Short-term fate of intertidal microphytobenthos carbon under enhanced nutrient availability: a $^{13}$C pulse-chase experiment

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Received: 25 October 2017 – Discussion started: 2 November 2017
Revised: 18 March 2018 – Accepted: 24 April 2018 – Published: 16 May 2018

Abstract. Shallow coastal waters in many regions are subject to nutrient enrichment. Microphytobenthos (MPB) can account for much of the carbon (C) fixation in these environments, depending on the depth of the water column, but the effect of enhanced nutrient availability on the processing and fate of MPB-derived C (MPB-C) is relatively unknown. In this study, MPB was labeled (stable isotope enrichment) in situ using $^{13}$C-sodium bicarbonate. The processing and fate of the newly fixed MPB-C was then traced using ex situ incubations over 3.5 days under different concentrations of nutrients (NH$_4^+$ and PO$_4^{3-}$: ambient, 2× ambient, 5× ambient, and 10× ambient). After 3.5 days, sediments incubated with increased nutrient concentrations (amended treatments) had increased loss of $^{13}$C from sediment organic matter (OM) as a portion of initial uptake (95 % remaining in ambient vs. 79–93 % for amended treatments) and less $^{13}$C in MPB (52 % ambient, 26–49 % amended), most likely reflecting increased turnover of MPB-derived C supporting increased production of extracellular enzymes and storage products. Loss of MPB-derived C to the water column via dissolved inorganic C (DIC) was minimal regardless of treatment (0.4–0.6 %). Loss due to respiration was more substantial, with effluxes of dissolved inorganic C (DIC) increasing with additional nutrient availability (4 % ambient, 6.6–19.8 % amended). These shifts resulted in a decreased turnover time for algal C (419 days ambient, 134–199 days amended). This suggests that nutrient enrichment of estuaries may ultimately lead to decreased retention of carbon within MPB-dominated sediments.

1 Introduction

Intertidal sediments are important sites for the processing of carbon (C) within estuaries, producing, remineralizing, and transforming considerable amounts of organic material prior to its export to the coastal shelf (Bauer et al., 2013). Algal production is a key source of C within the coastal zone and is primarily derived from microphytobenthos (MPB) in shallow photic sediments (Hardison et al., 2013; Middelburg et al., 2000). In addition to algal cells being a labile carbon source, MPB exudes large amounts of carbohydrates as extracellular polymeric substances (EPSs) (Goto et al., 1999) that allow for vertical migration and enhance sediment stability (Stal, 2010). A better understanding of the carbon pathways utilized during processing of algal cells and exudates within sediments is important for determining the quality and quantity of carbon exported from estuarine waters to continental shelves.

Application of rare isotope tracers can render fractionation effects and variability that affect natural abundance stable isotope techniques negligible and has been useful for elucidating pathways for the processing and loss of MPB-derived C (MPB-C) within estuarine sediments. Loss pathways for MPB-derived C include resuspension (Oakes and Eyre, 2014), fluxes of dissolved inorganic C (DIC) due to mineralization and respiration (Evrard et al., 2012; Oakes et al., 2012), fluxes of dissolved organic C (DOC) comprised of microbial exudates and products from cell lysis (Oakes et al., 2010a), and direct production of CO$_2$ (Oakes and Eyre, 2014). Stable isotope tracer studies have also enabled quantification of the trophic transfer of MPB-C (Middelburg et al., 2000; Miyatake et al., 2014; Nordström et al., 2014; Oakes...
et al., 2010a) and flux of newly produced C from sediments (Andersson et al., 2008; Oakes et al., 2012; Van Nugteren et al., 2009).

When $^{13}$C is combined with analysis of phospholipid-linked fatty acids (PLFAs), it becomes possible to trace C transfer into individual microbial groups that account for the living biomass within sediment organic C (OC) (Drenovský et al., 2004; Hardison et al., 2011; Oakes et al., 2012; Oakes and Eyre, 2014; Spivak, 2015). This allows for the quantification of microbial transfers of newly produced algal C between MPB and bacteria and the relative contributions of MPB and bacteria to microbial biomass in sediment OC. This technique has shown that EPS produced by MPB is readily utilized as a C source for heterotrophic bacteria (Oakes et al., 2010a; van Oevelen et al., 2006; Hardison et al., 2011). Pathways for processing of MPB-derived C have been reasonably well described, but the response of these pathways to local environmental changes remains a significant knowledge gap.

A major source of environmental change in coastal systems is nutrient over-enrichment (Cloern et al., 2001), which can affect the assimilation and flux pathways of MPB-derived carbon through (1) increased microbial biomass or an increase in production of extracellular enzymes resulting from relaxation of nutrient limitation, (2) increased algal production that drives elevated heterotrophic processes as bacteria utilize newly produced C, and (3) increased loss of C as DIC via respiratory pathways as heterotrophic processes dominate. MPB are able to use both porewater and water column nutrients, and, although MPB biomass can increase with elevated nutrient availability (Armitage and Fong, 2004; Cook et al., 2007), this is not always the case, with multiple studies finding no corresponding increase in MPB biomass (Alsterberg et al., 2012; Pascal et al., 2013; Piehler et al., 2010; Spivak and Ossolinski, 2016). Processing and mineralization of C are significantly affected by changes in the relationship between MPB and bacteria (Evvard et al., 2012). Both EPS production and bacterial utilization of newly produced EPS may decrease with increasing nutrient availability (Cook et al., 2007). Increased nutrient enrichment can lead to increased heterotrophy, as newly produced organic matter (OM) is mineralized (Fry et al., 2015), resulting in increased DIC production. Increased remineralization of newly produced MPB-C will result in greater loss of DIC from intertidal sediment via bacterial respiration (Hardison et al., 2011).

In this $^{13}$C pulse-chase study we aimed to quantify the short-term effects of increased nutrient concentrations on the processing pathways for MPB-derived C within subtropical intertidal sediments. The in situ MPB community was used to provide a pulse of labeled MPB-C of similar quantity and quality to normal production. Even application of separate label applications for each plot prior to incubation served to isolate the subsequent effect of increased nutrient availability on the processing of MPB-derived C. Pathways considered included transfer through sediment compartments (MPB, bacteria, uncharacterized and sediment OC) and loss via fluxes of DOC and DIC. We expected increased nutrient availability to stimulate production of MPB-C after initial labeling, resulting in decreased turnover times for MPB-C as well as a shift towards dominance of heterotrophic processes as bacteria utilized this additional labile C. We further hypothesized that enhanced heterotrophy would increase loss of newly fixed algal-derived C via respiration as DIC. Incorporation of $^{13}$C into biomarkers should reflect the shift towards heterotrophy, with quicker shifts towards increased bacterial utilization of newly produced algal C corresponding with increased nutrient load. Both DIC and DOC should be significant loss pathways for newly produced algal C as labile OM is readily processed by heterotrophs.

2 Methods

2.1 Study site

The study site was a subtropical intertidal shoal ~2 km upstream of the mouth of the Richmond River estuary in New South Wales, Australia (28°52′30″S, 153°33′26″E). The 6900 km$^2$ catchment has an annual rainfall of 1300 mm (Mcke et al., 2000) and an average flow rate of 2200 ML day$^{-1}$ (daily gauged flow adjusted for catchment area, averaged over years for which data was available; 1970–2013). Although the Richmond River estuary has highly variable flushing, salinity, and nutrient concentrations associated with frequent episodic rainfall events and flooding (Eyre, 1997; McKee et al., 2000), this study was undertaken during a dry period. The site experiences semidiurnal tides with a range of ~2 m. Samples were collected in the austral summer of January 2015 with an average site water temperature of 25.6 ± 2.3 °C. Sediment at depths of 0–2, 2–5, and 5–10 cm was dominated by fine sand (66–73 %), and sediment across 0–10 cm had an organic C content of 17.5 ± 0.02 mol C m$^{-2}$. Sediment molar C:N was lowest at 2–5 cm but comparable across all other depths (top scrape, TS, 14.4 ± 1.6, 0.13 ± 0.02 %OC; 0–2 cm 17.2 ± 1.7, 0.18 ± 0.07 %OC; 2–5 cm 10.9 ± 0.5, 0.23 ± 0.06 %OC; 5–10 cm 16.2 ± 2.2, 0.14 ± 0.03 %OC).

2.2 Overview

We labeled MPB with $^{13}$C via in situ application of $^{13}$C-labeled sodium bicarbonate to exposed intertidal sediments. Unincorporated $^{13}$C was flushed from the sediment during the next tidal inundation of the site. Sediment cores were collected and incubated in the laboratory over 3.5 days under four nutrient enrichment scenarios (ambient, minimal, moderate, and elevated) using pulsed nutrient additions. Incubation of cores ex situ allowed for explicit control of nutrient additions and examination of the short-term processing and fate (loss to overlying water) of MPB carbon. Sediments remained inundated during incubations with minimal water
exchange, as might be expected during neap tide at this site. Inundation also served to minimize C loss via physical re-suspension and export while we were examining sediment processing.

2.3 \( ^{13} \text{C} \) labeling

Bare sediment within a 2 m\(^2 \) experimental plot was \(^{13} \text{C} \) labeled when sediments were first exposed during the ebbing tide in the middle of the day by using motorized sprayers to evenly apply 99% NaH\(^{13}\text{CO}_3 \) onto individual 400 cm\(^2 \) subplots, closely following the method outlined in Oakes and Eyre (2014). Label applications were prepared using NaCl-amended Milli-Q matching site salinity (34.6), and 20 mL aliquots (1.7 mmol \(^{13} \text{C} \)) were applied to each individual subplot, resulting in application of 42.5 mmol \(^{13} \text{C} \) m\(^{-2} \). The use of individual aliquots of label ensured even \(^{13} \text{C} \) application across the sediment surface. Assimilation of label by the sediment community occurred over \( \sim 4 \) h with average light exposure of 1376 µE m\(^{-2} \) s\(^{-1} \), before tidal inundation removed the majority of unincorporated \(^{13} \text{C} \). Removal was confirmed by loss of 99.0% of the applied \(^{13} \text{C} \) from treatment applications within initial cores sampled in the field.

2.4 Collection of sediment cores

Prior to label application, three cores (9 cm diameter, 20 cm depth) were collected from unlabeled sediment surrounding the treatment plots and immediately extruded and sectioned (0–0.2 cm; TS; 0.2–2; 2–5; and 5–10 cm) to provide control natural abundance sediment OC \( \delta^{13} \text{C} \) values for sediment depths within the study site. Eleven hours after label application, at low tide, 35 sediment cores were similarly collected from the labeled plot using Plexiglas core liners. Immediately, three cores were extruded and sectioned as above to determine initial \(^{13} \text{C} \) uptake and grain size distribution for all sediment depths, as well as chlorophyll \( a \) (Chl \( a \)) concentrations in 0–1 cm sediments. All samples were placed within plastic ziplock bags, transported to the laboratory on ice, and stored frozen in the dark (\( \sim 20^\circ \text{C} \)). Plexiglas plates were used to seal the bottom of the core liners, and cores for incubation were then transported to the laboratory within 2 h of sampling. Site water (400 L) was collected and transported to the laboratory for use in incubations.

2.5 Nutrient amendment

Pulsed applications of nutrients for each treatment amendment were used to mimic a range of nutrient concentrations without exceeding sediment capacity for uptake. The treatment tanks were set up at ambient concentration (site water, DIN of 2.5 ± 0.04 µmol N L\(^{-1} \), measured on incoming tide) and with N (NH\(_4\)\(^+\)) and P (H\(_3\)PO\(_4\)) amendment to un-filtered site water at 2× (minimal treatment), 5× (moderate treatment), and 10× (elevated treatment) average water column concentrations near the study site (4 µmol L\(^{-1} \) NH\(_4\)\(^+\) and 5 µmol L\(^{-1} \) PO\(_4^{3-}\); Eyre (1997, 2000). To allow thorough mixing, the initial pulse of nutrients was added to both incubation tanks and bags holding replacement water for sampling, 1 h prior to cores being transferred into the incubation tanks. An additional pulse of NH\(_4\)\(^+\) was applied to incubation tanks at 1.5 days (after sample collection) to mimic the nutrient availability that occurs with regular inundation of tidal sediments. Silica (Si) was also added to all incubation tanks at 2.5 days (after sample collection) to ensure that isolation of the benthic diatom-dominated sediment from regular water turnover did not result in secondary limitation of Si. There was no significant accumulation of NH\(_4\)\(^+\) within treatment tank water, as nutrients were readily processed (Fig. S1 in the Supplement). Processing of newly fixed MPB-C occurred in the 13 h prior to incubation with nutrient amendments, but was likely similar across cores as they were kept in identical conditions prior to incubation before random allocation to treatments. Although MPB-C was not freshly fixed at 13 h and likely more refractory as a result, the available C was still relatively labile and readily processed across all treatments.

2.6 Benthic flux incubations

In the laboratory, cores were fitted with magnetic stir bars positioned 10 cm above the sediment surface, filled with \( \sim 2 \) L of site water, and randomly allocated to one of the four 85 L treatment tanks (ambient, minimal, moderate, elevated; eight cores per treatment). Water in the treatment tanks and cores was continuously recirculated, held at in situ temperature (25 ± 1°C) by a chiller on each tank, and aerated. Cores were stirred via a rotating magnet at the center of each treatment tank, which interacted with the magnetic stir bars. Stirring occurred at a rate below the threshold for sediment resuspension (Ferguson et al., 2003). Three sodium halide lamps suspended above the treatment tanks provided 824 ± 40 µE m\(^{-2} \) s\(^{-1} \) to the sediment–water interface within the cores on a 12 h-light–12 h-dark cycle which approximated the average light level measured at the sediment surface during inundation (941.4 ± 139 µE m\(^{-2} \) s\(^{-1} \)). Cores were allowed to acclimate in tanks for 6 h prior to the start of incubation to allow for the re-establishment of microclimates and anaerobic zonation that was potentially disturbed by coring. Cores remained open to the tank water until 30 min before sampling, when clear Plexiglas lids were fitted to each core liner to seal-in overlying water within the core for the duration of the incubation (\( \sim 15 \) h). Dissolved oxygen (±0.01 mg L\(^{-1} \)) and pH (±0.002 pH) were measured optically and electrically (Hach HQ40d multi-parameter meter) via a sampling port in the lid. Initial samples were taken 30 min after closure of the lids, dark samples were taken after \( \sim 12 \) h incubation with no light, and light samples were taken after 3 h of illumination following the end of the dark period. Light incubations were of shorter duration to prevent supersaturation of dissolved oxygen which would have compromised additional analyses required for a complemen-
tary study. During sampling, 50 mL of water was syringe filtered (prebomed Whatman GF/F filters) into prebomed 40 mL glass vials with Teflon-coated septa, sterilized with HgCl₂ (20 µL saturated solution), and refrigerated prior to analysis for concentration and δ¹³C of DIC and DOC. Sample water was simultaneously replaced by water held in δ to analysis for concentration and HgCl₂-busted 40 mL glass vials with Teflon-coated septa, sterilized 2876 P. M. Riekenberg et al.: Intertidal carbon processing national standards (NBS 19 and IAEA CH6). Precision for 2877 alongside glucose standards that are calibrated against inter-

2878 dynamics of δ incorporation confirmed the presence of a large number of pennate diatoms at the study site and diatom-specific PLFAs (e.g., 20:5(n − 3)) were detected, chromatographic peaks for these PLFAs were sometimes indistinct. The 16:1(n − 7) PLFA, which represents 27.4 % of total diatom PLFAs (Volkman et al., 1989), was consistently present across all samples and was used as a biomarker for diatoms, following correction for contributions from gram-negative bacteria, cyanobacteria, and sulfate-reducing bacteria, determined using 18:1 n7 as described in the calculations section below and in Oakes et al. (2016). Extraction of PLFAs used 40 g of freeze-dried sediment and a modified Bligh and Dyer technique. Sediment was spiked with an internal standard (500 µL of 1 mg mL⁻¹ tridecanoic acid, C₁₃₃; immersed in 30–40 mL of a 3 : 6 : 1 mixture of dichloromethane (DCM), methanol, and Milli-Q water; sonicated (15 min); and centrifuged (15 min, 9 g). The supernatant was removed into a separating funnel and the pellet was resuspended in 30–40 mL of the DCM : MeOH : Milli-Q mixture, sonicated, and centrifuged twice more to ensure complete removal of biomarkers. DCM (30 mL) and water (30 mL) were added to the supernatant, gently mixed, and phases were allowed to separate prior to removal of the bottom layer into a round bottom flask. The top layer was then rinsed with 15 mL of DCM, gently shaken, and phases were allowed to separate prior to addition to the round bottom flask. This extract was then concentrated under vacuum and separated using silica solid-phase extraction columns (Grace; 500 mg, 6.0 mL) by elution with 5 mL each of chloroform, acetone, and methanol. The fraction containing methanol was retained, reduced to dryness under N₂, methylated (3 mL 10 : 1 : 1 MeOH : HCl : CHCl₃, 80 °C, 2 h), quenched using first 3 and then 2 mL of 4 : 1 hexane : DCM, evaporated to ∼ 200 µL under N₂, transferred to a GC (gas chromatography) vial for analysis, and stored frozen (−20 °C). PLFA concentrations and δ¹³C values were measured using a non-polar 60 m HP5-MS column in a Trace GC coupled to a Delta V IRMS with a Thermo ConFlo III interface following the protocol outlined in Oakes et al. (2010a).

DIC and DOC concentrations and δ¹³C values were measured via continuous-flow wet oxidation isotope-ratio mass spectrometry using an Aurora 1030W total organic C analyzer coupled to a Thermo Delta V IRMS (Oakes et al., 2010b). Sodium bicarbonate (DIC) and glucose (DOC) of known isotopic composition dissolved in He-purged Milli-Q were used to correct for drift and verify both concentration and δ¹³C of samples. Reproducibility was ±0.2 mg L⁻¹ and ±0.1 %e for DIC and ±0.2 mg L⁻¹ and ±0.4 %e for DOC.

2.8 Calculations

Incorporation of δ¹³C into sediment OC, bacteria, and MPB (mmol δ¹³C m⁻²) was calculated as the product of excess δ¹³C (fraction ¹³C in sample – fraction ¹³C in control) and the mass of OC within each pool. For sediment, OC was the product of %C and dry mass per unit area. Percentages calculated from these pools are presented as portions of the sum of total δ¹³C contained within the sediment and the interpolated fluxes of DIC and DOC that were estimated to have occurred from 0 days until each sampling time.

Excess δ¹³C for PLFAs was determined only for 0–2, 2–5, and 5–10 cm depths, as there was inadequate sample mass for the 0–0.2 cm top scrape. Due to limitations of time and cost, PLFA samples were taken from only one of the two cores.
incubated for each treatment at each sampling period. PLFA excess $^{13}$C for both bacteria and diatoms was the product of excess $^{13}$C contained in the PLFA (fraction $^{13}$C in PLFA in sample − fraction $^{13}$C in PLFA in control) and the concentrations of C within respective biomarkers. Concentrations of PLFA C were calculated from their peak areas relative to the internal C$_{13}$ standard spike. Biomass of diatoms and bacteria were calculated using the method described by Oakes et al. (2016). Briefly, bacterial biomass was calculated as

$$\text{Biomass}_{\text{bacteria}} = \text{Biomass}_{i} \times (a \times b),$$  \hspace{1cm} (1)

where $a$ represents the average concentration of PLFA (0.056 g C PLFA per g C biomass; Brinch-Iversen and King, 1990) in bacteria and $b$ represents the average fraction of PLFA accounted for by corrected 16:1(n-7) (0.67; Volkman et al., 1996) in bacteria-dominated marine sediments (0.16, Osaka Bay, Japan; Rajendran et al., 1993, 1994). Biomass estimates for bacteria calculated using the minimum and maximum fraction values (16–19 % for 16:1(n-7); Volkman et al., 1990) in bacteria and 50 % of estimates (0.056 g C PLFA per g C biomass; Brinch-Iversen and King, 1990) in bacteria-dominated marine sediments (0.16, Osaka Bay, Japan; Rajendran et al., 1993, 1994) were within 50 % of estimates calculated using the method described by Oakes et al. (2016). Biomass estimates for diatoms calculated using the following formula:

$$\text{Biomass}_{\text{Diatom}} = \text{Biomass}_{\text{corrected}} \times (c \times d),$$  \hspace{1cm} (2)

where $c$ is the average fraction of diatom PLFAs accounted for by corrected 16:1(n-7) (0.67; Volkman et al., 1989) and $d$ is the average PLFA concentration in diatoms (0.035 g PLFA C per g of C biomass; Middelburg et al., 2000). Biomass estimates for diatoms calculated using minimum and maximum fraction values for 16:1(n-7) (18–33 %; Volkman et al., 1989) were within 50 % of estimates based on the average value. Microbial biomass is the sum of calculated diatom and bacterial biomass. Uncharacterized $^{13}$C was calculated as

$$^{13}\text{C}_{\text{uncharacterized}} = ^{13}\text{C}_{\text{sediment organic}} - ^{13}\text{C}_{\text{microbial biomass}},$$  \hspace{1cm} (3)

where $^{13}\text{C}_{\text{sediment organic}}$ represents total $^{13}$C in sediment organic carbon and $^{13}\text{C}_{\text{microbial biomass}}$ represents the $^{13}$C contained in microbial biomass within the same core.

Fluxes across the sediment–water interface were calculated from two measured concentrations, at the start and finish of each dark and light period (e.g., Oakes and Eyre, 2014), as a function of incubation time, core water volume, and sediment surface area. Dark flux rates were calculated using concentration data from the dark incubation period and light flux rates from the light incubation period. The following parameters were calculated from dark and light rates:

$$\text{Respiration (R)} = \text{Dark DO flux h}^{-1},$$  \hspace{1cm} (4)

$$\text{Net primary production (NPP)} = \text{Light DO flux h}^{-1},$$  \hspace{1cm} (5)

$$\text{Gross primary production (GPP)} = \text{NPP + R},$$  \hspace{1cm} (6)

$$\text{Production/respiration (P/R)} = \text{GPP} \times \text{daylight hours},$$  \hspace{1cm} (7)

To prevent the potential development of resource limitations during incubation, O$_2$ concentrations were not allowed to drop below 60 % saturation in the dark and light incubations were shortened (~3 h) to ensure that production was not allowed to become supersaturated.

Total $^{13}$C in DIC and DOC ($\mu$mol $^{13}$C) in the overlying water in the sediment core was calculated for the initial period, the end of the dark period, and the end of the light period as the product of excess $^{13}$C in labeled sample versus relevant natural abundance control, core volume, and concentration. Total excess flux of $^{13}$C as DIC or DOC ($\mu$mol $^{13}$C m$^{-2}$ h$^{-1}$) was then calculated as

$$\text{Excess}^{13}\text{C} = \text{Excess}^{13}\text{C}_{\text{start}} - \text{Excess}^{13}\text{C}_{\text{end}} / \text{SA} / t,$$  \hspace{1cm} (8)

where excess $^{13}$C$_{\text{start}}$ and excess $^{13}$C$_{\text{end}}$ represent excess $^{13}$C of DIC or DOC at the start and end of dark and light incubation periods, SA is sediment surface area, and $t$ is incubation period length (h). Net fluxes of excess $^{13}$C (excess $^{13}$C m$^{-2}$ h$^{-1}$) for DIC and DOC were calculated as

$$\text{Net flux} = (\text{dark flux} \times \text{dark hours}) + (\text{light flux} \times \text{light hours}) / 24 \text{h}.$$  \hspace{1cm} (9)

Total $^{13}$C lost via flux to the water column from initial labeling of each sampling period was interpolated from measured net flux values by calculating the area underneath the curve for each treatment. Because all $^{13}$C was contained within the cores, values for $^{13}$C budgets add up to 100 %. Starting values were estimated by looking at how much $^{13}$C remained in the sediment and how much was lost to the water column (initial $^{13}$C = $^{13}$C remaining + $^{13}$C lost).

2.9 Data analysis

The $^{13}$C remaining in sediment and MPB-C biomass was determined using Chl $a$ data for cores within all treatments for 0.5, 1.5, 2.5, and 3.5 days. We therefore used a two-way analysis of variance (ANOVA) to determine whether either $^{13}$C remaining in sediment or MPB-C biomass were affected by treatment and/or time. P/R ratios were determined for
1.5, 2.5, and 3.5 days to determine whether significant differences occurred between treatments within each time period (\( \alpha = 0.05 \)). Levene’s tests indicated that variances were homogeneous in all cases and there were no significant interactions between variables in either analysis. For significant effects of treatment or time, post hoc Tukey tests were used to identify significant differences between groups.

Total uptake for \(^{13}\text{C}\) into both MPB and bacteria and relative \(^{13}\text{C}\) uptake into MPB were determined for only a single core across all treatments from 0.5, 1.5, 2.5, and 3.5 days. To increase replication for statistical analysis, and therefore increase the power to detect a significant difference, we grouped data across times into two levels: before 1.5 days (including 0.5 and 1.5 days) and after 1.5 days (including 2.5 and 3.5 days). There was no pooling of data across treatments. A two-way ANOVA was then used to determine whether significant differences occurred among treatments within each pooled time period (\( \alpha = 0.05 \)). No significant interactions were observed for total uptake into MPB or bacteria, but there was a significant interaction observed for relative \(^{13}\text{C}\) uptake into MPB. For significant effects of interaction, treatment, and time, post hoc Tukey tests were used to identify significant differences between groups.

The data for \(^{13}\text{C}\) remaining in sediment OC were further examined by fitting an exponential decay function for each treatment across 3.5 days using the Exp2PMod1 function in OriginPro 2017 and \(^{13}\text{C}\) turnover estimates were then determined by solving for \( y = 0.05\% \) remaining \(^{13}\text{C}\) (a value close to 0) and \( x = 30 \) days for each treatment. Loss rate constants are reported as positive numbers following mathematical conventions associated with loss rates (Fig. 6).

### 3 Results

#### 3.1 Uptake of nutrient additions

Uptake of the added nutrients into the sediment was rapid and substantial, as indicated by decreases in dissolved inorganic nitrogen (\( \text{NH}_4^+ + \text{NO}_x \)) concentrations in the overlying core water to <1.2 ± 0.1 µM L\(^{-1}\) by 0.5 days. Across the incubation periods, elevated DIN concentrations in overlying water were occasionally observed (Fig. S1 in the Supplement) but corresponded with times when the cores were sealed for light and dark incubations, indicating that DIN production was a result of in-core processing rather than nutrient amendments.

#### 3.2 Sediment characteristics

Control sediment OC content was greater in the 2–5 cm depth (187.5 ± 27.7 µmol C g\(^{-1}\)) than at all other sediment depths (112.3 ± 11.4 µmol C g\(^{-1}\) in the TS, 149.8 ± 31.6 µmol C g\(^{-1}\) at 0–2 cm, and 120.1 ± 16.5 µmol C g\(^{-1}\) at 5–10 cm). Natural abundance \( \delta^{13}\text{C} \) values were most enriched in surface sediments (−18.7 ‰ in TS) and became progressively depleted within deeper sediments to −22.1 ‰ at 5–10 cm (Table 1). In the 0–2 cm depth of the control sediment, MPB-C biomass was 321.9 ± 42.0 mmol C m\(^{-2}\) and bacterial biomass was 500.4 ± 65.3 mmol C m\(^{-2}\) (Table 1).

#### 3.3 Initial \(^{13}\text{C}\) uptake

Uptake of \(^{13}\text{C}\) into sediment OC occurred rapidly and was observed in the first cores collected (11 h after labeling, after tidal flushing, and with cores sectioned in the field). At this time, prior to laboratory incubation and nutrient amendment, 1549 ± 140 µmol \(^{13}\text{C}\) m\(^{-2}\) had been incorporated into sediment OC. Sediment OC was \(^{13}\text{C}\) enriched across all sed-
iment depths at this time (Table 1), but 78% of the initially incorporated $^{13}$C was in the uppermost 2 cm of sediment (compared to 12.8% for 2–5 cm and 9.4% for 5–10 cm). Prior to incubation, $^{13}$C uptake into microbial biomass at 0–2 cm was dominated by MPB (92.7 ± 1.6%), despite their lower biomass (200.2 ± 26.5 nmol C m$^{-2}$) compared to bacteria (311.3 ± 56.4 nmol C m$^{-2}$) within the labeled cores. Conversely, bacteria dominated $^{13}$C uptake in 2–5 cm sediment (66.8 ± 17.2% of the $^{13}$C within microbial biomass). Although sediment OC at 5–10 cm was $^{13}$C enriched, minimal uptake was detected in microbial biomarkers.

### 3.4 Effect of nutrient additions on P/R

Average MPB biomass remained similar across treatments over the 3.5-day incubation (two-way ANOVA: treatment $F_{3,31} = 0.04$, $p = 0.99$; time $F_{3,31} = 0.1$, $p = 0.94$, Fig. S3 in the Supplement). However, there were changes in P/R ratio that varied among treatments. Examination of the effects of treatment and time on P/R showed no significant differences (two-way ANOVA: treatment $F_{3,23} = 3.0$, $p = 0.08$; time $F_{2,23} = 2.7$, $p = 0.11$), although the post hoc Tukey comparison between ambient and elevated treatments was nearly significant ($p = 0.0506$). For the ambient, minimal, and moderate treatments, P/R ratios were dominated by autotrophy and changed little over the first 2.5 days (1.5 ± 0.8, 1.2 ± 0.4, and 1.3 ± 0.1, respectively, Fig. 1b), as any increases in production were offset by increased respiration (Fig. 1a). By 3.5 days the minimal treatment had shifted into heterotrophy (0.6 ± 0.1) as a result of increased respiration and decreased production, whereas P/R ratios for the ambient and moderate treatments remained essentially unchanged (1.3 ± 0.2, 1.3 ± 0.4). P/R in the elevated treatment was initially high compared to all other treatments (2.2 ± 0.2 at 0.5 days), indicating strong dominance of autotrophic production (Fig. 1a and b). However, P/R in the elevated treatment decreased to 1.1 ± 0.4 after 3.5 days (Fig. 1b), indicating a strong shift away from autotrophy and towards dominance of heterotrophic processes as respiration increased and production decreased (Fig. 1a).

### 3.5 Incorporation of $^{13}$C into sediment organic carbon

#### 3.5.1 Uptake of $^{13}$C into 0–2 cm sediment

At 0.5 days, the $^{13}$C incorporated into sediment OC was predominantly contained in the 0–2 cm depth across all treatments (∼65–90%, Fig. 2) and was statistically similar across treatments (one-way ANOVA: $F_{3,7} = 4.2$, $p = 0.1$). By 3.5 days, $^{13}$C retention was lower within sediment from nutrient-amended treatments compared to the ambient treatment, whereas the $^{13}$C contained in the 0–2 cm depth in the ambient treatment was similar across 3.5 days (78.9 ± 8.8% 1.5 days, 77.0 ± 16.4% 2.5 days, 81.6 ± 4.4% 3.5 days); the $^{13}$C content decreased in the minimal, moderate, and elevated treatments to 70.3 ± 8.3, 73.6 ± 16.4, and 68.8 ± 7.6%, respectively (Fig. 2).

#### 3.5.2 Downward transport below 2 cm

Downward transport of newly labeled material to 2–5 cm depth was low across all treatments, but was higher for the elevated treatment at both 0.5 and 2.5 days. At 0.5 days there was less downward transport in minimal and moderate treatments compared to the ambient and elevated treatments. By 2.5 days downward transport was similar for ambient, minimal, and moderate treatments (10, 9, 10%, respectively; Fig. 2), but was considerably higher in the elevated treatment (28.4%). By 3.5 days, $^{13}$C incorporation into 2–5 cm sediment OC was similarly low for ambient, minimal, and moderate treatments (8.0 ± 2.1, 11.1 ± 0.1, and 8.7 ± 2.1%, respectively), but lower in the elevated treatment (4.8 ± 2.1%). At 0.5 days, downward transport into the 5–10 cm layer was a relatively small portion of initial $^{13}$C, but was higher in ambient and minimal treatments (8.7 ± 2.4 and 11.6 ± 1.5%) when compared to moderate and elevated treatments (2.3 ± 1.9 and 6.8 ± 0.1%, Fig. 2). Downward transport below 5 cm was similar (5–11%) for all treatments at 2.5 and 3.5 days.

### 3.6 $^{13}$C distribution amongst sediment compartments

#### 3.6.1 Microbial biomass

The total $^{13}$C content of MPB (nmol $^{13}$C m$^{-2}$; Fig. 4a) decreased significantly from before 1.5 to after 1.5 days for all treatments (two-way ANOVA: $F_{1,8} = 12.2$, $p = 0.008$), but
there was no significant difference among treatments (two-way ANOVA: $F_{3,8} = 2.7, p = 0.12$). The total $^{13}$C content of bacteria (mmol $^{13}$C m$^{-2}$; Fig. 4a) did not change significantly with time and was not significantly affected by treatment. The majority of the $^{13}$C assimilated into the cores was present in the 0–2 cm depth (0–2 cm 82.4 ± 5.8 %; 2–5 cm 9 ± 0.8 %; and 5–10 cm 5.2 ± 0.5 %; Fig. S4a, b, and c in the Supplement). $^{13}$C incorporation was largely dominated by bacteria across all treatments in sediments below 2 cm, with few exceptions. Increased bacterial contribution occurred more quickly and was more pronounced in nutrient-amended treatments at both 2–5 and 5–10 cm (Figs. S5 and S6 in the Supplement).

Total uptake of excess $^{13}$C (Fig. 4a), while informative about the amount of label contained within each core at each time period, is not as useful for comparison between microbial groups due to variations in the total amount of $^{13}$C assimilated between cores. It is important to consider the relative contribution to $^{13}$C uptake (Fig. 4b) of both microbial groups as each data point was sampled from separate cores that assimilated similar, but different, initial concentrations of newly fixed $^{13}$C. MPB contribution (%) to uptake of $^{13}$C decreased significantly after 1.5 days (two-way ANOVA: $F_{1,8} = 83.1, p < 0.0001$; Fig. 4a) but showed no significant difference between treatments ($F_{3,8} = 8.2, p = 0.008$), although there was a significant interaction between tested variables ($F_{3,8} = 8.2, p = 0.008$). Tukey tests found that MPB contributed less to microbial uptake of $^{13}$C in the elevated treatment than in the ambient treatment ($p = 0.01$) as well as the moderate treatment being lower than the minimal treatment ($p = 0.014$). MPB dominated the relative incorporation of $^{13}$C into microbial biomass at 0–2 cm in all treatments initially (0.5 days; 90 % ambient, 90 % minimal, 92 % moderate, and 92 % elevated; Fig. 4b) and throughout the 3.5-day incubation (81–90 % ambient, 82–91 % minimal, 74–92 % moderate, and 65–92 % elevated; Fig. 4b). The relative bacterial contribution to microbial $^{13}$C incorporation increased across all treatments as the incubations progressed, but increased in the moderate and elevated treatments at 2.5 and 3.5 days (Fig. 4b) corresponded with decreased $^{13}$C incorporation into MPB (Fig. 4a).

### 3.6.2 Uncharacterized

A portion of the $^{13}$C contained within sediment OC was uncharacterized, i.e., not contained within the viable microbial biomass measured using PLFA biomarkers. Initially (0.5 days) the uncharacterized pool accounted for less sed-
Figure 3. Excess $^{13}$C incorporation into microphytobenthos, bacteria, and uncharacterized OC as a percentage of $^{13}$C contained in sediment OC in 0–10 cm. There are no error bars as PLFAs were analyzed for only one replicate sample from each time period.

iment $^{13}$C within the nutrient-amended treatments (1–3 %) than within the ambient treatment (12 %; Fig. 3), indicating that there was more $^{13}$C contained in viable microbial biomass under increased nutrient availability after 12 h of incubation. By 3.5 days, increased contribution to the uncharacterized pool in the moderate and elevated treatments (29 % ambient, 32 % minimal, 41 % moderate, and 45 % elevated; Fig. 3) corresponded with decreased $^{13}$C contained in MPB (52 % ambient, 49 % minimal, 42 % moderate, and 26 % elevated). In contrast, changes in the $^{13}$C in the uncharacterized pool did not relate to $^{13}$C contained in bacteria, as the bacterial contribution to $^{13}$C remained relatively unchanged (17 % ambient, 14 % minimal, 15 % moderate, and 15 % elevated) and was similar among treatments at 3.5 days.

3.7 Loss of $^{13}$C from sediment OC

The total $^{13}$C remaining in sediment (Fig. 6) varied significantly among treatments (two-way ANOVA: $F_{3,31} = 5.7$, $p = 0.008$) and across sampling times ($F_{3,31} = 3.9$, $p = 0.03$). Throughout the study, there was generally less $^{13}$C remaining within the elevated treatment than in than either the ambient ($p = 0.008$) or minimal treatments ($p = 0.02$), and there was significantly less $^{13}$C remaining within the sediment at 3.5 days than at 0.5 days ($p = 0.02$). Rates of $^{13}$C loss from sediment OC to the water column were highest in the moderate and elevated treatments (total lost at 3.5 days: ambient 5 %, minimal, 7 %, moderate 11 %, and elevated 20 %; Figs. 5 and 6). Reflecting this, loss rate constants for the $^{13}$C remaining in sediment OC after accounting for losses of DI$^{13}$C and DO$^{13}$C across 3.5 days were equivalent for ambient and minimal treatments ($0.018 \pm 0.024$, $R^2 = 0.95$ and $0.021 \pm 0.001$, $R^2 = 0.99$, respectively; Fig. 6), but were higher for both moderate and elevated treatments ($0.0383 \pm 0.009$, $R^2 = 0.86$, 0.0566 $\pm 0.003$, $R^2 = 0.99$, respectively; Fig. 6). Since the intercept is known, i.e., the initial value equals 100 % at time 0, linear models where only the slopes were estimable were fitted to further analyze the differences between slopes. Assuming an exponential decay, the percentage remaining $^{13}$C ($Y$) was log$_{10}$ transformed and the value 2 was subsequently subtracted ($Z = \log_{10}(Y) - 2$), which implies that the intercept of $Z$ versus time equals 0. The model with different slopes for each treatment fit significantly better than the model with a single slope ($F$ test, $F_{3,28} = 9.84$, $P < 0.001$, Fig. S2 in the Supplement). The analysis was performed in R.

Across all treatments, most of the $^{13}$C loss from sediment during the incubation occurred via DIC fluxes (Fig. 5). Cumulative $^{13}$C export to the water column via DIC fluxes was
4.1 Loss pathways for $^{13}$C

Increased nutrient additions caused additional loss of $^{13}$C from sediment OC, largely driven by DIC fluxes to the water column (Figs. 5 and 6). Complete loss of newly produced C from sediment OC, as estimated from exponential decay functions, occurred more quickly in nutrient-amended treatments than in ambient (15 % increase for minimal, 210 % increase for moderate, and 310 % increase for elevated, Fig. 6). Increased loss rates indicated reduced turnover time for newly produced MPB-derived C under increased nutrient load (419 days ambient versus 199 days moderate and 134 days elevated). It should be noted that the loss rate constant for the minimal treatment (0.021 ± 0.001, $R^2 = 0.99$, 366 days) was comparable to that for the ambient treatment (0.018 ± 0.024, $R^2 = 0.95$, 419 days), indicating that a small nutrient addition may not cause significant decreases in C turnover time. Increased loss rates imply that C retention and burial in MPB-dominated photic sediments are greatest when nutrients are limiting and that increased nutrient availability alters the processing of MPB-C within the sediment. Increased nitrogen availability appears to have decreased the retention of C within MPB biomass (Fig. 3). Increased turnover of the newly fixed MPB-derived C from the sediment likely occurred as the net result of exudation of material and breakdown of cells. This increased turnover may have caused the increased efflux of MPB-derived C as exudates and cell components were increasingly available to support respiration. The focus of this study was the short-term fate of MPB-C, but our findings also show potential implications for longer-term retention. Our calculated retention times may be under or over-estimated due to their reliance on short-term data. However, the relative differences between treatments (decreased retention with increased nutrient amendment) are clear. The rationale for utilizing exponential functions in this study follows previous findings in Oakes et al. (2014) that $^{13}$C export from subtidal sediments at this site were well described by an exponential decay function across a longer time period (31 days). Additionally, the 30-day estimates provided within this study (18–58 %) fall across a range similar to that of other previous labeling experiments (30–50 %; Hardison et al., 2011; Oakes and Eyre, 2014; Oakes et al., 2012), leading the authors to conclude that the use of exponential functions to describe this relationship was valid in this study.

Across all treatments, DIC was the main loss pathway for MPB-C, DOC was a minor pathway, and loss via CO$_2$ was considered negligible (Oakes and Eyre, 2014) (Figs. 5 and 6). Loss of $^{13}$C via the DIC pathway appears to be stimulated by nutrient additions, resulting in increased export occurring earlier within incubations as a result of increased bacterial remineralization (Figs. 2 and 5). Increased DI$^{13}$C export represents the portion of DI$^{13}$C produced via respiration in excess of that which is recaptured and utilized by MPB to drive production. Given the close proximity of bacteria and MPB...
in the sediment, there is the potential for considerable utilization of the DI\(^{13}\)C arising from bacterial remineralization to support algal production. Relatively low fluxes of DI\(^{13}\)C to the water column in the ambient treatment across 2.5 days likely indicate more complete utilization and recycling of DI\(^{13}\)C to support algal production (Fig. 5). Export of DI\(^{13}\)C was considerably higher in both the moderate and elevated treatments, indicating production of DI\(^{13}\)C during bacterial remineralization in excess of utilization of DI\(^{13}\)C by MPB. Decreased recycling of DI\(^{13}\)C from remineralization in elevated treatments could develop due to (1) decreased DIC demand as algal production decreased after initial stimulation or (2) increased production of unlabeled DIC through remineralization of previously refractory organic material providing an alternative unlabeled source to support algal production.

Cumulative losses of DO\(^{13}\)C were low for all treatments across 3.5 days (<1.5% of total \(^{13}\)C, Fig. 5) and did not appear to change significantly with increased nutrient availability. Previous studies have also found that DOC fluxes are a relatively minor contributor to loss of MPB-derived carbon (Oakes et al., 2012; Oakes and Eyre, 2014), as observed in the current study, but DOC may be a significant export pathway in other settings. Produced DOC may be labile and respired to DIC prior to loss from the sediment, but this pathway was not greatly altered due to increased nutrient availability.

**4.2 Shifts in benthic metabolism**

Each nutrient amendment produced a different shift in benthic metabolism within the core incubations (Fig. 1) with no clear dose–effect relationship between increased nutrient availability and P/R observed among nutrient-amended treatments. Heterogeneity in both bacterial and MPB biomass are routinely observed within intertidal sediment and can lead to substantial variability between the production and respiration observed between cores (Eyre et al., 2005; Glud, 2008). Despite a background of variability between cores, both minimal and elevated treatments displayed a decrease in autotrophy. The minimal treatment shifted into heterotrophy (P/R < 1) and the elevated treatment stimulated initial algal production sufficiently to cause a subsequent spike in respiration. Increased respiration by 3.5 days was partially offset by maintained production that kept P/R above 1. In contrast, the moderate treatment maintained a steady P/R across 3.5 days, although substantial error bars indicate considerable variability between the cores within the treatment. MPB-dominated sediment is expected to be net autotrophic, with positive GPP (Tang and Kristensen, 2007) that may be further stimulated by nutrient inputs (Underwood and Kromkamp, 1999). Increased algal production of labile organic matter subsequently stimulates heterotrophic respiration, increasing oxygen consumption and lowering P/R (Glud, 2008; McGlathery et al., 2007). Quick increases in MPB productivity followed by increased respiration have been observed in response to pulses of organic matter in both oligotrophic and estuarine sediments (Eyre and Ferguson, 2005; Glud et al., 2008). Rapid increases in respiration rates, as reflected in the oxygen fluxes for the elevated treatment (Fig. 1a), are often associated with an increased supply of labile C and can occur at rates higher than expected for in situ temperature. This has been observed in subtropical sediments (Eyre and Ferguson, 2005) as well as polar and temperate systems (Banta et al., 1995; Rysgaard et al., 1998). Although the sediments in this study were not oligotrophic, the extent of the shift towards heterotrophy is still likely controlled by the amount and relative quality (C/N ratio) of the organic matter available for processing (Cook et al., 2009; Eyre et al., 2008). It is important to note that the elevated treatment did not shift to a P/R less than 1, but did display a considerable increase in respiration. The rapid increase in respiration in the elevated treatment suggests that the newly produced organic matter was readily bioavailable and quickly processed by bacteria as a result of increased nutrient availability.

**4.3 Retention of carbon within microphytobenthos biomass**

Within surface sediments, MPB biomass did not increase with increased nutrient load, despite apparent increases in productivity (Fig. S3 in the Supplement). Although MPB biomass did not change, by 3.5 days the \(^{13}\)C retained within MPB biomass in the nutrient-amended treatments appears to have decreased (Fig. 4a) indicating increased turnover of newly fixed C out of MPB biomass. This aligns with many previous reports that increased productivity does not neces-
sarily correspond with increased algal biomass (Alsterberg et al., 2012; Ferguson and Eyre, 2013; Ferguson et al., 2007; Hillebrand and Kahler, 2002; Piehler et al., 2010; Spivak and Ossolinski, 2016). Lack of change in MPB biomass, despite increased productivity, may occur as a result of grazing or secondary nutrient limitation (Hillebrand and Kahler, 2002), but these explanations are unlikely for the current study. Grazing is likely to have occurred at only a low level. There was very little fauna, including grazers, within sediment at the study site and, although any grazers such as copepods that were within the site water would have been included in the incubations, larger, mobile grazers were excluded. Secondary nutrient limitation of P or Si was avoided through additions of both elements at 0 days for P and 2.5 days for Si during incubation. It is more likely that the microbial community responded to pulses of increased nutrients through increased production of extracellular compounds (MPB: carbohydrates; bacteria: enzymes) rather than increasing their biomass (Thornton et al., 2010). This may be a strategy to optimally utilize intermittently available nutrient resources, given that increased cell numbers (biomass) within a biofilm community may otherwise increase competition among cells (Decho, 2000; Drescher et al., 2014). Allocation of additional N towards increased production of extracellular enzymes or storage molecules rather than new biomass may therefore benefit the community. Strong competition between MPB and bacteria for available N resulted in a minimal contribution from denitrification as a pathway for N loss likely as a result of limited availability of NO\textsubscript{3} for denitrifying bacteria (unpublished data).

4.4 \textsuperscript{13}C distribution within the sediment

4.4.1 Microbial biomass

Decreased autotrophy is somewhat reflected in the relative partitioning of \textsuperscript{13}C from newly produced algal C between MPB and bacteria within the individual treatments (Figs. 1 and 4b). Initially, uptake of \textsuperscript{13}C was strongly dominated by MPB amongst treatments, with minimal incorporation by bacteria. As incubations progressed, a shift towards increased relative contribution by bacteria was apparent in all treatments, but was more substantial in the elevated treatment (3.5 days; 19 % ambient, 18 % minimal, 26 % moderate, and 35 % elevated, Fig. 4b). This quicker shift towards bacterial dominance of \textsuperscript{13}C incorporation corresponded with the largest decrease in P/R ratios observed in this study, as increased respiration and decreased production caused the elevated treatment to become less autotrophic (Fig. 1). These corresponding factors are likely a result of a tight coupling and intense recycling between algal production and bacterial processing of newly produced MPB-derived C. EPS can be a large export pathway for newly fixed C from algal cells (up to 70.3 % Goto et al., 1999) and can provide a labile C source for heterotrophic or denitrifying bacteria. The \textsuperscript{13}C incorporated into bacteria represents the balance of respiration and uptake and is expected to become increasingly muddled by \textsuperscript{13}C being processed through other pathways (denitrification) as incubations progress. Therefore, this study only considered the transfer of MPB-C into bacteria at the 0.5-day sampling. However, given the low initial transfer of \textsuperscript{13}C to bacteria in all treatments over 24 h following labeling (0.5 days; 0.8 % h\textsuperscript{-1} ambient, 0.8 % h\textsuperscript{-1} minimal, 0.7 % h\textsuperscript{-1} moderate, 0.7 % h\textsuperscript{-1} elevated; Fig. 3), it appears that either production or utilization of EPS containing newly fixed C was relatively low in the current study, regardless of nutrient addition. This transfer was the net result of EPS production and bacterial remineralization and would have become increasingly muddled as \textsuperscript{13}C-containing detrital material accumulated as incubations progressed. Low EPS production at 0.5 days may indicate that N is not limiting for MPB in these sediments, as exuded EPS does not appear to be copious, as would be expected under severe N limitation (van den Meersche et al., 2004). Similarly low rates of C transfer from MPB to bacteria were previously reported for the site (0.83 % h\textsuperscript{-1}, Oakes and Eyre, 2014) and are towards the lower end of the range of EPS production rates for benthic diatoms (0.05 to 73 % h\textsuperscript{-1}; Underwood and Paterson, 2003). At 0.5 days nutrient availability appears to have had little effect on the initial transfer rates from MPB to bacteria, but appears to have decreased the turnover of MPB-C out of the microbial community, as contributions of \textsuperscript{13}C to the uncharacterized pool were lower in the nutrient-amended treatments (Fig. 3). By 3.5 days, increased nutrient availability appears to stimulate the transfer of \textsuperscript{13}C from microbial biomass in the uncharacterized pool, but had no effect on \textsuperscript{13}C in bacteria as the bacterial pool was equal across all treatments (15–18 %, Figs. 3 and 7).

4.4.2 Uncharacterized

A portion of the \textsuperscript{13}C incorporated into sediment OC was uncharacterized (i.e., not within microbial biomass). By 3.5 days, the portion of initially incorporated \textsuperscript{13}C that was within the uncharacterized pool varied substantially among the treatments (29–46 %, Fig. 7). This uncharacterized C is likely to represent a mixture of both labile and refractory OC (Veuger et al., 2012), including metabolic byproducts, senescent cells undergoing breakdown, EPS, extracellular enzymes, carbohydrates, and a variety of complex, molecularly uncharacterized organic matter (Hedges et al., 2000). Collectively, these molecules form a pool of labeled intracellular material remaining in sediment OC derived from both MPB and bacteria that is not characterized as microbial biomass when using PLFAs to estimate microbial biomass (e.g., \textsuperscript{13}C contained in storage products or enzymes that was not incorporated into phospholipids). Given that MPB can direct up to 70 % of their newly fixed C to bacteria (e.g., \textsuperscript{13}C containing storage material accumulated as incubations progressed. Low EPS production at 0.5 days may indicate that N is not limiting for MPB in these sediments, as exuded EPS does not appear to be copious, as would be expected under severe N limitation (van den Meersche et al., 2004). Similarly low rates of C transfer from MPB to bacteria were previously reported for the site (0.83 % h\textsuperscript{-1}, Oakes and Eyre, 2014) and are towards the lower end of the range of EPS production rates for benthic diatoms (0.05 to 73 % h\textsuperscript{-1}; Underwood and Paterson, 2003). At 0.5 days nutrient availability appears to have had little effect on the initial transfer rates from MPB to bacteria, but appears to have decreased the turnover of MPB-C out of the microbial community, as contributions of \textsuperscript{13}C to the uncharacterized pool were lower in the nutrient-amended treatments (Fig. 3). By 3.5 days, increased nutrient availability appears to stimulate the transfer of \textsuperscript{13}C from microbial biomass in the uncharacterized pool, but had no effect on \textsuperscript{13}C in bacteria as the bacterial pool was equal across all treatments (15–18 %, Figs. 3 and 7).

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MPB-derived carbon was transferred to intra- and extracellular carbohydrates within 30 days after an initial transfer rate of \( \sim 0.4\% \) into bacteria (2 days; Oakes et al., 2010a). In light of the higher transfer rates for \(^{13}\text{C}\) into bacteria observed in this study (0.7 to 0.9 \% h\(^{-1}\)), there is potential for a considerable portion of the uncharacterized pool to be accounted for by EPS.

When quantified, the uncharacterized C pool typically has a high C:N ratio (10 to 60; Cook et al., 2009; Eyre et al., 2016a), indicating that nitrogen availability may have a role in regulating its content and accumulation. Given that nitrogen limitation has been observed to suppress processing pathways of otherwise labile OM in soils (Jian et al., 2016; Schimel and Bennett, 2004), a similar mechanism may be possible in estuarine sediments. This mechanism may include a priming effect due to either increased production of extracellular enzymes or due to increased energy from labile C compounds allowing for the increased breakdown of sediment OM (Bianchi, 2011). Increased extracellular enzyme production would result in more complete utilization of sediment OM through promotion of hydrolysis (Arnosti, 2011; Huettel et al., 2014), a potentially limiting step during the breakdown of organic material. This would result in more complete utilization of \(^{13}\text{C}\) by microbial biomass and a smaller pool of uncharacterized C within sediment OC, as was observed in the nutrient-amended treatments at 0.5 days (Fig. 3). This is further supported by the increased turnover of MPB-C from microbial biomass into the uncharacterized pool observed within the nutrient-amended treatments (2.5 days, Fig. 3), indicating \(^{13}\text{C}\) that was previously incorporated into MPB was processed into the uncharacterized pool more quickly with increased nutrient availability. After 2.5 days, the \(^{13}\text{C}\) content of the uncharacterized pool was substantially larger for the elevated treatment (Figs. 3 and 7) and looks to have been largely sourced from MPB \(^{13}\text{C}\), given that bacterial contribution to sediment OM remained stable. Composition of the uncharacterized pool will be study-specific depending on the different biomarker techniques utilized to estimate microbial biomass incorporating different pools of material. The metabolic pathways and ecological strategies regulating the portion of \(^{13}\text{C}\) entering the uncharacterized pool warrant further investigation.

### 4.5 Downward transport

Increased nutrient availability reduced the downward transport of fixed \(^{13}\text{C}\), particularly within 2–5 cm, mainly as a result of increased export of MPB-C to the water column. In the ambient treatment, downward transport to 2–5 cm (10.0 \%) and 5–10 cm (9.2 \%) across 60 h was comparable to that reported by Oakes and Eyre (2014) for the same site (8.3 \% 2–5 cm, 14.9 \% 5–10 cm, 60 h). Oakes and Eyre (2014) suggested that resuspension resulting from a flood event limited the downward transport of \(^{13}\text{C}\), but a comparable and lower rate of downward transport at 60 h (12.1 \% 2–5 cm, 9 \% 5–10 cm, ambient treatment) was observed in the current study in the absence of marked freshwater inflow. Downward transport is not a large pathway for loss of \(^{13}\text{C}\) within this system as transport to sediment below 2 cm was minimal and ap-
peared further reduced in the elevated treatment (Fig. S4b and c in the Supplement). Decreased downward transport of MPB-derived C under increased nutrient load may reflect (1) decreased transport to depth as diatoms reduce migration downward to find nutrients (Saburova and Polikarpov, 2003) or (2) relaxation of the tight recycling and retention of newly fixed C between MPB and bacteria within surface sediments allowing for increased export of labile C to the water column (Cook et al., 2007). Decreased downward transport in this study likely reflects a combination of reduced algal transport of $^{13}$C to depth and increased loss of $^{13}$C from surface sediments to the water column.

5 Implications

This study has provided valuable insight into the processing of MPB-derived C under increased nutrient availability using multiple lines of evidence (budgeting $^{13}$C within sediment compartments and sediment–water effluxes, partitioning of C pools via biomarkers, and changes in P/R) and is among the first to have addressed this problem. However, some caveats on interpretation are important to note and are described as follows. (1) Ex situ incubation of sediment cores may not be directly comparable to processes occurring in situ and may overestimate C retention, as there is reduced potential for loss via sediment resuspension due to tidal movement, water currents, and grazing. (2) Removal of grazers may also increase MPB production and their release of exudates (Fouillard et al., 2014), which could enhance $^{13}$C transfer to bacteria. However, given the lack of apparent grazers at the site of the current study, and the low observed $^{13}$C transfer rate to bacteria (0.7–0.9 % h$^{-1}$ Fig. 4b) that was comparable to previously measured in situ rates in Oakes and Eyre (2014), grazers appear to have had little potential impact on sediment processing in this study.

The findings show that increased nutrient availability reduced C retention, but the main export pathway for algal carbon remained the same (primarily loss via DIC). Coastal environments are recognized as important sites for carbon storage. Although the focus has primarily been on vegetated environments (Duarte et al., 2005), which store the most carbon, unvegetated sediments also have capacity for longer-term retention (e.g., ~50 % after 21 days Hardison et al., 2011, 30 % after 30 days Oakes and Eyre, 2014; 31 % after 30 days Oakes et al., 2012). Based on N burial rates (and corresponding unpublished C burial rates) some coastal systems can have higher C burial rates in subtidal and intertidal macrophyte-free MPB sediments than in macrophyte-dominated sediments (Eyre et al., 2016b; Maher and Eyre, 2011), although this was shown in only one of the three estuaries studied. Increased nutrient loading into coastal settings has been implicated in historical decreases of long-term carbon storage through a shift from macrophyte-dominated systems (seagrass and mangrove) towards MPB-dominated systems (Macreadie et al., 2012) within coastal environments. Carbon storage potential within MPB-dominated sediments remains a significant knowledge gap within the carbon budgets of estuaries. The primary focus of this study was the short-term fate of MPB-C, but the significant decrease in retention observed with nutrient amendments suggests that short-term processes may have implications for longer-term retention. It is interesting to consider how these short-term changes may affect the longer-term retention (30 days) reported by previous studies (e.g., Oakes and Eyre, 2014), with the caveat that the substantial extrapolation required could introduce considerable error to estimates of retention. At 30 days, estimates of retention of C identified for ambient and minimal treatments were considerable in the current study (58 and 54 %); however, increased nutrient loading reduced this retention considerably (32 % moderate, 18 % elevated). Given that nutrient inputs have increased globally and bare photic sediment accounts for a large surface area within estuaries, these two factors could have resulted in substantial release of currently stored carbon and demonstrate the capacity for further substantial reduction of C storage potential globally if elevated nutrient inputs continue within estuarine systems.

Although MPB-dominated sediments probably have less decadal-scale long-term storage of C than macrophyte-dominated sediments, this study clearly demonstrates that the existing storage potential is further degraded by increased nutrient loading within MPB-dominated sediments. These sediments may lock away less C per area but are fairly ubiquitous within photic coastal and oceanic sediments and may contribute significantly to carbon storage within coastal systems due to this increased area. The observed increases in mobility of newly fixed algal carbon from intertidal sediments (Fig. 6) as a result of elevated anthropogenic nutrient loading will directly translate to increased carbon export to coastal oceans and reduced carbon storage potential within shallow photic estuarine sediments.

Data availability. We have not put this data set into a publicly available repository yet as other portions of the data set for this experiment are still being finalized for other manuscripts. These data are available on request from the corresponding author.

The Supplement related to this article is available online at https://doi.org/10.5194/bg-15-2873-2018-supplement.

Author contributions. PMR planned the experimental design and field work; performed the field work, isolation of biomarkers, and laboratory analysis; and wrote the manuscript. JMO planned the experimental design and field work, contributed to the data interpretation, and assisted with statistical analysis and writing of the
manuscript. BDE planned the experimental design and field work, contributed to the data interpretation, and assisted with the writing of the manuscript. The group of coauthors has approved the submission of this paper.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. We thank Jaap van der Meer for assistance with statistical analyses, Natasha Carlson-Perret and Jessica Riekenberg for field assistance, Iain Alexander for laboratory analysis, and Mathes Carvalho for isotope analysis. This study was funded by an Australian Research Council (ARC) Linkage Infrastructure, Equipment and Facilities grant to Bradley D. Eyre (LE0668495), an ARC Discovery Early Career Researcher Award to Joanne M. Oakes (DE120101290), an ARC Discovery grant to Bradley D. Eyre (DP160100248), and ARC Linkage grants to Bradley D. Eyre (LP110200975; LP150100451; LP150100519).

Edited by: Tina Treude
Reviewed by: two anonymous referees

References


www.biogeosciences.net/15/2873/2018/


