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Composition, production, and loss of carbohydrates in subtropical shallow subtidal sandy sediments: Rapid processing and long-term retention revealed by ^{13}C-labeling

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Abstract

The composition and production of carbohydrates (mannose, rhamnose, fucose, galactose, glucose, and xylose) and their transfer among sediment compartments (microphytobenthos [MPB], bacteria, and detritus) was investigated through in situ labeling with ^{13}C-bicarbonate. After 60 h, ^{13}C was found in all sediment compartments, demonstrating rapid transfer of fixed carbon from autotrophs to heterotrophs. Carbohydrates were a major carbon reservoir, accounting for 30% (day 0) to 15% (day 30) of the ^{13}C within sediments, and probably played a role in this transfer. Carbohydrate fractions were highly reactive (65–87%), less reactive (7–18%), and nonreactive (6–23%) over the experimental period. The rate of loss of the less reactive fraction (0.01–0.05 d^{-1}) was at least an order of magnitude lower than that for the highly reactive fraction (0.8–4.4 d^{-1}). Patterns of diagenesis estimated from label uptake and loss matched the carbohydrate composition observed in the sediment (glucose > galactose > rhamnose > fucose > xylose > mannose) and were similar to patterns reported previously. C : N ratios and δ^{13}C of sediment organic matter indicated an algal origin (MPB and phytoplankton). Although carbon was rapidly processed, loss from sediments was not immediate, and there was evidence of recycling into MPB and bacteria. Rapid transfer of carbon to and from carbohydrates has been found in various environments, including temperate, muddy, and intertidal sediments, and this study demonstrates the important role of carbohydrates in supporting heterotrophic production over extended periods (> 30 d) in subtropical shallow subtidal sands.

Carbohydrates are an important component of coastal sediments, comprising a considerable portion of the organic carbon (OC) pool (Arnosti and Holmer 1999; Burdige et al. 2000). Carbohydrates are the major product of photosynthesis and are present in both autotrophs and heterotrophs as structural materials (e.g., lignocellulose) and storage products (e.g., starch and glycogen). As a major component of the extracellular polymeric substances (EPS) secreted by microphytobenthos (MPB), carbohydrates play an important role in attachment, locomotion, and desiccation resistance of algal cells, and, at a system level, enhance sediment stability (Hoagland et al. 1993). More refractory carbohydrates can accumulate in the sediment; however, carbohydrates are generally considered to be a labile source of carbon for consumers (van Duyl et al. 1999; Bellinger et al. 2009), facilitating the transfer of carbon among trophic compartments.

There has been much interest in carbohydrate dynamics in coastal environments; however, most studies have been in temperate and mediterranean regions and focus on intertidal, mainly muddy, sediments. Few studies consider tropical or subtropical sediments (Marchand et al. 2005), despite the potential effect of warmer average temperatures on carbohydrate production, consumption, and transformation. Subtidal sandy sediments account for a large area of coastal habitat (70% of continental shelf area; Hall 2002) and may be important in determining the form and quantity of carbon exported from coasts to the ocean. The carbohydrate contents of sandy and muddy sediments are distinctly different, with higher organic matter content and higher carbohydrate concentrations typical of muddy sediments. However, although some studies have examined the composition and concentration of carbohydrates in sands (Jensen et al. 2005), only a few have considered trophic transfer and/or processing of carbohydrates in sandy sediments (Burdige et al. 2000; Cook et al. 2007; Evrard et al. 2008). Of these, none were in subtropical habitats and only Burdige et al. (2000) considered individual carbohydrates.

The composition and concentration of carbohydrates can be used to determine sources of organic matter to sediment (Taylor et al. 1999; de Brouwer and Stal 2001; Blasutto et al. 2005). Sources of carbohydrates to sediment can have characteristic compositional spectra dominated by glucose or galactose (terrestrial plants or macroalgae, respectively; Liebezeit 1987), xylose (angiosperms; Opsahl and Benner 1999), or rhamnose and fucose (MPB and/or bacteria; Liebezeit 1987; Marchand et al. 2005). Selective microbial degradation can alter compositional spectra by decreasing the relative abundance of labile carbohydrates such as glucose (Hedges et al. 1988; Hofmann et al. 2009) and xylose (Opsahl and Benner 1993, 1999), and increasing the abundance of the deoxysugars rhamnose and fucose (Opsahl and Benner 1999), which either may be more resistant to degradation, or may be produced in situ by bacteria (Hedges et al. 1988) and/or MPB (Nierop et al. 2001). The form in which carbohydrates are present can influence degradation rates. Glucose present as cellulose, for example, tends to have a slower turnover rate (Derrien et al. 2007) than glucose chrysolaminarin (a labile diatom

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storage product; D'Souza and Bhosle 2001). In general, however, the spectra of degraded organic matter tend to be relatively uniform (Opsahl and Benner 1999), whereas fresh, labile organic matter typically has high carbohydrate concentrations and a dominance of more labile carbohydrates (e.g., glucose, galactose, mannose; Kerherve et al. 2002), and low contribution of deoxysugars (D'Souza and Bhosle 2001). Carbohydrate composition and concentration can be further affected by nutrient availability (Underwood et al. 2004; Cook et al. 2007) and environmental conditions (e.g., light and salinity) that influence growth of carbohydrate sources (e.g., algae; Smith and Underwood 2000; Perkins et al. 2001; Abdullahi et al. 2006). Because of the competing influences on carbohydrate composition, spectra are best interpreted in combination with additional sediment characteristics such as C : N ratios (Khodse et al. 2008) and natural abundance stable isotope ratios (Marchand et al. 2005).

Much of the existing information on carbohydrate dynamics in coastal sediments has been obtained through studies of algal cultures (de Brouwer and Stal 2002; Underwood et al. 2004; Abdullahi et al. 2006) and ex situ manipulations (Smith and Underwood 2000; Cook et al. 2007; Hofmann et al. 2009). Laboratory-based studies provide useful information, but removal from the sediment community (algae, bacteria, fauna, etc.) and the environment (tides, light, temperature, etc.) affects carbohydrate production and fate. In addition, such studies are typically of short duration. Although carbohydrates can be rapidly utilized by bacteria (e.g., 50% within 24 h; Goto et al. 2001), carbon derived from carbohydrates may persist for some time. Cook et al. (2007) and Evrard et al. (2008), for example, saw negligible loss of fixed carbon from subtidal sandy sediments over 11 and 4 d, respectively, despite 50% of fixed carbon having been released as EPS, primarily carbohydrates. However, caution is advised when extrapolating findings of such ex situ studies to the environment. This demonstrates the need, therefore, for longer-term in situ studies of carbohydrates to gain a more complete understanding of their role in coastal carbon cycling.

Stable isotope labeling, in combination with compound-specific isotope analysis (CSIA) of carbohydrates, shows promise for allowing in situ assessment of carbohydrate dynamics. In stable carbon isotope labeling experiments, additions of the rare carbon isotope ^{13}C are used to trace carbon transfer among compartments within a system. CSIA separates compounds prior to isotope measurement and allows transfers of ^{13}C into individual compounds such as fatty acids (Middelburg et al. 2000), amino acids (Veuger et al. 2005), and carbohydrates (Bellinger et al. 2009) to be assessed. Because of the relatively recent development of CSIA for carbohydrates, only a few studies, four in terrestrial environments (Derrien et al. 2004; Glaser and Gross 2005; Bock et al. 2007) and one short-term study in coastal sediments (Bellinger et al. 2009), have combined stable isotope labeling and CSIA to assess carbohydrate dynamics. Gas chromatography (GC)–combustion–isotope ratio mass spectrometry has been the most common method used for CSIA of carbohydrates (Bellinger et al. 2009) but requires derivatization of carbohydrates. The calculations to correct for this reduce the accuracy and precision of the carbon isotope ratios ($\delta^{13}C$) determined. More recently, Boschker et al. (2008) demonstrated that high-performance liquid chromatography–isotope ratio mass spectrometry (HPLC-IRMS), which does not require sample derivatization, gives accurate and reproducible isotope signatures for individual carbohydrates.

The aim of the current study was to investigate, in situ, the fate of carbohydrates produced in subtropical shallow subtidal sandy sediments. We aimed to use the composition and concentration of carbohydrates to gain insight into sources of organic matter to the sediment, and to use a combination of ^{13}C-labeling and HPLC-IRMS for CSIA of carbohydrates, in situ, to determine rates of uptake and loss of carbon from individual carbohydrates in these sediments over a longer time period than has previously been investigated (30 d). Given the paucity of information on carbohydrate dynamics in shallow subtidal sandy sediments, particularly for subtropical regions, this is a valuable step towards understanding the role of carbohydrates in carbon cycling in this habitat.

Methods

Study site—The current study was done subtidally (approximately 1.5 m below average sea level) in Readings Bay, Brunswick River, subtropical Australia, at a site adjacent to that of Webb and Eyre (2004). Tides in this region are semidiurnal and have a tidal range from 2 m (spring tides) to < 1 m (neap tides). Sediment (upper 2 cm) at the site had an OC content of 3972 mmol C m^{-2} (0.14%) and a molar C : N ratio of 6.9 $\pm$ 0.7, and consisted primarily of fine (125–250 μm; 75%) and medium (250–500 μm; 20%) quartz sand grains. The site was net autotrophic (production : respiration $\sim$ 1.2; J. M. Oakes unpubl.). The MPB assemblage was examined by light microscopy (100×) and found to be dominated by pennate diatoms. No cyanobacteria were observed. Based on CO_2 fluxes, gross productivity averaged $\sim$ 105 mmol C m^{-2} d^{-1} (J. M. Oakes unpubl.).

^{13}C-labeling—Two experimental plots (1.2-m^2 area) were selected on physically similar areas of sediment that were free of macrophytes and animal burrows. These plots, and their overlying water, were enclosed using 30-cm-tall hexagonal perspex chambers (volume $\sim$ 360 liters). Approximately 0.6 mmol ^{13}C L^{-1} was introduced to each chamber via injection with $NaH^{13}CO_3$ (99% ^{13}C). When combined with the existing dissolved inorganic carbon (DIC) pool (1.9 mmol L^{-1}), this resulted in 23% of the DIC available to producers being in the form of $DI^{13}C$. Pumps circulated the ^{13}C-labeled water within the chambers for 24 h, allowing time for ^{13}C to be taken up by the sediment community. Chambers were then removed.

Sample collection—Immediately after chamber removal and at 1, 3, 10, 20, and 30 d thereafter, two cores of sediment (9-cm diameter × 20 cm deep, with 30 cm overlying water) were collected from each labeled experimental plot. At each time, two control cores for back-

ground isotope values were also collected from 5 to 8 m outside of labeled plots. Upon return to the laboratory, control and labeled cores were placed in separate tanks of unlabeled site water maintained at in situ temperature ($\pm$ 1°C) and light levels ($\pm$ 5%). Teflon-coated magnetic bars ~ 10 cm above the sediment surface stirred water within cores at a rate below the threshold for sediment resuspension. Cores remained in the tanks, unsealed, for a 24-h (12 h dark, then 12 h light) pre-incubation period. Cores were then sealed and incubated in the tanks for a further 24 h (12 h dark, then 12 h light). Pre-incubation and incubation of cores was part of a separate sediment–water exchange study and was not necessary for this pulse-chase study of carbohydrates. Incubation prevented monitoring of carbohydrate dynamics over the first 60 h following labeling. Transfers of carbon among sediment compartments can occur within hours (e.g., to meiofauna; Middelburg et al. 2000; Oakes et al. 2010), and these initial transfers could therefore not be investigated here. However, the focus of the current study was on longer-term carbohydrate processing and carbon transfer (60 h to 30 d), which have previously received little attention. Incubation of cores was advantageous in that it allowed monitoring of label uptake during the light period (light incubation in the laboratory), which would otherwise have been difficult given that label was added to the sediment using enclosed chambers.

The upper 2 cm of sediment was extruded and collected from one core from each plot at the end of the dark period (i.e., two replicates each of labeled and control samples from the dark period), and from the remaining cores at the end of the light period (i.e., two replicates each of labeled and control samples from the light period). Although bacteria are present throughout the 2-cm depth examined and MPB are concentrated in the upper few millimeters, this does not affect calculation of ^{13}C uptake because units are m^{-2}, and therefore not depth-specific. Calculations of loss are also not affected, as ^{13}C loss is calculated from relative changes in ^{13}C content. Sediment was homogenized and freeze-dried in preparation for carbohydrate extraction. Subsamples of lyophilized sediment collected at the end of the dark period were used to determine $\delta^{13}C$ of sediment OC, bacteria, and MPB.

Carbohydrate extraction—Carbohydrates were extracted using a method similar to that of Boschker et al. (2008), except that $SrCO_3$ was used instead of $BaCO_3$ to neutralize the samples after acid hydrolysis. Compared to $BaSO_4$, the $SrSO_3$ precipitate that is formed during neutralization of the hydrolysate is much more compact, leaving a greater volume of supernatant available for carbohydrate analysis. For each sample, approximately 500 mg of freeze-dried sediment was acidified with 0.5 mL of 11 mol L^{-1} H_2SO_4 and vortexed. After 1 h, the acid was diluted to 1 mol L^{-1} H_2SO_4 by adding 4.5 mL of Milli-Q H_2O, and the sample was hydrolyzed for 1 h at 120°C. Samples were then placed in crushed ice to stop the reaction, and the hydrolysate was transferred to a 50-mL centrifuge tube, neutralized (pH 5.5–6.0) with approximately 2 g of $SrCO_3$, and centrifuged (15 min, 4500 $\times$ g). The supernatant was transferred to an Eppendorf tube and frozen overnight to further precipitate $SrCO_3$, which was subsequently removed from thawed samples by centrifugation (7500 $\times$ g, 15 min). Samples were filtered through a 0.22-μm syringe filter into HPLC vials and stored frozen (−20°C) until analysis via HPLC-IRMS.

Biomarker extraction—Biomarkers to determine uptake of ^{13}C into bacteria and MPB were extracted from lyophilized sediments using a modified Bligh and Dyer method. An internal standard (500 μL of 500 μg mL^{-1} tridecanoic acid, C_{13}) was added to sediment at the beginning of the extraction procedure. Sediment was then sonicated (10 min) and centrifuged (1000 $\times$ g, 3 min) in 3 $\times$ 30–40 mL of 3 : 6 : 1 dichloromethane (DCM) : methanol : Milli-Q H_2O. Extracts were combined in a separating funnel. DCM (30 mL) and Milli-Q (30 mL) were added and phases allowed to separate. The bottom layer was drawn off into a round-bottom flask and the process repeated with 15 mL of DCM. Total extracts were reduced under vacuum and further separated using silicic acid columns (Alltech; 500 mg, 8.0 mL) by sequential elution with 5 mL each of chloroform, acetone, and methanol. The methanol fraction, containing phospholipids (PLFAs), was collected, reduced to dryness (N_2), and methylated (3 mL 10 : 1 : 1 MeOH : HCl : $CHCl_3$, 80°C, 2 h). The reaction was quenched with 3 mL Milli-Q, and the methylated fatty acid fraction was extracted using 3 mL then 2 mL ($\times$2) of 4 : 1 hexane : DCM and transferred to a GC vial for analysis.

Isotope analysis—Concentrations and isotope ratios of carbohydrates were determined using the method of Boschker et al. (2008). Analysis of carbohydrates was done using an HPLC system interfaced with a Delta V Advantage IRMS via an LC Isolink interface. Carbohydrates were separated on a Dionex PA-20 analytical column and were eluted isocratically with 1 mmol L^{-1} degassed NaOH at 300 μL min^{-1}. Data collection was controlled with Isodat 2.5 software (with Service Pack 1.13). The basic algorithm in Isodat was used for baseline corrections following analysis, with manual optimization as required. Carbohydrate standards as listed in Boschker et al. (2008) were injected at concentrations between 100 and 4000 μmol L^{-1} to check for consistency of isotope ratios (within <0.5‰ standard deviation) and for linearity of peak areas with carbohydrate concentrations ($R^2 > 0.96$).

PLFAs were analyzed for isotope ratios by GC–mass spectrometry using a Thermo Trace GC Ultra gas chromatograph coupled with a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS) via a Thermo Conflo III interface. A nonpolar 60-m HP5-MS column (0.32 mm i.d., 0.25 μm film thickness, J&W Scientific) was used with helium as a carrier gas. The GC oven was held at 60°C for 2 min followed by a ramp to 140°C at 25°C min^{-1}, then a ramp to 290°C at 3°C min^{-1}. The oven was then ramped to 300°C at 20°C min^{-1} and held there for 3 min.

Total nitrogen and, following acidification (5% HCl) in silver cups, $\delta^{13}C$ and %C of sediment organic matter were determined using a Thermo Finnigan Flash EA 112 interfaced via a Thermo Conflo III with a Thermo Delta V Plus IRMS. Molar C : N ratios were calculated from the

%C and %N values obtained for acidified and untreated sediment, respectively.

Isotope calculations and data analysis—Natural abundance isotope ratios of bacteria and MPB were calculated from control cores using $\delta^{13}C$ values of fatty acids specific to bacteria (i+a15:0) and diatoms (16:1(n-7)), corrected for fractionation factors of −5.0‰ (middle of range identified by Boschker et al. 1999) and −5.4‰ (Schouten et al. 1998), respectively.

Total uptake (incorporation) of ^{13}C into sediment organic matter, bacteria, MPB, and individual carbohydrates (μmol ^{13}C m^{-2}) was calculated as the product of excess ^{13}C (fraction ^{13}C in labeled sample – fraction ^{13}C in control) and mass of carbon derived from sediment organic matter, bacteria, MPB, or individual carbohydrates (Middelburg et al. 2000). Total ^{13}C in sediment organic matter was determined using %C estimates derived for OC during isotope analysis. For bacteria and MPB, the peak area of the C_{13} internal standard was used to calculate the concentration of individual PLFAs in sediment based on their peak areas. For bacteria, total biomass was determined using the biomarkers i15:0 and a15:0. Although the bacterial biomarker 18:1w7c was also detected, the peak was typically not distinct so was excluded from calculations. Bacterial biomass was therefore determined using the formula

$$\text{Biomass}_{\text{bacteria}} = \text{Biomass}_{\text{i+a15:0}} / (a \times b) \qquad (1)$$

where a is the average PLFA concentration in bacteria (0.056 g C PLFA per g C biomass; Brinch-Iversen and King 1990) and b is the average fraction of PLFA in sediments dominated by bacteria that is accounted for by i+a15:0 (0.16; Rajendran et al. 1993, 1994).

Biomass of MPB was determined using biomarkers specific to algae that gave distinct peaks (16:1(n-7), 20:5(n-3), 16:0, and 14:0; Bellinger et al. 2009) and was calculated using the formula

$$\text{Biomass}_{\text{MPB}} = \text{Biomass}_{\text{MPB biomarkers}} \times c/d \qquad (2)$$

where c is the average fraction of MPB PLFAs that is typically accounted for by the biomarkers considered (0.67; Volkman et al. 1989) and d is the average PLFA concentration in diatoms (0.035 g of PLFA carbon per g of carbon biomass; calculated by Middelburg et al. 2000). The average PLFA concentration given assumes a carbon to chlorophyll a ratio (C : Chl a) of 40, which is within the range recorded for the Brunswick River in summer (Ferguson et al. 2004). MPB biomass calculated using minimum and maximum values for c (62–71%, Volkman et al. 1989) and d (assuming C : Chl a = 30–60, Ferguson et al. 2004) was within ~ 30% of biomass calculated from average values.

Rates of ^{13}C uptake into individual carbohydrates were calculated from the increase in ^{13}C incorporation during light periods. A two-way analysis of variance (ANOVA) was used to determine if incorporation of ^{13}C into carbohydrates differed across days (six levels), with dark or light incubation (two levels), or with the two factors combined. The cores themselves, rather than the two labeled plots, were considered to be replicates. Because ^{13}C content of sediment OC, bacteria, and MPB was determined using only samples from the dark period, one-way ANOVAs were used to determine if ^{13}C incorporation into these compartments differed among days (six levels). Data were transformed (log(x)) prior to analysis to reduce heterogeneity of variances, and post hoc Tukey tests were used, where applicable, to identify significantly different groups of data. To determine rates of loss of ^{13}C from sediment compartments, data were fitted using a 2-G model (Westrich and Berner 1984), which assumes that a pool of organic matter consists of fractions (groups) that degrade exponentially at different rates, depending upon their reactivity. This model is represented by

$$G_T(t) = G_1[\exp(-k_1 t)] + G_2[\exp(-k_2 t)] + G_{NR} \qquad (3)$$

where G_T is the incorporation of ^{13}C within a specific compartment (e.g., glucose); t is time; G_1, G_2, and G_{NR} represent the initial incorporation of ^{13}C into highly reactive, less reactive, and nonreactive (over the timescale of the experiment) fractions of the compartment; and k_1 and k_2 represent first-order decay constants specific to fractions G_1 and G_2, respectively. Separate models were generated for dark cores from plot 1, dark cores from plot 2, light cores from plot 1, and light cores from plot 2. This gave four estimates of each model parameter, allowing calculation of means and standard errors associated with these estimates.

The isotope mixing program Isosource (Phillips and Gregg 2003) was used to estimate the contributions of MPB, phytoplankton, and terrestrial plant material to the sediment carbon pool.

Results

Sediment composition—Distinct chromatographic peaks of sufficient size for reliable determination of stable isotope ratios were identified for five carbohydrates: mannose, fucose, galactose, glucose, and xylose. A sixth peak consisted primarily of the carbohydrate rhamnose, but had a small contribution of co-eluting arabinose. However, because rhamnose is found in algae and bacteria, whereas arabinose is primarily in higher plants, changes in isotope ratio of the combined peak reflect in situ carbon uptake and loss from rhamnose. This peak, hereafter referred to as the rhamnose peak, was therefore also considered in the current study.

The six aforementioned carbohydrates were the dominant carbohydrates at the site and accounted for 10.2% (406 mmol C m^{-2}) of the total OC (3972 mmol C m^{-2}) in the upper 2 cm of sediment at the study site. Bacteria accounted for a further 3.9% (155 mmol C m^{-2}) and MPB a further 10.0% (396 mmol C m^{-2}) of the OC in sediment.

Of the carbohydrates identified, glucose, galactose, and rhamnose were the most abundant (average concentration of controls = 80–113 mmol C m^{-2}; Table 1), each representing 20%, 25%, and 27%, respectively, of the total carbon in carbohydrates (Fig. 1). Fucose was approximate-

Table 1. Concentration and natural abundance $\delta^{13}C$ observed for total OC, bacteria (total cell), MPB, and carbohydrates, and uptake rates for carbohydrates during light incubation. Standard errors are in parentheses. nd, not determined.

Sediment compartment	Concentration (mmol C m^{-2})	Natural abundance $\delta^{13}C$ (‰)	Uptake in light incubation (μmol ^{13}C m^{-2} h^{-1})
Organic carbon	3971.6(536.0)	−17.9(1.3)	nd
Bacteria	154.8(14.1)	−17.5(0.5)*	nd
MPB	396.1(34.6)	−15.4(1.8)*	nd
Mannose	24.8(1.6)	−15.7(2.0)	0.63
Fucose	49.2(2.9)	−16.8(1.0)	1.03
Rhamnose	80.0(4.8)	−18.1(0.7)	1.25
Galactose	105.0(6.1)	−18.2(0.5)	2.66
Glucose	113.0(7.6)	−17.9(0.4)	10.65
Xylose	34.2(2.4)	−15.2(1.3)	0.92

* Based on PLFA and corrected for offset between total cell and biomarker (5‰ and 5.4‰ for bacteria and MPB, respectively).

ly half as abundant (49 mmol C m^{-2}, 12%) whereas xylose and mannose were the least abundant carbohydrates (25–34 mmol C m^{-2}), representing 10% and 6%, respectively, of the carbon within carbohydrates (Fig. 1).

Natural abundance stable isotope ratios of sediment compartments—The natural abundance carbon isotope ratios ($\delta^{13}C$) of carbohydrates in control plots varied from an average of -18.2 ± 0.5‰ for galactose to -15.2 ± 1.3‰ for xylose (Table 1). The natural abundance $\delta^{13}C$ of total OC was -17.9 ± 1.3‰. Bacteria and MPB in control plots had $\delta^{13}C$ values of -17.5 ± 0.5‰ and -15.4 ± 0.4‰, respectively.

^{13}C incorporation into sediment compartments—At the end of the first dark incubation (60 h after initial label application = 36 h after chamber removal), 892 ± 32 μmol ^{13}C m^{-2} had been incorporated into sediment OC, equating to an uptake of 37 μmol ^{13}C m^{-2} h^{-1} during light hours. This equated to the replacement of approximately 0.02% of the carbon in sediment organic matter with ^{13}C. There was significant change in the ^{13}C content of sediment organic matter throughout the 30-d experiment (one-way ANOVA: $F_{5,6} = 16.135$, $p = 0.004$) with 85% of fixed carbon lost from sediment in the 0–2 cm layer by day 30 (127 ± 24 μmol ^{13}C m^{-2} remaining at this time). This decline fitted a 2-G model with 61% of sediment OC degrading at a rate of 2.06 d^{-1} (highly reactive fraction), 24% at a rate of 0.07 d^{-1}, and the remaining OC nonreactive over the timescale of the experiment (Table 2; Fig. 2A).

Of the overall ^{13}C incorporated into sediment biomass by the first sampling period, 11% (101 μmol ^{13}C m^{-2}) was in carbohydrates by the end of the first dark period. A further 206 μmol ^{13}C m^{-2} had been incorporated into carbohydrates by the end of the first light period, representing approximately 34% of the ^{13}C in sediments.

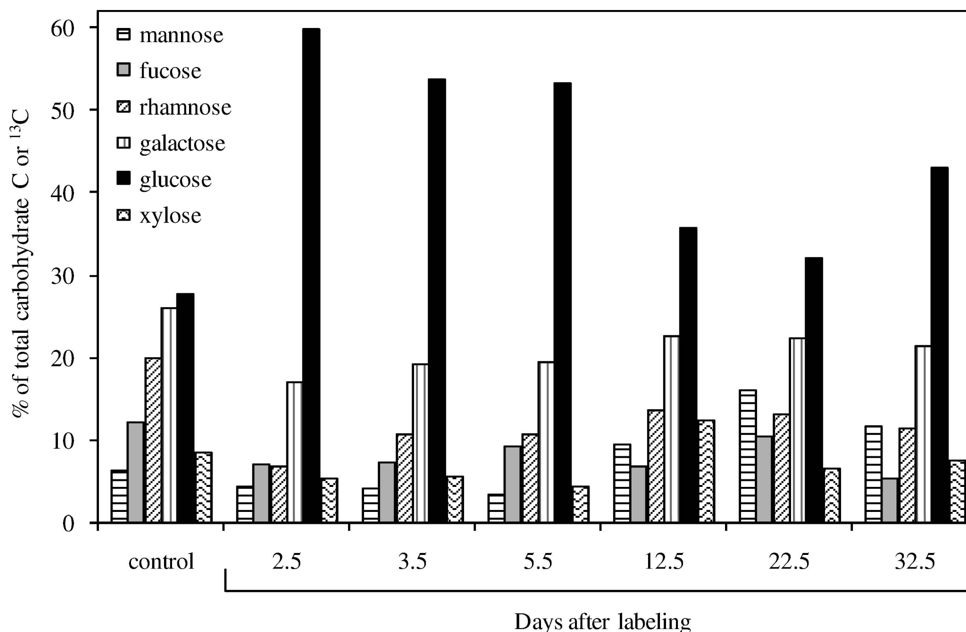

Fig. 1. Percentage of total carbohydrate carbon within individual carbohydrates in control sediments and percentage of ^{13}C incorporated into total carbohydrates that was within individual carbohydrates at different times following labeling.

Table 2. Output (*F* values and *p* values) of two-way ANOVAs, ratio of light uptake to decay of ^{13}C for carbohydrates, and parameters for 2-G models that fitted ^{13}C loss from carbohydrates and sediment OC. G_1, G_2, and G_{NR} are highly reactive, less reactive, and nonreactive fractions (over the experimental period). k_1 and k_2 are first-order decay rates specific to G_1 and G_2, respectively. Degrees of freedom for ANOVA factors are in column headings. Standard errors are in brackets (0 = error < 1). nd, not determined.

Compartment	Two-way ANOVA			2-G model						Ratio uptake : decay
	Day × incubation condition ($F_{5,12}$, *p* value)	Incubation condition ($F_{1,12}$, *p* value)	Day ($F_{5,12}$, *p* value)	G_1 (μmol C m^{-2})	k_1 (d^{-1})	G_2 (μmol C m^{-2})	k_2 (d^{-1})	G_{NR} (μmol C m^{-2})	R^2	
Organic carbon	nd	nd	nd	548(93)	2.06(0.54)	216(101)	0.07(0.01)	127(24)	0.97	nd
Mannose	0.36, 0.83	0.16, 0.70	1.23, 0.38	6(3)	4.38(1.02)	1(4)	0.01(0.01)	2(1)	0.97	0.22
Fucose	0.45, 0.77	0.61, 0.45	5.24, 0.01*	13(8)	1.50(0.29)	3(12)	0.05(0.01)	1(0)	0.99	0.88
Rhamnose	0.62, 0.66	1.70, 0.22	3.32, 0.05*	11(8)	0.81(0.18)	3(11)	0.03(0.01)	2(0)	0.99	2.25
Galactose	0.56, 0.70	0.47, 0.51	3.89, 0.04*	32(21)	1.27(0.25)	5(30)	0.03(0.01)	4(1)	0.99	2.69
Glucose	0.81, 0.55	1.35, 0.27	5.54, 0.01*	124(80)	1.38(0.25)	10(113)	0.04(0.01)	8(3)	1.00	8.88
Xylose	0.86, 0.52	0.36, 0.56	2.11, 0.16	9(7)	1.43(0.34)	1(10)	0.02(0.02)	1(0)	0.99	0.83

* Significant *p* value.

By the conclusion of the experiment, the contribution of ^{13}C in carbohydrates had declined to approximately 15% of the ^{13}C within the sediment organic matter. There was excess ^{13}C in all carbohydrates throughout the study. Over time, however, the distribution of ^{13}C among individual carbohydrates became generally more uniform (Fig. 1). Although glucose accounted for the greatest proportion of carbohydrate ^{13}C throughout the study, the contribution of glucose to the total ^{13}C within carbohydrates generally declined, from 60% at day 0 to approximately 40% at the conclusion of the study. In contrast, the contributions of mannose, rhamnose, and galactose generally increased to maxima of 17%, 14%, and 25%, respectively, at day 20. The maximum contribution of xylose (12%) was at day 10, but the contribution of xylose was otherwise relatively constant at 4–7%. The contribution of ^{13}C within fucose to total carbohydrate ^{13}C incorporation was relatively constant throughout the study (6–9%; Fig. 1).

Label incorporation by MPB was evident for the duration of the experiment, and initially accounted for 41% of the ^{13}C incorporated into sediment biomass (367 ± 2 μmol ^{13}C m^{-2}). The ^{13}C content of MPB declined throughout the experiment with only 53 μmol ^{13}C m^{-2} remaining in MPB by day 30 (Fig. 2B). However, throughout the experiment, ^{13}C within MPB accounted for a relatively constant proportion (41–63%) of the ^{13}C within sediment OC. It is important to note that ^{13}C within MPB represents label in the upper few millimeters of the sediment, where MPB is concentrated. Label not within MPB could have been in other compartments within the upper few millimeters of the sediment, or deeper within the sediment.

There was evidence of label uptake by bacteria throughout the 30-d experiment (Fig. 2B), but the contribution to ^{13}C in sediment OC was variable, accounting for between 5% (14 ± 9 μmol ^{13}C m^{-2}) of the ^{13}C in sediment at day 3 and 21% (54 ± 6 μmol ^{13}C m^{-2}) at day 10. There were significant differences in incorporation among days (one-way ANOVA: $F_{5,6} = 4.389$, $p = 0.022$). A post hoc Tukey test showed that incorporation was similar for days 1, 3, 20, and 30, and for days 0, 1, and 10. The latter of these groups of days had higher ^{13}C incorporation, demonstrating that bacterial ^{13}C decreased with time, but that there was a spike in incorporation at day 10. The contribution of ^{13}C in bacteria to ^{13}C in sediment OC, however, appeared to generally increase, but with a spike in contribution at day 10.

Throughout the experiment, the proportion of ^{13}C within sediment OC that could not be detected within MPB, bacteria, and carbohydrates varied from 11% to 35%. Although there was no distinct pattern to this variability, the greatest proportion of ^{13}C that could not be accounted for was towards the end of the experiment (31% and 35% on days 30 and 20, respectively).

Rates of uptake and loss of ^{13}C from carbohydrates—For all carbohydrates, there was no interacting effect of sampling day and incubation condition (light or dark) and no significant effect of incubation condition on excess ^{13}C content (Table 2). There was no statistically significant

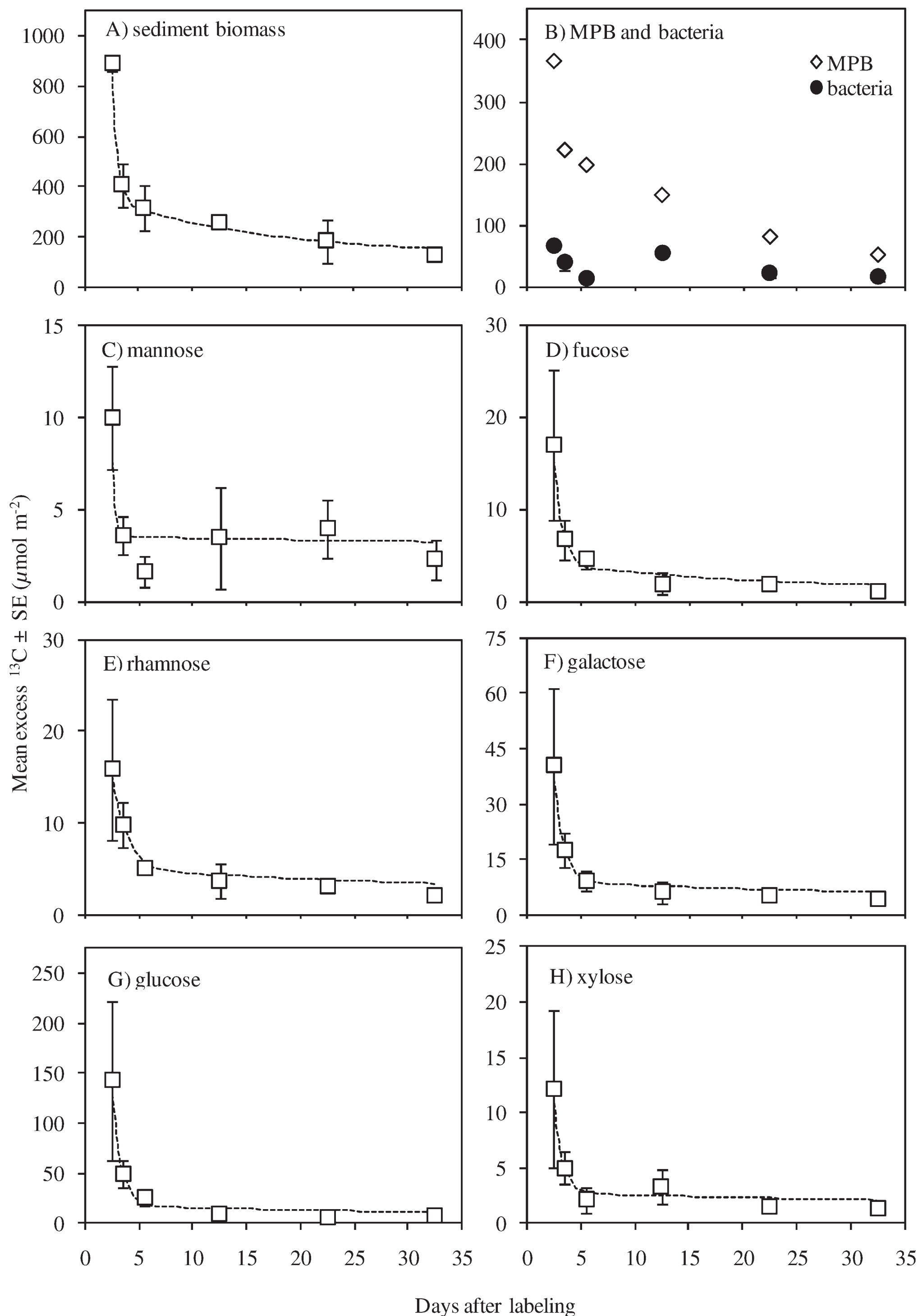

Fig. 2. Changes in excess ^{13}C incorporation throughout the study period in (A) sediment biomass; (B) bacteria and MPB; and the carbohydrates (C) mannose, (D) fucose, (E) rhamnose, (F) galactose, (G) glucose, and (H) xylose. Dashed lines show 2-G models that fit the data (parameters and R^2 values in Table 2). For carbohydrates, total mean of dark and light cores is shown (i.e., n = 4) as there was no statistically significant difference between these (*see* Table 2).

difference in the ^{13}C content of sediment in day 0 cores at the end of dark and light incubation. However, the mean ^{13}C content at the end of light incubation was slightly elevated. There may therefore have been some uptake of ^{13}C label during light incubation, indicating that some unincorporated $NaH^{13}CO_3$ may have remained in the sediment. For this reason, we calculated rates of uptake for carbohydrates from the change in label content during the light incubation. The greatest rate of uptake during this 12-h period was for glucose (10.6 μmol ^{13}C m^{-2} h^{-1}),

followed by galactose (Table 1). Uptake rates were similar for rhamnose (1.2 μmol ^{13}C m^{-2} h^{-1}), fucose (1.0 μmol ^{13}C m^{-2} h^{-1}), and xylose (0.9 μmol ^{13}C m^{-2} h^{-1}). The slowest rate of ^{13}C uptake was for mannose (0.6 μmol ^{13}C m^{-2} h^{-1}). By the end of the light incubation of day 0 cores, approximately 0.4% (rhamnose), 0.6% (fucose and xylose), 0.7% (galactose), 0.8% (mannose), and 2.5% (glucose) of the carbon within each carbohydrate had been replaced with ^{13}C.

Mean ^{13}C incorporation in carbohydrates generally declined throughout the experiment, with a rapid decline from day 0 to day 1. This loss of label was significant for all carbohydrates except mannose and xylose (Fig. 2; Table 2), which not only had high error associated with mean ^{13}C values at day 0, but also showed increases in ^{13}C content at day 10, and days 10 and 20, respectively. Because there was no significant effect of incubation condition on excess ^{13}C, 2-G models to describe ^{13}C loss from carbohydrates were determined for average excess ^{13}C values for sediments collected at the end of both the light and dark incubation periods. Loss of ^{13}C from all carbohydrates could be fitted using 2-G models (R^2 = 0.97–1.00 in all cases; Table 2). The highly reactive fraction of material (G_1) accounted for between 65% (mannose) and 87% (glucose) of each carbohydrate and degraded (or was transformed) at rates (k_1) of 0.81 d^{-1} (rhamnose) to 4.38 d^{-1} (mannose). The less reactive fraction (G_2) represented 7% (glucose) to 18% (rhamnose) of each carbohydrate. Rate constants (k_2) for this fraction were more than an order of magnitude lower than for the highly reactive fraction, varying from 0.01 d^{-1} (mannose) to 0.07 d^{-1} (glucose). The nonreactive fraction (G_{NR}) represented 6% (fucose) to 23% (mannose) of each carbohydrate (Table 2).

Discussion

To our knowledge, although studies have looked at carbohydrate composition of temperate shallow sediments (Liebezeit 1987; Khodse et al. 2008) and diagenesis in tropical intertidal muds (Marchand et al. 2005), this is the first to examine the composition, production, and loss of carbohydrates in shallow subtidal sands in the subtropics. Furthermore, this is the first application of a HPLC-IRMS technique (Boschker et al. 2008) to monitor carbon transfer to and from carbohydrates in a long-term (30 d) in situ ^{13}C-labeling experiment.

Sediment organic matter sources—The composition and concentration of carbohydrates provides information on the source of sediment organic matter. Carbohydrates in sediments can account for a substantial portion of the dissolved and particulate OC (Arnosti and Holmer 1999; Burdige et al. 2000; Jensen et al. 2005) and are typically dominated by glucose. In the current study, however, carbohydrates represented only 10% of total OC. This is a far smaller contribution to OC than was observed by Cook et al. (2007) for temperate shallow subtidal sands (29%), the most comparable sediments to the ones studied here, in terms of texture and overlying water depth, for which such information is available. The contribution of glucose to total carbohydrates in the current study was also relatively small, accounting for a smaller proportion (28%) of total carbohydrates than has been observed in temperate intertidal muds (Taylor et al. 1999; Boschker et al. 2008), tropical subtidal estuarine sediments (Khodse et al. 2008), or temperate coarse subtidal sands (10-m depth; Jensen et al. 2005). The relatively uniform composition of carbohydrates we observed was similar to that of deeper-water (25–295 m) temperate sediments receiving refractory allochthonous inputs (Jensen et al. 2005). The observed low carbohydrate : OC ratio and uniform compositional spectrum of carbohydrates suggest that organic matter within the sediments studied is highly degraded (Khodse et al. 2008). This could indicate input of degraded allochthonous material (i.e., pelagic algae or terrestrial detritus). However, given that the system studied was autotrophic, this more likely reflects rapid degradation of in situ production, which may relate to the higher temperature, bacterial productivity, and biomass typical of lower latitudes (Eyre and Balls 1999). This is further demonstrated by the greater contribution of rhamnose to the sediments we studied than to the temperate subtidal sands studied by Jensen et al. (2005). Rhamnose can be produced by bacteria or MPB (Hedges et al. 1988; Nierop et al. 2001). Increased contributions of rhamnose may therefore reflect higher bacterial biomass and productivity and/or greater MPB production related to the greater light availability (shallower depth) and/or warmer temperatures typical of the subtropical sediments studied.

Organic matter C : N and stable isotope ratios can assist with interpretation of carbohydrate compositions. Ratios of C : N are not well characterized for MPB (Cook et al. 2009), but sediment organic matter C : N in the current study was similar (6.9) to that expected for marine algae (6.6; Redfield et al. 1963) and well below that for terrestrial plants (> 20; Hamilton and Hedges 1988). Allochthonous inputs to the sediment would therefore primarily be from pelagic algae, with little contribution from terrestrial plants. Natural abundance stable isotope ratios further support this. The $\delta^{13}C$ value of sediment OC (−17.9‰) was intermediate to that inferred for MPB (−15.4‰) and that reported for pelagic algae (−18‰ to −24‰; Michener and Schell 1994) and terrestrial plant material (−23‰ to −30‰; Michener and Schell 1994). Isotope mixing calculations based on these end-member ranges revealed MPB, phytoplankton, and terrestrial plant contributions to sediment carbon of 56% ± 20%, 32% ± 20%, and 12% ± 5%, respectively. The composition and concentration of carbohydrates, combined with sediment C : N ratios and $\delta^{13}C$ values of sediment and MPB, therefore demonstrate that OC within the sediments studied is derived primarily from in situ production by MPB. This is supplemented by substantial inputs of carbon derived from pelagic algae, which is degraded before, or rapidly upon, entry to the sediment. Terrestrial detritus makes limited, if any, contribution.

Natural abundance stable isotope ratios of sediment compartments—Few studies have reported natural abun-

dance stable isotope ratios of individual carbohydrates (Moers et al. 1993; Boschker et al. 2008; Bellinger et al. 2009). Our study determined $\delta^{13}C$ values of bulk sediment OC, individual carbohydrates, and PLFAs of bacteria and algae, providing an opportunity to explore isotopic differences among these. Carbohydrates are generally enriched up to ~ 8–9‰ (van Dongen et al. 2002; Teece and Fogel 2007) and PLFAs depleted by 1–16‰ (van Dongen et al. 2002) relative to their source. However, isotopic differences vary considerably depending on species and growth conditions (van Dongen et al. 2002). Mannose (Teece and Fogel 2007) and glucose (van Dongen et al. 2002; Teece and Fogel 2007), for example, are depleted compared to bulk material in some algae. In the current study, carbohydrate $\delta^{13}C$ values were similar to, or enriched compared to, bulk sediment OC. Galactose, rhamnose, and glucose all had $\delta^{13}C$ values (−17.9‰ to −18.2‰) similar to that of OC, whereas fucose, mannose, and xylose were enriched by up to 2.7‰ compared to sediment OC. The biomarker PLFAs for bacteria (i+a15:0) and MPB (16:1(n-7)), however, were depleted relative to sediment OC by 4.6‰ and 2.9‰, respectively. These general patterns are similar to those previously observed, but the enrichment of carbohydrates was smaller than expected. This may relate to atypical processes during laboratory incubations, but is more likely due to natural growth conditions that affect fractionation (van Dongen et al. 2002) and/or selective degradation of carbohydrate fractions that are relatively enriched (though this is the reverse of expected trends).

Carbohydrate production—Isotope labeling allowed us to investigate in situ carbohydrate production. There had been substantial incorporation of ^{13}C into bulk sediment OC by the time the first sample was taken (36 h after chamber removal). At this time, similar proportions of ^{13}C were in carbohydrates (30%) and MPB (40%). The carbohydrates we studied included both intra- and extracellular fractions, so rapid incorporation of ^{13}C into carbohydrates as well as MPB was not unexpected. However, the similar label content of MPB and carbohydrates indicates that at least some portion of labeled carbohydrate was external to MPB and excretion of carbohydrates (presumably as EPS) must have occurred. The carbohydrates we considered (mannose, rhamnose, galactose, glucose, xylose, and fucose) account for 3–11% of the total carbon in diatoms and cyanobacteria cells (Aguilar et al. 1999). Based on the label content of MPB at the first sampling period, up to 40 μmol ^{13}C m^{-2} of the ^{13}C within sediment OC at this time is therefore attributable to carbohydrates within MPB. Approximately ~ 198 μmol ^{13}C m^{-2} of the ^{13}C within carbohydrates was therefore external to MPB, confirming EPS production.

Assuming that all ^{13}C in sediment OC was initially fixed by MPB and transferred via EPS secretion, there had been a transfer of ~ 60% of fixed ^{13}C from MPB to EPS by the first sampling period (524 μmol ^{13}C m^{-2} of ^{13}C in sediment OC was external to MPB). This is at the upper end of the reported range (Underwood and Paterson 2003), as expected for autotrophic sediments (Cook et al. 2009). High transfers of carbon may relate to faster production due to elevated temperatures, and the high bacterial biomass and productivity typical of lower latitudes (Eyre and Balls 1999).

Carbon transfer among sediment compartments—Tracing ^{13}C over 30 d provided insight into transfer and recycling of carbon among sediment compartments via carbohydrates over a longer time than has previously been considered (Goto et al. 2001; Cook et al. 2007; Bellinger et al. 2009).

The turnover rate of MPB in the current study was high, indicated by a low biomass : production ratio of 5.5 d (Sundbäck et al. 1996 reported 2–44 d for sands). In the absence of recycling of ^{13}C into MPB, a major part of ^{13}C should therefore be removed from MPB via senescence, grazing, and resuspension within 5.5 d of the end of label application. Such losses are reflected in the marked reduction in MPB ^{13}C within 3 d. Evidence of ^{13}C-label in MPB at day 30, however, indicates recycling of carbon back into MPB. This is most likely due to capture of DIC, produced by heterotrophs, during light periods. Diatoms can assimilate carbon from DIC during the dark (Mortain-Bertrand et al. 1988); however, a study in intertidal sands ~ 80 km north of the Brunswick River showed negligible uptake of ^{13}C-labeled DIC by MPB over a 4-h dark period (Oakes 2007). Diatoms are also capable of heterotrophic dissolved OC (DOC) uptake (Smith and Underwood 2000), but do not compete well with obligate heterotrophs (Znachor and Nedoma 2010). This is therefore likely to be a far less important route for carbon recycling into MPB.

The spike in ^{13}C content of bacteria at day 10, despite a general decline throughout the experiment, may also indicate carbon recycling. Rapid ^{13}C transfer to bacteria has been observed (Middelburg et al. 2000; Bellinger et al. 2009), but our longer-term study suggests that bacteria utilize an additional OC source, probably material released from a more refractory fraction. In addition, a general increase in the ^{13}C within sediments that was not accounted for by the measured compartments (from ~ 12% to 30%) indicates ^{13}C transfer to detritus and possibly higher heterotrophs throughout the experiment. Carbon from these sources may also have become available to bacteria as they eventually acquired ^{13}C label.

Carbohydrate diagenesis—The final composition of carbohydrates within sediment is a function not only of their delivery and production, but also of the processes that affect their loss and degradation. The ratio between the rate of ^{13}C incorporation into carbohydrates (uptake during light incubation; Table 1) and the overall rate of loss of ^{13}C from carbohydrates provided insight into carbohydrate diagenesis and was calculated as follows:

$$\text{Turnover ratio} = \text{uptake during light}/(\%G_1 \times k_1 + \%G_2 \times k_2) \qquad (4)$$

where $\%G_1$ and $\%G_2$ represent the percentages of total carbohydrate within G_1 and G_2 fractions (Table 2), and k_1 and k_2 are their decay rates, respectively. A smaller ratio

represents a high decay rate relative to incorporation—the carbohydrate will represent a smaller proportion of the carbohydrates that ultimately remain in the sediment. Based on these ratios (Table 2), the contribution of carbohydrates produced in situ to the total pool within sediments was expected to be greatest for glucose, followed by galactose, rhamnose, fucose, and xylose. Mannose should have the smallest contribution. This corresponded well with our observations (Fig. 1) and further highlights the contribution of in situ production to sediments at the study site. Discrepancies between the predicted composition of carbohydrates, based on observed production and loss, and the composition of carbohydrates that was measured reflect the input of allochthonous material. This allochthonous material evidently had a relatively low glucose content (i.e., the measured contribution of glucose was lower than expected), as would be expected for degraded detritus. Alternatively, such discrepancies may result from variations in degradation rates of less reactive pools over longer timescales than we considered. However, the patterns of diagenesis we predict generally match those previously reported (Cowie and Hedges 1984; Opsahl and Benner 1999; Woulds et al. 2010). Therefore, although there may be some differences in the inputs and underlying processes and/or the extent to which they occur in subtropical shallow subtidal sands, the relative susceptibilities of carbohydrates to degradation is similar, regardless of habitat, sediment type, and climate.

Carbon loss from sediments—Similar to previous studies (Goto et al. 2001; Bellinger et al. 2009), we saw loss of fixed carbon from surface sediment OC, with 85% of ^{13}C removed over 30 d. This loss may be due to transfer of carbon to deeper sediment layers that were not considered in the current study, or to the water column.

One potential mechanism for downward transfer of carbon is metazoan consumption. Higher heterotrophs were not considered in the current study, but can derive substantial carbon via MPB fixation (Oakes et al. 2010). However, transfer of MPB-derived carbon into heterotrophs is proportional to biomass (Middelburg et al. 2000). Because higher heterotrophs typically have a low contribution to sediment OC compared to, for example, bacteria (Middelburg et al. 2000), it is likely that they have a reduced role in carbon transfer in our study.

Carbon loss to the water column may occur via resuspension (Bellinger et al. 2009) or as DOC or DIC (Goto et al. 2001). Although Goto et al. (2001) also reported substantial, rapid loss of carbohydrates from sandy sediments (50% within 24 h) this was in contrast to studies by Cook et al. (2007) and Evrard et al. (2008) who reported minimal loss of carbohydrate carbon from shallow subtidal sands over 11 and 4 d, respectively. In each of the aforementioned cases, however, studies were ex situ and, in the case of Goto et al. (2001) involved the use of sediment slurries, which disrupt sediment layers, complicating the application of results to the environment. In the current study, sediments remained in situ until shortly prior to sampling, and were therefore subject to the influences of grazing, currents, etc., that affect removal of carbon from sediment. The different environment from which samples were collected may also affect results. Sediments at lower latitudes, such as the subtropical sands considered here, are characterized by high bacterial biomass and production (Alongi 1994) compared to the temperate sands previously considered. This might affect rates of transfer of carbohydrates, and their production and fate.

Implications—There were distinct differences between trends we observed and those reported for other environments. Although algae are typically characterized by high glucose concentration and appeared to be the main source of sediment OC, the uniform carbohydrate composition we observed suggests that the organic matter within sediments was highly degraded. The productivity of MPB was comparable to that of sands studied previously (Middelburg et al. 2000) and was at the upper range of productivity observed for muddy, photic, subtidal sediments in the upper reaches of the Brunswick River estuary (Eyre and Ferguson 2005) and sandy sediments in the lower estuary (Ferguson et al. 2003). This high productivity is likely reflected in the rapid transfer of fixed carbon to carbohydrates at rates at the upper end of expected ranges. High productivity, however, was balanced by high respiration, most likely because of the high temperature (to which respiration is more sensitive) and high bacterial biomass and production typical of subtropical sediments. High production led to bacterial demand for labile carbohydrates excreted by MPB, reflected in the rapid transfer of ^{13}C to bacteria. Degradation by bacteria resulted in the observed uniform composition and low concentration of carbohydrates. Although there was substantial loss of carbon from sediment, some carbon persisted for extended periods. This suggests that increased demand for carbon, due to high bacterial activity, led to the observed recycling of carbon into MPB and bacterial compartments.

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