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Transformation and fate of microphytobenthos carbon in subtropical, intertidal sediments: long-term carbon retention revealed by ¹³C-labeling

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Abstract

Microphytobenthos (MPB) are ubiquitous in coastal sediments, but the fate of their production (carbon biomass) is poorly defined. The processing and fate of MPB-derived carbon in subtropical intertidal sediments was investigated through in situ labeling with 13 C-bicarbonate. Of the added 13 C, 100 % was fixed within ~ 4 h, suggesting that MPB productivity was limited by inorganic carbon availability. Although there was rapid transfer of ¹³C to bacteria (within 12h), a relatively small fraction of ¹³C was transferred to heterotrophs (up to 12.5% of total fixed ¹³C into bacteria and 0.01% into foraminifera). MPB was the major reservoir for ¹³C throughout the study, suggesting that production of extracellular polymeric substances was limited and/or MPB recycled ¹³C. This re-10 tention of ¹³C was reflected in remarkably slow estimated turnover times for the MPB community (66–100 d). Over 31 d, ~ 70 % of the 13 C was lost from sediments. This was primarily via resuspension (~ 55%), enhanced by elevated freshwater flow following rainfall. A further $\sim 14\%$ was lost via fluxes of dissolved inorganic carbon during inundation. However, ¹³C losses via dissolved organic carbon fluxes from inundated 15 sediments (0.5%) and carbon dioxide fluxes from exposed sediments (< 0.1\%) were minimal. The retention of $\sim 30\%$ of the carbon fixed by MPB within one tidal exposure after > 30 d, despite high resuspension, demonstrates the potentially substantial longer-term retention of MPB-derived carbon in unvegetated sediments and suggests that MPB may contribute to carbon burial ("blue carbon").

1 Introduction

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Sediments at the land–ocean interface are sites of rapid organic matter transformation. Due to the high availability of light and nutrients (Heip et al., 1995), benthic communities in coastal sediments are often dominated by microscopic photoautotrophs (microphytobenthos, or MPB). MPB are highly productive and fix inorganic carbon from overlying water or air (in intertidal sediments). This carbon may undergo various transformations





before being ultimately lost to the overlying watercolumn or buried within sediments (Fig. 1). As well as being directly consumed, MPB can exude much of their production (up to 73%; Goto et al., 2001) as extracellular polysaccharides (EPS), particularly where there is low nutrient availability (Cook et al., 2007). MPB and their EPS pros vide a labile carbon source for bacteria (Bellinger et al., 2009; Oakes et al., 2010b) and higher order consumers (Middelburg et al., 2000; Oakes et al., 2010a). Ultimately, MPB, EPS, and organic matter containing MPB-derived carbon may be lost via resuspension (de Jonge and van Beusekom, 1995; Hanlon et al., 2006) or removed by mobile consumers. Alternatively, processing within sediments can result in loss of MPB-derived carbon via fluxes of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) 10 or carbon dioxide (CO2; exposed sediments). DOC fluxes can include EPS (Smith and Underwood, 2000), but DOC can also be released from algae via cell lysis by viruses, bacteria, or grazers (Agustí and Duarte, 2013). Remineralization of organic matter containing MPB-derived carbon results in fluxes of inorganic carbon (CO₂, HCO₃⁻, CO₃²⁻)

that contribute to losses of fixed carbon. All these inorganic carbon forms can diffuse 15 to overlying water as DIC while sediment is inundated, but only CO₂ is released from sediment exposed to air. These various transformation pathways for the carbon fixed by MPB affect the quality and quantity of carbon inputs to the coastal ocean.

Despite the potential importance of MPB processing pathways in coastal sediments, these have not been well defined. Stable isotope labeling is a powerful approach for 20 tracing carbon in coastal systems, and has been used in a number of studies to trace assimilation of MPB-derived carbon by bacteria and higher consumers (e.g., Middelburg et al., 2000; Oakes et al., 2010b; Evrard et al., 2012). More recently carbon stable

isotope labeling has also been used to trace pathways of loss for algal derived car-

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bon, including fluxes of DIC, DOC (Oakes et al., 2011, 2012; Evrard et al., 2010) and CO₂ (Maher et al., 2013). This approach has been used in a few studies in intertidal muds and sands and shallow subtidal sands in temperate regions, but there has been only one study in the subtropics, which looked at shallow subtidal sands (Oakes et al.,





2012). No studies have investigated the transformation and fate of MPB-derived carbon in subtropical intertidal sediments.

The processing of MPB-derived carbon in subtropical sediments may be fundamentally different to processing in temperate sediments. Differences in tidal movement may

- affect rates of sediment resuspension and flushing, and temperature differences may affect the rate and relative importance of carbon transformation pathways (Middelburg et al., 1996). In addition, the higher biomass and productivity of bacteria that is typical of sediments in warmer climates (Alongi, 1994) may also affect the rate and pathways of carbon processing. The only study to have assessed the processing of MPB-derived
- ¹⁰ carbon in subtropical sediment (shallow subtidal sands; Oakes et al., 2012) reported marked differences in the fate of carbon (greater burial and retention) compared to previous studies of sands and muds in the intertidal zone within temperate areas. However, it was not clear whether these differences reflected variations in climate and associated microbial communities, subtidal vs. intertidal differences, or some other factor.
- An impediment to understanding MPB carbon processing across all systems is the relatively short time scale over which studies have been done (e.g. 4–6 d; Middelburg et al., 2000; Evrard et al., 2010). We recently showed that 30% of the carbon fixed by MPB within 1 d remained in subtropical shallow subtidal sands after 30 days (Oakes et al., 2012). There has recently been considerable focus on the carbon sink potential of coastal habitats ("Blue carbon"), but the focus has been on vegetated habitats (Duarte
- et al., 2005; McLeod et al., 2011; Duarte et al., 2013). Although MPB lack the extensive below-ground biomass of other habitats, the long-term retention of carbon fixed by MPB in unvegetated subtropical sands observed previously suggests that MPB-derived carbon may accumulate within sediments, thereby contributing to carbon sequestration.
- ²⁵ In this study we aimed to combine in situ stable isotope labeling with measurements of DOC, DIC, and CO₂ fluxes and assimilation in sediment compartments to quantify the pathways for transformation and ultimate fate of MPB-derived carbon in subtropical intertidal sediments over the longer-term (31 d). This study provides an opportunity to gain insight into processing of intertidal MPB carbon and compare similar environ-





ments in different climate zones, and a nearby (within ~ 40 km) subtidal environment. Few studies have looked at processing of MPB carbon in situ (Middelburg et al., 2000; Bellinger et al., 2009; Oakes et al., 2010b, 2012), and only the latter two of these have considered such an extended time period. We hypothesized that the carbon fixed

⁵ by MPB in subtropical intertidal sediments would be more susceptible to removal by resuspension compared to that in subtropical subtidal sediments. We further hypothesized that subtropical intertidal sediments would have greater potential for retaining MPB-derived carbon than temperate intertidal sediments, due to greater processing and recycling by the more active microbial community.

10 2 Methods

2.1 Site description

The study site was an intertidal shoal in the lower Richmond River Estuary, subtropical New South Wales, Australia (28°52′25.01″ S, 153°33′20.43″ E). The river has a catchment of ~ 6900 km² with annual rainfall of ~ 1300 mm (McKee and Eyre, 2000) and an average flow rate of ~ 2200 ML d⁻¹ (daily gauged flow adjusted for catchment area, averaged over years for which data was available; 1970–2013). The catchment is subject to frequent episodic rainfall events, and associated flooding, resulting in highly variable estuary flushing, salinity, and nutrient concentrations (Eyre and Twigg, 1997; Eyre, 2000). For details of the hydrology and biogeochemistry of the Richmond River, refer to McKee and Eyre (2000) and Eyre and Twigg (1997).

Sediment at the site was sandy mud, with the surface layer (0–2 cm depth) dominated by fine sand (125–250 μ m; 68 %) and very fine sand (63–125 μ m; 23 %). Deeper sediments (2–10 cm) were also dominated by fine sand (~ 78 %), but had a lower contribution of very fine sand (~ 12 %) and a greater contribution of medium sand (~ 10 %).

²⁵ Based on O₂ fluxes in control cores, the site was net autotrophic (p/r = 2.23). Examination by light microscopy (100×) showed that the microphytobenthos was dominated





by diatoms. No cyanobacteria were observed. Gross productivity (GPP) of inundated sediments based on O_2 fluxes in control cores was $4.5 \pm 1.5 \text{ mmol Cm}^{-2} \text{h}^{-1}$ (assuming $O_2 : \text{C} = 1 : 1$). Surface sediments (0–2 cm) had an organic carbon (OC) content of $146.8 \pm 18.7 \text{ gm}^{-2}$. Sediment organic matter at depths of 0–2 cm, 2–5 cm and 5–10 cm had molar C : N ratios of 15.8, 14.1 and 11.1, respectively.

2.2 ¹³C-labeling

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At the beginning of low tide two experimental plots $(1 \text{ m} \times 1 \text{ m})$ were established within 10 m of one another on the intertidal shoal by pushing aluminium frames 3 cm into the sediment such that their upper surface was flush with the sediment. String stretched across each frame divided the plots into grids of $20 \text{ cm} \times 20 \text{ cm}$ squares. An equal quantity of ¹³C-labeled NaHCO₃ (99 % ¹³C) dissolved in filtered (GF/F) site water was applied to the sediment surface within each square using a motorized sprayer. This ensured even application of ¹³C across each plot, equivalent to 11.5 mmol ¹³C m⁻². The ¹³C-labeled plots remained exposed to air for ~ 4 h of daylight before tidal inundation.

⁵ One tidal inundation was allowed to occur before the first sampling to remove any ¹³Cenriched sodium bicarbonate that had not been incorporated by MPB. Frames were left in place for the duration of the study.

2.3 Sample collection

 During low tide when the sediments were exposed at 0.5, 1, 3, 10, 20 and 30 d after ¹³C-labeling, one core of sediment (9 cm diameter × ~ 20 cm depth) was collected from each plot using a Plexiglas core liner for incubation in the laboratory. On each occasion, two cores of sediment were also collected from 5–10 m outside of each plot for incubation to determine background (control) isotope values and fluxes. On the first two sampling occasions, an additional set of cores was collected from within and outside of each plot. These cores were immediately split into sediment depth layers of 0–2 cm, 2–5 cm, and 5–10 cm, which were stored frozen prior to analysis of ¹³C within





sediment organic carbon (OC), fauna, MPB and bacteria. To minimize site disturbance, PVC pipes filled with site sediment (90 mm diameter, 20 cm long) were placed in the holes left following core removal on each sampling occasion.

Because the site was intertidal, each core was incubated in the dark and the light with water overlying the sediment (inundated conditions), and in the dark and light with water drained from the core (exposed conditions). Cores were incubated for ~ 5 to 6 h under each set of conditions (dark inundated, light inundated, dark exposed, and light exposed). Incubations began immediately upon return to the laboratory, under conditions reflecting those in the field at the time (e.g., light exposed incubations occurred during daylight hours when there was a low tide at the study site). Control cores and ¹³C-labeled cores were incubated in separate chambers of water maintained at approx-

imately in situ temperature $(18.5 \pm 1 \degree C)$ and light levels $(1200 \mu mol photons m^{-2} s^{-1} \pm 5 \%)$.

- For inundated incubations, cores were gently filled with site water, taking care not to disturb the sediment surface, and capped with gastight Plexiglas lids containing sampling ports. An external rotating magnet operated magnetic stir bars suspended ~ 5 cm above the sediment surface within each core, maintaining water circulation below the sediment resuspension threshold. At the start and end of each incubation period, the dissolved oxygen concentration within each core was measured (±0.01 mg
- ²⁰ L-1; Hach HQ40d, luminescent DO probe) and ~ 50 mL of water was removed from each core. Sample water was syringe filtered (precombusted GF/F) into precombusted 40 mL glass vials with Teflon-coated septa, killed ($200 \mu L 50: 50 w/v ZnCl_2$) and refrigerated, without headspace, until analysis for $\delta^{13}C$ and concentrations of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). Additional water samples
- ²⁵ were collected for a separate study. Sample water was replaced, as it was withdrawn, with site water from a collapsible reservoir.

For exposed incubation, any water overlying the sediment was carefully siphoned off, taking care to avoid disturbing the sediment surface, and weighted containers and foam pads were used to raise the sediment within each core sleeve so that the sediment





surface was within $\sim 1 \text{ cm}$ of a gastight Plexiglas lid containing two septa. Weighted containers were used to prevent the cores from floating within the incubation chambers, whilst the foam pads, which fitted tightly within the core sleeves, allowed for finer adjustment of sediment height whilst preventing drainage of interstitial porewater. At

the start and end of each exposed incubation period 2 mL samples of the air overlying the sediment within each core was withdrawn into a gastight syringe through one of the septa in the core lid. However, samples for the first three sampling periods were lost. Sampled air was transferred to 12 mL exetainers prefilled with degassed water, which were held upside down as air was injected. Displaced water escaped via a second
 syringe tip placed through the septa in each exetainer lid.

At the conclusion of the incubation procedure, sediment was extruded and sectioned (0-2 cm, 2-5 cm, and 5-10 cm depths) for analysis of sediment OC, fauna, and phospholipid-derived fatty acid (PLFA) biomarkers for MPB and bacteria.

2.4 Sample analysis

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¹⁵ Stable isotope analysis of all background (control) samples was done separately to analysis of samples collected from ¹³C-labeled plots.

The concentrations and δ^{13} C of DOC and DIC were measured via continuous-flow wet-oxidation isotope-ratio mass spectrometry using an Aurora 1030W total organic carbon analyzer coupled with a Thermo Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) as described by Oakes et al. (2011).

The δ^{13} C of CO₂(g) (0.1 % reproducibility) was determined using a preconcentration system attached to a Thermo Trace GC Ultra gas chromatograph with a Porapak Q column (30 m × 0.32 mm) coupled to a Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) via a Conflo III interface. The sample headspace was purged directly into the preconcentration system using helium flow and was concentrated at -65 °C using

the preconcentration system using helium flow and was concentrated at -65°C using HaySep Q as a chemical sieve, and desorbed at 150°C onto the GC column. The GC oven was held at 40°C, and the flow rate of the helium carrier gas was 3 mLmin⁻¹. Because variations in CO₂(g) concentration were within the range of instrument er-





ror, a constant $CO_2(g)$ concentration was assumed (typical atmospheric concentration, 15.6 μ mol L⁻¹).

Macrofauna at the site had low abundance and were highly mobile, preventing assessment of their use of MPB. We focused on small fauna retained on 63 µm to 500 µm
sieves (meiobenthos). Due to the laborious nature of this work, samples were only analysed for one replicate on day 3, 10, and 30 and only for surface sediments. Fauna were sorted under a dissecting microscope. Biomass was determined by manually removing (under a dissecting microscope) and weighing all meiobenthos, sorted by taxa, from a known quantity of homogenized sediment. For dominant taxa, based on biomass, individual organisms were manually removed from the sediment under a dissecting microscope to obtain sufficient material for isotope analysis.

Samples of sediment and fauna were lyophilized, homogenized, weighed into silver cups, and acidified (5% HCl) prior to the determination of δ^{13} C (±0.2‰) and % C (error ~ 1.0% of measured value) using a Thermo Finnigan Flash Elemental Analyzer

- 15 112 interfaced via a Thermo Conflo III with a Thermo Delta V Plus Isotope Ratio Mass Spectrometer (EA-IRMS). The % N of unacidified subsamples of sediment was also determined via EA-IRMS, allowing calculation of molar C : N ratios for sediment OC. Helium dilution of the carrier stream was turned off for foraminifera samples to reduce the required mass.
- ²⁰ Phospholipid biomarkers (PLFAs) to determine ¹³C uptake into MPB and bacteria were extracted from lyophilized sediments following addition of an internal standard (tridecanoic acid, C₁₃), using a modified Bligh and Dyer method (Oakes et al., 2010b). PLFA concentrations and δ^{13} C were determined using a Thermo Trace GC Ultra gas chromatograph coupled with a Thermo Delta V Plus IRMS via a Thermo Conflo III interface (Oakes et al., 2010b). The column used was a 60 m perpelor HPE MS (18)W
- interface (Oakes et al., 2010b). The column used was a 60 m nonpolar HP5-MS (J&W Scientific, 0.32 mm i.d., 0.25 μm film thickness).





2.5 Calculations

PLFA δ^{13} C values were corrected for the addition of a carbon atom during methylation as described by Jones et al. (2003). Natural abundance δ^{13} C values for bacteria and MPB were calculated using δ^{13} C values of PLFAs specific to bacteria and diatoms from 5 control cores, as described by Oakes et al. (2010b).

Total ¹³C uptake into sediment OC, fauna, bacteria, and MPB (μmol ¹³C m⁻²) was calculated as described by Oakes et al. (2012) based on the quantity of excess ¹³C in each sample and the carbon biomass of each compartment. Carbon biomass of sediment and fauna was the product of % C and total dry mass per unit area. For fauna, the total number of individuals per m² was estimated based on the number of individuals within sediment subsamples of known volume. Total mass was determined by multiplying this estimate by the average mass of an individual, based on the mass of a known number of individuals.

Concentrations of PLFA biomarkers for bacteria (i15:0 and a15:0) and MPB (16:1(n-

- ¹⁵ 7) and 20:5(n-3)) were calculated based on their peak areas relative to the C₁₃ internal standard. Total biomass of bacteria and MPB was calculated as described by Oakes et al. (2010b). The fraction of MPB PLFAs represented by the considered biomarkers was estimated to be 0.37 (Volkman et al., 1989).
- Transfer of ¹³C into DOC and DIC in the water column and CO₂in air 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 ¹³C in DOC, DIC, or CO₂ (fraction ¹³C in labeled sample – fraction ¹³C in equivalent control), water or air volume within the core, and the concentration of DOC or DIC, or assumed concentration of CO₂ (15.6 µmol L⁻¹). The total flux of excess ¹³C in DOC, DIC, or CO₂ during dark or light incubation was then calculated as follows:

Excess¹³C flux =
$$\left(\text{Excess}^{13}\text{C}_{\text{start}} - \text{Excess}^{13}\text{C}_{\text{end}}\right)/\text{SA}/t$$
 (1)





where excess ${}^{13}C_{start}$ and excess ${}^{13}C_{end}$ 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 ${}^{13}C m^{-2} d^{-1}$) of DOC, DIC, or CO₂ were calculated as follows:

5 Net flux = (dark flux/dark hours) + (light flux/light hours) $\times t$

where *t* represents the number of hours of sediment inundation (for DOC and DIC fluxes) or exposure (CO₂ fluxes). We interpolated between measured net flux values and estimated the total quantity of ¹³C lost via fluxes of DOC, DIC, and CO₂ from the end of labeling up until each sampling period by determining the area under the curve. A 2-G model (Westrich and Berner, 1984) determined the rate of ¹³C loss from 0–

10 cm sediment from each experimental plot, as follows:

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

where G_{T} represents ¹³C incorporation into sediment OC, *t* is time, G_1 , G_2 , and G_{NR} represent ¹³C incorporation 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 for G_1 and G_2 , respectively.

3 Results

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3.1 Estuary conditions

Around 35 d before sampling began a rainfall event increased freshwater flow rates at the head of the estuary to a maximum of 24 248 MLd⁻¹. Although flow rates at the head of the estuary decreased to typical levels (~ 2000 MLd⁻¹) and remained so for the first three sampling occasions (up to 4 d after label application, Fig. 2), a further rain event then resulted in marked increases in flow to 39 757 MLd⁻¹ within 9 d of label application



(2)

(3)

(Fig. 2). Flow rates remained elevated for the fourth sampling occasion (37 952 MLd⁻¹), but declined rapidly thereafter. However, flow remained elevated above typical levels for the remainder of the study period, with rates of ~ $3000-4000 \text{ MLd}^{-1}$ in the final days of the study. These variations in flow rate are typical of the estuary (Eyre and Twigg, 1997; Eyre, 2000) and corresponded with variations in salinity (0.91 to 24.04) and concentrations of dissolved inorganic nitrogen (1.02 to 18.11 µmolL⁻¹) and dissolved inorganic phosphorus (0.37 to 1.36 µmolL⁻¹) over the duration of the study (Fig. 2).

3.2 Sediment organic carbon distribution

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Sediment OC content was higher by volume in shallower sediments (average over the study of $611 \pm 78 \,\mu\text{mol}\,\text{CmL}^{-1}$ in 0–2 cm sediments) than in deeper sediments ($418 \pm 46 \,\mu\text{mol}\,\text{CmL}^{-1}$ in 2–5 cm sediments, $333 \pm 23 \,\mu\text{mol}\,\text{CmL}^{-1}$ in 5–10 cm sediments). The total OC content of surface sediments decreased by ~ 24 % over the duration of the study.

On average, MPB and bacteria together accounted for ~ 89% of the OC in 0–2 cm sediments (Table 1) and ~ 77% of the OC in 2–5 cm sediments. However, whereas MPB and bacteria had similar biomass in surface sediments, there was a marked decline in MPB biomass in deeper sediments, with MPB accounting for ~ 24% of OC in 2–5 cm sediments, and only ~ 3% of OC in deeper sediments. Bacteria represented ~ 44% and 54% of the OC in 0–2 cm and 2–5 cm sediments, respectively, but accounted for only 11.6% of the OC in 5–10 cm sediments. MPB and bacteria in surface sediments showed similar declines in biomass over the duration of the study, with respective losses over 31 d of ~ 35% and ~ 40%.

A total of 51 species of meiobenthos were recorded, and these were primarily foraminiferans. Only two species (*Elphidium advenum* and *Ammonia beccarii*) were common to most samples and were present at sufficient biomass for isotope analysis ($\sim 2000 \,\mu\text{mol}\,\text{Cm}^{-2}$ and $\sim 1000 \,\mu\text{mol}\,\text{Cm}^{-2}$, respectively). These species together accounted for $62 \pm 5 \,\%$ of the total mass of fauna in 0–10 cm sediment, but represented



< 1 % of the sediment OC pool (Table 1). There was a similar contribution of *E. advenum* and *A. beccarii* to total meiobenthos in 0–2 (~ 22 %) and 2–5 cm sediment (~ 31 %), but *E. advenum* was more abundant than *A. beccarii* in 5–10 cm sediments (45 % vs 34 % of meiobenthos mass).

The fraction of sediment OC that was not within the compartments considered in the current study (i.e. uncharacterized OC) increased with sediment depth (Table 1). Whereas only $\sim 11\%$ of sediment OC was uncharacterized in 0–2 cm sediments, we were unable to account for $\sim 23\%$ and $\sim 86\%$ of OC at 2–5 and 5–10 cm (Table 1).

3.3 Natural abundance stable isotopes

Sediment OC δ¹³C values were similar for 0–2 cm and 2–5 cm sediments (average = -16.0% and -16.3%, respectively, but enriched in deeper sediments (-14.4%). Values of δ¹³C for MPB and bacteria varied little with depth (Table 1), and δ¹³C values for bacteria were 0.5 to 2% depleted, on average, compared to MPB (Table 1). *E. advenum* and *A. beccarii* in 0–2 cm sediments had δ¹³C values of -16.4% and -15.4%, within the range of bacteria and bulk sediment OC. In contrast, δ¹³C values for uncharacterized surface sediment OC were relatively depleted (~ -23% to -25%; Table 1), but were more enriched (-14.3%) in 5–10 cm sediments (-14.3%).

Control δ^{13} C values of water column DOC were relatively depleted (-30.6 ± 0.5 ‰). DIC fluxes had δ^{13} C values of -1.5 ± 1.8 ‰ throughout the study period.

20 3.4 ¹³C incorporation, burial, and transfer

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By 12 h after label application $12.0 \pm 4.5 \text{ mmol}^{13}\text{C} \text{ m}^{-2}$ had been incorporated into OC at sediment 0–10 cm deep, giving an uptake rate for ${}^{13}\text{C}$ of $1.0 \pm 0.4 \text{ mmol}^{13}\text{C} \text{ m}^{-2} \text{ h}^{-1}$. However, given that unincorporated label would have been removed with the first tidal inundation, ${}^{13}\text{C}$ uptake most likely occurred during ~ 4 h after label application (before tidal inundation), giving an adjusted uptake rate of $3.0 \pm 1.1 \text{ mmol}^{13}\text{C} \text{ m}^{-2}\text{ h}^{-1}$. Rates of carbon fixation at the site apparently have the potential to be far higher than this,



however, as 100 % of the added ^{13}C was assimilated, indicating that MPB at the site was carbon limited.

For the first 4 d following label addition, MPB was the major reservoir for fixed ¹³C, accounting for 77.6 to 100% of the total ¹³C remaining within sediment OC (77.4 to 89.6% of the initially fixed ¹³C; Fig. 3). The importance of MPB declined thereafter, however, with MPB accounting for 15.0% of the ¹³C remaining in sediment OC, and 4.6% of the initially fixed ¹³C, by day 31 (Fig. 3).

Despite their higher overall biomass (Table 1), bacteria had a far smaller role in ¹³C incorporation than MPB, accounting for between 1.6 and 8.8 % of the ¹³C within sediment OC within the first 3 d following label addition (Fig. 3). Thereafter, the importance of bacteria generally increased, with bacteria accounting for a peak of 30.5 % of the ¹³C within sediment OC (13.8 % of fixed ¹³C) at 20 d after label addition.

The proportion of ¹³C remaining in sediments that was accounted for by MPB and bacteria generally increased during the study to 74.6% at day 31 (Fig. 3). Fauna ac-¹⁵ counted for 0.01 to 0.03% of the ¹³C remaining within the sediment OC at the times samples were analyzed (4 d, 10 d, and 31 d).

There was a general increase in the ¹³C content of deeper sediments (2–5 cm and 5–10 cm) over time (Fig. 3), but this transfer of ¹³C to deeper sediments did not match the larger decline in ¹³C content of surface sediments. This reflected the overall loss of ¹³C from 0–2 cm sediments. The transfer of ¹³C to deeper sediments was relatively slow; a total of 3.69 % of the fixed ¹³C was within 2–10 cm sediments 12 h after label addition, equating to a burial rate of 0.3 % h⁻¹. By the conclusion of the study, 75.3 % of the remaining ¹³C was within deeper sediments (2–10 cm; Fig. 3), representing burial over 31 d of around 23.2 % of the ¹³C that was initially fixed by MPB.

25 3.5 ¹³C loss from sediments

By the end of the study, 69.2% of the added 13 C had been lost from 0–10 cm sediment (Fig. 4). However, only 13.6% of the added 13 C was estimated to be lost via



the pathways we considered, and this was almost entirely due to fluxes of ¹³C as DIC (Fig. 4). Over the 31 day study, 1572.7 μ mol ¹³C m⁻² (13.1% of added ¹³C) was lost from the sediment as DIC, with ~ 80% of this loss occurring over the first ~ 4 d (Fig. 4). There was generally uptake of DIC into sediment during the light (-2943.0 ± 3213.4 μ mol m⁻² h⁻¹) and release during the dark (1406.6±1711.1 μ mol m⁻² h⁻¹). This corresponded with higher fluxes of ¹³C abelled DIC in the dark than in the light.

Far less ¹³C was lost from sediments as CO₂ or DOC. DOC fluxes accounted for loss of only 58.34 μ mol ¹³C m⁻² (0.48 % of fixed ¹³C). This was not surprising as there was a net uptake of DOC (-85.6 ± 149.7 μ mol m⁻² h⁻¹) into sediments. In this case, losses of ¹³C via DOC fluxes indicate an exchange of DOC between the sediment and the

of ¹³C via DOC fluxes indicate an exchange of DOC between the sediment and the overlying water. DOC fluxes were highly variable, with generally smaller DOC uptake in the light (-23.9 ± 278.0 µmol m⁻² h⁻¹) than in the dark (-147.4 ± 189.3 µmol m⁻² h⁻¹). The greatest rate of loss of ¹³C as DOC was recorded during the first dark incubation period. At all times thereafter, there was greater loss of ¹³C as DOC in the light than in the dark.

Fluxes of CO₂ were only measured for the last three time periods, but at these times the loss of ¹³C via CO₂ was only 0.2% to 5.1% of the loss of ¹³C via DIC. Assuming that fluxes of ¹³C in CO₂ at all times represented ~ 5% of the ¹³C lost as DIC (the maximum observed in the current study), a total of 0.02% of the fixed ¹³C (~ 3 µmol ¹³C m⁻²) would have been lost via fluxes of CO₂ from exposed sediments.

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The 55.6 % of added ¹³C that was lost from sediments and which was not accounted for by the pathways that were directly measured was assumed to represent the fraction of fixed carbon that was lost from sediments via resuspension, burial to deeper sediments than those considered here, or removal by mobile animals (Fig. 4).

²⁵ The decline in ¹³C content of OC in 0–10 cm sediments over the duration of the current study could be fitted with a 2-G model ($R^2 = 0.89 \pm 0.08$; Fig. 4). Of the total sediment OC, 22.2±7.2 % was highly reactive (fraction G_1), with a degradation rate (k_1) of 0.022±0.018 d⁻¹). A further 47.7±8.1 % was less reactive (fraction G_2), degrading



at a rate (k_2) of 0.002 ± < 0.001 d⁻¹). The remaining 30.0 ± 0.8 % of the sediment OC was nonreactive over the timescale of the experiment (fraction G_{NR}).

4 Discussion

4.1 Site characteristics

- The microbial community at the study site was highly abundant; MPB and bacteria to-5 gether comprised ~ 90 % of the OC in surface (0-2 cm) sediments (Table 1). Based on O_2 fluxes in control cores (assuming O_2 : C = 1:1 and 12 h of production per day), the estimated GPP of the MPB community ($\sim 240 \,\mathrm{gCm}^{-2} \,\mathrm{vr}^{-1}$) was towards the upper end of values reported for MPB in intertidal and shallow coastal sediments (Colijn and de Jonge, 1984; Barranguet et al., 1998 and references therein). The productivity of the 10 MPB was similar throughout the study despite variations in MPB biomass, but the ratio of biomass to productivity was high, indicating that turnover was slow. During the first 10 d of the study the turnover time of MPB was \sim 100 d, and even at the conclusion of the study, with 40 % lower MPB biomass, the turnover time was still \sim 66 d. This is remarkably slow compared to estimates for temperate subtidal and intertidal sand and 15 mud (1.3–62 d; Sundbäck et al., 1996; Middelburg et al., 2000; Evrard et al., 2010) and subtropical shallow subtidal sand and mud (0.6 to 21.7; Ferguson et al., 2003;
- 5.5 d; Oakes et al., 2010b). As noted by Middelburg et al. (2000), similar rates of MPB production can correspond with substantial differences in MPB biomass. For example,
- MPB in subtropical shallow subtidal sediments have been reported to be similarly productive, but with far lower biomass than in the current study (Ferguson et al., 2003; Oakes et al., 2010b). This demonstrates that MPB biomass is not a reliable indicator of productivity.

The long turnover time of MPB at the study site may reflect inorganic carbon limitation and substantial recycling of fixed carbon by MPB. Inorganic carbon availability has been reported to limit primary production by MPB in laboratory studies (Admiraal et al.,





1982; Cook and Røy, 2006) and epilithic microalgae in situ (coral biofilms; Larkum et al., 2003), particularly when productivity is high. At the study site, the uptake of all of the added ¹³C suggests that inorganic carbon limited MPB productivity, at least during exposure. This probably relates to the high biomass of MPB, leading to intense competition for resources. The estimated productivity of MPB during exposure, based on ¹³C uptake $(3.0 \pm 1.1 \text{ mmol m}^{-2} \text{ h}^{-1})$, was similar to the productivity of inundated sed-iments based on O₂ fluxes (see "Site description"; ~ $3 \text{ mmol m}^{-2} \text{ h}^{-1}$). This is despite the potential stimulation of the carbon-limited exposure is usually somewhat lower. This may reflect depletion of inorganic carbon within porewater during emersion, and limited diffusion of new CO₂ into sediment porewater. Given that our estimate of GPP over the year was based on inundated fluxes, true GPP of the study site may be as low as half of that estimated, as sediments would be exposed to air for approximately half

15 of each day.

Although MPB and bacteria represented most of the OC within surface sediments, a further 10.7% was uncharacterized. This would likely be comprised of both labile and refractory components including extracellular organic carbon exuded by MPB and bacteria, and allochthonous OC. Isotope mixing suggests a δ^{13} C value for uncharacterized are refracted on the surface of δ^{13} C value for uncharacterized.

- ²⁰ terized OC of ~ -25‰ (Table 1). This is within the range of δ^{13} C values for phytoplankton and terrestrial plants (Michener and Schell, 1994), confirming a contribution of allochthonous OC. Given the relatively depleted natural abundance δ^{13} C value for DOC at the site (~ -30‰), allochthonous OC may also be the source of the DOC released from control sediments. The relatively high C : N ratio of sediment OC was well
- ²⁵ above a typical Redfield ratio. This could reflect substantial re-working of sediment OC, leading to enrichment of the C : N ratio, but more likely reflects a contribution of terrestrial material, as episodic rainfall events increase the flow of freshwater at the study site, and can enhance the delivery of terrestrial OC (Eyre and Twigg, 1997). This deposition of new material would offset losses of OC and is reflected in the lower apparent





rate of loss of total OC from sediments (25 %) compared to MPB (40 %) and bacteria (45 %).

The contribution of uncharacterized OC to the total sediment pool increased with sediment depth (Table 1), but its source was less clear. The δ^{13} C value of uncharacterized OC was considerably enriched in deeper (5–10 cm) sediments (Table 1), suggesting that there has been (i) burial of MPB-derived carbon, (ii) burial of relatively ¹³C-enriched allochthonous OC (e.g. seagrass; Jones et al., 2003), or (iii) substantial re-working of OC resulting in preferential removal of ¹²C- or ¹³C-depleted compounds (e.g. lipids; DeNiro and Epstein, 1977).

- ¹⁰ Given the considerable contribution of bacteria to sediment OC at all sediment depths (11.6% to 53.6% of total OC), they are likely to be the main contributor to carbon processing at the study site. The relatively enriched δ^{13} C values of bacteria suggested that there was a substantial contribution of MPB-derived carbon, but the uncharacterized OC fraction also contributed, leading to the depletion of δ^{13} C values
- for bacteria compared to MPB. Assuming a trophic fractionation of 0.5‰ for carbon (McCutchan et al., 2003), MPB and the uncharacterized OC fraction contributed 82.9 and 17.1%, respectively, of the carbon for bacteria in 0–2 cm sediments, and 74.2 and 25.8% in 2–5 cm sediments. The carbon source for bacteria in 5–10 cm sediment could not be resolved, as the δ^{13} C value was depleted compared to both potential sources.
- ²⁰ However, given the greater biomass of bacteria in shallow sediment, the δ^{13} C value of bacteria in 5–10 cm sediments was likely dominated by bacteria towards the upper extent of this layer, which would have access to more depleted uncharacterized OC (Table 1).

4.2 ¹³C transfer and burial

Given that an earlier study ~ 120 km north of the site of the current study showed that there was no carbon fixation in muddy or sandy sediments in the dark (Oakes, 2007), all ¹³C within the sediments was assumed to have been initially fixed by MPB. There was rapid transfer of ¹³C to heterotrophs, with 4.5 % of the fixed ¹³C found within



bacteria 12 h after label addition, but the total quantity of ¹³C transferred was limited. Throughout the study, only 1.2% to 12.5% of the total fixed ¹³C was found in bacteria, and up to 0.01% in fauna. At best, 28.4% of the ¹³C remaining within sediment OC at any one time was within bacteria (at 10 d), but this only equated to just over half
of the ¹³C within MPB at this time. This was similar to the proportion of remaining fixed carbon that was in bacteria 10 d after ¹³C-labeling of MPB in subtropical subtidal sands (Oakes et al., 2010b). However, whereas this and a number of other studies (Middelburg et al., 2000; Woulds et al., 2007; Oakes et al., 2010b) have found ¹³C incorporation by sediment compartments to be roughly proportional to biomass this was not the case in the current study. Whereas the biomasses of MPB and bacteria were similar in 0–2 cm sediments, the majority of fixed ¹³C within sediments was in MPB at all sampling times. Even in the 2–5 cm sediments, where bacterial biomass exceeded that of MPB, MPB contained a greater portion of ¹³C at all times. Until at

least 4 d after label addition, 78% or more of the ¹³C remaining within sediment OC was within MPB, and by the end of the study (at 31 d) 15% of the ¹³C was still within MPB. This corresponds with the slow turnover rate estimated for MPB. An exponential curve fitted to the data ($R^2 = 0.98$) predicted that ¹³C would remain within MPB until > 80 d after label addition (Fig. 5).

Possible explanations for the apparent slow turnover and ¹³C retention of MPB include (i) substantial recycling of fixed carbon by MPB via mobilization of their own EPS (Stal, 2003) or internal storage compounds (Bellinger et al., 2009), (ii) limited grazing of MPB by higher heterotrophs (supported by low biomass and ¹³C content of fauna), and (iii) efficient recapture of ¹³C remineralized by bacteria, but most likely relates to (iv) limited production of EPS. A substantial portion of the carbon fixed by MPB can be
rapidly exuded as EPS (up to 70.3 % h⁻¹; Goto et al., 1999). In the current study, however, only 10.5 % of the ¹³C within sediment OC had been transferred from MPB within 12 h, equating to an EPS production rate at the lower end of the range reported for marine benthic diatoms and sediment biofilms (0.9 % h⁻¹; Underwood and Paterson, 2003). Given that EPS can play an important role in the transfer of carbon from au-





totrophs to heterotrophs (Oakes et al., 2010b), low production of EPS is likely to have contributed to the limited transfer of ¹³C to bacteria and higher heterotrophs (Fig. 1). The production of EPS assists with diatom movement and attachment and increases with nutrient limitation (Cook et al., 2007), functioning to maintain Redfield C : N whilst

- ⁵ protecting cells from potential damage by excess energy (Stal, 2003). Around the time of the current study, rain events most likely ensured that nutrients were in plentiful supply, both in the watercolumn and in sediments, due to degradation of recently deposited allochthonous material. Excessive production of EPS by MPB would therefore be unnecessary and, given that inorganic carbon availability is sometimes limited, may be undesirable. Rather, it is possible that fixed carbon is stored intracellularly as chryso-
- ¹⁰ undesirable. Rather, it is possible that fixed carbon is stored intracellularly as chrysolaminaran (Bellinger et al., 2009), for use when carbon limits MPB production (e.g., during exposure; Fig. 1).

Although 75.3% of the ¹³C remaining at the end of the study (31 d) was in sediments at 2–10 cm depth, representing 23.2% of the ¹³C that was initially fixed, the rate of downward transport of ¹³C was slower than has been seen in previous studies. Oakes et al. (2012) observed transfer of 12.9% of fixed carbon to 2–5 cm and 18.6% to 5–10 cm within 60 h in shallow subtidal subtropical sands, and Middelburg et al. (2000) and Evrard et al. (2010) reported similar transport of fixed carbon to 2– 5 cm in temperate mud and sand within 3 d. However, in the current study a far smaller proportion of the initially fixed ¹³C was at these depths even 31 d after label addition (8.3% and 14.9% to 2–5 cm and 5–10 cm, respectively). In contrast to our earlier study of subtidal sands (Oakes et al., 2012), considerable removal of ¹³C via resuspension

- in the current study would have limited the carbon pool available for downward transport (Fig. 1). The low biomass of fauna would also have limited the potential for carbon ²⁵ burial through bioturbation, subduction, and consumption by mobile animals. However,
- the intertidal study of Middelburg et al. (2000) also reported substantial loss of fixed carbon via resuspension, but had greater burial. Sediment flushing and mixing, which is influenced by sediment porosity and tidal movement, can transport fixed carbon to deeper sediments. The grain size of our sediment was intermediate to that of sediment





at two sites studied by Middelburg et al. (2000). However, the tidal amplitude ($\sim 5 \text{ m}$) at the site studied by Middelburg et al. (2000) was far greater than in the current study ($\sim 2 \text{ m}$). Although this would have contributed to sediment resuspension, this may also have enhanced sediment mixing and, therefore, burial of carbon that had not been resuspended. Increased flow rates and scouring at the time of the current study may also have reduced the potential for new sediments to deposit at the study site, thereby limiting burial through this mechanism.

4.3 ¹³C loss

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An important attribute of this study is that the ¹³C-labeled sediment community was exposed to natural environmental conditions for the majority of the study, allowing for natural loss processes to occur. The overall loss of ¹³C from sediments and the budget for loss and retention over the duration of the study therefore incorporates the effect of these in situ processes. A further characteristic of the current study is that it is one of very few (e.g. Oakes et al., 2012) to have directly measured so many loss pathways. However, we were still unable to account for loss of ~ 50% of the carbon initially fixed by MPB. This carbon was most likely lost via resuspension of surface sediment OC, particularly given the elevated freshwater flow rates at the study site, but this was not directly quantified in the current study. Although burial to sediments below 10 cm and/or removal by mobile consumers are also possible pathways for loss that were not considered, the general lack of fauna in the sediments, and limited burial of ¹³C below 5 cm suggests that these pathways are probably relatively minor contributors to carbon loss.

Resuspension of sediment and associated material, including MPB, is generally driven by wind- or tide-generated turbulence (deJonge and van Beusekom, 1995) but in the current study was probably also strongly driven by enhanced freshwater flow rates following a rain event (Eyre and Twigg, 1997). Significant rainfall events are common in the Richmond River catchment (Eyre and Twigg, 1997; Eyre, 2000) and the enhanced freshwater flows that occurred during the period of the current study are therefore typi-



cal of the system. These episodic freshwater flows are also typical of any (sub) tropical systems (Eyre, 1998, 2000; Eyre and Balls, 1999). However, it is likely that a smaller portion of the carbon fixed by MPB would be lost from sediments via resuspension under drier conditions. Similarly, a larger proportion of the carbon fixed by MPB would be lost from sediments via resuspension during larger floods (see Eyre and Twigg, 1997; Eyre, 2000).

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The susceptibility of surface sediments to resuspension depends on a number of factors including water depth and flow rate, tidal height, activity of fauna and characteristics of the sediment including the presence or absence of a stabilizing biofilm (MacIntyre et al., 1996). In intertidal sediments, tidal movements can cause resuspension of a substantial part of the MPB community (up to 50% or more in temperate mudflats; de Jonge and van Beusekom, 1995) and EPS (~ 60%; Hanlon et al., 2006).

Migration of MPB can buffer against their resuspension, but given that MPB were the major reservoir for fixed carbon throughout the current study, and there was similar loss of MPB and bacterial biomass from the sediments, it is likely that resuspension of MPB was the major conduit for loss of fixed carbon via this pathway. Alternatively, resuspension may have removed EPS before it was assimilated by heterotrophs.

Few studies have specifically assessed rates of loss of MPB-derived carbon from sediment. In subtropical subtidal sands we saw very little loss of fixed carbon from sub-

- tidal sands via resuspension over 30 d in a previous study (< 3 %; Oakes et al., 2012). In contrast, Middelburg et al. (2000) reported loss via resuspension of 34 % of carbon fixed by MPB from intertidal sands within 5.6 d, reflecting the influence of the turbid tidal edge. Although we observed similar loss of fixed carbon overall in subtropical intertidal sediments in the current study, the rate of loss was slower, occurring primarily over
- 10 d. As discussed previously, this may relate to the lower tidal movement at the site of the current study, compared to that at the site studied by Middelburg et al. (2000). Given that losses due to resuspension were almost certainly enhanced by elevated river flows in the current study, it is likely that the loss to resuspension would be lower again under more typical flow conditions. Although other factors may also be important,





the pattern of faster loss of MPB carbon with increased tidal movement across three studies (Middelburg et al., 2000; Oakes et al., 2012; current study) suggests that tidal movement has a major influence on MPB carbon fate.

- Of the pathways measured directly, DIC flux during inundation was the most important for loss of carbon fixed by MPB (Fig. 1), but still accounted for only ~ 19% of the total lost ¹³C, or ~ 13% of the carbon that was initially fixed. In temperate and subtropical subtidal sands, respiration has been reported to have a greater contribution to removal of MPB-derived carbon (14% over 4 d and 63% over 30 d, respectively; Evrard et al., 2010; Oakes et al., 2012). This difference is not surprising, given the considerable removal of fixed carbon by resuspension in the current study, which would limit
- the carbon available for respiration. However, in temperate intertidal sands that are similarly susceptible to resuspension, respiration was estimated to account for 40% of the total loss of MPB-derived carbon, although it should be noted that this was not measured directly (Middelburg et al., 2000). The limited role of respiration for transfor-
- ¹⁵ mation and loss of MPB-derived carbon in the current study site is further evidence that limited MPB-derived carbon is transferred to heterotrophs, including bacteria, over at least 31 d. The contribution of recycling to the retention of carbon by MPB is supported by the reduced flux of ¹³C-labelled DIC in the light, particularly early in the study. This clearly reflects the re-capture of respired MPB-derived carbon by MPB for use in photermities and event is the second study. The study of the second study of the second study.
- tosynthesis and would contribute to the apparent slow turnover of MPB and long-term retention of MPB-derived carbon within the sediment.

Fluxes of DOC were a relatively minor contributor to loss of MPB-derived carbon in the current study (Fig. 1). Given that tidal flushing and resuspension can remove a substantial portion of the EPS produced by MPB (e.g. ~ 60 %; Hanlon et al., 2006),

²⁵ our ex situ core incubations may have underestimated DOC fluxes. However, this is unlikely, given the apparently low rate of EPS production. Furthermore, the two studies that have specifically considered this loss pathway (in temperate and subtropical shallow subtidal sands, respectively; Cook et al., 2007; Oakes et al., 2012) also reported limited loss of MPB-derived carbon via DOC fluxes. This may reflect rapid use of DOC





within the sediment by bacteria, particularly in the current study, due to the greater bacterial biomass typical of more tropical sediments, and intense competition for labile EPS due to its limited production.

- Fluxes of CO₂ from sediments exposed at low tide are a further potential loss path-⁵ way but contributed very little to loss of MPB-derived carbon in the current study (up to 5% of DIC fluxes at the times measured; Fig. 1). Differences in fluxes of inorganic carbon from intertidal sediments during inundation and exposure may relate to variations in the activity of fauna during the tidal cycle. For example, bioirrigation by fauna occurs only during tidal inundation and can enhance exchange of solutes between sediment and the overlying water (Forster et al., 1999; Hedman et al., 2011). At the site
- of the current study, however, little macrofauna was observed at the site, and variations in the activity of biota are therefore unlikely to explain the differences in fluxes of DIC and CO_2 from inundated and exposed sediments. Chemical factors offer a more likely explanation. Whereas only CO_2 is released from sediments during exposure,
- DIC consists of CO₂, HCO₃⁻ and CO₃²⁻, all of which be transferred to overlying water (Cook et al., 2004). Therefore, if the production of DIC is constant across a tidal cycle, the ability of a greater fraction of DIC to transfer to water could lead to greater fluxes of inundated DIC than exposed CO₂ from intertidal sediments. However, the few studies that have compared fluxes of CO₂ and DIC from intertidal sediments during exposure and inundation have produced variable results. Whereas Gribsholt and Kris-
- ²⁰ exposure and inundation have produced variable results. Whereas Gribsholt and Kristensen (2003), Cook et al. (2004), and Faber et al. (2012) all found that exposed CO_2 fluxes were at least ~ 2× lower than inundated DIC fluxes in unvegetated sediments, Alongi et al. (1999) found little difference in tropical intertidal sediments, and Gribsholt and Kristensen (2003) found that exposed CO_2 fluxes were higher than inundated DIC
- fluxes in a vegetated marsh. In the current study, we saw very little loss of ¹³C via CO₂ fluxes compared to DIC fluxes. However, it should be noted that previous studies have looked at total fluxes of DIC and CO₂, whereas we compared fluxes derived from recent (within 31 d) MPB carbon fixation. Regardless, even assuming that fluxes of CO₂ were half as important as fluxes of DIC, as reported for bare sediments (Gribsholt and CO₂).





Kristensen, 2003; Cook et al., 2004), CO_2 fluxes would still only account for loss over 31 d of ~ 5.5 % of the ¹³C initially fixed by MPB.

4.4 Carbon storage implications

Despite differences in carbon processing pathways, particularly the influence of resuspension, we observed similar substantial retention of MPB-derived carbon in subtidal (Oakes et al., 2010b, 2012) and intertidal subtropical sites (this study; Fig. 1). Approximately 30 % of the carbon that had been fixed by the MPB community in each of these environments within one exposed period was still present within sediment OC after ~ 30 d. In the current study, 75.3 % of this remaining carbon had been buried below 2 cm, suggesting that there could be a substantial contribution of MPB to long-term carbon sequestration in unvegetated sediments. This is particularly remarkable given that the burial and retention of MPB-derived carbon in the current study was observed under conditions of high flow, when resuspension would be enhanced. This clearly demonstrates the potential of MPB in unvegetated subtropical sediments to contribute to coastal carbon storage.

Only two previous studies of which we are aware (Oakes et al., 2010b, 2012) have considered the processing of MPB-derived carbon over such an extended time period (~ 30 d). However, a comparison of the ¹³C remaining in sediments at the end of shorter-term studies with the ¹³C remaining at a similar time in the longer-term studies shows that there is some variation in carbon retention. Whereas we estimate that ~ 75% of the ¹³C incorporated in our study remained in sediments after 4 d, Middelburg et al. (2000) found ~ 67% of MPB-derived carbon remaining in temperate intertidal mud and ~ 20% in temperate intertidal sand after a similar time. In the study by Bellinger et al. (2009), ~ 60% of the carbon fixed by MPB remained within muds after

36 h, although there was not a clear trend of carbon loss over time. The greater carbon retention we observed in subtropical sediments may reflect the higher productivity and/or biomass of bacteria in warmer climate sediments, leading to greater recycling of MPB-derived carbon. There is currently considerable interest in carbon burial in



coastal ecosystems ("Blue Carbon"; Duarte et al., 2005; McLeod et al., 2011; Duarte et al., 2013), but the potential of MPB to contribute to this has not been considered (mostly focused on seagrass, mangroves and saltmarshes). In the current study, the retention of a substantial portion of fixed carbon after 31 d (30.0%), with most of this

- (75.3%) buried below 2 cm, suggests that MPB in subtropical intertidal sediments may contribute substantially to carbon burial in unvegetated sediments. Considering that previous studies in a variety of sediment types (intertidal or subtidal, muddy or sandy) and in both temperate and subtropical regions have also shown that carbon fixed by MPB within hours remains within sediments after a number of days, the contribution of MPB to carbon storage may be widespread.
- ¹⁰ MPB to carbon storage may be widespread.

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Table 1 Mean natural abundance carbon stable isotope ratios (δ^{13} C) biomass (standard errors
in breakets), and $\frac{9}{2}$ of and impart example earbor $\frac{9}{2}$ ($\frac{9}{2}$), represented by and impart examples the product of the section of the sectio
in blackets), and % of sediment organic carbon (% OC) represented by sediment compartments
at depths of 0–2 cm, 2–5 cm and 5–10 cm, based on control samples. Note that δ ''C values for
bacteria and MPB are for whole cells. Units are per area, not volume. δ^{13} C of uncharacterized
material estimated using isotope mixing models.

	0–2 cm			2–5 cm			5–10 cm		
Compartment	δ ¹³ C	Biomass (mmol C m ⁻²)	% OC	δ ¹³ C	Biomass (mmol C m ⁻²)	% OC	δ ¹³ C	Biomass (mmol C m ⁻²)	% OC
Sediment OC	-16.0 (1.0)	12221.8 (1557.2)	100	-16.3 (0.3)	12 550.3 (1369.3)	100	-14.4 (0.2)	16 637.8 (1151.8)	100
Microphytobenthos	-14.3 (0.6)	5518.6 (461.9)	45.1 (3.8)	-13.0 (0.3)	2972.9 (962.4)	23.7 (7.7)	-14.4 (0.5)	481.9 (82.1)	2.9 (0.5)
Bacteria	-15.6 (0.7)	5404.1 (593.2)	44.2 (4.8)	-15.0 (0.7)	6726.5 (2526.3)	53.6 (20.1)	-14.9 (0.4)	1929.6 (865.8)	11.6 (5.2)
Elphidium advenum	-16.4	2.1 (0.9)	0.01 (0.01)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ammonia beccarii	-15.4	1.2 (0.6)	0.02 (0.01)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Uncharacterized	-24.8	1295.8 (1729.2)	10.7 (6.1)	-22.7	2850.9 (3030.4)	22.72 (21.5)	-14.3	14 227.3 (1443.3)	85.5 (5.22)





Fig. 1. Conceptual model showing pathways for the transfer of carbon fixed by microphytobenthos in subtropical intertidal sediments during inundation and exposure. Thicker arrows indicate pathways that, in the current study, were determined or estimated to be more dominant over 31 days.







Fig. 2. Variations in flow rate of the Richmond River and variations in salinity, dissolved inorganic nitrogen concentration (DIN), and dissolved inorganic phosphorus concentration (DIP) at the study site during the study period.



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Fig. 4. Carbon budget showing cumulative percentage (mean \pm SE) of the ¹³C that was initially incorporated by microphytobenthos that remained in total sediment organic carbon (0–10 cm depth) and that had been lost via dissolved organic carbon and dissolved inorganic carbon or via unknown pathways since the end of ¹³C-labeling. Note that fluxes of CO₂were too low to be shown. Arrows indicate ¹³C expected to remain in total sediment organic carbon at each sampling time, based on 2-G modeling.







Fig. 5. Exponential curve predicting percentage of ¹³C remaining in sediment organic carbon that is expected to be within MPB over time. Data points show values measured over the 31 day study.

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