

# Fate of macroalgae in benthic systems: carbon and nitrogen cycling within the microbial community

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**ABSTRACT:** High nutrient loading to coastal bays is often accompanied by the presence of bloom-forming macroalgae, which take up and sequester large amounts of C and N while growing. This pool is temporary, however, as nuisance macroalgae exhibit a bloom and die-off cycle, influencing the biogeochemical functioning of these systems in unknown ways. The objective of this study was to trace the C and N from senescing macroalgae into relevant sediment pools. A macroalgal die-off event was simulated by the addition of freeze-dried macroalgae (*Gracilaria* spp.), pre-labeled with stable isotopes (<sup>13</sup>C and <sup>15</sup>N), to sediment mesocosms. The isotopes were traced into bulk sediments and partitioned into benthic microalgal (BMA) and bacterial biomass using microbial biomarkers to quantify the uptake and retention of macroalgal C and N. Bulk sediments took up label immediately following the die-off, and macroalgal C and N were retained in the sediments for at least 2 wk. Approximately 6 to 50 % and 2 to 9 % of macroalgal N and C, respectively, were incorporated into the sediments. Label from the macroalgae appeared in both bacterial and BMA biomarkers, suggesting that efficient shuttling of macroalgal C and N between these communities may serve as a mechanism for retention of macroalgal nutrients within the sediments.

**KEY WORDS:** Stable isotopes · Macroalgae · Benthic microalgae · Bacteria · Biomarker · Coastal eutrophication

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## INTRODUCTION

Macroalgal blooms are increasingly recognized as a symptom of eutrophication in shallow coastal systems worldwide (Duarte 1995, Valiela et al. 1997). Their proliferation has been linked to increased nutrient loadings (Hauxwell et al. 2001, Bintz et al. 2003), and in many systems macroalgae have replaced slower-growing seagrasses and perennial macrophytes (Duarte 1995, Valiela et al. 1997, Hauxwell et al. 2001, Bintz et al. 2003, McGlathery et al. 2007). In temperate systems blooms usually develop in spring and collapse in mid to late summer, when high temperatures and self-shading negatively affect algal productivity (Peckol & Rivers 1995, McGlathery et al. 1997, Brush & Nixon 2003, Higgins et al. 2008). The deleterious effects these blooms have on the surrounding system while alive

and following die-off have been studied extensively (e.g. Sfriso et al. 1992, Raffaelli 2000, Hauxwell et al. 2001, Cummins et al. 2004, Nuzzi & Waters 2004).

Blooms can attain biomasses up to 10 kg wet weight m<sup>-2</sup> (Gordon & McComb 1989, Pavoni et al. 1992, Valiela et al. 1992, Morand & Briand 1996, Astill & Lavery 2001). While growing, macroalgae take up and sequester significant quantities of nutrients, often at similar magnitudes to nutrient loading, thereby serving effectively as a nutrient filter (Thybo-Christesen et al. 1993, Valiela et al. 1997, McGlathery et al. 1996, 2001, Pedersen et al. 2004). However, these blooms are not long-lived and, therefore, do not serve as a permanent nutrient reservoir. Although the fate of senescent macroalgal biomass is not fully known, it likely greatly impacts nutrient cycling dynamics within these systems.

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Studies of macroalgal bloom decay have demonstrated rapid breakdown of biomass, resulting in release of both inorganic and organic nutrients to the water column (Buchsbaum et al. 1991, Tyler et al. 2001, Castaldelli et al. 2003, García-Robledo et al. 2008), supporting phytoplankton and bacterial metabolism (Sfriso et al. 1992, Nedergaard et al. 2002). Fewer studies have focused on macroalgal decay within the sediments (Nedergaard et al. 2002, Lomstein et al. 2006, Rossi 2007, García-Robledo et al. 2008), where heterotrophic bacterial densities are significantly higher than in the water column (Deming & Baross 1993, Schmidt et al. 1998, Ducklow 2000). In addition, most of the sediment studies have been conducted in low or no light environments, even though light is typically available to shallow sediments where macroalgal die-offs occur and sediment biogeochemistry is largely affected by benthic microalgal (BMA) activity (Underwood & Kromkamp 1999). While nutrients associated with senescent macroalgal blooms are recycled and can have a positive feedback on phytoplankton production in the water column, nutrients released during macroalgal decay in the sediments may support BMA and bacterial production, which could intercept the return of nutrients to the overlying water column. Thus if shallow-water sediments behave as a nutrient filter, the response by phytoplankton may be reduced, and benthic production could effectively buffer the system from further eutrophication. In order to better constrain the input and retention of macroalgae-associated nutrients in the sediments, we used a dual stable isotope labeling approach to track macroalgal C and N into bulk sediments and the sediment microbial community after a simulated macroalgal die-off.

## MATERIALS AND METHODS

**Site description.** Sediments and macroalgae were collected from 2 lagoons along the Delmarva Peninsula, USA: Hog Island Bay, Virginia (HIB) and Isle of Wight Bay, Maryland (IWB; Fig. 1). These bays are typical of temperate lagoons along the US East Coast. Both are shallow, on average less than 2 m deep at mean low water, and are characterized by ephemeral macroalgal blooms (Goshorn et al. 2001, McGlathery

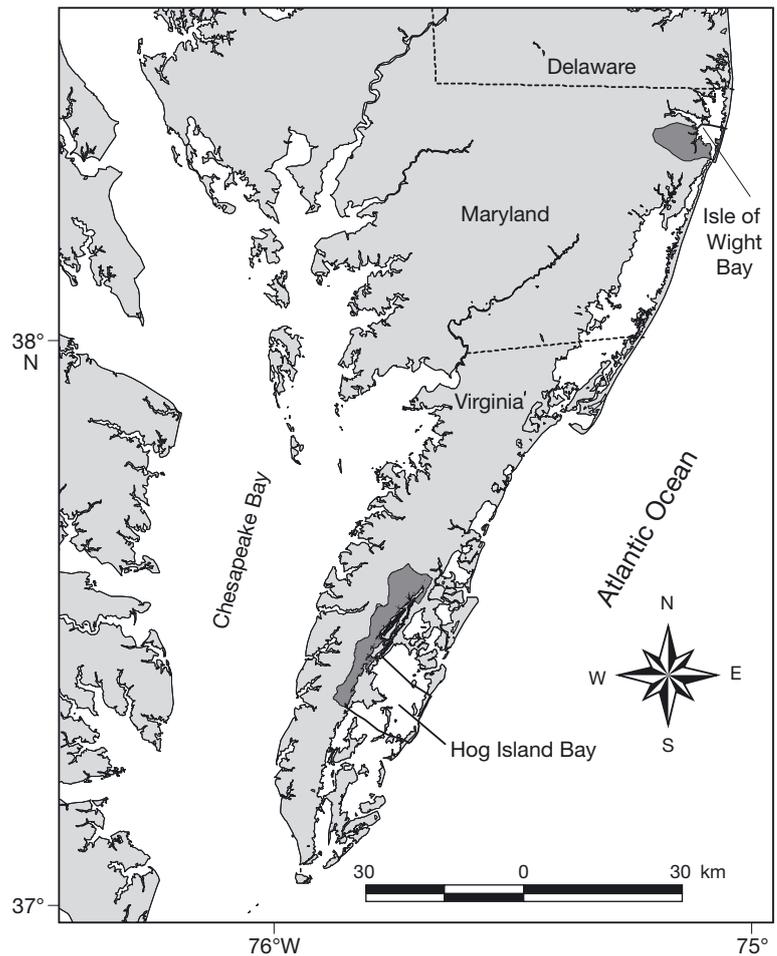


Fig. 1. Sediments and macroalgae were collected from 2 coastal lagoons: Hog Island Bay, VA, and Isle of Wight Bay, MD. Dark grey shaded areas indicate the watersheds of each lagoon. Hog Island Bay is located within the Virginia Coast Reserve LTER network

et al. 2001, Thomsen et al. 2006); however, there are important differences between the lagoons. External loading of nutrients (nitrogen) to HIB ( $14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ; Anderson et al. 2010) is lower than for IWB ( $65 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ; Boynton et al. 1996), and as a result, macroalgal densities in HIB are lower and only dominant at select sites during brief portions of the year (Boynton et al. 1996, Goshorn et al. 2001, McGlathery et al. 2001, Stanhope et al. 2009, present study).

**Experimental design.** A flow-through mesocosm array was set up at the Virginia Institute of Marine Science (VIMS) Eastern Shore Laboratory (ESL) in Wachapreague, VA. Each mesocosm ( $0.61 \times 0.61 \text{ m}$ , diameter  $\times$  height) was constructed of translucent fiberglass to allow maximum light penetration (87% visual light transmission; Solar Components). Three mesocosms were filled with sediments from one location in each lagoon in June 2006 to a depth of  $\sim 20 \text{ cm}$  using intact sediments extruded from cores taken at

the field sites. The field sites were chosen based on the presence of macroalgal blooms in previous monitoring efforts. Care was taken not to include any macroalgae or visible macrofauna in the collected cores. At the ESL, the mesocosms were placed in shallow water baths under 30% shade cloth to control temperature and light. Ambient, filtered (10  $\mu\text{m}$ ) seawater, pumped from the creek adjacent to the ESL, was delivered gravimetrically to each mesocosm at a rate that achieved a water column flushing time of  $\sim 2$  d, similar to the flushing time observed at the study sites (Oertel 2001). The water column was circulated using mini-jet pumps (Aquatic Ecosystems, Inc.) secured on the inner tank wall  $\sim 18$  cm above the sediment surface to avoid sediment resuspension. The mesocosms were equilibrated for 2 wk before beginning the experiment. Water column temperature and salinity were measured in each mesocosm throughout the experiment using a YSI datasonde.

Macroalgae (*Gracilaria* spp.) were collected from both lagoons in May 2006 and returned to the laboratory for isotopic labeling. The macroalgae were cleaned of epiphytes and epifauna, rinsed with 0.7  $\mu\text{m}$  filtered seawater, and placed in separate aquaria inside a greenhouse. Filtered (0.7  $\mu\text{m}$ ) seawater was added to each tank and aerated during labeling. The algae were starved for 10 d and then fertilized daily for 14 d with a solution containing 50 atom % (at.%)  $^{15}\text{N-NH}_4^+$  (as  $\text{NH}_4\text{Cl}$ ) and 98 at. %  $^{13}\text{C-HCO}_3^-$  (as  $\text{NaHCO}_3$ ). Rates of N and C addition were estimated to sustain tissue N at 3% and C at 25% of dry weight (DW) with a growth rate of 5%  $\text{d}^{-1}$  following the procedure of Tyler & McGlathery (2006). To insure that P was not limiting, P was added to provide a 10:1 ratio of N:P. At the end of the labeling period, the algae were rinsed with filtered (0.7  $\mu\text{m}$ ) seawater, patted dry, and freeze-dried as intact tissues. The final isotopic enrichments of the dead macroalgae were approximately 30 at. %  $^{15}\text{N}$  and 9 at. %  $^{13}\text{C}$ . After the mesocosm equilibration period, the intact, isotopically labeled, freeze-dried algal tissues were added to the surface of sediments in corresponding mesocosms from HIB or IWB, at ambient densities observed in each system (HIB: 84  $\text{g DW m}^{-2}$ ; IWB: 184  $\text{g DW m}^{-2}$ ; Table 1). The intact macroalgae settled to the sediment surface, but retained some buoyancy for the first 2 d due to the gentle water circulation; after 1 wk, macroalgae were no longer visible.

**Sediment sampling.** The mesocosms were sampled 1 d prior to the macroalgal additions to capture baseline

conditions (Day 0) and on Days 1, 2, 7, and 14 after the additions. At each sampling, surface sediments (0 to 1 cm), collected using 2 acrylic cores (5.7 cm i.d.), were reserved for bulk (total organic C [TOC], total N [TN]), amino acid, and fatty acid analyses. Sediments from both cores were combined in pre-combusted glass jars, immediately frozen at  $-4^\circ\text{C}$ , and frozen at  $-80^\circ\text{C}$  within 3 d. The remaining sediment in the cores was placed carefully back into the holes in the mesocosm sediments. Surface sediments from a third acrylic core were collected and processed immediately for determination of bulk density, organic content, and water content. Sediments were also collected for chlorophyll *a* (chl *a*) concentrations using a cut-off syringe (1.1 cm i.d.). Samples were sectioned into 0 to 0.3 cm and 0.3 to 1.0 cm horizons, placed into 15 ml centrifuge tubes, immediately frozen at  $-4^\circ\text{C}$ , and analyzed within 1 mo. A different region of the sediment surface was sampled each time to avoid resampling any sediments.

**Bulk sediment analyses.** Sediments for percent water and organic matter (OM) were processed immediately. A known volume of sediment was weighed, dried at  $40^\circ\text{C}$  and reweighed for water content and bulk density. The dried sediments were combusted for 4 h at  $500^\circ\text{C}$  to obtain ash-free dry weight. Samples were analyzed for benthic chl *a* concentrations according to the method of Pinckney et al. (1994), a modification of Lorenzen (1967). The sediment pellet was sonicated in 90% acetone, vortexed, and extracted for 24 h at  $-4^\circ\text{C}$ . The supernatant was passed through a 0.45  $\mu\text{m}$  filter and read on a Shimadzu UV-1601 UV visible spectrophotometer ( $\lambda = 665, 750$  nm). Chl *a* concentrations ( $\text{mg m}^{-2}$ ) for the 0 to 0.3 and 0.3 to 1.0 cm horizons were calculated according to the equations in

Table 1. Environmental parameters measured in the field and in the mesocosms. Except for peak macroalgal biomass, field values were combined for 2 sampling dates in June 2006 at 3 sites in each lagoon ( $n = 6$ ). Mesocosm values are presented as the mean ( $\pm$ SE) across all 5 sampling days during the experiment ( $n = 15$ ). Peak macroalgal biomass values for the field correspond to the maximum biomass measured from May through October 2006 at multiple sites across Hog Island Bay (HIB;  $n = 9$ ) and Isle of Wight Bay (IWB;  $n = 5$ ). Mesocosm values correspond to the mass of freeze-dried, labeled macroalgae added to each mesocosm ( $n = 3$ ). n/a: not applicable

Parameter	Field		Mesocosm	
	HIB	IWB	HIB	IWB
Temperature ( $^\circ\text{C}$ )	28.3 (0.1)	28.6 (0.2)	22.2 (1.2)	23.0 (1.2)
Salinity (psu)	31.3 (0.01)	29.3 (0.08)	30.1 (0.7)	29.9 (0.7)
Sediment organic matter (%)	2.59 (0.77)	0.77 (0.10)	2.01 (0.18)	1.04 (0.07)
Chlorophyll <i>a</i> ( $\text{mg chl } a \text{ m}^{-2}$ )	56.2 (14.5)	89.5 (19.7)	26.7 (3)	109 (7)
<b>Peak macroalgal biomass (<math>\text{g DW m}^{-2}</math>)</b>				
Range	0–192	29–538	n/a	n/a
Mean	52 (24)	176 (98)	83 (2)	183 (2)

Lorenzen (1967) and added to obtain the concentration for the 0 to 1 cm horizon.

For bulk sediment TOC, TN, and isotopic measurements, sediments were freeze-dried, ground and homogenized, acidified with 10% HCl to remove inorganic C (Hedges & Stern 1984), and analyzed for  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (EA-IRMS, Sercon) at the University of California at Davis Stable Isotope Facility. Stable isotope ratios for C ( $R = ^{13}\text{C}/^{12}\text{C}$ ) and N ( $R = ^{15}\text{N}/^{14}\text{N}$ ) were used to calculate  $\delta$ -values in units of per mil (‰):

$$\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$ . Samples were expressed relative to international standards Pee Dee Belemnite (C) and atmospheric  $\text{N}_2$ .  $\delta X$  was used to calculate at.%  $X$ , which was used to calculate excess  $X$  (absolute amount of incorporated  $^{13}\text{C}$  or  $^{15}\text{N}$ ):

$$\text{at.}\% X = [100 \times R_{\text{standard}} \times (\delta X_{\text{sample}}/1000 + 1)] / [1 + 6R_{\text{standard}} \times (\delta X_{\text{sample}}/1000 + 1)] \quad (2)$$

$$\text{excess } X (\text{nmol } X \text{ g DW}^{-1}) = [(\text{at.}\% X_{\text{sample}} - \text{at.}\% X_{\text{control}})/100] \times \text{concentration}_{\text{sample}} \quad (3)$$

where concentrations were expressed in moles C or N relative to sediment dry weight. The control (unlabeled) samples were collected on Day 0, before macroalgae were added to the mesocosms.

**Hydrolyzable amino acids.** Hydrolyzable amino acids (HAAs) were extracted and analyzed according to the method presented in Veuger et al. (2005). Freeze-dried sediment (1 g) was rinsed with 2N HCl and Milli-Q water to remove dissolved amino acids. The sediment pellet was then hydrolyzed with 6N HCl at 110°C for 20 h. Following purification by cation exchange chromatography, amino acids were derivatized with isopropanol and pentafluoropropionic anhydride and further purified by solvent extraction. Concentrations and stable isotope ratios for C ( $R = ^{13}\text{C}/^{12}\text{C}$ ) and N ( $R = ^{15}\text{N}/^{14}\text{N}$ ) of the derivatized D- and L-amino acids were measured at the Netherlands Institute of Ecology (NIOO) by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) on an HP 6890 GC (Chirasil L-Val column) with a Thermo type III combustion interface and a Thermo Delta Plus IRMS.  $\delta$ - and at.%  $X$  values were calculated according to Eqs. (1) & (2) and used to calculate excess  $X$  according to Eq. (3), where amino acid concentrations were expressed in moles C or N relative to sediment dry weight. Carbon isotopic values of amino acids were corrected for the C atoms added during derivatization using a mass balance approach following Veuger et al.

(2006). The sum of concentrations of, and/or excess label incorporated in, all amino acids analyzed will be referred to as total HAAs (THAAs).

The ratio of excess  $^{13}\text{C}$  or  $^{15}\text{N}$  incorporation into D-alanine (D-Ala), a bacterial-specific amino acid, relative to L-alanine (L-Ala), an amino acid made by all organisms, was calculated as:

$$\text{D/L-Ala ratio (D/L-Ala)} = (\text{excess } X \text{ in D-Ala}) / (\text{excess } X \text{ in L-Ala}) \quad (4)$$

where  $X$  was  $^{13}\text{C}$  or  $^{15}\text{N}$ . During hydrolysis, some racemization of L-Ala to D-Ala takes place, resulting in a D/L-Ala value of ~0.017 (Veuger et al. 2007b). We corrected values of excess isotope in D-Ala for this racemization according to Veuger et al. (2007a), whereas values of D/L-Ala have been left uncorrected. Instead, the D/L-Ala value of 0.017 will be indicated graphically in our results to reflect racemization during hydrolysis (Veuger et al. 2007b). We estimated the bacterial contribution to total  $^{13}\text{C}$  or  $^{15}\text{N}$  incorporation according to Veuger et al. (2007a):

$$\text{Bacterial contribution (\%)} = [(\text{excess } X_{\text{D/L-Ala}} - 0.017) / (\text{bacterial D/L-Ala} - 0.017)] \times 100\% \quad (5)$$

where  $X$  was  $^{13}\text{C}$  or  $^{15}\text{N}$ . Bacterial D/L-Ala represents the D/L-Ala abundance ratio for bacteria. The upper bound of the ratio ranges from 0.05 for Gram-negative (G-) bacteria, to 0.1 for Gram-positive (G+) bacteria and cyanobacteria (Veuger et al. 2007b). Previous work suggests that G+ bacteria are more prominent in deeper (anaerobic) sediments (Moriarty & Hayward 1982, Gontang et al. 2007). Since the present study used sandy photic sediments, we assumed that the contribution from G+ bacteria was negligible. Additionally, photosynthetic pigment analyses obtained by HPLC of sediments from the HIB field sites showed low zeaxanthin:chlorophyll ratios, suggesting that cyanobacterial contribution to the microbial community was negligible (M. Waters pers. comm.). As a result, we further assumed that their contribution to the D/L-Ala ratio of the total microbial community was negligible, and we estimated the bacterial D/L-Ala ratio for our sediments at 0.05. This will also be indicated graphically in our results. The lower bound of D/L-Ala, when bacteria do not take up any label, is 0.017, which represents abiotic racemization of L-Ala (Veuger et al. 2007b). Thus excess  $^{13}\text{C}$  and  $^{15}\text{N}$  D/L-Ala ratio values should fall between these upper and lower limits, with higher values indicating a higher bacterial contribution to the total label uptake since only bacteria incorporate label into D-Ala.

**Phospholipid-linked fatty acids.** Total fatty acids were analyzed according to a modified Bligh & Dyer (1959) method (Poerschmann & Carlson 2006, Canuel

et al. 2007). Wet sediments (~12 g) were extracted using an accelerated solvent extractor (ASE) system (Dionex ASE 200) adapted for in-cell silica gel chromatography. Each sample was extracted twice on the ASE: neutral lipids were collected following extraction with a 9:1 (v:v) hexane:acetone mixture at 50°C, and then polar lipids were collected following extraction with a 8:2 (v:v) methanol:chloroform solution at 80°C. Neutral and polar lipid fractions were saponified using KOH-CH<sub>3</sub>OH for 2 h at 110°C. Saponified samples were then extracted under basic and acidic conditions. The acid-extracted fractions were methylated with BF<sub>3</sub>-CH<sub>3</sub>OH to form fatty acid methyl esters (FAMES). The neutral FAMES included neutral and glycolipids, while the polar FAMES represented the phospholipid-linked fatty acids (PLFAs). FAME concentrations were measured by gas chromatography with flame ionization detection (GC-FID; DB-5 column, HP 5890) and quantified using methyl heneicosanoate as an internal standard. Peak identities were verified using reference standards as well as coupled gas chromatography mass spectrometry (GC-MSD; HP 6890). Fatty acids are designated A:B $\omega$ C, where A is the total number of carbon atoms, B is the number of double bonds, and C is the position of the first double bond from the aliphatic ' $\omega$ ' end of the molecule. The prefixes 'i' and 'a' refer to iso- and anteiso-methyl branched fatty acids, respectively (see Canuel et al. 1995 and references therein). Stable C isotope ratios ( $R = {}^{13}\text{C}/{}^{12}\text{C}$ ) for PLFAs were measured at NIOO with a GC-C-IRMS system consisting of a Trace GC Ultra gas chromatograph (BPX70 column) coupled to a Delta Plus Advantage IRMS through a GC/C-III interface and were used to calculate  $\delta^{13}\text{C}$  (Eq. 1) and at. %<sup>13</sup>C (Eq. 2). Excess <sup>13</sup>C was calculated according to Eq. (3), where concentrations were FAME concentrations expressed in moles C relative to dry weight. Actual PLFA isotopic values were derived from the FAME isotopic compositions by correcting for the isotopic composition of the C added during derivatization using a mass balance approach. We analyzed the concentrations and isotopic content of total PLFAs as well as branched odd fatty acids (BrFAs; summed excess <sup>13</sup>C in iso- and anteiso-C<sub>13:0</sub>, C<sub>15:0</sub>, C<sub>17:0</sub>, and C<sub>19:0</sub>), representative of heterotrophic bacterial biomass (Perry et al. 1979), and polyunsaturated fatty acids (PUFA; summed excess <sup>13</sup>C in C<sub>20:4</sub>, C<sub>20:5</sub>, C<sub>22:5</sub>, and C<sub>22:6</sub>), representative of algal biomass (Volkman et al. 1998).

**Field monitoring.** Concurrent with the mesocosm experiment, we conducted field measurements of various water column and sediment parameters at the sediment collection sites within each lagoon on 2 sampling dates. Triplicate measurements of water temperature and salinity were taken using a YSI datasonde during each sampling. Triplicate samples for sediment per-

cent OM and benthic chl *a* concentrations were also collected and measured as described above for the mesocosm experiment. We also monitored macroalgal biomass from May through October 2006. Macroalgal samples ( $n = 3$ ) were collected at multiple sites across HIB ( $n = 9$ ) and IWB ( $n = 5$ ) by randomly tossing a cylinder (0.42 m i.d.) and collecting the total biomass contained within the cylinder. The algae were dried at 40°C and normalized to the cylinder area for biomass ( $\text{g DW m}^{-2}$ ). The triplicate biomass values for each site were averaged to obtain a site biomass estimate. Since this experiment was designed to simulate a die-off event following a bloom, we report the ranges over the growing season of maximum biomass estimates for each site as well as the mean  $\pm$  SE for all sites within each lagoon (Table 1).

**Data analysis.** We applied repeated measures ANOVA to examine the effects of lagoon (HIB vs. IWB) and time (Days 0, 1, 2, 7, 14) on the sediment parameters using the Mixed procedure in SAS 9.1 (SAS Institute). In all models, a first-order ante-dependence error structure (Kenward 1987) was used to model the within-subject covariance structure. We present results from the Type III test of fixed effects from the ANOVA model. To further explain the effects of time, post hoc contrasts were used to compare the isotopic enrichment parameters for Days 1 and 2 with Days 7 and 14. Unless otherwise noted, mesocosm values presented are means  $\pm$  1 SE for 3 replicates.

## RESULTS

### Field and experimental conditions

Temperatures and salinities in the mesocosms were similar to values measured at the field sites during June (Table 1). Though temperatures in the mesocosms were lower than in the field by ~6°C, the values were well within the ranges of water temperatures typically observed throughout a day and between days in these shallow lagoons (Giordano 2009). Between-lagoon differences in organic content and benthic chl *a* concentrations in mesocosm sediments were also similar to those observed at the field sites (Table 1). The surface (0–1 cm) sediment organic content within HIB mesocosms was approximately twice as high as in IWB. These values represent means  $\pm$  SE ( $n = 15$ ) over 5 sampling days, since sediment OM did not accumulate in either treatment over the course of the experiment (Table 2). In addition, benthic chl *a* concentrations were lower at HIB in the surface sediments than at IWB (Table 1). Again, these values represent means  $\pm$  SE ( $n = 15$ ), since benthic chl *a* did not accumulate in either treatment over the experiment (Table 2). Simi-

Table 2. Mean ( $\pm$ SE) values for bulk sediment parameters ( $n = 3$ ) in Hog Island Bay (HIB) and Isle of Wight Bay (IWB). Last 3 columns show p-values for repeated-measures ANOVA used to test for differences in lagoons over time for various sediment pools. Significant p-values are indicated in **bold**. THAAs: total hydrolyzable amino acids (AAs); PLFAs: phospholipid-linked fatty acids; OC: organic content

Parameter	Lagoon	Experimental day					ANOVA		
		0	1	2	7	14	Lagoon	Time	Interaction
Organic matter (%)	HIB	1.82 (0.48)	2.52 (0.70)	1.84 (0.11)	1.75 (0.31)	2.12 (0.22)	<b>0.0008</b>	0.6708	0.6958
	IWB	1.20 (0.26)	0.93 (0.21)	0.96 (0.04)	0.89 (0.12)	1.22 (0.14)			
Chlorophyll <i>a</i> (mg chl $a$ $m^{-2}$ )	HIB	15.0 (2.5)	29.0 (3.4)	28.1 (8.6)	29.0 (6.8)	33.7 (8.6)	<b>0.0010</b>	0.3187	0.9870
	IWB	93.7 (7.7)	108 (24)	112 (21)	110 (4)	124 (16)			
THAAs ( $\mu$ mol AA $mg$ $OC^{-1}$ )	HIB	3.44	3.95 (0.70)	2.29 (0.04)	1.91 (0.17)	2.24 (0.11)	0.7074	<b>0.0023</b>	0.3591
	IWB	3.74	3.28 (0.28)	2.90 (0.15)	2.16 (0.13)	2.35 (0.27)			
PLFAs ( $\mu$ g PLFA $mg$ $OC^{-1}$ )	HIB	14.5 (5.4)	13.9 (3.6)	9.13 (1.78)	19.7 (1.5)	14.9 (1.9)	0.7033	0.1893	0.4235
	IWB	15.7 (2.3)	11.9 (0.7)	14.2 (3)	13.9 (1.2)	19.5 (5.0)			

larly, organic content at the field sites was higher in HIB, while benthic chl *a* concentrations were higher in IWB (Table 1). Peak macroalgal biomass from May through October 2006 ranged across the field sites from 0 to 192 and 29 to 538 g DW  $m^{-2}$  for HIB ( $n = 9$ ) and IWB ( $n = 5$ ), respectively (Table 1). The biomass of dead macroalgae added to the mesocosms fell within these ranges; approximately twice as much algae was added to the IWB treatments to reflect the higher macroalgal densities there.

### Bulk isotopes

All sediment pools displayed similar isotopic enrichment patterns. Excess  $^{13}C$  and  $^{15}N$  in bulk sediments (0–1 cm) are presented in Fig. 2.  $^{13}C$  appeared in mesocosms from both lagoons immediately following the addition of macroalgae (Day 1,  $22.03 \pm 0.29$  and  $62.76 \pm 21.91$  nmol  $^{13}C$  g  $DW^{-1}$  for HIB and IWB, respectively) and excess  $^{13}C$  values were similar on Day 2. Individual replicates (data not shown) peaked on either Day 7 or 14, which accounted for the large variance associated with the means for those days. Nevertheless, post hoc contrasts indicated that excess  $^{13}C$  values were significantly higher on Days 7 and 14 than on Days 1 and 2 (Table 3, Fig. 2a). Excess  $^{15}N$  followed the same pattern as  $^{13}C$ : label first appeared on Day 1 ( $28.94 \pm 5.96$  and  $79.61 \pm 36.27$  nmol  $^{15}N$  g  $DW^{-1}$  for HIB and IWB, respectively), and values peaked on either Day 7 or 14. Again, post hoc contrasts showed higher excess  $^{15}N$  on Days 7 and 14 than Days 1 and 2 (Table 3, Fig. 2b). There were no significant lagoon differences in either excess  $^{13}C$  or  $^{15}N$  for bulk sediments (Table 3), and both lagoons showed similar patterns of initial uptake on Day 1 and maximum values on Day 7 or 14. Maximum isotopic enrichments were well above natural abundance levels ( $\sim 200\%$   $\delta^{13}C$  and  $>4000\%$

$\delta^{15}N$  relative to  $-14\%$   $\delta^{13}C$  and  $\sim 10\%$   $\delta^{15}N$ ). Given the maximum isotopic enrichments for each mesocosm and the masses of  $^{13}C$  and  $^{15}N$  that were added to each mesocosm as macroalgal material, we estimated that  $6.4 \pm 1.4$  and  $35.1 \pm 10.3\%$  of the added macroalgal  $^{13}C$  and  $^{15}N$  were incorporated into the sediments (0–1 cm) for HIB. In IWB,  $2.9 \pm 0.9$  and  $8.8 \pm 2.1\%$  of the macroalgal  $^{13}C$  and  $^{15}N$  were incorporated into the sediments.

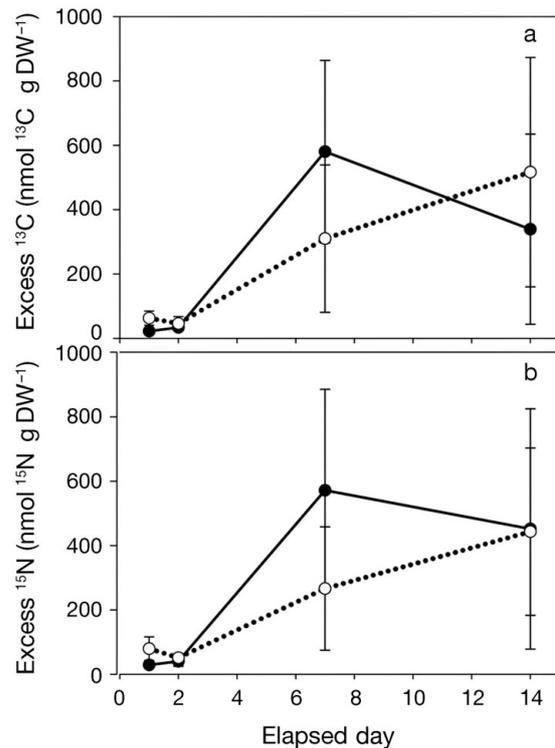


Fig. 2. Bulk sediment isotopic enrichments for Hog Island Bay (●, solid lines) and Isle of Wight Bay (○, dotted lines). Values are reported as means  $\pm$  1 SE ( $n = 3$ ) for excess (a)  $^{13}C$  and (b)  $^{15}N$

Table 3. Results of 2-factor repeated measures ANOVA used to test for differences in lagoons over time for isotopic enrichments ( $^{13}\text{C}$  or  $^{15}\text{N}$ ) of various sediment pools and percent enrichment of select sediment pools out of the bulk sediment enrichment. Results for the post hoc contrast for Days 1 and 2 versus Days 7 and 14 are shown to the right. One outlier was removed from the Isle of Wight Bay (IWB)  $^{15}\text{N}$  total hydrolyzable amino acid (THAA) analyses (Day 2) and the Hog Island Bay  $^{13}\text{C}$  phospholipid-linked fatty acid (PLFA) analyses (Day 1) due to methodological errors, which accounts for the reduced degrees of freedom for those parameters. Significant p-values are indicated in **bold**

Parameter	Isotope	Lagoon			Time			Interaction			Post hoc contrast		
		df	F	p	df	F	p	df	F	p	df	F	p
Bulk sediments	$^{13}\text{C}$	4	0.01	0.9163	12	6.98	<b>0.0057</b>	12	3.29	0.0580	12	19.16	<b>0.0009</b>
	$^{15}\text{N}$	4	0.42	0.5532	12	5.76	<b>0.0112</b>	12	0.8	0.5168	12	15.61	<b>0.0019</b>
THAA	$^{13}\text{C}$	4	0.25	0.6460	12	3.68	<b>0.0434</b>	12	1.57	0.2487	12	10.37	<b>0.0074</b>
	$^{15}\text{N}$	4	0.92	0.3921	11	3.63	<b>0.0485</b>	11	1.56	0.2542	11	9.93	<b>0.0092</b>
D-Ala	$^{13}\text{C}$	4	1.14	0.3461	12	2.21	0.1399	12	1.03	0.4147	12	5.16	<b>0.0424</b>
	$^{15}\text{N}$	4	2.76	0.1721	11	1.98	0.1755	11	1.05	0.4086	11	4.89	<b>0.0492</b>
D/L-Ala	$^{13}\text{C}$	4	5.92	0.0718	12	5.49	<b>0.0132</b>	12	1.21	0.3473	12	15.7	<b>0.0019</b>
	$^{15}\text{N}$	4	7.72	<b>0.0499</b>	11	17.83	<b>0.0002</b>	11	5.25	<b>0.0171</b>	11	16.21	<b>0.0020</b>
PLFA	$^{13}\text{C}$	4	0.79	0.4247	11	3.32	0.0605	11	1.06	0.4037	11	6.93	<b>0.0233</b>
Bacterial FA	$^{13}\text{C}$	4	1.11	0.3507	11	3.39	0.0575	11	1.08	0.3961	11	5.83	<b>0.0344</b>
Algal FA	$^{13}\text{C}$	4	0.68	0.4566	11	6.84	<b>0.0072</b>	11	1.61	0.2426	11	17.11	<b>0.0017</b>
THAA/Bulk (%)	$^{13}\text{C}$	4	6.38	0.0649	12	2.86	0.0815	12	0.57	0.6474	12	5.62	<b>0.0353</b>
	$^{15}\text{N}$	4	1.35	0.3102	11	7.8	<b>0.0046</b>	11	0.3	0.8255	11	19.83	<b>0.0010</b>
D-Ala/Bulk (%)	$^{13}\text{C}$	4	13.48	<b>0.0214</b>	12	13.08	<b>0.0004</b>	12	3.03	0.0712	12	33.01	<b>&lt;0.0001</b>
	$^{15}\text{N}$	4	6.29	0.0662	11	16.59	<b>0.0002</b>	11	3.64	<b>0.0483</b>	11	15.59	<b>0.0023</b>
PLFA/Bulk (%)	$^{13}\text{C}$	4	0.58	0.4905	11	1.65	0.2338	11	1.55	0.2569	11	0.96	0.3476

### Hydrolyzable amino acids

All biomarker concentrations were normalized to sediment TOC to account for the differences in organic content between HIB and IWB. THAA concentrations were 3.22 and 3.74  $\mu\text{mol AA mg OC}^{-1}$  on Day 0 for HIB and IWB, respectively, and decreased slightly over the course of the experiment; there were no significant lagoon differences (Table 2). THAA represented a stable fraction of the sediment organic content, comprising ~13% of the TOC and ~30% of the TN. Excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in the THAA pool (summed excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in the individual amino acids) were lower than in the bulk pool, but displayed similar patterns, showing enrichments well above natural abundance; for THAA, maximum values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were >500‰ and >6000‰, whereas background values were approximately -15‰ and 20‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Excess  $^{13}\text{C}$  appeared in both lagoons immediately following the macroalgal addition (Day 1,  $10.34 \pm 1.43$  and  $26.10 \pm 13.46$   $\text{nmol }^{13}\text{C g DW}^{-1}$  for HIB and IWB, respectively) and peaked on either Day 7 or 14 (Fig. 3a). Excess  $^{15}\text{N}$  followed the same pattern as  $^{13}\text{C}$ : label first appeared on Day 1 ( $13.80 \pm 2.87$  and  $35.73 \pm 12.02$   $\text{nmol }^{15}\text{N g DW}^{-1}$  for HIB and IWB, respectively), and values peaked on either Day 7 or 14 (Fig. 3b). There were no significant lagoon differences for excess  $^{13}\text{C}$  or  $^{15}\text{N}$  in THAA; however, there were significant time effects. Post hoc contrasts indicated that

excess  $^{13}\text{C}$  and  $^{15}\text{N}$  on Days 7 and 14 were significantly higher than on Days 1 and 2 (Table 3, Fig. 3a,b). Across all sampling days, excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in THAA accounted for an average of ~40% (range = 11 to 75%) of excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in bulk sediments, although the  $^{13}\text{C}$  and  $^{15}\text{N}$  accounted for a greater fraction of the  $^{13}\text{C}$  and  $^{15}\text{N}$  in bulk sediment on Days 1 and 2 than on Days 7 and 14 (Tables 3 & 4).

Excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in individual amino acids provided additional information about the fate of macroalgal C and N within the sediment microbial pool. Excess  $^{13}\text{C}$  (corrected for hydrolysis-induced racemization) appeared in D-Ala, a bacterial-specific amino acid, on Day 1 in sediments from both bays (HIB:  $0.05 \pm 0.01$   $\text{nmol }^{13}\text{C g DW}^{-1}$ ; IWB:  $0.05 \pm 0.02$   $\text{nmol }^{13}\text{C g DW}^{-1}$ ) and peaked on Day 7 or 14 (Fig. 3c, Table 3). Similarly,  $^{15}\text{N}$  appeared on Day 1 (HIB:  $0.06 \pm 0.03$   $\text{nmol }^{15}\text{N g DW}^{-1}$ ; IWB:  $0.08 \pm 0.04$   $\text{nmol }^{15}\text{N g DW}^{-1}$ ) and peaked on Day 7 or 14 (Fig. 3d, Table 3). While the absolute values for excess  $^{13}\text{C}$  and  $^{15}\text{N}$  were low on Days 1 and 2 and increased throughout the experiment, the proportion of excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in the bulk sediment pool that appeared in D-Ala was highest on Days 1 and 2 and then decreased through Day 14 (Tables 3 & 4).

Additionally, the D/L-Ala ratio of excess isotope (either  $^{13}\text{C}$  or  $^{15}\text{N}$ , not corrected for hydrolysis-induced racemization) indicated the relative importance of bacterial uptake to total label uptake (Veuger et al. 2005;

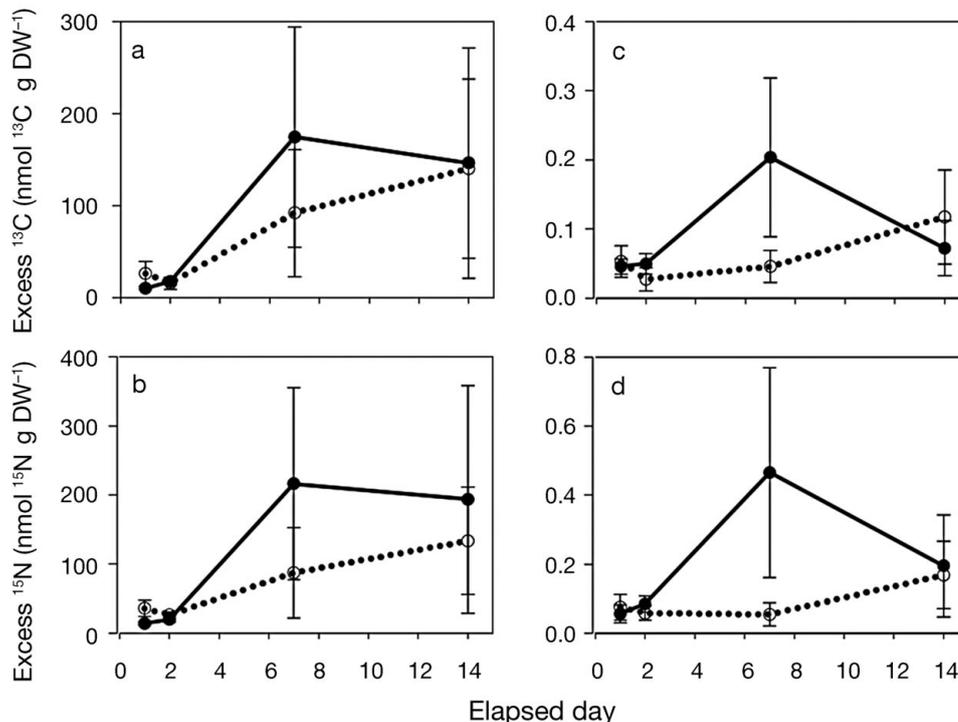


Fig. 3. Amino acid isotopic enrichments for Hog Island Bay (solid lines) and Isle of Wight Bay (dotted lines). Values are means  $\pm$  1 SE (n = 3) for excess (a)  $^{13}\text{C}$  and (b)  $^{15}\text{N}$  in total hydrolyzable amino acids and excess (c)  $^{13}\text{C}$  and (d)  $^{15}\text{N}$  in D-Ala

Fig. 4). The values for excess  $^{13}\text{C}$  and  $^{15}\text{N}$  in D/L-Ala showed similar patterns (Fig. 4a,b). Excess  $^{13}\text{C}$  in D/L-Ala peaked for both bays on Day 1 (HIB:  $0.053 \pm 0.006$ ; IWB:  $0.042 \pm 0.007$ ) and decreased by Day 14 (HIB:  $0.031 \pm 0.005$ ; IWB:  $0.029 \pm 0.002$ ; Fig. 4a). This represented a decrease from Day 1 to Day 14 from ~100 to

41 % bacterial  $^{13}\text{C}$  incorporation for HIB and 77 to 36 % for IWB (Fig. 4a, right axis). Excess  $^{15}\text{N}$  in D/L-Ala peaked on Day 2 for HIB and Day 1 for IWB (HIB:  $0.048 \pm 0.003$ ; IWB:  $0.033 \pm 0.005$ ) and decreased by Day 14 (HIB:  $0.028 \pm 0.003$ ; IWB:  $0.027 \pm 0.0004$ ). This represented a decrease over the 14 d from ~100 to 33 %

bacterial  $^{15}\text{N}$  incorporation for HIB and 49 to 32 % for IWB (Fig. 4b, right axis). Even though excess  $^{13}\text{C}$  D/L-Ala in HIB showed a trend of being higher than IWB, the treatments were not significantly different; however, excess  $^{15}\text{N}$  in D/L-Ala was significantly higher for sediments from HIB than IWB (Table 3, Fig. 4).

Table 4. Fraction (%) of excess isotope ( $^{13}\text{C}$  or  $^{15}\text{N}$ ) in total hydrolysable amino acids (THAAs), D-Ala, and phospholipid-linked fatty acids (PLFAs) out of excess isotope in bulk sediment. Values are presented as the mean ( $\pm$ SE, n = 3) for each lagoon on each experimental day. HIB: Hog Island Bay; IWB: Isle of Wight Bay

Parameter Lagoon	Isotope	Experimental day			
		1	2	7	14
THAAs/Bulk (%)					
HIB	$^{13}\text{C}$	47.1 (7.1)	51.0 (4.5)	26.4 (7.6)	50.9 (12.5)
	$^{15}\text{N}$	47.7 (1.5)	56.1 (9.1)	33.8 (5.1)	39.1 (2.7)
IWB	$^{13}\text{C}$	37.1 (6.5)	38.9 (10.3)	30.3 (5.4)	31.3 (5.5)
	$^{15}\text{N}$	49.3 (8.3)	42.1 (10.9)	30.7 (2.5)	33.2 (4.7)
D-Ala/Bulk (%)					
HIB	$^{13}\text{C}$	0.21 (0.05)	0.14 (0.03)	0.04 (0.01)	0.06 (0.03)
	$^{15}\text{N}$	0.19 (0.07)	0.23 (0.03)	0.07 (0.01)	0.05 (0.01)
IWB	$^{13}\text{C}$	0.08 (0.01)	0.06 (0.03)	0.02 (0.01)	0.04 (0.01)
	$^{15}\text{N}$	0.10 (0.02)	0.10 (0.04)	0.03 (0.01)	0.04 (0.01)
PLFA/Bulk (%)					
HIB	$^{13}\text{C}$	3.46 (0.17)	2.94 (0.57)	3.53 (0.97)	4.89 (3.79)
IWB	$^{13}\text{C}$	2.85 (1.01)	3.43 (1.09)	3.05 (0.59)	0.22 (0.01)

### Phospholipid-linked fatty acids

Total PLFA concentrations, normalized to sediment TOC, remained steady throughout the experiment, and sediments from both lagoons had similar concentrations (~15  $\mu\text{g}$  PLFA  $\text{mg OC}^{-1}$ ; Table 2). PLFA represented ~1 % of the sediment TOC for HIB and IWB. Excess  $^{13}\text{C}$  in total PLFAs were

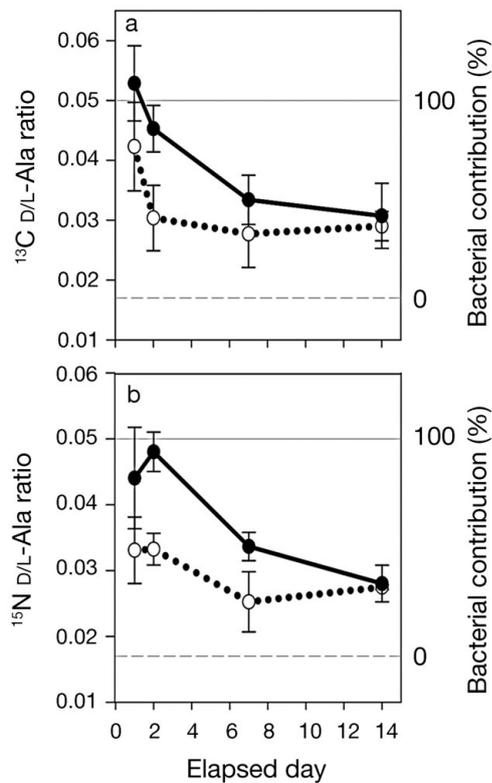


Fig. 4. Ratio of excess  $^{13}\text{C}$  (a) or  $^{15}\text{N}$  (b) in D/L-Ala for Hog Island Bay (●, solid lines) and Isle of Wight Bay (○, dotted lines). Values are means  $\pm$  1 SE ( $n = 3$ ). Horizontal dashed gray lines represent the racemization background (0.017). Horizontal solid gray lines represent bacterial D/L-Ala abundance ratio (0.05). Values on the right y-axes correspond to estimates of bacterial contribution to total label incorporation

lower than in bulk and THAA pools, but followed a similar pattern. Label first appeared on Day 1 (HIB:  $0.77 \pm 0.03$  nmol  $^{13}\text{C}$  g DW $^{-1}$ ; IWB:  $2.14 \pm 1.34$  nmol  $^{13}\text{C}$  g DW $^{-1}$ ) and peaked on Day 7 or 14 (Fig. 5a). There were no lagoon effects, but excess  $^{13}\text{C}$  values were significantly lower on Days 1 and 2 than on Days 7 and 14 (Table 3, Fig. 5a). Averaged over the entire experiment, excess  $^{13}\text{C}$  in PLFAs accounted for  $\sim 3\%$  (range: 1.4 to 3.5%) of excess  $^{13}\text{C}$  in bulk sediments for both lagoons (Tables 3 & 4). Linear regressions of excess  $^{13}\text{C}$  in PLFAs vs. excess  $^{13}\text{C}$  in THAAs showed good agreement (HIB:  $r^2 = 0.72$ ,  $p = 0.001$ ; IWB:  $r^2 = 0.96$ ,  $p < 0.001$ ), suggesting that microbial biomarkers in each compound class tracked one another.

Excess  $^{13}\text{C}$  in groups of individual PLFAs provided additional information about the importance of specific sediment microbial pools in the cycling of macroalgal C and N. Excess  $^{13}\text{C}$  appeared in BrFAs, representative of heterotrophic bacterial biomass (Perry et al. 1979), on Day 1 for both bays (HIB:  $0.03 \pm 0.02$  nmol  $^{13}\text{C}$  g DW $^{-1}$ ; IWB:  $0.12 \pm 0.04$  nmol  $^{13}\text{C}$  g DW $^{-1}$ ) and peaked

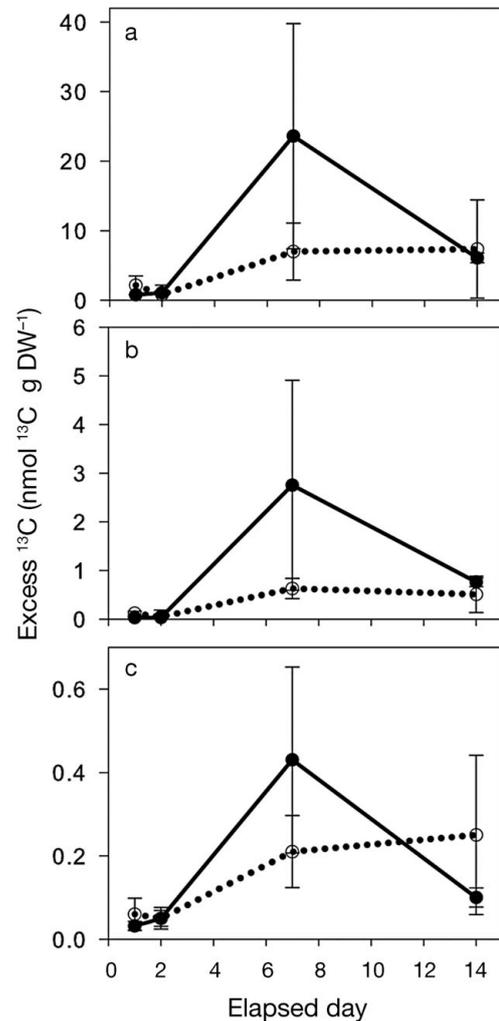


Fig. 5. Phospholipid-linked fatty acid (PLFA) isotopic enrichments for Hog Island Bay (●, solid lines) and Isle of Wight Bay (○, dotted lines). Values are means  $\pm$  1 SE ( $n = 3$ ) for excess  $^{13}\text{C}$  in (a) total PLFA, (b) branched odd fatty acids, which represent heterotrophic bacteria, and (c) polyunsaturated fatty acids, which represent algae

on Day 7 or 14 (Fig. 5b; Table 3). Values of excess  $^{13}\text{C}$  in BrFAs were linearly related to excess  $^{13}\text{C}$  in D-Ala, another bacterial-specific biomarker (HIB:  $r^2 = 0.95$ ,  $p < 0.0001$ ; IWB:  $r^2 = 0.93$ ,  $p < 0.0001$ ). Excess  $^{13}\text{C}$  also appeared in PUFAs, representative of algal biomass (Volkman et al. 1998), on Day 1 (HIB:  $0.03 \pm 0.01$  nmol  $^{13}\text{C}$  g DW $^{-1}$ ; IWB:  $0.06 \pm 0.04$  nmol  $^{13}\text{C}$  g DW $^{-1}$ ) and peaked on Day 7 or 14 (Fig. 5c, Table 3). Overall,  $^{13}\text{C}$  enrichments in PUFAs were lower than for BrFAs. Linear regressions of excess  $^{13}\text{C}$  in BrFAs and PUFAs suggested that this trend was consistent across all time points and the fatty acid groups showed good agreement (HIB:  $r^2 = 0.92$ ,  $p < 0.0001$ ; IWB:  $r^2 = 0.86$ ,  $p < 0.0001$ ).

## DISCUSSION

We conducted this experiment to simulate the die-off of a macroalgal bloom, an annual event common to many coastal lagoons worldwide (Sfriso et al. 1992, Valiela et al. 1997). We selected field sites for collecting sediment and macroalgae within 2 lagoons representative of coastal bays along the US East Coast. Both sites have sandy sediments, are similar in water depth (~1 m deep at mean low water), and are exposed to similar light levels. Although the organic content of the sediments differed significantly between the bays, the values still fell within a relatively narrow range of 0.6 to 3.8%, typical of many coastal bays. Differences in benthic chl *a* concentrations in the mesocosms, which were consistent with differences in the field, were more pronounced than organic content and likely influenced the processing of macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$  in the sediments.

Since this experiment investigated the fate of dead macroalgae, we freeze-dried the macroalgae prior to adding it to the mesocosms. Although previous macroalgal decomposition studies have used frozen macroalgae (Buchsbbaum et al. 1991, Nedergaard et al. 2002, Castaldelli et al. 2003, García-Robledo et al. 2008), we were unable to use frozen macroalgae for logistical reasons (i.e. lack of a freezer at our remote field station). Other studies have used buried live macroalgae (Franke et al. 2006, Rossi 2007); we did not feel that burial of the algae in the sediments adequately represented the natural die-off process, which occurs at the sediment–water interface. Numerous phytoplankton fate studies have used freeze-dried material (Moodley et al. 2000, Aberle & Witte 2003, Witte et al. 2003). After 1 wk, macroalgae were no longer visible in our mesocosms, indicating that all of the macroalgae was decomposed, remineralized, respired, or fragmented into particle sizes too small to see (either in the water column or sediments), so we are confident that freeze-drying was appropriate for the purposes of tracking macroalgal C and N into the sediments.

### Label incorporation into bulk sediments

Previous studies have shown that some decomposition of macroalgae occurs in the water column, resulting in release of inorganic and organic nutrients, consumption of dissolved oxygen, and release of toxic sulfides. These processes have been associated with phytoplankton blooms, fish and other faunal kills, as well as seagrass declines (Buchsbbaum et al. 1991, Sfriso et al. 1992, Hauxwell et al. 2001, Nedergaard et al. 2002, Cummins et al. 2004). The fate of macroalgal biomass deposited to the sediments, however, is less

clear. Our bulk sediment isotope data suggest that a fraction of macroalgal-derived  $^{13}\text{C}$  and  $^{15}\text{N}$  appeared in the sediments; however, a smaller percentage of added label appeared in IWB sediments than in HIB sediments, and less  $^{13}\text{C}$  appeared than  $^{15}\text{N}$  for both lagoons. In HIB, maximum values of ~6 and 35% of macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$  were observed in the sediments. In IWB, ~3 and 9% of macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$  were incorporated into the sediments. We were careful not to include visible macroalgal fragments in our samples, so labeling in the sediments should have represented either detrital (macroalgal) particulate or dissolved organic matter (DOM) or transfer of  $^{13}\text{C}$  and  $^{15}\text{N}$  to other active pools within the sediments, rather than direct sampling of macroalgal fragments. This is further supported by the uncoupling of  $^{13}\text{C}$  and  $^{15}\text{N}$  transferred to the sediments, which suggests that macroalgal C and N were transferred disproportionately. We believe that these stoichiometric and lagoonal differences in C and N transfer were related to microbial processes, as discussed below.

Sediments showed immediate enrichment in macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$  (Day 1), followed by an increase for 1 to 2 wk. While the general trends were similar, replicates were variable suggesting heterogeneity within and/or across mesocosms. Some showed highest enrichments on Day 7; others did not peak until Day 14. This variability likely resulted from patchiness associated with the bloom deposition in our mesocosms or heterogeneity in the sediment microbial communities. Similar patchiness has been observed in field studies (Holmquist 1997, Sfriso & Marcomini 1999, McGlathery et al. 2001). Despite the observed variability, our statistical analyses confirmed that bulk isotopic enrichments at the end of the experiment (Days 7 & 14) were higher than at the beginning of the experiment (Days 1 & 2) for both lagoons, demonstrating accumulation and retention of macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$  in the sediments following the simulated die-off.

Patterns of  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment in THAAs and PLFAs tracked the bulk sediment enrichments. These pools showed immediate isotopic enrichment on Day 1 and peak enrichments on Days 7 or 14. The labeled THAAs and PLFAs in sediments collected on Days 1 and 2 likely represented rapid uptake by microbes, although some microscopic macroalgal detritus may have remained after removal of all visible fragments. The isotopically labeled PLFAs observed in the sediments on Days 7 and 14 most likely represented living microbial biomass given rapid rates of PLFAs turnover (Parkes 1987). THAAs on Days 7 and 14 may have included macroalgal detritus, but we observed no visible fragments of macroalgae by then, suggesting that the biomass had been decomposed.

### Bacterial incorporation of macroalgal label

Overall, a smaller fraction of macroalgal  $^{13}\text{C}$  appeared in the bulk sediments than  $^{15}\text{N}$  for both lagoons. This is consistent with expected preferential uptake of N by the microbial community since N is typically the element limiting microbial production and, therefore, is likely to be taken up efficiently, while C is relatively easily lost by respiration (Kirchman 2000, Howarth & Marino 2006). More directly, the isotopic enrichments of specific amino acids and fatty acids provided an explicit indication of the communities in the sediments that were taking up macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$ . Enrichment of D-Ala and BrFAs clearly demonstrated transfer of macroalgal label to bacterial biomass through decomposition. The correlations between excess  $^{13}\text{C}$  in D-Ala and BrFAs provided strong evidence for bacterial  $^{13}\text{C}$  uptake, corroborating their use as 2 independent proxies for bacterial biomass. Appearance of the labels on Day 1 showed an immediate response by bacteria to the addition of fresh OM. This rapid response has been shown in previous studies investigating decomposition of macroalgae (Buchsbaum et al. 1991, Nedergaard et al. 2002, Castaldelli et al. 2003, Franke et al. 2006). For example, just minutes after macroalgae were added to sediments, Franke et al. (2006) measured oxygen consumption rates nearly 18 times higher in sediments with macroalgae than in nearby sediments without macroalgae. While others have investigated metabolic responses to the addition of macroalgae, the present study is the first to demonstrate explicitly the incorporation of macroalgal C and N into sediment bacterial biomass following a simulated die-off event.

Not only did the bacteria in the present study respond rapidly to the addition of macroalgal biomass, but the bacterial biomarkers also showed prolonged enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  throughout the 2 wk experiment. The prolonged enrichment of bacterial biomarkers may reflect incorporation into a refractory pool after cell death, which accumulated in the sediments. However, the PLFAs were not likely part of the detrital pool given the rapid turnover of PLFAs after cell death (Parkes 1987, Veuger et al. 2006), and at the time scale of our experiment, we believe accumulation of labeled detrital (bacterial) amino acids was negligible (Veuger et al. 2006). Instead, we believe the continued enrichment of the bacterial biomarkers on Days 7 and 14 is consistent with microbially mediated decomposition of macroalgae. The isotopic labels could persist even longer in the sediments if recycling of macroalgal detritus by bacteria was coupled with BMA metabolism (see 'Bacterial-BMA coupling'). Regardless of the mechanism(s) responsible for the high isotopic enrichments in the bacterial pool, macroalgal  $^{13}\text{C}$

and  $^{15}\text{N}$  clearly persisted in the sediments for at least 2 wk following the simulated die-off.

Interestingly, there were no significant between-lagoon differences in excess isotope levels in bulk sediments, THAAs, or PLFAs, even though twice as much dead macroalgae was added to the IWB mesocosms. We believe that the lower relative enrichments in IWB sediments may be explained by slower decomposition rates. The rates and extent of decomposition are influenced by a variety of environmental, biological, and physical factors (Wakeham & Canuel 2006). In addition to differences in the amount of macroalgal biomass added to the treatments, the distinguishing characteristic of the lagoon treatments was the sediments and, by default, perhaps the microbial communities within the sediments. It is possible that the IWB sediment bacteria that processed the macroalgal biomass may have been limited in a way that the HIB sediment bacteria were not. For example, some studies have determined hydrolysis to be the rate-limiting step in decomposition (Arnosti 2004 and references therein), either because hydrolysis rates are slow or because the organism required to produce a specific enzyme to break down specific macromolecules is absent or in low abundance. Regardless of the mechanism responsible for limiting retention of macroalgal  $^{13}\text{C}$  and  $^{15}\text{N}$  in IWB sediments, this difference could profoundly affect system-wide nutrient cycling. Since macroalgae in both lagoon treatments had visibly disappeared within days following the additions, more macroalgal decomposition likely took place in the IWB water column, which could promote phytoplankton growth and further eutrophication of the system. We measured elevated water column concentrations of  $\text{NH}_4^+$ , dissolved inorganic phosphorus (DIP), and dissolved organic N in mesocosms from both lagoons following addition of macroalgae (data not shown), indicating remineralization of macroalgal detritus in the water column. DIP concentrations were significantly higher in IWB than HIB, possibly reflecting higher rates of remineralization there in response to higher macroalgal biomass. Our results corroborate numerous observations in natural systems demonstrating elevated concentrations of dissolved inorganic and organic nutrients from decomposition of macroalgal material in the water column following a die-off (e.g. Sfriso et al. 1992, Hauxwell et al. 2001, Tyler et al. 2001), but our findings further demonstrate that a fraction of macroalgal detritus is also decomposed and retained within the sediments.

### Bacterial-BMA coupling

The ratio of isotopic enrichments in D/L-Ala provides information on the relative contribution of bacteria and



stored (temporarily) as microbial biomass. Even though macroalgal distributions may be patchy, the work of Franke et al. (2006) has shown that the effects of a macroalgal die-off may be expansive in the sediments of some systems. In their study, macroalgal DOM was distributed well beyond the deposition location in systems that experienced advective flow, fueling heterotrophic bacteria throughout the sediments.

### Summary and implications for eutrophied systems

The extent to which sediments act as a sink for macroalgal C and N depends largely on the amount of biomass transferred to the sediments as well as recycling processes within the sediments. In our experiment, less than half of the macroalgae was incorporated into the sediments from HIB, and even less for sediments from IWB. The isolation of our mesocosms from the hydrodynamic regime typically found in the environment may have biased the amount of macroalgal biomass that was transferred to the sediments relative to what occurs in the environment. On the one hand, we may have overestimated transfer of macroalgal material to the sediments if wave and tidal action disperse the macroalgal detritus and decrease deposition onto the sediment surface. On the other hand, our estimate may have been conservative if hydrodynamic mixing of the sediment surface entrains macroalgae into the sediments. In either case, it is clear from other laboratory and field studies that some fraction of macroalgae associated with blooms is transferred to the sediments following die-off (Sfriso et al. 1992, Lomstein et al. 2006, García-Robledo et al. 2008). We suggest that further research investigating the influence of hydrodynamic forcings and sediment resuspension on macroalgal decomposition in the sediments is warranted. Nevertheless, the present study suggests that uptake and recycling of C and N by BMA and bacteria within the sediments may serve as a temporary reservoir for a fraction of the C and N that was previously stored as macroalgal biomass. While bacteria are the primary agents of macroalgae decomposition, BMA intercept the return of nutrients to the water column, thereby diminishing phytoplankton uptake and the positive feedback to further eutrophication.

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