2012

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Publication details
Published version available from:
http://dx.doi.org/10.4319/lo.2012.57.06.1846
Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: A $^{13}$C-labeling study

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Acknowledgements

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

Microphytobenthos (MPB) in photic sediments are highly productive but the fate of this production remains uncertain. Over 33 d, tracing of $^{13}$C from added bicarbonate in subtropical shallow subtidal sand showed rapid transfer of MPB-derived carbon to deeper sediment; below 2 cm (31% within 60 h) and 5 cm (18%). Despite their high turnover (5.5 d) and only representing ~8% of sediment organic carbon, MPB represented up to 35% of the $^{13}$C retained in sediments, demonstrating substantial carbon recycling. Carbon was rapidly transferred to heterotrophs, but their contribution to sediment $^{13}$C was similar to their biomass contribution (<0.1% to 3.8%), with the exception of the foraminifera Cellanthus craticulatus, which accounted for up to 33% of the $^{13}$C within sediment.

There was little loss of MPB-derived carbon via dissolved organic carbon (DOC) effluxes (3%) or resuspension (minimal). Respiration was the major loss pathway (63%), reflecting the high microbial biomass typical of lower latitudes. Given that MPB take up dissolved inorganic carbon (DIC) from overlying water, and the carbon they fix is released as DIC, MPB in subtropical sands are unlikely to substantially alter the form of carbon transported offshore (i.e., there is no conversion to DOC), but processing within the sediment may alter its $\delta^{13}$C value. Given that 31% of fixed carbon remained in sediments after 33 d, subtropical sands may act as a carbon sink, thereby affecting the quantity of carbon transported offshore.
Introduction

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

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

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

A further gap in current understanding of coastal carbon cycling is the fate of MPB-derived carbon. Following transformation and/or burial, MPB-derived carbon may be lost to the water column via resuspension, fluxes of dissolved inorganic carbon (DIC).
following mineralization or respiration (Goto et al. 2001; Evrard et al. 2012), and/or fluxes of dissolved organic carbon (DOC) derived from exudates (Smith and Underwood 2000) and, to a lesser extent, cell lysis during grazing. These pathways may be an important component of coastal carbon cycling. For example, sediment fluxes can contribute significantly to the DOC in coastal waters (Maher and Eyre 2011), and MPB has been proposed as a major source (Maher and Eyre 2010). However, the pathways for loss of MPB-derived carbon from sediments have not been well-characterized.

For temperate, photic, subtidal, sandy sediments Cook et al. (2007) and Evrard et al. (2008) reported negligible loss of MPB-derived carbon to overlying water over 6 and 4 d, respectively. Cook et al. (2007) recovered only 1-5% of fixed carbon as DOC. In contrast, Oakes et al. (2010a) reported loss from in situ surficial subtropical photic sands of 85% of MPB-derived carbon over 33 d. The pathways responsible for this loss were not determined, but this highlights that loss to the water column may be the main fate of carbon fixed by MPB in this environment. Alternatively, carbon may have been buried deeper within sediments (below 2 cm; Oakes et al. 2010a). The greater loss of carbon from subtropical sands compared to temperate sands may relate solely to the longer period of study or the methods used. For example, given that the sediment studied by Cook et al. (2007) and Evrard et al. (2008) was ex situ, and therefore was not subject to natural environmental flows and the effects of mobile grazers, loss of fixed carbon may have been underestimated. In support of this, Middelburg et al. (2000) reported substantial loss of fixed carbon (~53%) over 4 d from in situ temperate sands, albeit in the intertidal zone. This was estimated to be primarily due to resuspension (~60%; Middelburg et al. 2000), but losses of carbon as DIC and DOC were not measured. The
different loss rates for carbon in subtropical and temperate sands may also reflect
characteristic differences between sands at these latitudes. High bacterial biomass and
productivity is typical of lower latitudes (Alongi 1994; Eyre and Balls 1999) and this may
account for the greater loss of fixed carbon observed for subtropical sands.

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

Methods

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

Study site

The subtidal study site (~1.5 m below average sea level) in the Brunswick River, subtropical Australia, was described by Oakes et al. (2010a). The site was net autotrophic (production:respiration ~1.2 over 24 h; J. M. Oakes unpubl.). Gross productivity based on CO$_2$ fluxes averaged ~105 mmol C m$^{-2}$ d$^{-1}$ (J.M. Oakes unpubl.). The organic carbon (OC) content of sediment to 10 cm depth was 18.6 mol C m$^{-2}$. Surface sediments (0-2 cm) had a higher OC content (0.18%) and a lower molar C:N ratio (6.9 ± 0.7) than deeper 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 permeable. Sediment at all depths, was comprised primarily of fine (125-250 µm; 72-79%) and medium (250-500 µm; 17-24%) quartz sand grains.

$^{13}$C-labeling

$^{13}$C-labeling of three experimental plots (1.2 m$^2$) was achieved by $^{13}$C-labeling the water column DIC pool (23% $^{13}$C) in benthic chambers using NaH$^{13}$CO$_3$, as described in
Oakes et al. (2010a). Pumps maintained laminar flow of water across the sediment within the chambers for a 24 h period. Chambers were then removed.

Sample collection

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

At the beginning and end of the dark incubation period and at the end of the light incubation period, ~50 mL of water was removed from each core to determine concentrations and carbon stable isotope ratios (δ¹³C) of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). Samples were syringe filtered (precombusted GF/F), leaving no headspace, into pre-combusted 40 mL glass vials with Teflon-coated septa and were killed (200 µL 50:50 w:v ZnCl₂) and refrigerated until analysis. Sample
water was replaced, as it was withdrawn, with site water from a collapsible reservoir. Additional water samples, not included in this study, were also collected.

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

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

### Sample analysis

DOC and DIC concentrations and $\delta^{13}C$ were measured, as described by Oakes et al. (2010b, 2011), via continuous flow wet-oxidation isotope ratio mass spectrometry using an Aurora 1030W total organic carbon analyzer coupled to a Thermo Delta V Plus Isotope Ratio Mass Spectrometer (IRMS). Sodium bicarbonate (DIC) and glucose (DOC) of known isotope composition dissolved in helium-purged milli-Q were used for drift correction and to verify concentrations and $\delta^{13}C$ values. Reproducibility for DOC and DIC, respectively, was ± 0.3 mg L$^{-1}$ and ± 0.4 mg L$^{-1}$ (concentrations) and ± 0.08‰ and ± 0.10‰ ($\delta^{13}C$).
PLFA biomarkers to determine $^{13}$C uptake into bacteria and MPB were extracted from lyophilized sediments using a modified Bligh and Dyer method, as described by Oakes et al. (2010a). An internal standard (tridecanoic acid, C$_{13}$) was added at the beginning of the extraction procedure. Extracts were analyzed via gas chromatography-isotope ratio mass spectrometry, as described by Oakes et al. (2010a).

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

**Calculations**

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

Total uptake (incorporation) of $^{13}$C into sediment OC, foraminifera, bacteria, and MPB ($\mu$mol $^{13}$C m$^{-2}$) was calculated as the product of excess $^{13}$C (fraction $^{13}$C in labeled sample – fraction $^{13}$C in control) and the mass of OC in each compartment. For sediment and foraminifera, OC mass was the product of %C and total dry mass per unit area. For foraminifera, counts of individuals within sediment subsamples were scaled up to provide an estimate of the total number of individuals per m$^2$ at each depth. Total mass was
determined by multiplying this estimate by the average mass of an individual, which was
determined by weighing a known number of individuals.

Concentrations of PLFA biomarkers for bacteria and MPB were calculated based
on their peak areas relative to that of the C\textsubscript{13} internal standard. Total biomass of bacteria
and MPB was calculated as described by Oakes et al. (2010\textit{a}), based on the bacterial
biomarkers i\textsubscript{15}:0 and a\textsubscript{15}:0, and the algal biomarkers 16:1\textit{n}(7) and 20:5\textit{n}(3), which
gave distinct chromatographic peaks. The average fraction of MPB PLFAs that is
typically accounted for by the biomarkers considered is 0.37 (Volkman et al. 1989).

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

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

where excess $^{13}\text{C}_{\text{start}}$ and excess $^{13}\text{C}_{\text{end}}$ represent excess $^{13}$C at the beginning and end of
the dark or light incubation period, SA is the sediment surface area within a core, and $t$
represents hours of dark or light incubation. Net fluxes (excess $^{13}$C m\textsuperscript{-2} d\textsuperscript{-1}) of DOC or
DIC were calculated as follows:

$$\text{Net flux} = \text{dark flux} / \text{dark hours} + \text{light flux} / \text{light hours} \times 24 \text{ hours}$$  \hspace{1cm} (2)

We interpolated between measured net flux values and estimated the total quantity of $^{13}$C
lost via fluxes of DOC and DIC from the end of labeling up until each sampling period
by determining the area under the curve.
Data analysis

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

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

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

where $G_T$ is the incorporation of $^{13}$C within sediment OC, $t$ is time, $G_1$, $G_2$, and $G_{NR}$ represent the incorporation of $^{13}$C into highly reactive, less reactive and non-reactive (over the timescale of the experiment) fractions of sediment OC, and $k_1$ and $k_2$ represent first-order decay constants specific to $G_1$ and $G_2$, respectively.
The isotope mixing program IsoSource (Phillips and Gregg 2003) calculates all feasible combinations of multiple possible sources to a mixture, based on isotope ratios, and was therefore used to estimate the likely contribution of MPB, phytoplankton and terrestrial plants to DOC effluxes from the sediment.

**Results**

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

**Sediment organic carbon composition and depth distribution**

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

**Natural abundance stable isotope ratios**

Sediment OC was more enriched in $^{13}$C in 0-2 cm sediment than in deeper sediments (-17.9‰ vs. ~-19.5‰; Table 1). At all depths, mean $\delta^{13}$C values of bacteria
were depleted by up to 0.9‰ compared to MPB (Table 1). Across depths, sediment OC was depleted in $^{13}$C by 1.6‰ to 5.6‰ compared to MPB and bacteria. Water column DIC and DOC in control cores had mean $\delta^{13}$C values of 2.6‰ and -23.3‰, respectively.

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

By the time the first sediment was collected (60 h after label addition), 1492 $\mu$mol $^{13}$C m$^{-2}$ was in sediment OC 0-10 cm deep. Allowing for measured loss of $^{13}$C as DOC and DIC during the dark incubation period preceding sediment sample collection, 1809 ± 110 $\mu$mol $^{13}$C m$^{-2}$ was in 0-10 cm sediments 48 h after label addition. This equated to an incorporation of 75 $\mu$mol $^{13}$C m$^{-2}$ light h$^{-1}$ until this point, with ~0.01% of sediment OC replaced with $^{13}$C. True rates of $^{13}$C incorporation are likely to have been somewhat higher, however, as we were unable to account for losses of $^{13}$C from the sediment during the pre-incubation period. Based on $^{13}$C incorporation, and allowing for the partial labeling of available DIC (23% $^{13}$C), total carbon fixation was estimated to be ~328 $\mu$mol C m$^{-2}$ light h$^{-1}$. This is far lower than the gross primary productivity for the study site based on O$_2$ and CO$_2$ fluxes (~4000 $\mu$mol m$^{-2}$ h$^{-1}$; J. M. Oakes unpubl.). Fixed $^{13}$C was detected in deeper sediments (2-5 and 5-10 cm deep) from the first sampling time (Fig. 1). Within 60 h of label addition 31.5% of the incorporated $^{13}$C in sediments was below 2 cm and 18.6% was between 5 and 10 cm. Assuming fixation of carbon in deeper sediments was negligible, this gives transport rates of 10 $\mu$mol $^{13}$C m$^{-2}$ h$^{-1}$ and 6 $\mu$mol $^{13}$C m$^{-2}$ h$^{-1}$ to these depths, respectively. Considering that the added $^{13}$C accounted for 23% of the total water column DIC available to MPB, fixed carbon was
transported to sediment below 2 cm at a rate of 41 \mu\text{mol} \text{C} m^{-2} h^{-1}, and to sediment below
5 cm at a rate of 24 \mu\text{mol} m^{-2} h^{-1}.

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

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

MPB in surface sediments (0-2 cm) initially accounted for a far greater portion of
the $^{13}$C fixed into sediment OC than did MPB in deeper sediments (Fig. 1). Within each
sediment depth layer, the contribution of MPB to the $^{13}$C in sediment OC far outweighed
the contribution of MPB biomass to sediment OC. Whereas MPB biomass represented
only 6.6 to 9.0% of the OC within 0-2, 2-5, and 5-10 cm sediment (Table 1), the average
contribution of MPB to $^{13}$C within OC at each sediment depth throughout the study
period was 34.8 ± 3.9%, 27.3 ± 3.7%, and 15.8 ± 3.5%, respectively. Throughout the
study, there was a marked decrease in the $^{13}$C content of MPB in 0-2 cm sediment. By 33
d after label addition, only 2.9% of the $^{13}$C initially fixed into sediment OC remained in
this compartment (Fig. 1). The incorporation of $^{13}$C into MPB in 2-5 and 5-10 cm
sediments varied relatively little throughout the study (Fig. 1) and was significantly lower than in 0-2 cm sediments (three-way ANOVA: $F_{2,35}=14.068$, $p=0.000$).

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

There was evidence of label uptake into all three foraminifera species by the time the first sediment sample was taken (Fig. 1). The greatest incorporation of $^{13}$C, per unit biomass, was by *Cellanthus craticulatus*, which dominated the foraminifera (Table 1). The greatest uptake of $^{13}$C by *C. craticulatus* was generally in sediment at 2-5 cm (Fig. 1), where *C. craticulatus* accounted for up to $\sim 5\%$ of the total $^{13}$C initially fixed into sediments (Fig. 1). Despite representing only $0.8\%$ to $3.8\%$ of the biomass within each sediment depth layer (Table 1), *C. craticulatus* accounted for, on average, $9.6 \pm 2.1\%$, $31.2 \pm 5.9\%$, and $9.7 \pm 2.9\%$ of the $^{13}$C within sediment OC at 0-2, 2-5, and 5-10 cm, respectively. In contrast, the contribution of *Ammonia beccarii* and *Elphidium advenum* to the $^{13}$C in sediment OC was similar to their contribution to biomass (Fig. 1, Table 1). The incorporation of $^{13}$C into *A. beccarii* was greater in 5-10 cm sediment than in
shallower sediments. At any one time, *E. advenum* accounted for less than 0.01% of the total $^{13}$C initially incorporated into sediment OC (Fig. 1).

Loss of $^{13}$C from sediments

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

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

Loss of $^{13}$C from sediment to the water column was dominated by fluxes of DIC, with very little $^{13}$C lost as DOC (Fig. 3). Most of the loss of $^{13}$C as DIC occurred over the first few days of the study, with nearly 50% of the $^{13}$C initially incorporated into sediments having been lost to the water column as DIC within 6 days of label addition (Fig. 3). By the conclusion of the study ~63% (59% to 67%) of incorporated $^{13}$C had been lost to the water column as DIC, and ~3% (1.4% to 4.0%) as DOC. Approximately 31% of the $^{13}$C that was initially incorporated by MPB remained within the sediment OC pool. Of this, ~12% (4.0% to 33.1%) was in sediment at 0-2 cm, ~10% (4.6% to 17.7%) was at 2-5 cm, and ~9% (3.0% to 13.6%) was at 5-10 cm (Fig. 3). Based on excess $^{13}$C per
volume, this was dominated by OC within surface sediments. At the conclusion of the
study, only ~3% of the initially incorporated $^{13}$C could not be accounted for by the
pathways and compartments considered.

Discussion

The $^{13}$C-labeling experiment described in the current study was primarily done in
situ, with cores removed to the laboratory shortly before incubation for sediment fluxes.
For most of the study period, the sediment studied was therefore subject to natural loss
and transfer processes, including grazing by mobile consumers, bioturbation, and
physical mixing and resuspension by water movement. As these processes can not be
adequately replicated in a laboratory environment, in situ experimentation was essential
for our focus on the longer-term fate of MPB-derived carbon. Although the fate and
transformation of MPB-derived carbon has been investigated in situ (Middelburg et al.
2000; Bellinger et al. 2009), this was done in temperate, intertidal sediments, which are
subject to fundamentally different environmental conditions compared to the subtropical
subtidal sediment we studied. In addition, the study by Middelburg et al. (2000) used a
mass-balance approach to estimate losses to respiration and resuspension and did not
estimate DOC losses, and Bellinger et al. (2009) did not measure DIC and DOC fluxes.
In comparison, the current study directly quantified losses to both DOC and DIC. It is
important to note that our experimental procedure may have affected shorter-term
processes, as the first samples collected had been within chambers and cores (i.e.,
isolated from in situ conditions) for 60 h. Similarly, cores collected at other early time
points had a proportionally small period during which they were exposed to in situ
conditions. This may have affected our observations of carbon cycling in the short-term, particularly carbon loss. However, for cores collected towards the end of the study, which were primarily exposed to in situ conditions, the quantity of $^{13}$C remaining within sediment OC matched that expected based on earlier observations. Similarly, our budget accounted for ~97% of the added $^{13}$C over 33 d. Any effect of our experimental treatment on short-term carbon cycling processes therefore appears to be negligible.

**Natural abundance isotope ratios**

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

Natural abundance $\delta^{13}$C of DIC at the study site (2.56 ± 0.59‰) was within the range of coastal DIC (Schidlowski 2000), however, natural abundance $\delta^{13}$C of DOC (-23.31 ± 1.03‰) is more difficult to put in context and to interpret. Assuming that DOC faithfully reflects the $\delta^{13}$C value of the primary producer from which the carbon is ultimately derived, isotope mixing calculations (IsoSource; Phillips and Gregg 2003)
based on natural abundance $\delta^{13}$C values for MPB (-15.4‰, this study), pelagic algae (-18‰ to -24‰, Michener and Schell 1994), and terrestrial plants (-23‰ to -30‰) suggest that the carbon within DOC in the lower Brunswick estuary is ultimately derived primarily from carbon fixed by phytoplankton (45 ± 37%, standard deviation) and terrestrial plants (38 ± 37%). MPB appear to have a smaller contribution (18 ± 27%) but may contribute anywhere from 0% to 46% of the DOC. This may represent direct release from primary producers, or release of DOC by bacteria that have consumed carbon that was fixed by these primary producers. The usefulness of this analysis, however, is questionable as, rather than faithfully reflecting its source, preferential use of more labile DOC components within sediments (Chipman et al. 2010) can result in fluxes of DOC being comprised of a very enriched (e.g., carbohydrates) or depleted (e.g., lipids) fraction of the source organic matter (Maher and Eyre 2011).

$^{13}$C incorporation and transfer within sediments

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

Compared to previous studies in temperate intertidal sands (Middelburg et al. 2000) and temperate subtidal sands (Evrard et al. 2008, 2010), there was greater downward transport of $^{13}$C in the current study. In the current study, 12.9% of fixed $^{13}$C was in sediment OC at a depth of 2-5 cm 60 h after label application, and 18.6% of fixed
\(^{13}\)C was transferred to sediments 5-10 cm deep. In comparison, it took longer (3 d) for a similar fraction of \(^{13}\)C to enter 2-5 cm sediment in the studies of temperate sands (Middelburg et al. 2000; Evrard et al. 2008), and there was negligible transfer of \(^{13}\)C below 5 cm. This may reflect differences in methodology and/or location (temperate vs. subtropical, intertidal vs. subtidal, differences in sediment composition), and associated variation in rates of bioturbation, active migration of MPB and heterotrophs, and physical mixing of sediments by water movement. A further explanation for differences in \(^{13}\)C incorporation in deeper sediments is differences in rates of chemoautotrophy. Relatively little is known of the importance of chemoautotrophy in coastal sands, however, Evrard et al. (2008) identified chemoautotrophy as being responsible for a relatively small portion of \(^{13}\)C uptake at depth in temperate sands. In the current study the delay between label application and sample collection prevented us from identifying any contribution of chemoautotrophy to \(^{13}\)C uptake into deeper sediments. However, there was no evidence of \(^{13}\)C uptake by bacteria in the absence of, or in excess of, \(^{13}\)C uptake by MPB.

Considerable loss via resuspension (60\%) in the study by Middelburg et al. (2000) may have reduced the carbon pool available for downward transport, and the ex situ nature of the study by Evrard et al. (2008) isolated sediments from natural water movement and bioturbation, which may have limited carbon burial. The different depth distribution of \(^{13}\)C in the study by Middelburg et al. (2000) compared to the current study may reflect differences in water flow and sediment mixing in intertidal sands, compared to the subtidal sediments we studied. However, the \(^{13}\)C depth distribution observed by Middelburg et al. (2000) was similar to that observed for subtidal temperate sands by Evrard et al. (2008). The differences in \(^{13}\)C depth distribution between studies of
temperate sands, and that seen in the current study, hints at the possibility that different
processes influence carbon burial in temperate and subtropical sands. However, further
replicated studies across climate zones are required to determine if this is the case.

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

MPB-derived carbon appears to have been recycled by MPB, bacteria and foraminifera, with considerable $^{13}$C detected in all of these compartments throughout the experimental period. Particularly for *Cellanthus craticulatus* in sediment at 0-5 cm, and for bacteria at all depths, there was no apparent decline in $^{13}$C content throughout the experiment. Whereas the fraction of fixed $^{13}$C that was within bacteria was similar to their contribution to sediment OC, the fraction of $^{13}$C within MPB and *C. craticulatus* far outweighed their contribution to sediment OC. This suggests that MPB and *C. craticulatus* have a greater role than bacteria in the long-term retention of fixed carbon. The low biomass:productivity ratio for MPB at the study site (~5.5 d; Oakes et al. 2010a), possibly due to fast turnover in response to high grazing pressure (Herman et al. 2000), may mean that MPB need to intercept DIC produced through respiration, in addition to water column DIC, to satisfy their carbon demand. For foraminifera, the carbon source can vary with species (Oakes et al. 2010c). Foraminifera can obtain carbon from MPB and phytodetritus (Moodley et al. 2002, Oakes et al. 2010c), as well as from bacteria (Mojtahid et al. 2011), macrophyte detritus (Oakes et al. 2010c) and dissolved organic matter (DeLaca 1982). Larger foraminifera can contain symbiotic algae, allowing them to directly access inorganic carbon as a source (Lee 1995). Larger foraminifera were not observed in the current study, but foraminifera in the family Elphidiidae (which includes the genera *Cellanthus* and *Elphidium*) can contain chloroplasts extracted from diatoms which can remain photosynthetically active, fixing carbon for their hosts, for weeks or months (Lee 1995). The high accumulation of $^{13}$C in *C. craticulatus* compared
to *Ammonia beccarii* and *Elphidium advenum*, relative to their biomass, may therefore indicate the presence of functional chloroplasts in *C. craticulatus*. However, given that the $^{13}$C content of *C. craticulatus* in surface sediments did not noticeably decline with time, in contrast to MPB, it appears that *C. craticulatus* retain fixed carbon for a longer period than MPB and/or also access MPB-derived carbon. Given that rates of active migration for benthic foraminifera can be high (0.4 to 184.3 mm d$^{-1}$; Bornmalm et al. 1997), particularly in shallow-water sediments and at higher temperatures, *C. craticulatus* may play an important role in the transfer of fixed carbon to deeper sediments.

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

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

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

Given that only ~3% of the carbon fixed by MPB was not found within sediments or accounted for by fluxes of DOC and DIC by the end of the study, resuspension
apparently played only a minor role in the loss of MPB-derived carbon from the subtropical subtidal sands studied. This may be due to low water movement over the sediment surface, the observed rapid transfer of carbon to deeper sediments, high production by MPB of EPS (Oakes et al. 2010a) that binds and stabilizes sediment, or the absence of macrofauna, which can destabilize sediment (Herman et al. 2000). Regardless, the apparently low resuspension rate suggests that losses of carbon from sediment in the current study reflect processing of fixed carbon by the sediment community.

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

It is clear that not all of the DIC produced via respiration of MPB-derived carbon was released from sediments. DIC was the major pathway for $^{13}$C recycling into MPB,
implying that DIC produced through respiration in the sediment is used by MPB in addition to DIC from the overlying water column. This is supported by the observation that gross primary production, based on O$_2$ and CO$_2$ fluxes, was far greater than was estimated based on MPB uptake of $^{13}$C from overlying water. DIC produced through respiration may be particularly important for MPB in deeper sediments, where DIC diffusion from overlying water would be limited. This is evident in the maintenance of $^{13}$C incorporation in MPB within the 5-10 cm sediment layer (Fig. 1).

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

**Implications**

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


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Table 1: Mean natural abundance carbon stable isotope ratios ($\delta^{13}$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 $\delta^{13}$C values for bacteria and microphytobenthos are for whole cells. Units are per area, not volume. $\delta^{13}$C of uncharacterized material was estimated using isotope mixing models.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>0-2 cm</th>
<th></th>
<th>2-5 cm</th>
<th></th>
<th>5-10 cm</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{13}$C</td>
<td>Biomass (mmol C m$^{-2}$)</td>
<td>% OC</td>
<td>$\delta^{13}$C</td>
<td>Biomass (mmol C m$^{-2}$)</td>
<td>% OC</td>
</tr>
<tr>
<td>Sediment organic carbon</td>
<td>-17.9(1.3)</td>
<td>4471.2(246.8)</td>
<td>100</td>
<td>-19.4(0.5)</td>
<td>5576.5(261.4)</td>
<td>100</td>
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<td>Microphytobenthos</td>
<td>-15.4(0.4)</td>
<td>402.4(0.3)</td>
<td>9.0</td>
<td>-13.8(0.5)</td>
<td>385.4(34.3)</td>
<td>6.9</td>
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<tr>
<td>Bacteria</td>
<td>-16.3(0.5)</td>
<td>164.8(10.3)</td>
<td>3.7</td>
<td>-14.5(0.2)</td>
<td>415.2(103.1)</td>
<td>7.4</td>
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<td><em>Cellanthus craticulatus</em></td>
<td>-10.0(0.6)</td>
<td>169.1(0.2)</td>
<td>3.8</td>
<td>-18.3(&lt;0.1)</td>
<td>42.2(0.0)</td>
<td>0.8</td>
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<tr>
<td><em>Ammonia beccarii</em></td>
<td>-16.2(0.5)</td>
<td>9.4(&lt;0.1)</td>
<td>0.2</td>
<td>-16.4(1.3)</td>
<td>32.1(0.1)</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Elphidium advenum</em></td>
<td>-15.6(&lt;0.1)</td>
<td>1.2(&lt;0.1)</td>
<td>&lt;0.1</td>
<td>-20.4(0.4)</td>
<td>0.2(0.0)</td>
<td>&lt;0.1</td>
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<tr>
<td>Uncharacterized</td>
<td>-18.6</td>
<td>3724.3(247.0)</td>
<td>83.3</td>
<td>-20.3</td>
<td>4701.4(283.1)</td>
<td>84.3</td>
</tr>
</tbody>
</table>
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 ± 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 ± 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 ± standard error).
Figure 1
Figure 2
Figure 3