

2012

Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: a ^{13}C -labeling study

Joanne Margaret Oakes
Southern Cross University

Bradley D. Eyre
Southern Cross University

Jack J. Middelburg
Utrecht University

Publication details

Postprint of: Oakes, JM, Eyre, BD & Middelburg, JJ 2012, 'Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: a ^{13}C -labeling study', *Limnology and Oceanography*, vol. 57, no. 6, pp. 1846-1856.

Published version available from:

<http://dx.doi.org/10.4319/lo.2012.57.06.1846>

1

2 Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal
3 sands: A ¹³C-labeling study

4

5

6

7 Joanne M. Oakes,^{a,*} Bradley D. Eyre,^a Jack J. Middelburg^{b,c}

8

9

10

11

12 * Corresponding author: joanne.oakes@scu.edu.au

13

14 Running head: Microphytobenthos carbon fate in sands

^aCentre for Coastal Biogeochemistry, Southern Cross University, Lismore, New South Wales, Australia

^b Department of Ecosystems, Royal Netherlands Institute for Sea Research, Yerseke, The Netherlands

^c Faculty of Geosciences, Utrecht University, Utrecht, The Netherlands

1 **Acknowledgements**

2 We thank Pete Squire, Melissa Bautista, Terry Lewins, Michael Brickhill, and
3 Simon Turner for field assistance, Kym Haskins and Max Johnston for extraction of
4 biomarkers, Melissa Gibbes for fauna extraction, and Matheus Carvalho for isotope
5 analysis. This study was funded by Australian Research Council (ARC) Discovery grants
6 to B.D.E. and J.J.M. (DP0663159) and B.D.E., J.M.O., and J.J.M. (DP0878568), an ARC
7 Linkage grant to B.D.E. (LP0667449), and ARC Linkage Infrastructure, Equipment and
8 Facilities grants to B.D.E. and J.M.O. (LE0989952, LE0668495), and an ARC Discovery
9 Early Career Researcher Award to J.M.O. (DE120101290). We thank two anonymous
10 reviewers for their constructive feedback.

11

1 **Abstract**

2 Microphytobenthos (MPB) in photic sediments are highly productive but the fate
3 of this production remains uncertain. Over 33 d, tracing of ^{13}C from added bicarbonate in
4 subtropical shallow subtidal sand showed rapid transfer of MPB-derived carbon to deeper
5 sediment; below 2 cm (31% within 60 h) and 5 cm (18%). Despite their high turnover
6 (5.5 d) and only representing ~8% of sediment organic carbon, MPB represented up to
7 35% of the ^{13}C retained in sediments, demonstrating substantial carbon recycling. Carbon
8 was rapidly transferred to heterotrophs, but their contribution to sediment ^{13}C was similar
9 to their biomass contribution (<0.1% to 3.8%), with the exception of the foraminifera
10 *Cellanthus craticulatus*, which accounted for up to 33% of the ^{13}C within sediment.
11 There was little loss of MPB-derived carbon via dissolved organic carbon (DOC) effluxes
12 (3%) or resuspension (minimal). Respiration was the major loss pathway (63%),
13 reflecting the high microbial biomass typical of lower latitudes. Given that MPB take up
14 dissolved inorganic carbon (DIC) from overlying water, and the carbon they fix is
15 released as DIC, MPB in subtropical sands are unlikely to substantially alter the form of
16 carbon transported offshore (i.e., there is no conversion to DOC), but processing within
17 the sediment may alter its $\delta^{13}\text{C}$ value. Given that 31% of fixed carbon remained in
18 sediments after 33 d, subtropical sands may act as a carbon sink, thereby affecting the
19 quantity of carbon transported offshore.

20

21

1 **Introduction**

2 Sediments within the photic zone may be colonized by highly productive
3 microscopic photosynthetic organisms (Cahoon 1999), collectively referred to as
4 microphytobenthos (MPB), and may therefore play an important role in intercepting and
5 modifying carbon inputs from the coast to the ocean.

6 The functional and trophic importance of MPB in coastal systems is widely
7 recognized. MPB can affect movement of nutrients, oxygen and carbon across the
8 sediment-water interface (Cahoon 1999; Eyre and Ferguson 2005). Particularly where
9 nutrients are limited (Cook et al. 2007), much of the carbon fixed by MPB is secreted as
10 extracellular polysaccharides (EPS; up to 73% of productivity; Goto et al. 2001),
11 primarily carbohydrates (Underwood et al. 2004; Oakes et al. 2010a). EPS reduces
12 resuspension by stabilizing sediments (Cahoon 1999) and, in addition to direct grazing on
13 MPB, can be a significant labile carbon source for consumers (Middelburg et al. 2000).

14 Trophic transfer of carbon fixed by MPB has received considerable attention,
15 particularly in intertidal silty and muddy sediments (Middelburg et al. 2000; Moens et al.
16 2002; Oakes et al. 2010c). MPB are also an important carbon source in sands (Herman et
17 al. 2000; Middelburg et al. 2000; Evrard et al. 2010), but studies have largely been in
18 temperate regions. Carbon cycling in the subtropics may be fundamentally different, due
19 to the high bacterial biomass at low latitudes (Alongi 1994). However, the role of MPB
20 as a carbon source for consumers in subtropical sands is poorly understood.

21 A further gap in current understanding of coastal carbon cycling is the fate of MPB-
22 derived carbon. Following transformation and/or burial, MPB-derived carbon may be lost
23 to the water column via resuspension, fluxes of dissolved inorganic carbon (DIC)

1 following mineralization or respiration (Goto et al. 2001; Evrard et al. 2012), and/or
2 fluxes of dissolved organic carbon (DOC) derived from exudates (Smith and Underwood
3 2000) and, to a lesser extent, cell lysis during grazing. These pathways may be an
4 important component of coastal carbon cycling. For example, sediment fluxes can
5 contribute significantly to the DOC in coastal waters (Maher and Eyre 2011), and MPB
6 has been proposed as a major source (Maher and Eyre 2010). However, the pathways for
7 loss of MPB-derived carbon from sediments have not been well-characterized.

8 For temperate, photic, subtidal, sandy sediments Cook et al. (2007) and Evrard et
9 al. (2008) reported negligible loss of MPB-derived carbon to overlying water over 6 and
10 4 d, respectively. Cook et al. (2007) recovered only 1-5% of fixed carbon as DOC. In
11 contrast, Oakes et al. (2010a) reported loss from in situ surficial subtropical photic sands
12 of 85% of MPB-derived carbon over 33 d. The pathways responsible for this loss were
13 not determined, but this highlights that loss to the water column may be the main fate of
14 carbon fixed by MPB in this environment. Alternatively, carbon may have been buried
15 deeper within sediments (below 2 cm; Oakes et al. 2010a). The greater loss of carbon
16 from subtropical sands compared to temperate sands may relate solely to the longer
17 period of study or the methods used. For example, given that the sediment studied by
18 Cook et al. (2007) and Evrard et al. (2008) was ex situ, and therefore was not subject to
19 natural environmental flows and the effects of mobile grazers, loss of fixed carbon may
20 have been underestimated. In support of this, Middelburg et al. (2000) reported
21 substantial loss of fixed carbon (~53%) over 4 d from in situ temperate sands, albeit in
22 the intertidal zone. This was estimated to be primarily due to resuspension (~60%;
23 Middelburg et al. 2000), but losses of carbon as DIC and DOC were not measured. The

1 different loss rates for carbon in subtropical and temperate sands may also reflect
2 characteristic differences between sands at these latitudes. High bacterial biomass and
3 productivity is typical of lower latitudes (Alongi 1994; Eyre and Balls 1999) and this may
4 account for the greater loss of fixed carbon observed for subtropical sands.

5 Stable isotopes at natural abundance are a popular tool for determining carbon
6 flows in aquatic systems, but interpretation can be complicated by fractionation and
7 variability and/or similarity in source signatures (Peterson 1999). Deliberate application
8 of the rare carbon isotope, ^{13}C , can overcome these problems, allowing trophic transfers
9 of carbon to be traced and quantified (Middelburg et al. 2000; Oakes et al. 2010c). More
10 recently, it has been possible to trace ^{13}C into fluxes of DOC and DIC. This has been
11 demonstrated for natural abundance $\delta^{13}\text{C}$ (Raymond and Bauer 2001; Oakes et al. 2010b;
12 Maher and Eyre 2011), and in ^{13}C -labeling experiments (Andersson et al. 2008; Oakes et
13 al. 2011; van den Meersche et al. 2011). In the current study, we aimed to use a
14 combination of ^{13}C -labeling, compound-specific isotope analysis of biomarkers, and
15 isotope analysis of DOC and DIC to investigate the fate of MPB-derived carbon in
16 subtropical photic subtidal sandy sediments. Specifically, we aimed to determine rates of
17 carbon transfer within sediment compartments and rates of carbon loss to the water
18 column. We hypothesized that DIC fluxes would be a major pathway for loss of MPB-
19 derived carbon, due to the high bacterial biomass typical of the subtropical sediments
20 studied, and the high affinity of bacteria for the labile DOC exuded by MPB. Because we
21 were particularly interested in pathways of carbon loss, the experiment was done in situ,
22 allowing natural loss processes to occur, and over an extended time period (33 d). Given
23 the paucity of information on the transformation and fate of carbon fixed by MPB,

1 particularly in subtropical photic sands, this is a valuable step towards understanding the
2 role of MPB in coastal carbon cycling.

3

4 **Methods**

5 Two of the experimental plots used in the current study, and the cores collected
6 from these plots, were also used by Oakes et al. (2010a). However, the current study
7 utilized an additional ^{13}C -labeled plot (i.e., three plots in total).

8

9 *Study site*

10 The subtidal study site (~1.5 m below average sea level) in the Brunswick River,
11 subtropical Australia, was described by Oakes et al. (2010a). The site was net autotrophic
12 (production:respiration ~1.2 over 24 h; J. M. Oakes unpubl.). Gross productivity based on
13 CO_2 fluxes averaged ~105 mmol C m⁻² d⁻¹ (J.M. Oakes unpubl.). The organic carbon
14 (OC) content of sediment to 10 cm depth was 18.6 mol C m⁻². Surface sediments (0-2
15 cm) had a higher OC content (0.18%) and a lower molar C:N ratio (6.9 ± 0.7) than deeper
16 sediments (0.16%, 9.7 ± 0.8 at 2-5 cm; 0.15%, 9.8 ± 0.6 at 5-10 cm). The sands were not
17 permeable. Sediment at all depths, was comprised primarily of fine (125-250 μm ; 72-
18 79%) and medium (250-500 μm ; 17-24%) quartz sand grains.

19

20 *^{13}C -labeling*

21 ^{13}C -labeling of three experimental plots (1.2 m²) was achieved by ^{13}C -labeling the
22 water column DIC pool (23% ^{13}C) in benthic chambers using $\text{NaH}^{13}\text{CO}_3$, as described in

1 Oakes et al. (2010a). Pumps maintained laminar flow of water across the sediment within
2 the chambers for a 24 h period. Chambers were then removed.

3

4 *Sample collection*

5 Immediately after chamber removal and at 1, 3, 10, 20, and 30 d thereafter, two
6 sediment cores (9 cm diameter × 20 cm deep, with 30 cm overlying water) from each
7 labeled experimental plot and two control cores from 5 to 8 m outside of each plot (for
8 background isotope values) were collected. In the laboratory, control and labeled cores
9 were placed in separate tanks of unlabeled site water at in situ temperature ($\pm 1^\circ\text{C}$) and
10 light levels ($\pm 5\%$, $\sim 400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at sediment surface, 12 h) and were pre-
11 incubated (12h dark, then 12 h light), then sealed and incubated (12 h dark then 12 h
12 light) as described by Oakes et al. (2010a). Magnetic bars ~ 10 cm above the sediment
13 gently stirred water within cores. Pre-incubation was intended to allow sediment
14 microhabitats to re-establish, thus minimizing the effects on fluxes due to sediment
15 disturbance, but use of chambers and pre-incubation prevented monitoring of ^{13}C losses
16 over the first 48 h, and sediment transfers over the first 60 h, following labeling. The
17 focus of the study was therefore on longer-term transfer and loss processes.

18 At the beginning and end of the dark incubation period and at the end of the light
19 incubation period, ~ 50 mL of water was removed from each core to determine
20 concentrations and carbon stable isotope ratios ($\delta^{13}\text{C}$) of dissolved organic carbon (DOC)
21 and dissolved inorganic carbon (DIC). Samples were syringe filtered (precombusted
22 GF/F), leaving no headspace, into pre-combusted 40 mL glass vials with Teflon-coated
23 septa and were killed ($200 \mu\text{L}$ 50:50 w:v ZnCl_2) and refrigerated until analysis. Sample

1 water was replaced, as it was withdrawn, with site water from a collapsible reservoir.

2 Additional water samples, not included in this study, were also collected.

3 Sediment was extruded and sectioned (0-2 cm, 2-5 cm, and 5-10 cm depths) for
4 one core from each plot and for one of each pair of control cores at the end of the dark
5 incubation, and from remaining cores at the end of the light incubation. A known portion
6 of each sediment sample was freeze-dried for $\delta^{13}\text{C}$ analysis of sediment OC and
7 phospholipid-derived fatty acid (PLFA) biomarkers for bacteria and MPB. The remainder
8 was stored frozen (-20°C) for separation of fauna.

9 Low abundance of macrofauna at the study site prevented assessment of their use
10 of MPB. We therefore focused on the meiobenthos, which, at the time of sampling, was
11 dominated by three species of foraminifera (*Cellanthus craticulatus*, *Ammonia beccarii*,
12 and *Elphidium advenum*; 95-98% of total meiobenthos biomass). Foraminifera were
13 picked, by hand, from sediment samples to obtain sufficient material for isotope analysis.

14

15 *Sample analysis*

16 DOC and DIC concentrations and $\delta^{13}\text{C}$ were measured, as described by Oakes et
17 al. (2010b, 2011), via continuous flow wet-oxidation isotope ratio mass spectrometry
18 using an Aurora 1030W total organic carbon analyzer coupled to a Thermo Delta V Plus
19 Isotope Ratio Mass Spectrometer (IRMS). Sodium bicarbonate (DIC) and glucose (DOC)
20 of known isotope composition dissolved in helium-purged milli-Q were used for drift
21 correction and to verify concentrations and $\delta^{13}\text{C}$ values. Reproducibility for DOC and
22 DIC, respectively, was $\pm 0.3 \text{ mg L}^{-1}$ and $\pm 0.4 \text{ mg L}^{-1}$ (concentrations) and $\pm 0.08\text{‰}$ and
23 $\pm 0.10\text{‰}$ ($\delta^{13}\text{C}$).

1 PLFA biomarkers to determine ^{13}C uptake into bacteria and MPB were extracted
2 from lyophilized sediments using a modified Bligh and Dyer method, as described by
3 Oakes et al. (2010a). An internal standard (tridecanoic acid, C_{13}) was added at the
4 beginning of the extraction procedure. Extracts were analyzed via gas chromatography-
5 isotope ratio mass spectrometry, as described by Oakes et al. (2010a).

6 Foraminifera and sediment samples were placed dried in silver cups (60°C to
7 constant weight), acidified (10% HCl), re-dried (60°C), then analyzed for $\delta^{13}\text{C}$ and %C
8 (% of OC in the unacidified sample) using a Thermo Finnigan Flash EA 112 interfaced
9 via a Thermo ConFlo III with a Thermo Delta V Plus IRMS. Helium dilution of the
10 carrier stream was turned off for foraminifera samples to reduce the required mass.

11

12 *Calculations*

13 PLFA $\delta^{13}\text{C}$ values were corrected for the addition of a carbon atom during
14 methylation as described by Jones et al. (2003). Natural abundance $\delta^{13}\text{C}$ values for
15 bacteria and MPB were calculated using $\delta^{13}\text{C}$ values of PLFAs specific to bacteria and
16 diatoms from control cores, as described by Oakes et al. (2010a).

17 Total uptake (incorporation) of ^{13}C into sediment OC, foraminifera, bacteria, and
18 MPB ($\mu\text{mol } ^{13}\text{C m}^{-2}$) was calculated as the product of excess ^{13}C (fraction ^{13}C in labeled
19 sample – fraction ^{13}C in control) and the mass of OC in each compartment. For sediment
20 and foraminifera, OC mass was the product of %C and total dry mass per unit area. For
21 foraminifera, counts of individuals within sediment subsamples were scaled up to provide
22 an estimate of the total number of individuals per m^2 at each depth. Total mass was

1 determined by multiplying this estimate by the average mass of an individual, which was
2 determined by weighing a known number of individuals.

3 Concentrations of PLFA biomarkers for bacteria and MPB were calculated based
4 on their peak areas relative to that of the C₁₃ internal standard. Total biomass of bacteria
5 and MPB was calculated as described by Oakes et al. (2010a), based on the bacterial
6 biomarkers i15:0 and a15:0, and the algal biomarkers 16:1(n-7) and 20:5(n-3), which
7 gave distinct chromatographic peaks. The average fraction of MPB PLFAs that is
8 typically accounted for by the biomarkers considered is 0.37 (Volkman et al. 1989).

9 Total ¹³C transfer into water column DOC and DIC was calculated for the
10 beginning and end of the dark incubation period and for the end of the light period as the
11 product of excess ¹³C in DOC or DIC (fraction ¹³C in labeled sample – fraction ¹³C in
12 equivalent control), core volume and the concentration of DOC or DIC. The total flux of
13 excess ¹³C in DOC or DIC during dark or light incubation was then calculated as follows:

$$14 \quad \text{Excess } ^{13}\text{C flux} = \text{Excess } ^{13}\text{C}_{\text{start}} - \text{Excess } ^{13}\text{C}_{\text{end}} / SA / t \quad (1)$$

15 where excess ¹³C_{start} and excess ¹³C_{end} represent excess ¹³C at the beginning and end of
16 the dark or light incubation period, SA is the sediment surface area within a core, and *t*
17 represents hours of dark or light incubation. Net fluxes (excess ¹³C m⁻² d⁻¹) of DOC or
18 DIC were calculated as follows:

$$19 \quad \text{Net flux} = \text{dark flux} / \text{dark hours} + \text{light flux} / \text{light hours} \times 24 \text{ hours} \quad (2)$$

20 We interpolated between measured net flux values and estimated the total quantity of ¹³C
21 lost via fluxes of DOC and DIC from the end of labeling up until each sampling period
22 by determining the area under the curve.

23

1 *Data analysis*

2 Sediment OC, foraminifera, bacteria and MPB excess ^{13}C values were determined
3 for the end of both dark and light periods. We therefore used three-way analyses of
4 variance (ANOVAs) to determine if these data should be treated separately for each
5 depth or for each time. Factors of depth, day, and light exposure (dark or light) were used
6 to test for an effect of light exposure alone or in combination with other factors
7 (interaction with depth and/or day). As Levene's test was significant in each case
8 (variances were heterogeneous), alpha was set at 0.01 to reduce the possibility of falsely
9 rejecting the null hypothesis. Significant three-way interactions were explored using two-
10 way ANOVAs, and significant two-way interactions were explored using one-way
11 ANOVAs. For example, where there was depth \times day interaction, one-way ANOVAs
12 were used to determine if there was a difference among depths on each individual day.
13 Where ANOVAs indicated a significant main effect, post hoc Tukey tests showed which
14 groups were statistically different.

15 A 2-G model (Westrich and Berner 1984) was used to determine the rate of ^{13}C
16 loss from total sediment OC (0-10 cm) from each experimental plot using the average of
17 light and dark sediment $\delta^{13}\text{C}$ values (as these were not significantly different) at each
18 time, as follows:

19
$$G_T(t) = G_1 [\exp (-k_1 t)] + G_2 [\exp (-k_2 t)] + G_{\text{NR}} \quad (3)$$

20 where G_T is the incorporation of ^{13}C within sediment OC, t is time, G_1 , G_2 , and G_{NR}
21 represent the incorporation of ^{13}C into highly reactive, less reactive and non-reactive
22 (over the timescale of the experiment) fractions of sediment OC, and k_1 and k_2 represent
23 first-order decay constants specific to G_1 and G_2 , respectively.

1 The isotope mixing program IsoSource (Phillips and Gregg 2003) calculates all
2 feasible combinations of multiple possible sources to a mixture, based on isotope ratios,
3 and was therefore used to estimate the likely contribution of MPB, phytoplankton and
4 terrestrial plants to DOC effluxes from the sediment.

6 **Results**

7 Three-way ANOVAs showed that there was no significant effect of light exposure
8 during laboratory incubation on excess ^{13}C in sediment OC, bacteria, foraminifera, or
9 MPB. Light and dark excess ^{13}C values were therefore combined for subsequent
10 calculations.

12 *Sediment organic carbon composition and depth distribution*

13 Sediment OC content was greater in shallower sediments, with 223.56 ± 12.34
14 $\mu\text{mol C mL}^{-1}$ in 0-2 cm sediment compared to $185.88 \pm 8.71 \mu\text{mol C mL}^{-1}$ in 2-5 cm
15 sediment and $172.12 \pm 7.39 \mu\text{mol C mL}^{-1}$ in 5-10 cm sediments. MPB, bacteria and
16 foraminifera together accounted for 16.9%, 15.8%, and 18.1% of the sediment OC,
17 respectively, in these sediment depths (Table 1). The contribution of MPB and
18 foraminifera to sediment OC was greatest in surface sediments (Table 1), whereas
19 bacteria made the greatest contribution to OC in deeper sediments (Table 1).

21 *Natural abundance stable isotope ratios*

22 Sediment OC was more enriched in ^{13}C in 0-2 cm sediment than in deeper
23 sediments (-17.9‰ vs. $\sim -19.5\text{‰}$; Table 1). At all depths, mean $\delta^{13}\text{C}$ values of bacteria

1 were depleted by up to 0.9‰ compared to MPB (Table 1). Across depths, sediment OC
2 was depleted in ^{13}C by 1.6‰ to 5.6‰ compared to MPB and bacteria.

3 Water column DIC and DOC in control cores had mean $\delta^{13}\text{C}$ values of 2.6‰ and
4 -23.3‰, respectively.

5

6 *Incorporation of ^{13}C and transfer among sediment compartments*

7 By the time the first sediment was collected (60 h after label addition), 1492 μmol
8 $^{13}\text{C m}^{-2}$ was in sediment OC 0-10 cm deep. Allowing for measured loss of ^{13}C as DOC
9 and DIC during the dark incubation period preceding sediment sample collection, 1809 \pm
10 110 $\mu\text{mol }^{13}\text{C m}^{-2}$ was in 0-10 cm sediments 48 h after label addition. This equated to an
11 incorporation of 75 $\mu\text{mol }^{13}\text{C m}^{-2} \text{ light h}^{-1}$ until this point, with ~0.01% of sediment OC
12 replaced with ^{13}C . True rates of ^{13}C incorporation are likely to have been somewhat
13 higher, however, as we were unable to account for losses of ^{13}C from the sediment during
14 the pre-incubation period. Based on ^{13}C incorporation, and allowing for the partial
15 labeling of available DIC (23% ^{13}C), total carbon fixation was estimated to be ~328 μmol
16 $\text{C m}^{-2} \text{ light h}^{-1}$. This is far lower than the gross primary productivity for the study site
17 based on O_2 and CO_2 fluxes (~4000 $\mu\text{mol m}^{-2} \text{ h}^{-1}$; J. M. Oakes unpubl.).

18 Fixed ^{13}C was detected in deeper sediments (2-5 and 5-10 cm deep) from the first
19 sampling time (Fig. 1). Within 60 h of label addition 31.5% of the incorporated ^{13}C in
20 sediments was below 2 cm and 18.6% was between 5 and 10 cm. Assuming fixation of
21 carbon in deeper sediments was negligible, this gives transport rates of 10 $\mu\text{mol }^{13}\text{C m}^{-2}$
22 h^{-1} and 6 $\mu\text{mol }^{13}\text{C m}^{-2} \text{ h}^{-1}$ to these depths, respectively. Considering that the added ^{13}C
23 accounted for 23% of the total water column DIC available to MPB, fixed carbon was

1 transported to sediment below 2 cm at a rate of $41 \mu\text{mol C m}^{-2} \text{ h}^{-1}$, and to sediment below
2 5 cm at a rate of $24 \mu\text{mol m}^{-2} \text{ h}^{-1}$.

3 Significantly more excess ^{13}C was measured in sediment OC at 0-2 cm than at 2-5
4 cm and 5-10 cm early in the study (at 3 and 4 days after label application, two-way
5 ANOVA: $F_{2,5}=12.515$, $p=0.001$ and $F_{2,5}=8.005$, $p=0.005$, respectively; Fig. 1). However,
6 by 13 d after label application transport of ^{13}C to deeper sediment, combined with ^{13}C
7 loss from surface sediments to the water column, led to similar excess ^{13}C in sediment
8 OC across depths ($p > 0.01$; depth \times sampling day interaction, three-way ANOVA:
9 $F_{10,35}=3.836$, $p=0.001$; Fig. 1).

10 The sediment compartments considered in the current study (MPB, bacteria, and
11 the three species of foraminifera) accounted for approximately half ($51.6 \pm 5.8\%$) of the
12 ^{13}C within sediment OC across all sampling times.

13 MPB in surface sediments (0-2 cm) initially accounted for a far greater portion of
14 the ^{13}C fixed into sediment OC than did MPB in deeper sediments (Fig. 1). Within each
15 sediment depth layer, the contribution of MPB to the ^{13}C in sediment OC far outweighed
16 the contribution of MPB biomass to sediment OC. Whereas MPB biomass represented
17 only 6.6 to 9.0% of the OC within 0-2, 2-5, and 5-10 cm sediment (Table 1), the average
18 contribution of MPB to ^{13}C within OC at each sediment depth throughout the study
19 period was $34.8 \pm 3.9\%$, $27.3 \pm 3.7\%$, and $15.8 \pm 3.5\%$, respectively. Throughout the
20 study, there was a marked decrease in the ^{13}C content of MPB in 0-2 cm sediment. By 33
21 d after label addition, only 2.9% of the ^{13}C initially fixed into sediment OC remained in
22 this compartment (Fig. 1). The incorporation of ^{13}C into MPB in 2-5 and 5-10 cm

1 sediments varied relatively little throughout the study (Fig. 1) and was significantly lower
2 than in 0-2 cm sediments (three-way ANOVA: $F_{2,35}=14.068$, $p=0.000$).

3 The initial incorporation of fixed ^{13}C into bacteria in 0-2 and 2-5 cm sediment was
4 similar (but note the different volumes encompassed by the given depth ranges; Fig. 1). A
5 smaller portion of fixed ^{13}C was in 5-10 cm sediment at the first sampling period (Fig. 1).
6 The contribution of bacteria to the ^{13}C in OC within each sediment depth layer was $6.7 \pm$
7 1.3% , $11.9 \pm 2.2\%$ and $10.7 \pm 3.5\%$, respectively, for sediment at depths of 0-2, 2-5 and
8 5-10 cm. This was similar to the contribution of bacterial biomass to OC within each
9 sediment layer (Table 1). The ^{13}C content of bacteria was similar across sediment depths
10 ($p>0.01$) but varied significantly among days (three-way ANOVA: $F_{5,35}=3.559$, $p=0.007$;
11 Fig. 1). This was dominated by a general decrease in the ^{13}C content of bacteria in 0-2 cm
12 sediments throughout the study period (Fig. 1).

13 There was evidence of label uptake into all three foraminifera species by the time
14 the first sediment sample was taken (Fig. 1). The greatest incorporation of ^{13}C , per unit
15 biomass, was by *Cellanthus craticulatus*, which dominated the foraminifera (Table 1).
16 The greatest uptake of ^{13}C by *C. craticulatus* was generally in sediment at 2-5 cm (Fig.
17 1), where *C. craticulatus* accounted for up to ~5% of the total ^{13}C initially fixed into
18 sediments (Fig. 1). Despite representing only 0.8% to 3.8% of the biomass within each
19 sediment depth layer (Table 1), *C. craticulatus* accounted for, on average, $9.6 \pm 2.1\%$,
20 $31.2 \pm 5.9\%$, and $9.7 \pm 2.9\%$ of the ^{13}C within sediment OC at 0-2, 2-5, and 5-10 cm,
21 respectively. In contrast, the contribution of *Ammonia beccarii* and *Elphidium advenum*
22 to the ^{13}C in sediment OC was similar to their contribution to biomass (Fig.1, Table 1).
23 The incorporation of ^{13}C into *A. beccarii* was greater in 5-10 cm sediment than in

1 shallower sediments. At any one time, *E. advenum* accounted for less than 0.01% of the
2 total ^{13}C initially incorporated into sediment OC (Fig. 1).

3

4 *Loss of ^{13}C from sediments*

5 The decline in ^{13}C content of 0-10 cm sediments throughout the study period was
6 substantial and could be fitted with a 2-G model ($R^2 = 0.98$; Fig. 2). A highly reactive
7 fraction of sediment OC (G_1 ; $60.67 \pm 5.95\%$) was lost at a rate of $1.51 \pm 0.20 \text{ d}^{-1}$ (k_1), and
8 a less reactive fraction (G_2 ; $17.18 \pm 5.39\%$) degraded at a rate of $0.02 \pm <0.01 \text{ d}^{-1}$ (k_2).
9 The remaining sediment OC was non-reactive over the timescale of this experiment (G_{NR} ;
10 $24.09 \pm 3.65\%$).

11 There was generally an efflux of DIC from sediment in the dark (1677 ± 252
12 $\mu\text{mol C m}^{-2} \text{ h}^{-1}$) and an uptake in the light ($-2319 \pm 278 \mu\text{mol C m}^{-2} \text{ h}^{-1}$), resulting in net
13 DIC uptake. For DOC, there were effluxes in both the dark and the light ($596 \pm 235 \mu\text{mol}$
14 $\text{C m}^{-2} \text{ h}^{-1}$ and $482 \pm 211 \mu\text{mol C m}^{-2} \text{ h}^{-1}$, respectively).

15 Loss of ^{13}C from sediment to the water column was dominated by fluxes of DIC,
16 with very little ^{13}C lost as DOC (Fig. 3). Most of the loss of ^{13}C as DIC occurred over the
17 first few days of the study, with nearly 50% of the ^{13}C initially incorporated into
18 sediments having been lost to the water column as DIC within 6 days of label addition
19 (Fig. 3). By the conclusion of the study ~63% (59% to 67%) of incorporated ^{13}C had been
20 lost to the water column as DIC, and ~3% (1.4% to 4.0%) as DOC. Approximately 31%
21 of the ^{13}C that was initially incorporated by MPB remained within the sediment OC pool.
22 Of this, ~12% (4.0% to 33.1%) was in sediment at 0-2 cm, ~10% (4.6% to 17.7%) was at
23 2-5 cm, and ~9% (3.0% to 13.6%) was at 5-10 cm (Fig. 3). Based on excess ^{13}C per

1 volume, this was dominated by OC within surface sediments. At the conclusion of the
2 study, only ~3% of the initially incorporated ^{13}C could not be accounted for by the
3 pathways and compartments considered.

4

5 **Discussion**

6 The ^{13}C -labeling experiment described in the current study was primarily done in
7 situ, with cores removed to the laboratory shortly before incubation for sediment fluxes.
8 For most of the study period, the sediment studied was therefore subject to natural loss
9 and transfer processes, including grazing by mobile consumers, bioturbation, and
10 physical mixing and resuspension by water movement. As these processes can not be
11 adequately replicated in a laboratory environment, in situ experimentation was essential
12 for our focus on the longer-term fate of MPB-derived carbon. Although the fate and
13 transformation of MPB-derived carbon has been investigated in situ (Middelburg et al.
14 2000; Bellinger et al. 2009), this was done in temperate, intertidal sediments, which are
15 subject to fundamentally different environmental conditions compared to the subtropical
16 subtidal sediment we studied. In addition, the study by Middelburg et al. (2000) used a
17 mass-balance approach to estimate losses to respiration and resuspension and did not
18 estimate DOC losses, and Bellinger et al. (2009) did not measure DIC and DOC fluxes.
19 In comparison, the current study directly quantified losses to both DOC and DIC. It is
20 important to note that our experimental procedure may have affected shorter-term
21 processes, as the first samples collected had been within chambers and cores (i.e.,
22 isolated from in situ conditions) for 60 h. Similarly, cores collected at other early time
23 points had a proportionally small period during which they were exposed to in situ

1 conditions. This may have affected our observations of carbon cycling in the short-term,
2 particularly carbon loss. However, for cores collected towards the end of the study, which
3 were primarily exposed to in situ conditions, the quantity of ^{13}C remaining within
4 sediment OC matched that expected based on earlier observations. Similarly, our budget
5 accounted for ~97% of the added ^{13}C over 33 d. Any effect of our experimental treatment
6 on short-term carbon cycling processes therefore appears to be negligible.

7

8 *Natural abundance isotope ratios*

9 We previously determined that OC in surface sediments (0-2 cm) at our study site
10 is derived primarily from MPB (~56%) and pelagic algae (~32%), with only a small
11 contribution of terrestrial plant material (~12%; Oakes et al. 2010a). In the current study,
12 $\delta^{13}\text{C}$ of OC in deeper sediments was depleted (~-19.5‰; Table 1) compared to surface
13 sediments ($-17.9 \pm 1.3\text{‰}$), indicating an increased contribution of ^{13}C -depleted OC,
14 possibly derived from terrestrial plant material (-23‰ to -30‰; Michener and Schell
15 1994). This reflects the greater biomass of relatively ^{13}C -enriched MPB ($-15.4 \pm 0.4\text{‰}$) in
16 surface sediments combined with rapid processing of relatively ^{13}C -enriched labile MPB
17 (Oakes et al. 2010a) and pelagic algae-derived carbon in surface sediments, compared to
18 more refractory ^{13}C -depleted terrestrial organic matter, prior to OC burial.

19 Natural abundance $\delta^{13}\text{C}$ of DIC at the study site ($2.56 \pm 0.59\text{‰}$) was within the
20 range of coastal DIC (Schidlowski 2000), however, natural abundance $\delta^{13}\text{C}$ of DOC (-
21 $23.31 \pm 1.03\text{‰}$) is more difficult to put in context and to interpret. Assuming that DOC
22 faithfully reflects the $\delta^{13}\text{C}$ value of the primary producer from which the carbon is
23 ultimately derived, isotope mixing calculations (IsoSource; Phillips and Gregg 2003)

1 based on natural abundance $\delta^{13}\text{C}$ values for MPB (-15.4‰, this study), pelagic algae (-
2 18‰ to -24‰, Michener and Schell 1994), and terrestrial plants (-23‰ to -30‰
3 (Michener and Schell 1994)) suggest that the carbon within DOC in the lower Brunswick
4 estuary is ultimately derived primarily from carbon fixed by phytoplankton ($45 \pm 37\%$,
5 standard deviation) and terrestrial plants ($38 \pm 37\%$). MPB appear to have a smaller
6 contribution ($18 \pm 27\%$) but may contribute anywhere from 0% to 46% of the DOC. This
7 may represent direct release from primary producers, or release of DOC by bacteria that
8 have consumed carbon that was fixed by these primary producers. The usefulness of this
9 analysis, however, is questionable as, rather than faithfully reflecting its source,
10 preferential use of more labile DOC components within sediments (Chipman et al. 2010)
11 can result in fluxes of DOC being comprised of a very enriched (e.g., carbohydrates) or
12 depleted (e.g., lipids) fraction of the source organic matter (Maher and Eyre 2011).

13

14 *^{13}C incorporation and transfer within sediments*

15 We were unable to account for loss of ^{13}C that occurred before the first water
16 sample was taken (48 h after label application). Given that MPB-derived carbon can be
17 rapidly processed (Middelburg et al. 2000) it is therefore likely that we underestimated
18 ^{13}C incorporation. However, the rates of transfer and loss calculated from our starting
19 point (48 h) are independent of the accuracy of this incorporation rate.

20 Compared to previous studies in temperate intertidal sands (Middelburg et al.
21 2000) and temperate subtidal sands (Evrard et al. 2008, 2010), there was greater
22 downward transport of ^{13}C in the current study. In the current study, 12.9% of fixed ^{13}C
23 was in sediment OC at a depth of 2-5 cm 60 h after label application, and 18.6% of fixed

1 ^{13}C was transferred to sediments 5-10 cm deep. In comparison, it took longer (3 d) for a
2 similar fraction of ^{13}C to enter 2-5 cm sediment in the studies of temperate sands
3 (Middelburg et al. 2000; Evrard et al. 2008), and there was negligible transfer of ^{13}C
4 below 5 cm. This may reflect differences in methodology and/or location (temperate vs.
5 subtropical, intertidal vs. subtidal, differences in sediment composition), and associated
6 variation in rates of bioturbation, active migration of MPB and heterotrophs, and physical
7 mixing of sediments by water movement. A further explanation for differences in ^{13}C
8 incorporation in deeper sediments is differences in rates of chemoautotrophy. Relatively
9 little is known of the importance of chemoautotrophy in coastal sands, however, Evrard
10 et al. (2008) identified chemoautotrophy as being responsible for a relatively small
11 portion of ^{13}C uptake at depth in temperate sands. In the current study the delay between
12 label application and sample collection prevented us from identifying any contribution of
13 chemoautotrophy to ^{13}C uptake into deeper sediments. However, there was no evidence
14 of ^{13}C uptake by bacteria in the absence of, or in excess of, ^{13}C uptake by MPB.

15 Considerable loss via resuspension (60%) in the study by Middelburg et al. (2000)
16 may have reduced the carbon pool available for downward transport, and the ex situ
17 nature of the study by Evrard et al. (2008) isolated sediments from natural water
18 movement and bioturbation, which may have limited carbon burial. The different depth
19 distribution of ^{13}C in the study by Middelburg et al. (2000) compared to the current study
20 may reflect differences in water flow and sediment mixing in intertidal sands, compared
21 to the subtidal sediments we studied. However, the ^{13}C depth distribution observed by
22 Middelburg et al. (2000) was similar to that observed for subtidal temperate sands by
23 Evrard et al. (2008). The differences in ^{13}C depth distribution between studies of

1 temperate sands, and that seen in the current study, hints at the possibility that different
2 processes influence carbon burial in temperate and subtropical sands. However, further
3 replicated studies across climate zones are required to determine if this is the case.

4 In the current study, initial burial of ^{13}C occurred within chambers and cores, and
5 therefore occurred in the absence of natural water flow. Given that the sands were not
6 permeable, advective flow would not be important in carbon burial in this environment.
7 The minimal loss of carbon via resuspension observed in the current study also suggests
8 that water movement (i.e., physical mixing) had little effect on sediments and their
9 carbon distribution. Given that bioturbation would be limited by the low abundance of
10 fauna at the site, it is likely that high rates of MPB and heterotroph migration (i.e.,
11 migration by bacteria and/or meiobenthos) were responsible for the high rate of
12 downward transport for ^{13}C in the current study. This may reflect the high heterotrophic
13 activity typical of lower latitudes (Boer et al. 2009). Active migration by MPB can occur
14 to considerable depths within sands (Underwood 2002; Saburova and Polikarpov 2003)
15 and this may account for the considerable ^{13}C content of MPB in 2-5 cm and, to a lesser
16 extent, 5-10 cm sediments. This may be assisted by the greater light availability at low
17 latitudes, which may make it possible for MPB in subtropical sands to persist at greater
18 sediment depths. MPB may also move to deeper, aphotic layers of sandy sediments to
19 access nutrients (Saburova and Polikarpov 2003). Regardless of the mechanism involved,
20 the rapid downward transport of carbon observed in the current study has implications for
21 the ultimate fate of carbon fixed by MPB. Labile OC will be respired, but the depth at
22 which this occurs, and the pathways leading to its respiration, will depend on mixing

1 regime. The efficient burial of MPB-derived carbon is likely to contribute to its long-term
2 retention within subtropical sandy sediments.

3 MPB-derived carbon appears to have been recycled by MPB, bacteria and
4 foraminifera, with considerable ^{13}C detected in all of these compartments throughout the
5 experimental period. Particularly for *Cellanthus craticulatus* in sediment at 0-5 cm, and
6 for bacteria at all depths, there was no apparent decline in ^{13}C content throughout the
7 experiment. Whereas the fraction of fixed ^{13}C that was within bacteria was similar to
8 their contribution to sediment OC, the fraction of ^{13}C within MPB and *C. craticulatus* far
9 outweighed their contribution to sediment OC. This suggests that MPB and
10 *C. craticulatus* have a greater role than bacteria in the long-term retention of fixed
11 carbon. The low biomass:productivity ratio for MPB at the study site (~5.5 d; Oakes et al.
12 2010a), possibly due to fast turnover in response to high grazing pressure (Herman et al.
13 2000), may mean that MPB need to intercept DIC produced through respiration, in
14 addition to water column DIC, to satisfy their carbon demand. For foraminifera, the
15 carbon source can vary with species (Oakes et al. 2010c). Foraminifera can obtain carbon
16 from MPB and phytodetritus (Moodley et al. 2002, Oakes et al. 2010c), as well as from
17 bacteria (Mojtahid et al. 2011), macrophyte detritus (Oakes et al. 2010c) and dissolved
18 organic matter (DeLaca 1982). Larger foraminifera can contain symbiotic algae, allowing
19 them to directly access inorganic carbon as a source (Lee 1995). Larger foraminifera
20 were not observed in the current study, but foraminifera in the family Elphidiidae (which
21 includes the genera *Cellanthus* and *Elphidium*) can contain chloroplasts extracted from
22 diatoms which can remain photosynthetically active, fixing carbon for their hosts, for
23 weeks or months (Lee 1995). The high accumulation of ^{13}C in *C. craticulatus* compared

1 to *Ammonia beccarii* and *Elphidium advenum*, relative to their biomass, may therefore
2 indicate the presence of functional chloroplasts in *C. craticulatus*. However, given that
3 the ^{13}C content of *C. craticulatus* in surface sediments did not noticeably decline with
4 time, in contrast to MPB, it appears that *C. craticulatus* retain fixed carbon for a longer
5 period than MPB and/or also access MPB-derived carbon. Given that rates of active
6 migration for benthic foraminifera can be high (0.4 to 184.3 mm d⁻¹; Bornmalm et al.
7 1997), particularly in shallow-water sediments and at higher temperatures, *C. craticulatus*
8 may play an important role in the transfer of fixed carbon to deeper sediments.

9 The ^{13}C within sediment OC that we were unable to account for may have been
10 incorporated by meiobenthos that we did not consider, occasional macrofauna, or
11 senescent MPB, bacteria, or fauna. Extracellular compounds (e.g., EPS; Goto et al. 2001)
12 can also be a major reservoir for MPB-derived carbon. For example, intra- and
13 extracellular carbohydrates together contained 15% to 30% of the ^{13}C incorporated into
14 0-2 cm sediment OC at our site (Oakes et al. 2010a). Depending on their contribution to
15 total carbohydrates, extracellular carbohydrates may therefore contribute significantly to
16 the uncharacterized portion of the ^{13}C within sediment OC in the current study.

17

18 *Loss of ^{13}C from sediments*

19 We previously observed that of 85% of the carbon fixed by MPB was lost from
20 surface sediments (0-2 cm) over 33 d (Oakes et al. 2010a). However, the loss pathways
21 were not determined and were therefore the focus of the current study.

22 Given that only ~3% of the carbon fixed by MPB was not found within sediments
23 or accounted for by fluxes of DOC and DIC by the end of the study, resuspension

1 apparently played only a minor role in the loss of MPB-derived carbon from the
2 subtropical subtidal sands studied. This may be due to low water movement over the
3 sediment surface, the observed rapid transfer of carbon to deeper sediments, high
4 production by MPB of EPS (Oakes et al. 2010a) that binds and stabilizes sediment, or the
5 absence of macrofauna, which can destabilize sediment (Herman et al. 2000). Regardless,
6 the apparently low resuspension rate suggests that losses of carbon from sediment in the
7 current study reflect processing of fixed carbon by the sediment community.

8 MPB are a potential source of water column DOC (Porubsky et al. 2008).
9 However, DOC derived from MPB represented only a small fraction of the total DOC
10 efflux from sediments in the current study (0.4% during the first dark incubation, when
11 excess ^{13}C flux as DOC was highest) and was a minor pathway for loss of ^{13}C fixed by
12 MPB (2.64% over 33 d). The DOC released from sediments at the study site may be
13 primarily derived from carbon fixed by MPB before label addition, but is more likely to
14 be derived from alternative sources, including phytoplankton and terrestrial organic
15 matter, as suggested by natural abundance carbon stable isotope ratios of water column
16 DOC. Given that almost all of the substantial DOC pool exuded by MPB at the study site
17 (Oakes et al. 2010a) was consumed by heterotrophs and is therefore apparently highly
18 labile, the DOC that is released from sediments is presumably dominated by more
19 refractory components. This is reflected in the relatively depleted natural abundance $\delta^{13}\text{C}$
20 value of DOC. Greater loss of MPB-derived carbon via DOC fluxes may be expected
21 where MPB productivity is high, but heterotrophic activity is low.

22 It is clear that not all of the DIC produced via respiration of MPB-derived carbon
23 was released from sediments. DIC was the major pathway for ^{13}C recycling into MPB,

1 implying that DIC produced through respiration in the sediment is used by MPB in
2 addition to DIC from the overlying water column. This is supported by the observation
3 that gross primary production, based on O₂ and CO₂ fluxes, was far greater than was
4 estimated based on MPB uptake of ¹³C from overlying water. DIC produced through
5 respiration may be particularly important for MPB in deeper sediments, where DIC
6 diffusion from overlying water would be limited. This is evident in the maintenance of
7 ¹³C incorporation in MPB within the 5-10 cm sediment layer (Fig. 1).

8 Release of DIC from sediments was the major pathway for loss of MPB-derived
9 carbon from the sands studied. This may reflect, in part, the high bacterial productivity
10 typical of subtropical sediments (Alongi 1994), although this would be offset by MPB
11 productivity. Where MPB productivity is lower, there may be even greater loss of MPB-
12 derived carbon via respiration.

13

14 *Implications*

15 This study demonstrates the key role of MPB as a resource for benthic
16 heterotrophs in shallow photic subtidal sandy sediments in the subtropics. Given that
17 MPB take up DIC from the overlying water and the carbon they fix is released as DIC,
18 MPB is unlikely to substantially alter the form of carbon transported offshore (i.e., there
19 is no conversion to DOC), although processing within the sediment may alter its $\delta^{13}\text{C}$
20 value. However, given that 35% of fixed carbon was retained over 33 d, the sediments
21 may act as a carbon sink, affecting the quantity of carbon that is transported offshore.

1 **References**

- 2 Alongi, D. M. 1994. The role of bacteria in nutrient recycling in tropical mangrove and
3 other coastal benthic ecosystems. *Hydrobiologia* **285**: 19-32.
- 4 Andersson, J. H., C. Woulds, M. Schwartz, G. L. Cowie, L. A. Levin, K. Soetaert, and J.
5 J. Middelburg. 2008. Short-term fate of phytodetritus in sediments across the
6 Arabian Sea oxygen minimum zone. *Biogeosciences* **5**: 43-53.
- 7 Bellinger, B. J., G. J. C. Underwood, S. E. Ziegler, and M. R. Gretz. 2009. Significant of
8 diatom-derived polymers in carbon flow dynamics within estuarine biofilms
9 determined through isotopic enrichment. *Aquat. Microb. Ecol.* **55**: 169-187.
- 10 Bornmalm, L., B. H. Corliss, and K. Tedesco. 1997. Laboratory observations of rates and
11 patterns of movement of continental margin benthic foraminifera. *Mar.*
12 *Micropalaentol.* **29**: 175-184.
- 13 Cahoon, L. B. 1999. The role of benthic microalgae in neritic ecosystems. *Oceanogr.*
14 *Mar. Biol.* **37**: 47-86.
- 15 Chipman, L., D. Podgorski, S. Green, J. Kostka, W. Cooper, and M. Huettel. 2010.
16 Decomposition of plankton-derived dissolved organic matter in permeable coastal
17 sediments. *Limnol. Oceanogr.* **55**: 857-871.
- 18 Cook, P. L. M., B. Veuger, S. Böer, and J. J. Middelburg. 2007. Effect of nutrient
19 availability on carbon and nitrogen incorporation and flows through benthic algae
20 and bacteria in near-shore sandy sediment. *Aquat. Microb. Biol.* **49**: 165-180.
- 21 DeLaca, T. E. 1982. Use of dissolved amino acids by the foraminifer *Notodendrodes*
22 *antarctikos*. *Am. Zool.* **22**: 683-690.
- 23 Evrard, V. K., P. L. M. Cook, B. Veuger, M. Huettel, and J. J. Middelburg. 2008.

- 1 Tracing carbon and nitrogen incorporation pathways in the microbial community
2 of a photic subtidal sand. *Aquat. Microb. Ecol.* **53**: 257-269.
- 3 Evrard, V., M. Huettel, P. L. M. Cook, K. Soetaert, C. H. R. Heip, and J. J. Middelburg.
4 2012. Relative importance of phytodetritus deposition and microphytobenthos
5 for the heterotrophic community of a shallow subtidal sandy sediment. *Mar. Ecol.*
6 *Prog. Ser.* **455**: 13-21.
- 7 Evrard, V., K., K. Soetaert, C. H. R. Heip, M. Huettel, M. A. Xenopoulos, and J. J.
8 Middelburg. 2010. Carbon and nitrogen flows through the benthic food web of a
9 photic subtidal sandy sediment. *Mar. Ecol. Prog. Ser.* **416**: 1-16.
- 10 Eyre, B., and P. Balls. 1999. A comparative study of nutrient behavior along the salinity
11 gradient of tropical and temperate estuaries. *Estuaries* **22**: 313-326.
- 12 Eyre, B. D., and A. J. P. Ferguson. 2005. Benthic metabolism and nitrogen cycling in a
13 subtropical east Australian Estuary (Brunswick): Temporal variability and
14 controlling factors. *Limnol. Oceanogr.* **50**: 81-96.
- 15 Goto, N., O. Mitamura, and H. Terai, H. 2001. Biodegradation of photosynthetically
16 produced extracellular organic carbon from intertidal benthic algae. *J. Exp. Mar.*
17 *Biol. Ecol.* **257**: 73-86.
- 18 Herman, P. M. J., J. J. Middelburg, J. Widdows, C. H. Lucas, and C. H. R. Heip. 2000.
19 Stable isotopes as trophic tracers: Combining field sampling and manipulative
20 labeling of food resources for macrobenthos. *Mar. Ecol. Prog. Ser.* **204**:79-92.
- 21 Jones, W. B., L. A. Cifuentes, and J. E. Kaldy. 2003. Stable carbon isotope evidence for
22 coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon.
23 *Mar. Ecol. Prog. Ser.* **255**: 15-25.

- 1 Lee, J. J. 1995. Living sands: The symbiosis of protists and algae can provide good
2 models for the study of host/symbiont interactions. *BioScience* **45**: 252-261.
- 3 Maher, D., and B. D. Eyre. 2011. Insights into estuarine benthic dissolved organic carbon
4 (DOC) dynamics using $\delta^{13}\text{C}$ -DOC values, phospholipid fatty acids and dissolved
5 organic nutrient fluxes. *Geochim. Cosmochim. Acta* **75**: 1889-1902.
- 6 Maher, D. T., and B. D. Eyre. 2010. Benthic fluxes of dissolved organic carbon in three
7 temperate Australian estuaries: Implications for global estimates of benthic DOC
8 fluxes. *J. Geophys. Res.* **115**: G04039, doi: 10.1029/2010JG001433.
- 9 Michener, R. H., and D. M. Schell. 1994. Stable isotope ratios as tracers in marine
10 aquatic food webs, p. 138-157. *In* K. Lajtha and R. H. Michener [eds.], *Stable*
11 *isotopes in ecology and environmental science*. Blackwell Scientific.
- 12 Middelburg, J. J., C. Barranguet, H. T. S. Boschker, P. M. J. Herman, T. Moens, and C.
13 H. R. Heip. 2000. The fate of intertidal microphytobenthos carbon: An in situ
14 ^{13}C -labeling study. **45**: 1224-1234.
- 15 Moens, T., C. Luyten, J. J. Middelburg, P. M. J. Herman, and M. Vincx. 2002.
16 Tracing organic matter sources of estuarine tidal flat nematodes with stable
17 carbon isotopes. *Mar. Ecol. Prog. Ser.* **234**: 127-137.
- 18 Mojtahid, M., M. V. Zubkov, M. Hartman, and A. J. Gooday. 2011. Grazing of intertidal
19 benthic foraminifera on bacteria: Assessment using pulse-chase radiotracing. *J.*
20 *Exp. Mar. Biol. Ecol.* **399**: 25-34.
- 21 Oakes, J. M., B. D. Eyre, J. J. Middelburg, and H. T. S. Boschker. 2010a. Composition,
22 production, and loss of carbohydrates in subtropical shallow subtidal sandy
23 sediments: Rapid processing and long-term retention revealed by ^{13}C -labeling.

- 1 Limnol. Oceanogr. **55**: 2126-2138.
- 2 Oakes, J. M., B. D. Eyre, D. J. Ross, and S. D. Turner. 2010b. Stable isotopes trace
3 estuarine transformations of carbon and nitrogen from primary- and secondary-
4 treated paper and pulp mill effluent. Environ. Sci. Technol. **44**: 7411-7417.
- 5 Oakes J. M., R. M. Connolly, and A. T. Revill. 2010c. Isotope enrichment in mangrove
6 forests separates microphytobenthos and detritus as carbon sources for animals.
7 Limnol. Oceanogr. **55**: 393-402.
- 8 Oakes. J. M., M. D. Bautista, D. Maher, W. B. Jones, and B. D. Eyre. 2011. Carbon self-
9 utilization may assist *Caulerpa taxifolia* invasion. Limnol. Oceanogr. **56**: 1824-
10 1831.
- 11 Peterson, B. J. 1999. Stable isotopes as tracers of organic matter input and transfer in
12 benthic food webs: A review. Acta Oecol. **20**: 479-487.
- 13 Phillips, D. L., and J. W. Gregg. 2003. Source partitioning with stable isotopes: Coping
14 with too many sources. Oecologia **136**: 261-269.
- 15 Porubsky, W. P., L. E. Velasquez, and S. B. Joye. 2008. Nutrient-replete benthic
16 microalgae as a source of dissolved organic carbon to coastal waters. Estuaries
17 and Coasts **31**: 860-876.
- 18 Raymond, P. A., and J. E. Bauer. 2001. Use of ^{14}C and ^{13}C natural abundances for
19 evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: A
20 review and synthesis. Org. Geochem. **32**: 469-485.
- 21 Saburova, M. A., and I. G. Polikarpov. 2003. Diatom activity within soft sediments:
22 behavioural and physiological processes. Mar. Ecol. Prog. Ser. **251**: 115-216.
- 23 Schidlowski, M. 2000. Carbon isotopes and microbial sediments, p. 84-104. *In* R. E.

- 1 Riding and S. M. Awramik [eds.], Microbial sediments. Springer-Verlag.
- 2 Smith, D. J., and G. J. C. Underwood. 2000. The production of extracellular
3 carbohydrates by estuarine benthic diatoms: The effects of growth phase and light
4 and dark treatment. *J. Phycol.* **36**: 321-333.
- 5 Underwood, G. J. C. 2002. Adaptations of tropical marine microphytobenthic
6 assemblages along a gradient of light and nutrient availability in Suva Lagoon,
7 Fiji. *Eur. J. Phycol.* **37**: 449-462.
- 8 Underwood, G. J. C., M. Boulcott, and C. A. Raines. 2004. Environmental effects on
9 exopolymer production by marine benthic diatoms: dynamics, changes in
10 composition, and pathways of production. *J. Phycol.* **40**: 293-304.
- 11 van den Meersche, K., K. Soetaert, and J. J. Middelburg. 2011. Plankton dynamics in an
12 estuarine plume: A mesocosm ¹³C and ¹⁵N tracer study. *Mar. Ecol. Prog. Ser.* **429**:
13 29-43.
- 14 Volkman, J. K., S. W. Jeffrey, P. D. Nichols, G. I. Rogers, and C. D. Garland. 1989. Fatty
15 acid and lipid composition of 10 species of microalgae used in mariculture. *J.*
16 *Exp. Mar. Biol. Ecol.* **128**: 219-240.
- 17 Webb, A. P., and B. D. Eyre. 2004. Effect of natural populations of burrowing
18 thalassinidean shrimp on sediment irrigation, benthic metabolism, nutrient fluxes
19 and denitrification. *Mar. Ecol. Prog. Ser.* **268**: 205-220.
- 20 Westrich, J. T., and R. A. Berner. 1984. The role of sedimentary organic matter in
21 bacterial sulfate reduction: The G model tested. *Limnol. Oceanogr.* **29**: 236-249.

Table 1: Mean natural abundance carbon stable isotope ratios ($\delta^{13}\text{C}$), biomass (standard errors in brackets), and % of sediment organic carbon (% OC) represented by sediment compartments, based on control samples collected across all sampling times. Note that $\delta^{13}\text{C}$ values for bacteria and microphytobenthos are for whole cells. Units are per area, not volume. $\delta^{13}\text{C}$ of uncharacterized material was estimated using isotope mixing models.

Compartment	0-2 cm			2-5 cm			5-10 cm		
	$\delta^{13}\text{C}$	Biomass (mmol C m ⁻²)	% OC	$\delta^{13}\text{C}$	Biomass (mmol C m ⁻²)	% OC	$\delta^{13}\text{C}$	Biomass (mmol C m ⁻²)	% OC
Sediment organic carbon	-17.9(1.3)	4471.2(246.8)	100	-19.4(0.5)	5576.5(261.4)	100	-19.5(0.4)	8606.1(369.7)	100
Microphytobenthos	-15.4(0.4)	402.4(0.3)	9.0	-13.8(0.5)	385.4(34.3)	6.9	-15.6(0.2)	568.9(64.3)	6.6
Bacteria	-16.3(0.5)	164.8(10.3)	3.7	-14.5(0.2)	415.2(103.1)	7.4	-15.7(0.7)	797.5(119.5)	9.3
<i>Cellanthus craticulatus</i>	-10.0(0.6)	169.1(0.2)	3.8	-18.3(<0.1)	42.2(0.0)	0.8	-18.2	128.8(0.4)	1.5
<i>Ammonia beccarii</i>	-16.2(0.5)	9.4(<0.1)	0.2	-16.4(1.3)	32.1(0.1)	0.6	-18.3	47.4(0.1)	0.6
<i>Elphidium advenum</i>	-15.6(<0.1)	1.2(<0.1)	<0.1	-20.4(0.4)	0.2(0.0)	<0.1	-17.9(0.1)	1.2(<0.1)	<0.1
Uncharacterized	-18.6	3724.3(247.0)	83.3	-20.3	4701.4(283.1)	84.3	-20.3	7062.3(393.8)	82.1

Figure legends

Figure 1: Excess ^{13}C incorporation in microphytobenthos, bacteria, and foraminifera (*Cellanthus craticulatus*, *Ammonia beccarii*, and *Elphidium advenum*) at sediment depths of 0-2, 2-5, and 5-10 cm throughout the study period as a percentage of the ^{13}C initially incorporated into sediment organic carbon (mean \pm standard error). Note some bars and error bars are too small to be seen. Scale on y-axis varies with sediment depth. *Elphidium advenum* incorporation is too low to be seen.

Figure 2: Excess ^{13}C incorporation in total sediment organic carbon (0-10 cm sediment depth) throughout the study period as a percentage of the ^{13}C initially incorporated into sediment OC (mean \pm standard error). Line represents the 2-G model predicting ^{13}C loss from total sediment organic carbon.

Figure 3: Carbon budget showing excess ^{13}C within sediment organic carbon at 0-2, 2-5, and 5-10 cm at each sampling time, and the cumulative excess ^{13}C lost via fluxes of DIC and DOC from the end of ^{13}C -labeling until each sampling time, as a percentage of the ^{13}C initially incorporated into sediment organic carbon (mean \pm standard error).

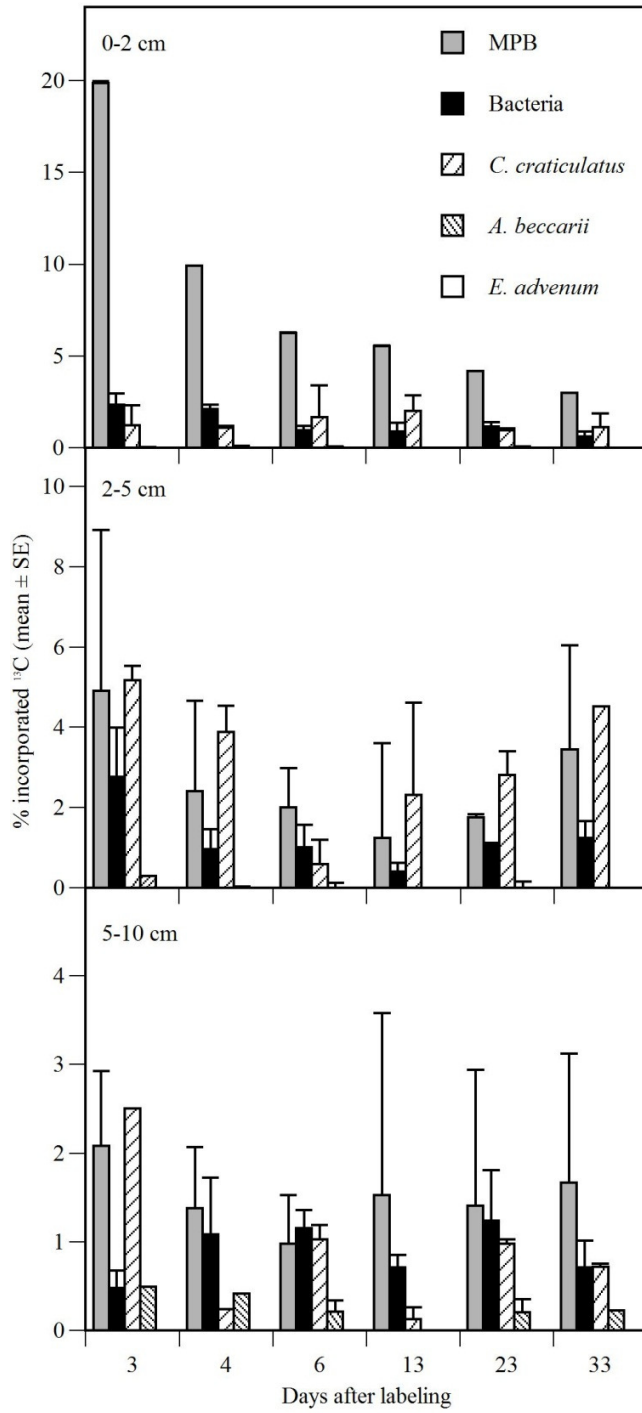


Figure 1

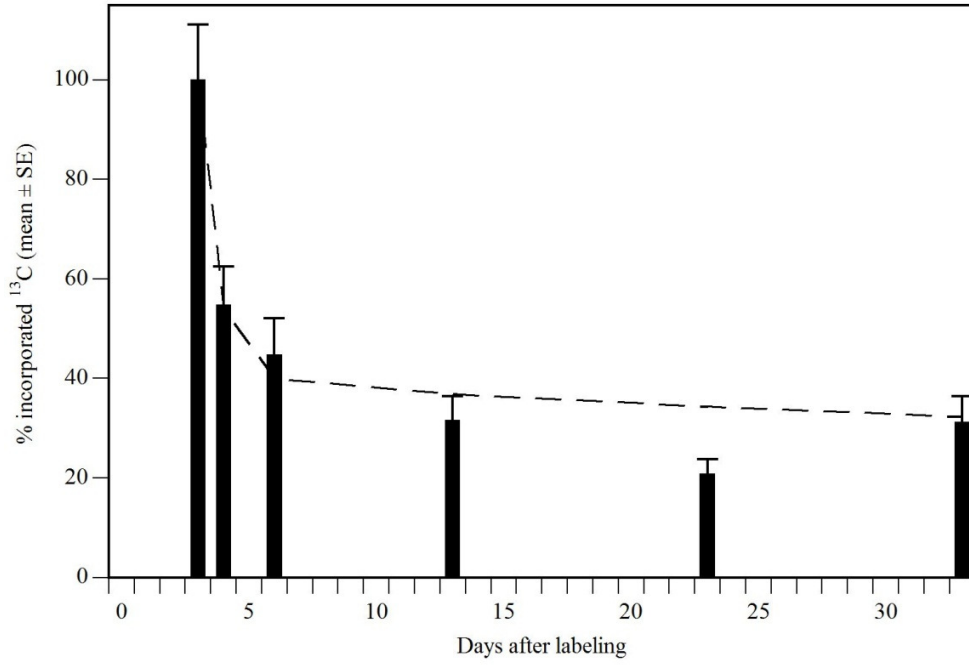


Figure 2

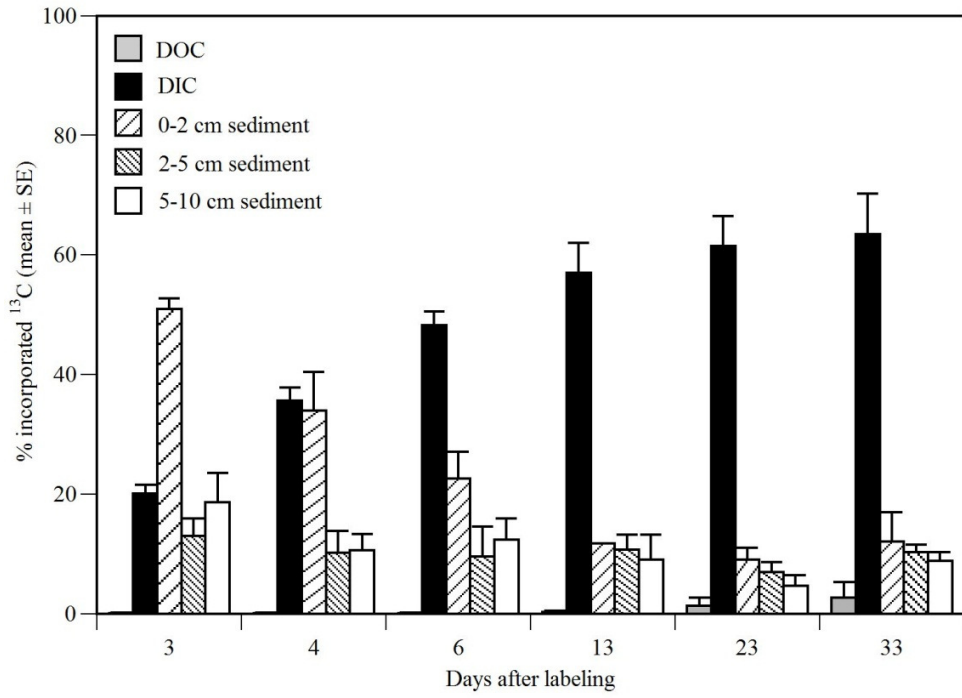


Figure 3