, consisting of mineral salts without plant leachate, were inoculated and incubated with the other treatments. DOC, DIC, and cell biomass were sampled initially and after 36 h to measure bacterial metabolism without plant leachate addition. Separate bottles were used for DIC measurements to prevent the exchange of respired CO_2 with the atmosphere during experiments.

Measurement of bacteria. Bacteria were filtered onto precombusted GF/F filters (Whatman) for concentration and stable isotope analyses. Filters were spotted with a solubilized platinum (Fisher) catalyst and dried at 60°C overnight. Twenty-five percent of each filter was removed for analysis. Filter pieces were combusted to CO_2 , which was then cryogenically distilled. The concentrations were measured manometrically, and stable isotope values were measured on a Delta-S mass spectrometer (Finnigan Corp.). Total bacterial carbon was corrected for carbon contamination in GF/F filters. Samples (10 ml) for bacterial cell numbers were removed from bioassay bottles every 6 h, preserved with 2% formaldehyde, and refrigerated until counted by acridine orange and epifluorescence microscopy (10).

DIC. Microbially respired CO_2 was estimated as the difference in the amounts of DIC measured at the beginning and end of incubations. CO_2 was stripped from acidified samples under vacuum by the method of Kroopnick (16). The $^{13}\text{C}/^{12}\text{C}$ ratio of CO_2 was then measured on a mass spectrometer (Finnigan Corp.).

When CO_2 was distilled from the *S. alterniflora* addition experiment (experiment 1), a yellow gas coeluted with CO_2 . The yellow gas was separated from respired CO_2 by distillation into cold pentane. Subsequent comparative studies have shown that there was a slight fractionation of DIC during this process. A correction for this fractionation was made by using a linear regression between samples collected along an estuarine salinity gradient and analyzed by cryogenic distillation with and without pentane.

DOC. For stable isotope analysis of DOC, 25 ml of the experimental medium was sampled at the beginning and end of each experiment. Samples were filtered through a precombusted GF/F filter and then through a 0.2- μm -pore-size filter (Millipore) which had been leached in 10% HCl overnight. Stable isotope values and concentrations of DOC in the filtrate were measured by a sealed-tube combustion method (8).

Blank corrections. All experiments (experiments 1 through 4) were corrected for bacterial growth and metabolism attributed to organic contamination in the mineral salts medium. Control concentrations (no plant leachate addition) were directly subtracted from the carbon concentrations in treatment flasks. Stable isotope values from the unamended control treatments were used in mass balance calculations to derive the stable isotope values due solely to bacterial growth on the carbon from plant leachate additions. These values represented 2 to 3% of the values from experimental treatments.

Some DOC was retained by filters and would have led to erroneous stable isotope measurements and overestimations of bacterial carbon yields. Consequently, we used two procedures to estimate DOC sorption effects. First, we collected the 0.2- μm filtrate which sorbed to GF/F filters prior to inoculation. These blanks were 2 and 6 μmol of C in the green *T. angustifolia* and brown *T. angustifolia* experiments (experiments 3 and 4), respectively. Second, we measured colloidal DOC absorbed to filters after bacterial growth. After the bioassay was harvested onto a GF/F filter, the filtrate was passed through a 0.2- μm -pore-size filter to remove bacteria that had not been retained on the GF/F filter. The 0.2- μm filtrate was filtered through a second GF/F filter that was then combusted. The amounts of carbon that sorbed to the second GF/F filter after growth were 1 and 2 μmol of C for green (experiment 3) and senescent *T. angustifolia* (experiment 4), respectively. These values represent a range of carbon concentrations due to the sorption of leachates on GF/F filters.

Calculations. Stable isotope ratios are reported as $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$ where $X = ^{13}\text{C}$ and $R = ^{13}\text{C}/^{12}\text{C}$. The isotope mass balances for experiments were calculated as follows: $\delta^{13}\text{C}_{\text{DOCfinal}} = [(\delta^{13}\text{C}_{\text{DOCinitial}} \times M_{\text{DOCfinal}}) + (\delta^{13}\text{C}_{\text{DIC}} \times M_{\text{DIC}}) + (\delta^{13}\text{C}_{\text{Biomass}} \times M_{\text{Biomass}})] / (M_{\text{DOCfinal}} + M_{\text{DIC}} + M_{\text{Biomass}})$, where mass (M) and $\delta^{13}\text{C}_x$ were measured values. The calculated initial $\delta^{13}\text{C}$ values were compared directly with the measured initial $\delta^{13}\text{C}_{\text{DOC}}$ values.

The fractionation factor (18) for the substrate (plant leachate) remaining after bacterial growth is an estimate of the kinetic isotope effect. The fractionation factor (ϵ) was calculated as $\epsilon = (\delta^{13}\text{C}_{\text{DOCfinal}} - \delta^{13}\text{C}_{\text{DOCinitial}}) / (\ln[\text{DOC}_{\text{final}}] / [\text{DOC}_{\text{initial}}])$. The calculated fractionation factor is independent of the degree of DOC utilization if the substrate behaves as a homogeneous pool.

Statistics. The treatment effects on the variables DIC, DOC, and biomass were assessed by one-way analysis of variance. To normalize treatments for the variable amounts of leachate added, concentrations were analyzed as $[\text{DIC}/\text{DOC}_{\text{initial}}]$, $[\text{Biomass}/\text{DOC}_{\text{initial}}]$, and $[\text{DOC}_{\text{final}}/\text{DOC}_{\text{initial}}]$ and arcsine trans-

FIG. 1. $\delta^{13}\text{C}$ values of final carbon pools relative to that of initial DOC in four growth experiments. Data are means $\pm$ standard deviations. *, significantly different ($P < 0.05$) from the values of other treatments. ■, *S. alterniflora* (experiment 1); □, green *T. angustifolia* (experiment 2); ▨, green *T. angustifolia* (experiment 3); ▩, senescent *T. angustifolia* (experiment 4).

formed (15). Changes in isotope values due to microbial metabolism were analyzed as $\delta^{13}\text{C}_{\text{DOCfinal}} - \delta^{13}\text{C}_{\text{DOCinitial}}$, $\delta^{13}\text{C}_{\text{Biomass}} - \delta^{13}\text{C}_{\text{DOCinitial}}$, and $\delta^{13}\text{C}_{\text{DICfinal}} - \delta^{13}\text{C}_{\text{DOCinitial}}$. Scheffe's mean test was used to analyze differences among treatments (15).

RESULTS

The results for the $\delta^{13}\text{C}$ of the DOC pool were significantly different among experiments 1 through 4 ($F = 57.5$; $P < 0.001$; $n = 10$) (Fig. 1). The final DOC pool in the *S. alterniflora* addition experiment (experiment 1) was significantly different (Scheffe's; $P < 0.05$) from the rest of the treatments, as was the *T. angustifolia* addition in experiment 2 (Scheffe's; $P < 0.05$). When the isotope values of the leachate were compared with the whole-plant values, the DOC derived from *T. angustifolia* (Table 1 [experiments 2, 3, and 4]) was enriched in ^{13}C (relative to whole plants) by 0.5 to 2.2‰, whereas leachate from *S. alterniflora* was depleted in ^{13}C by 2.8‰.

Microbial use of DOC was reflected in the isotopic values of bacterial biomass and DIC. Bacterial growth on DOC did not significantly change the $\delta^{13}\text{C}$ of the DOC pool in the green and senescent *T. angustifolia* (experiments 3 and 4, respectively) treatments. These experiments showed the most consistent replication of isotope values, while experiments 1 and 2 showed some unexpected features. In the green *T. angustifolia* treatment (experiment 2) (Scheffe's; $P < 0.05$), the stable isotope value of respired DIC was significantly different from that of the initial DOC pool ($F = 74.66$; $P < 0.0001$; $n = 10$) (Fig. 1). The isotope values from the other treatments reflected the isotope values of the initial DOC pool (experiments 1, 3, and 4) (Fig. 1). The changes in the $^{13}\text{C}/^{12}\text{C}$ ratio of bacterial biomass were significantly different among the different experimental treatments ($F = 21.71$; $P < 0.001$; $n = 10$) (Fig. 1). The largest change in stable isotope values of the bacterial biomass occurred in the *S. alterniflora* treatment (experiment 1) (Scheffe's; $P < 0.05$) (Fig. 1). The poor mass balance in the *S. alterniflora* experiment (experiment 1) may be due to errors in blank corrections with the pentane slush or yellow gas that was not quantified. The $\delta^{13}\text{C}$ of unaccounted-for carbon would have been -22.3‰ in experiment 1. In the *T. angustifolia* treatments (experiments 2, 3, and 4), the bacterial biomass reflected the initial DOC pool. There was no significant fractionation between the initial DOC pool and bacterial biomass in the green *T. angustifolia* and senescent *T. angustifolia* treat-

TABLE 1. Mass balance in four bacterial growth experiments with DOC leached from estuarine plants^a

Parameter	C (μM)				δ ¹³ C			
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 1	Expt 2	Expt 3	Expt 4
Whole plant					-13.0	-27.8	-27.4	-27.2
Initial DOC	3,140 ± 71	2,591 ± 18	4,315 ± 36	9,089 ± 186	-15.8 ± 0.3	-25.6 ± 0.1	-26.9 ± 0.1	-26.3 ± 0.2
Final DOC	628 ± 5	1,053 ± 89	3,129 ± 69	6,843 ± 159	-14.8 ± 0.1	-27.8 ± 0.5	-27.3 ± 0.1	-27.1 ± 0
Final cell biomass	799 ± 239	585 ± 29	636 ± 35	1,306 ± 177	-13.0 ± 0.9	-25.2 ± 0.2	-26.1 ± 0.2	-24.7 ± 0.1
Final respired CO ₂	1,287 ± 189	803 ± 6	606 ± 54	972 ± 139	-15.7 ± 0.1	-23.0 ± 0.2	-27.2 ± 0.1	-26.6 ± 1.0
Final recovery ^b	2,714 (86)	2,441 (94)	4,371 (101)	9,121 (100)	-14.7 (1.1)	-25.6 (0)	-27.1 (-0.2)	-26.7 (-0.3)

^a Experiments 1 and 2 (*S. alterniflora* and *T. angustifolia*, respectively) were conducted in 1990 with two replicates per experiment, and experiments 3 and 4 (green *T. angustifolia* and senescent *T. angustifolia*, respectively) were conducted in 1991 with three replicates per experiment. Each pool was independently measured. Data are means ± standard deviations.

^b Data are total C at the end of each experiment (DOC + cell biomass + respired CO₂), with parenthetical values showing the percentages of the initial DOC, and the weighted average δ ¹³C_{DOCfinal} [(δ ¹³C_{DOCfinal} × M_{DOCfinal}) + (δ ¹³C_{DIC} × M_{DIC}) + (δ ¹³C_{Biomass} × M_{Biomass})] / (M_{DOCfinal} + M_{DIC} + M_{Biomass}), where M is mass and δ ¹³C_x are measured values, with parenthetical values showing the per mille changes from initial DOC values.

ments (experiments 3 and 4, respectively) or either green *T. angustifolia* treatment (experiments 2 and 3).

The amounts of DOC used for microbial growth were significantly different among plant leachates ($F = 792.3$; $P < 0.0001$). DOC consumption was high in the *S. alterniflora* leachate treatment (Scheffe's; $P < 0.05$) (experiment 1), and significantly smaller amounts of DOC were used by bacteria growing in the brown and green *T. angustifolia* treatments (Scheffe's; $P < 0.05$) (experiments 3 and 4). However, bacterial biomass was the same for all treatments ($F = 1.2$; $P < 0.4$); bacterial respiration after growth on different plant leachates varied significantly ($F = 74.7$; $P < 0.0001$). Respiration was significantly higher in the *S. alterniflora* leachate experiment ($P = 0.05$).

Reasonable recoveries were obtained from parallel bioassay and DIC incubations. The recoveries (respired CO₂, cell biomass, and residual DOC combined) were 86 to 94% of the initial plant leachate added to the *S. alterniflora* and *T. angustifolia* experiments in 1990 (Table 1). One hundred percent of the carbon added as DOC was accounted for in the green and senescent *T. angustifolia* treatments of 1991. The ¹³C mass balance for experiments predicted the measured δ ¹³C value for initial DOC within 1‰ (Table 1).

Bacterial growth efficiencies were high. In experiments 1 and 2, growth efficiency [bacterial carbon yield/(DOC_{initial} - DOC_{final})] ranged from 32 to 37% of the *T. angustifolia* and *S. alterniflora* leachates consumed and respired carbon was about 52% of the DOC consumed (Table 1). The final yields of cells at early log growth phase in experiments 1 and 2 were 1.53×10^8 and 8.2×10^7 cells per ml, respectively. In experiments 3 and 4, respiration accounted for 43 to 51% of the DOC consumed. The growth efficiency was 54 to 58% for green and senescent *T. angustifolia* treatments. The final yields of bacterial cells were 6.4×10^7 and 1.2×10^8 cells per ml in experiments 3 and 4, respectively.

In general, the fractionation factors were small and positive (Fig. 2), averaging +1.8‰ for products and +1.4‰ for DOC. The positive fractionation factors are unusual and may indicate the selective use of molecules enriched in δ ¹³C.

DISCUSSION

Microbial metabolism of DOC can be elucidated by using a mass balance approach which employs both mass and stable isotope analyses. One can measure how bacterial respiration and production change the δ ¹³C value of the undegraded DOC pool. Deviations from the expected mass balance indicate areas of unusual metabolic properties or methodological

weakness. In this study, we measured each of the components necessary for a mass balance. For example (Table 1), assume that the initial DOC of green *T. angustifolia* (experiment 3) equals the residual DOC plus the bacterial biomass plus the respired CO₂. The measured value for the initial DOC was -26.9‰. The predicted value for the initial DOC calculated from the mass balance of carbon concentrations and δ ¹³C of the product pools was -27.1‰. This close agreement between the direct measurement and the calculated value indicates that all of the δ ¹³C present initially was measured in the product pools. Similarly, 86 to 100% of the DOC added in experiments could be accounted for after bacterial growth (Table 1).

Differences in stable isotope values were found between whole plants and the dissolved organic matter leached from them. The *S. alterniflora* leachate (-15.8‰) was more depleted in ¹³C than the *S. alterniflora* plant (-13.0‰). Coffin et al. (5) also found that the leachate from *S. alterniflora* was depleted in ¹³C. Others (2) have found that leachates of C₄ plants which included lipids, carbohydrates, and amino acids were depleted by 1 to 4‰ relative to whole-plant material. The *T. angustifolia* leachate was slightly more enriched than the whole plant, which is similar to the results for a leachate from a C₃ plant (2, 6). Senescence did not affect the isotope values of the *T. angustifolia* plant or leachate. Haines (9) also found very little difference in δ ¹³C between standing dead marsh plants and green marsh plants in a Georgia saltmarsh.

FIG. 2. Fractionation factors (ε) calculated for products (respired CO₂ and bacterial biomass) and residual DOC for bacterial growth experiments with DOC leached from estuarine plants (as identified in the legend to Fig. 1). Data are the means ± standard deviations of three replicates.

Bacterial cells growing on a single carbon compound generally reflect the $\delta^{13}\text{C}$ of the source (3, 6, 11). In this study, bacteria growing on a suite of compounds leached from *T. angustifolia* had the same isotope value as the leachate. The $\delta^{13}\text{C}$ of bacteria growing on the *T. angustifolia* leachate was the same as the stable isotope signature of the *T. angustifolia* leachate. The senescence of *T. angustifolia* did not alter the stable isotope signature of the bacterial biomass relative to DOC. However, cells grown on the *S. alterniflora* leachate were significantly enriched ($>2\text{‰}$) in ^{13}C . Biomass enriched in ^{13}C could be due to the selective assimilation of isotopically enriched amino acids. Macko and Estep (17) found that when a microorganism was grown on glycine and NO_3^- , the $\delta^{13}\text{C}$ of cells was 6 to 9‰ heavier than that of the starting material. *S. alterniflora* has an average of 120 $\mu\text{mol/g}$ (fresh weight) of the osmoregulatory amino acid betaine (*N,N,N*-trimethylglycine) (4). Preferential metabolism of an amino acid like glycine betaine (12, 17, 22) could result in a bacterial biomass enriched in ^{13}C .

Previous studies using one bacterial species grown on a single carbon source have reported that CO_2 is usually more depleted in ^{13}C than is the substrate (1, 3). However, in this study, the stable isotope ratio of respired CO_2 was either the same as that of the initial DOC pool or was significantly enriched in ^{13}C (Fig. 1). When a microbial community grows on mixed substrates, the respired CO_2 reflects a weighted average of the various carbon pools and the multiple metabolic pathways involved in their catabolism. The stable isotope value of the CO_2 from mixed substrates probably depends upon the type of molecule being catabolized, the proportional use of compounds due to microbial selectivity, and any kinetic isotope effects (3). In comparison, studies with higher organisms also found that the respired CO_2 reflected the $\delta^{13}\text{C}$ of the mixed diet (1, 19, 20).

Selectivity by microbes for a portion of the DOC pool can be reflected in the fractionation factor. In a typical isotope effect, the products are depleted in ^{13}C relative to the initial substrate (18). Any deviation from this pattern suggests selective use of the substrate pool or incomplete measurement of the components involved. In the *T. angustifolia* treatments, the products (DIC + biomass) were enriched in ^{13}C by an average of +1.5‰. The reverse isotope effect for the *T. angustifolia* treatments (Fig. 2) suggests selective use of a portion of the *T. angustifolia* DOC.

The bacterial growth efficiency (biomass C produced/DOC assimilated) was considerably higher than those calculated for bacteria with natural concentrations of DOC (14), perhaps reflecting nonlimiting growth conditions in the bioassays. Growth efficiency ranged from 32 to 37% for *S. alterniflora* and *T. angustifolia* (experiments 1 and 2), and respired carbon was about 52% of the DOC consumed. Growth efficiency was about 55% for green and senescent *T. angustifolia*. In experiments 3 and 4, respiration accounted for 43 to 51% of the DOC consumed. Studies in which *Escherichia coli* was grown on glucose have shown growth efficiencies (3) and respiration yields (3, 23) similar to those in experiments 1 and 2.

Stable isotope ratios have been used to study organic matter flow in a variety of ecosystems (20). When using carbon stable isotopes, the most valuable information is gleaned from systems in which a wide range of isotope values occur. For example, in the Parker River estuary, the stable isotope values of the major carbon sources range from -13.0‰ for *S. alterniflora* to -43.7‰ for oligohaline phytoplankton (7). However, $\delta^{13}\text{C}$ -DOC measurements range only from -21.0 to -34.0‰ because bulk DOC contains DOC from the mixing of water from freshwater (-28‰) and oceanic end (-22‰) members. A

large portion of the terrestrial and oceanic DOC is flushed through the system because it is mineralized by bacteria relatively slowly. This large background mixture of relatively refractory DOC is supplemented by relatively fresh inputs of labile DOC from local sources, such as phytoplankton and marsh grasses. We hypothesize that estuarine bacteria would assimilate the more labile DOC preferentially and thus would have $\delta^{13}\text{C}$ values reflecting these sources.

When natural bacterial assemblages are grown on organic matter leached from plant matter sources, they reflect the stable isotope composition of the source (5, 6) (this study). The fractionation factors are small, suggesting that although there may be selectivity for certain carbon molecules, there is little fractionation among the microbial biomass, respired CO_2 , and the initial substrate pool. The average change in the $\delta^{13}\text{C}$ from that of the initial DOC pool was -0.6 , 1.4 , and 0.5‰ for DOC remaining, bacterial biomass, and respired CO_2 , respectively. These shifts are small relative to the range of isotope ratios (-13.0 to -45.0‰) in the sources of DOC found in estuarine water (17). Because there is little isotopic shift from the source to the bacterial biomass or respired CO_2 produced, one can infer from the analysis of microbial $\delta^{13}\text{C}$ values which source(s) supports bacterial production at different locations in estuaries.

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