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Carbon and nitrogen dynamics in shallow photic systems: Interactions between macroalgae, microalgae, and bacteria

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Abstract

We tracked carbon (C) and nitrogen (N) uptake into sediments in the presence and absence of benthic macroalgae using dual stable isotope tracers in combination with compound-specific isotope analyses of hydrolyzable amino acids and phospholipid-linked fatty acids to quantify the uptake and retention of C and N within bulk sediments, benthic microalgae (BMA), and heterotrophic bacteria. Stable isotope tracers (as $^{15}NH_4^+$ and $H^{13}CO_3^-$) were added to mesocosms either via the surface water or pore water for the first 14 d of the 42-d experiment. Macroalgae and sediments exposed to ambient light and dark cycles rapidly took up label from both sources and retained label for at least 4 weeks after isotope additions ended. BMA dominated sediment uptake of ^{13}C and ^{15}N, initially accounting for 100% of total uptake. Over time, heterotrophic bacterial uptake became relatively more important, increasing from 0% on day 1 to 20–50% on day 42, indicating a close coupling between BMA and bacterial production. In treatments with macroalgae, sediment ^{13}C and ^{15}N uptake was ~ 40% lower than treatments without macroalgae, likely because of shading of the sediment surface by macroalgae, which decreased BMA production, which in turn decreased bacterial production. Overall, sediments served as a sink for C and N through uptake and retention by the microbial community, but retention was lower in the presence of macroalgae.

In shallow coastal systems where the majority of the sediment surface exists within the euphotic zone, benthic primary producers such as macrophytes, macroalgae, and benthic microalgae (BMA) often dominate nutrient cycling dynamics directly through uptake and immobilization or indirectly by altering the chemical and physical environment (McGlathery et al. 2004; Pedersen et al. 2004). Coastal bays are particularly vulnerable to nutrient enrichment because of their position along the coast, where human populations and associated anthropogenic nutrient loadings are rapidly increasing (NRC 2000). Numerous studies suggest that increased nutrient loading to shallow coastal systems may result in shifts in autotrophic community structure, but related shifts in biogeochemical cycles are less clear.

Macroalgal blooms represent a symptom of eutrophication in shallow systems worldwide (Sfriso et al. 1992; Hauxwell et al. 2001). The deleterious effects of macroalgae have been studied extensively and include replacement of seagrass (Hauxwell et al. 2001) and decreased diversity and biomass within the faunal and fish communities (Wennhage and Pihl 2007), which may translate to decreased food availability for upper trophic levels (Raffaelli 2000). These blooms have also been shown to affect biogeochemical cycling. For example, macroalgae directly affect nutrient cycles by immobilizing nutrients, often in excess of their growth demands (Peckol et al. 1994). Indeed, in eutrophied systems with large amounts of macroalgal biomass, water quality often appears good because macroalgae are so efficient at removing nutrients from the water column (Valiela et al. 1997).

Because they reside at the sediment surface, macroalgae also have the potential to influence nutrient cycling at the sediment–water interface, a zone of intense biogeochemical activity mediated by autotrophic and heterotrophic microbes. However, to date, few studies have focused on the effect of macroalgae on the sediment microbial community because of the methodological challenges inherent in studying microbial communities in a sediment matrix. Benthic flux studies have revealed that macroalgae play a major role in regulating nutrient cycling at the sediment surface. For example, McGlathery et al. (2001) used dissolved inorganic carbon (DIC) fluxes to document that BMA production increased after a macroalgal die-off, suggesting competition between macroalgae and BMA for light, nutrients, or both. Tyler et al. (2003) found that macroalgae uncoupled sediment–water column processes by controlling the exchange of dissolved inorganic and organic nitrogen between the sediments and water column. Dalsgaard (2003) measured lower denitrification rates in the presence of macroalgae, presumably because macroalgae outcompeted sediment denitrifiers for water column nitrate. Although benthic fluxes such as these have been able to generate information about the net results of processes occurring at the sediment–water interface, it has been challenging to further describe the microbial ''black box'' within the sediments using flux data alone.

To address this gap, we used a novel dual stable isotope tracer approach combined with compound-specific isotope analyses of microbial biomarkers to track explicitly both

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carbon (C) and nitrogen (N) uptake into sediment microbes (BMA, heterotrophic bacteria) and bulk sediments in the presence and absence of macroalgae. Because benthic autotrophs may use nutrients from both the water column and sediment pore water, we designed an experimental apparatus that allowed introduction of dissolved nutrients via surface water or pore water so that we could assess differences in uptake by the algal communities.

Methods

Site description—Sandy sediments (84% sand; 24% water) and macroalgae (*Gracilaria vermiculophylla*) were collected from Hog Island Bay, Virginia (HIB; Fig. 1), located in the Virginia Coast Reserve, a Long-Term Ecological Research site. HIB is a shallow coastal lagoon (< 2 m deep at mean low water [MLW]), typical of temperate lagoons along the U.S. East Coast and is dominated by benthic autotrophs (McGlathery et al. 2001; Thomsen et al. 2006). We collected sediments and macroalgae from a midlagoon shoal site where localized blooms of macroalgae have previously developed and dominated benthic production during the warmer months. Throughout the rest of the year when macroalgal biomass is low, BMA dominate (McGlathery et al. 2001; Anderson et al. 2003; Thomsen et al. 2006).

Experimental design—A flow-through outdoor mesocosm array was assembled at the Virginia Institute of Marine Science Eastern Shore Laboratory (ESL). In preparation for this experiment, we designed and tested an experimental apparatus that allowed for addition of nutrients simultaneously via surface water (SW) and pore water (PW). The "perfusionator" consisted of a 60-cm inner diameter (i.d.) × 60-cm-high translucent fiberglass cylinder that includes a reservoir for PW at the base of the sediment column (Fig. 2). Discussion of the design and performance of the perfusionator can be found in Hardison et al. (2011). Twelve perfusionators were filled to a depth of ~ 15 cm with intact sediments extruded from cores (13.3 cm i.d.) taken from the field site ("shoal"; < 1 m MLW) in May 2007 (Fig. 1). Care was taken not to transfer any macroalgae to the mesocosms. At the ESL, the perfusionators were placed in shallow water baths under shade cloth (30% light attenuation) to control temperature and light. The water column above the sediments was connected to a flow-through seawater system, supplied with filtered seawater from the adjacent creek, and was stirred continuously with a mini–jet pump to keep the water column well mixed. Once connected to the seawater system, the mesocosms were left to equilibrate for 2 weeks before beginning the experiment.

Our experiment consisted of an incomplete factorial design made up of three factors, each with two levels (Fig. 2): (1) light (ambient vs. dark), (2) isotope delivery source (via the SW or PW), and (3) macroalgae (presence vs. absence of live macroalgae). All factors were crossed except the dark plus macroalgae treatment because, for logistical purposes, only light treatments received a macroalgal addition. Dark treatments were used to exclude light-dependent isotopic incorporation. Each treatment was run in duplicate.

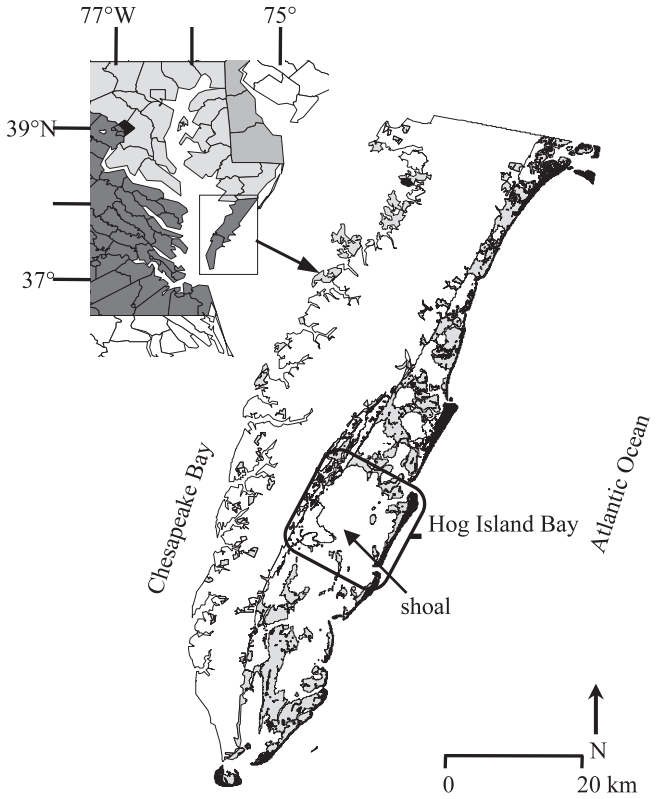

Fig. 1. Sediments and macroalgae were collected from a midlagoon shoal site in Hog Island Bay, Virginia, located along the Delmarva Peninsula, U.S.A. Hog Island Bay is part of the Virginia Coast Reserve, a Long-Term Ecological Research (LTER) site. Figure adapted from Anderson et al. (2010).

For the nutrient additions, C and N were added to each mesocosm simultaneously via the SW and PW. However, for each treatment, *isotopically labeled* C and N were only delivered via one source (i.e., for the PW treatments, isotopically labeled nutrients were added through the PW and unlabeled nutrients were added through the SW, and vice versa for the SW treatments; Fig. 2). All feed water was drawn from a creek adjacent to the ESL, pumped through a series of sand, bag (10 μm), and cartridge (5 and 1 μm) filters to remove particulate material, and exposed to ultraviolet light to kill bacteria. The filtered feed water was amended either with labeled ($H^{13}CO_3^- + {}^{15}NH_4^+$) or unlabeled ($H^{12}CO_3^- + {}^{14}NH_4^+$) nutrients in a mixing chamber before delivery to each perfusionator. Nutrients were added to either the SW or PW feed lines using a high-precision metering pump. For the SW treatments, $(^{15}NH_4)_2SO_4$ (25 atom % [at%] ^{15}N) and $NaH^{13}CO_3$ (99 at% ^{13}C) solutions were added to the SW feed line, with a target isotopic enrichment of the NH_4^+–N pool of 25 at% and DIC of 9 at%. Surface water NH_4^+ concentrations were ~ 25 μmol L^{-1} above background (2–4 μmol L^{-1}), and DIC concentrations were increased by ~ 9%. For the PW treatments, $(^{15}NH_4)_2SO_4$ (50 at% ^{15}N) and $NaH^{13}CO_3$ (99 at% ^{13}C) were metered into the PW feed line to achieve

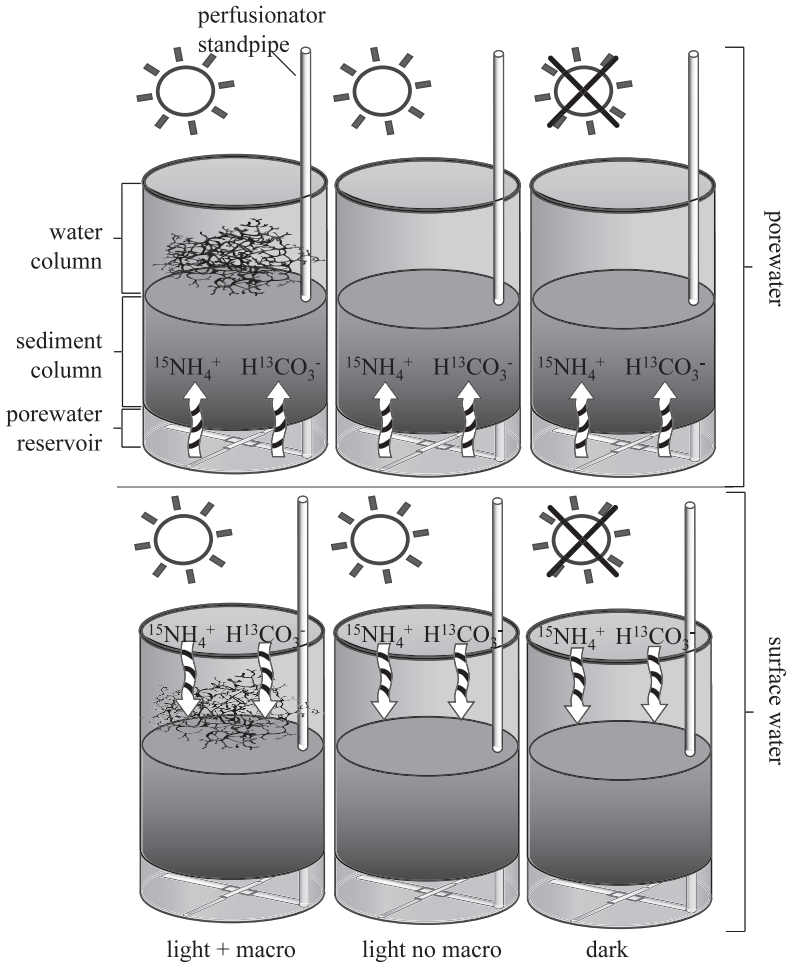

Fig. 2. Experimental design: isotopes were delivered either through the pore water (PW) or surface water (SW), and mesocosms were exposed to ambient light or kept dark. Two light treatments received a live macroalga (*Gracilaria vermiculophylla*) addition. $n = 2$ for six treatments. The perfusionators are translucent fiberglass cylinders that contain a pore water reservoir beneath the sediment column. Amended pore water is introduced to the reservoir via a stand pipe (Hardison et al. 2011).

30 at% ^{15}N enrichment of NH_4^+–N and 9 at% ^{13}C enrichment of DIC in sediment PW. NH_4^+ and DIC were added to achieve PW concentrations of $\sim$ 200 μmol L^{-1} and $\sim$ 2.5 mmol L^{-1}, respectively, reflecting concentrations in the natural pore water that was being replaced. Unlabeled nutrients were added at the same rates as the isotopically labeled nutrients to the corresponding mesocosms. Nutrient-amended feed water was delivered directly to the perfusionator water column gravimetrically at a rate of $\sim$ 43 L d^{-1}, or a SW residence time of $\sim$ 2 d. Fine-scale control of the SW flow rate at each mesocosm was achieved using intravenous (IV) drippers, which were calibrated daily. PW additions were delivered through a standpipe into the perfusionator reservoir located below the sediment column (Fig. 2) at a rate of $\sim$ 15 L d^{-1}, or a PW residence time of $\sim$ 1.8 d. Fine-scale control of the PW flow rate into each perfusionator was achieved using an IV dripper located at each standpipe, which was also calibrated daily. Isotopes were added for the first 14 d of the 42-d experiment. For the remainder of the experiment (i.e., the "postlabeling" period), unlabeled nutrients were added via the SW and PW for all treatments.

Macroalgae collected live from HIB in May 2007 were returned to the laboratory, cleaned of epiphytes and epifauna, rinsed with 0.7-μm filtered seawater, and placed in aquaria in a greenhouse. Filtered (0.7-μm) seawater was added to each aquarium and kept aerated while the algae were starved for 10 d to ensure depletion of internal stored nutrients and rapid uptake of nutrients once in the mesocosms. Live macroalgae were added to the light plus macro treatments in densities observed naturally (124.8 $\pm$ 1.6 g dry weight [dry wt] m^{-2}; Thomsen et al. 2006; Hardison et al. 2010).

Sampling—Nutrient, isotope, and macroalgal additions began on day 0, and isotopes were added through day 14. The mesocosms were sampled before the additions to capture baseline conditions on days 1, 3, 7, and 14 during the isotope-labeling period and on days 16, 21, 29, and 42 during the postlabeling period. At each sampling, surface

sediments (0–1 cm) were collected with the use of two acrylic cores (5.7 cm i.d.) and reserved for bulk (total organic C [TOC], total N [TN]), amino acid, and fatty acid analyses. Sediments from both cores were combined in precombusted glass jars, immediately frozen at −20°C, and frozen at −80°C within 3 d. The remaining sediment in the cores was placed carefully back into the holes in the mesocosm sediments. Sediments (0–1 cm) were also collected for chlorophyll *a* (Chl *a*) concentrations using a cut-off syringe (1.1 cm i.d.). Samples were placed in 15-mL centrifuge tubes, immediately frozen at −20°C, and analyzed within 1 month. A different region of the sediment surface was sampled each day to avoid artifacts associated with resampling any sediments. Each sampling removed ~ 52 cm^2 of the sediment surface, which summed to 15% of the sediment surface over 8 sample days.

Macroalgae were removed from each mesocosm, patted dry, and weighed on days 7, 14, 21, 29, and 42. Wet mass was converted to dry weight using percent water (72%) determined from *G. vermiculophylla* collected in the field, and dry weight values were normalized to the mesocosm sediment surface area (0.29 m^2). Before addition to the mesocosms, and when weighed for determination of growth, a small piece of macroalgal biomass was removed and reserved at −20°C for isotopic analysis.

Bulk analyses—Samples were analyzed for benthic Chl *a* concentrations according to a modification of the method of Lorenzen (1967; Pinckney et al. 1994). The sediment pellet was sonicated in 90% acetone, vortexed, and extracted for 24 h at −20°C. The supernatant was passed through a 0.45-μm filter and read on a Shimadzu Ultraviolet (UV)-1601 UV Visible spectrophotometer (λ = 665, 750 nm). Chl *a* concentrations (mg m^{-2}) were calculated according to the equations in Lorenzen (1967).

For bulk sediment TOC, TN, and isotopic measurements, sediments were freeze-dried, ground, acidified to remove inorganic C, and analyzed for ^{13}C and ^{15}N with an elemental analyzer coupled to a Thermo Delta V Plus isotope ratio mass spectrometer (EA-IRMS). Macroalgae were dried at 40°C, ground, and also analyzed for ^{13}C and ^{15}N. Isotopic enrichments were measured as "delta" (δ) values,

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

where X = ^{13}C or ^{15}N, and R is the ratio of heavy to light isotope. The $\delta^{13}C$ and $\delta^{15}N$ values were expressed relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2. δX was converted to at% X, which was used to calculate excess X (i.e., the mass of ^{13}C or ^{15}N in excess of natural abundance),

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

$$\text{excess } X\ \left[\text{nmol } X(\text{g dry wt})^{-1}\right] = \left[\left(\text{at\% } X_{\text{sample}} - \text{at\% } X_{\text{control}}\right)/100\right] \times \text{concentration}_{\text{sample}} \qquad (3)$$

where concentrations are moles C or N relative to sediment or macroalgal dry weight (g dry wt). Control (unlabeled) samples were collected before the isotopic additions.

Total hydrolyzable amino acids—Hydrolyzable amino acids (HAA) were analyzed on a subset of sediment samples according to the method presented in Veuger et al. (2005). Briefly, freeze-dried sediment (1 g) was rinsed with 2 mol L^{-1} HCl and Milli-Q water, and the sediment pellet was hydrolyzed with 6 mol L^{-1} HCl at 110°C for 20 h. After 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 and N of the derivatized D- and L-amino acids were measured by gas chromatography combustion IRMS (GC-c-IRMS) on a Hewlett Packard 6890 GC with a Thermo type III combustion interface and a Thermo Delta Plus IRMS. δX, at% X, and excess X values were calculated (Eqs. 1–3), where concentration was amino acid concentration expressed in moles C or N relative to sediment dry weight. Carbon isotopic values of amino acids were corrected for C atoms added during derivatization using a mass balance approach. The sum of concentrations of, the excess label incorporated in, or both amounts of all amino acids analyzed will be referred to as total hydrolyzable amino acids (THAA). The ratio of excess ^{13}C or ^{15}N incorporation into D-alanine (D-Ala), a bacteria-specific amino acid, relative to L-alanine (L-Ala), a common amino acid in all organisms, was calculated as:

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

During hydrolysis, some racemization of L-Ala to D-Ala takes place. This typically results in a D:L-Ala ratio of 0.015–0.02 (Veuger et al. 2007*b*). For the present study, we used 0.014, which corresponds to the average ^{13}C D:L-Ala value for SW during the first few days of the experiment. We corrected values of excess isotope in D-Ala for this racemization according to Veuger et al. (2007*a*), whereas values of D:L-Ala were left uncorrected. Instead, the D:L-Ala racemization background of 0.014 will be indicated graphically in our results. We estimated the bacterial contribution to total ^{13}C or ^{15}N incorporation according to Veuger et al. (2007*b*):

$$\text{Bacterial contribution (\%)} = [(\text{excess } X \text{ D : L-Ala} - 0.014)/(\text{bacterial D : L-Ala} - 0.014)] \times 100\% \qquad (5)$$

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 bacteria to 0.1 for Gram positive (G+) bacteria and cyanobacteria (Veuger et al. 2007*b*). Previous work suggests that G+ bacteria are more prominent in deeper (anaerobic) sediments (Moriarty and Hayward 1982; Gontang et al. 2007). Because our study used sandy, photic sediments, we assumed the contribution from G+

bacteria to be negligible. Additionally, photosynthetic pigment analyses obtained by high-performance liquid chromatography (HPLC) of sediments from the mesocosms showed low zeaxanthin : chlorophyll ratios, suggesting that cyanobacterial contributions to the microbial community, and to the D : L-Ala ratio, were minimal (M. Waters pers. comm.), so we estimated the bacterial D : L-Ala ratio for our sediments to be 0.05. This will also be indicated graphically in our results.

Phospholipid-linked fatty acids—Total fatty acids were analyzed on a subset of sediment samples according to a modified Bligh and Dyer (1959) method (Poerschmann and Carlson 2006). Wet sediments (~ 12 g) were extracted using an accelerated solvent extractor system (Dionex ASE 200) adapted for in-cell silica gel chromatography. Each sample was extracted twice on the ASE: neutral lipids were collected after extraction with 9 : 1 (v : v) hexane : acetone at 50°C, then polar lipids were collected after extraction with 8 : 2 (v : v) methanol : chloroform at 80°C. The polar lipid fraction was saponified using KOH–CH_3OH for 2 h at 110°C then extracted under basic and acidic conditions. The acid-extracted fractions were methylated with BF_3–CH_3OH to form fatty acid methyl esters (FAMEs). Polar FAMEs represented the phospholipid-linked fatty acids (PLFAs). PLFA concentrations were measured by GC 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 and coupled GC mass spectrometry (HP 6890 GC-MSD). Stable C isotope ratios for PLFA were measured at the University of California Davis Stable Isotope Facility using a Thermo GC-c-IRMS system composed of a Trace GC Ultra GC (DB-5 column) coupled to a Delta Plus Advantage IRMS through a GC-c-III interface. These isotope values were used to calculate $\delta^{13}C$, at% ^{13}C, and excess ^{13}C (Eqs. 1–3), where concentrations were FAME concentrations expressed in moles C relative to dry weight sediment. Actual PLFA isotopic values were derived from the FAME isotopic compositions by correcting for the $\delta^{13}C$ of the C added during derivatization using a mass balance approach.

We analyzed ^{13}C uptake into total PLFAs and specific groups of PLFAs. Excess ^{13}C in polyunsaturated fatty acids (PUFA: $C_{20:5\omega3}$, $C_{20:4\omega6}$, $C_{22:5\omega3}$, $C_{22:5\omega6}$) represented uptake into BMA biomass (Volkman et al. 1998), whereas excess ^{13}C in branched odd fatty acids (BrFA: iso- and anteiso-branched $C_{13:0}$, $C_{15:0}$, $C_{17:0}$, $C_{19:0}$) represented heterotrophic bacterial uptake (Boschker et al. 2000). The ratio of excess ^{13}C in BrFA relative to the sum of BrFA and PUFA (bacteria-to-algae ratio, BAR) was calculated as:

$$\mathrm{BAR} = \left(\text{excess } ^{13}\mathrm{C} \text{ in BrFA}\right) / \left(\text{excess } ^{13}\mathrm{C} \text{ in BrFA} + \text{excess } ^{13}\mathrm{C} \text{ in PUFA}\right) \quad (6)$$

This ratio ranges from 0, which represents 100% BMA (0% bacterial) uptake, to 1, or 0% BMA (100% bacterial) uptake of label.

Data analysis—We applied repeated measures analysis of variance (ANOVA) to examine the effects of isotope delivery source (PW vs. SW), light (ambient vs. dark), macroalgae (presence vs. absence), and time (days) 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. Unless otherwise noted, values presented are means ± 1 SE for duplicates.

Results

Analysis of isotopic enrichments of PW and SW DIC and NH_4^+ (data not shown) confirmed that isotope delivery differed between SW and PW treatments. Among treatments receiving delivery from either SW or PW, there were no systematic differences in isotope delivery to mesocosms, suggesting that source enrichments were consistent between treatments.

Macroalgae and bulk sediments—Macroalgal growth was nearly linear throughout the experiment, increasing from 125 g dry wt m^{-2} on day 0 to 308 and 513 g dry wt m^{-2} on day 42 for SW and PW, respectively (Fig. 3a). This represented an average growth rate of 5–6% d^{-1}. There was no significant difference in macroalgal biomass between SW and PW treatments throughout the experiment. For both SW and PW treatments, excess ^{13}C in macroalgae increased throughout the labeling period, peaked on day 14 or 21, and decreased through day 42 (Fig. 3b). There was no significant difference in excess ^{13}C between isotope delivery sources (Table 1), although there was a trend of SW values exceeding PW values (Fig. 3b). Excess ^{15}N in macroalgae also became enriched throughout the labeling period, peaked on day 21, and decreased through day 42 (Fig. 3c). Again, there was no significant isotope source difference, although there was a trend of PW values generally exceeding SW values.

Averaged across time, TOC and TN concentrations in sediment were 248 ± 13 μmol C (g dry wt)$^{-1}$ and 23 ± 1 μmol N (g dry wt)$^{-1}$, respectively (SE; $n = 36$ treatment means). All treatments began with similar benthic Chl *a* content (14.8 ± 4.5 mg Chl *a* m^{-2}); however, throughout the experiment, benthic Chl *a* concentrations in ambient light mesocosms increased significantly relative to dark mesocosms (Fig. 4; Table 1). There was no significant macroalga effect among light treatments. Overall, benthic Chl *a* increased in the light treatments, although with high variability.

In both SW and PW treatments, sediments in the ambient light treatments were more enriched than the dark treatments (Figs. 5a,b, 6a,b; Table 1), reaching levels well above natural abundance (max $\delta^{13}C \sim 2000‰$ and $\delta^{15}N \sim 20{,}000‰$ vs. background $\delta^{13}C \sim -20‰$ and $\delta^{15}N \sim 10‰$). Excess ^{13}C in light treatments increased during the labeling period, peaked during the postlabeling period on day 21, then decreased through day 42 (Fig. 6a,b). Among the light treatments, excess ^{13}C in treatments with macroalgae was significantly lower than in treatments without macroalgae (Fig. 6a,b; Table 1). The same patterns were observed for

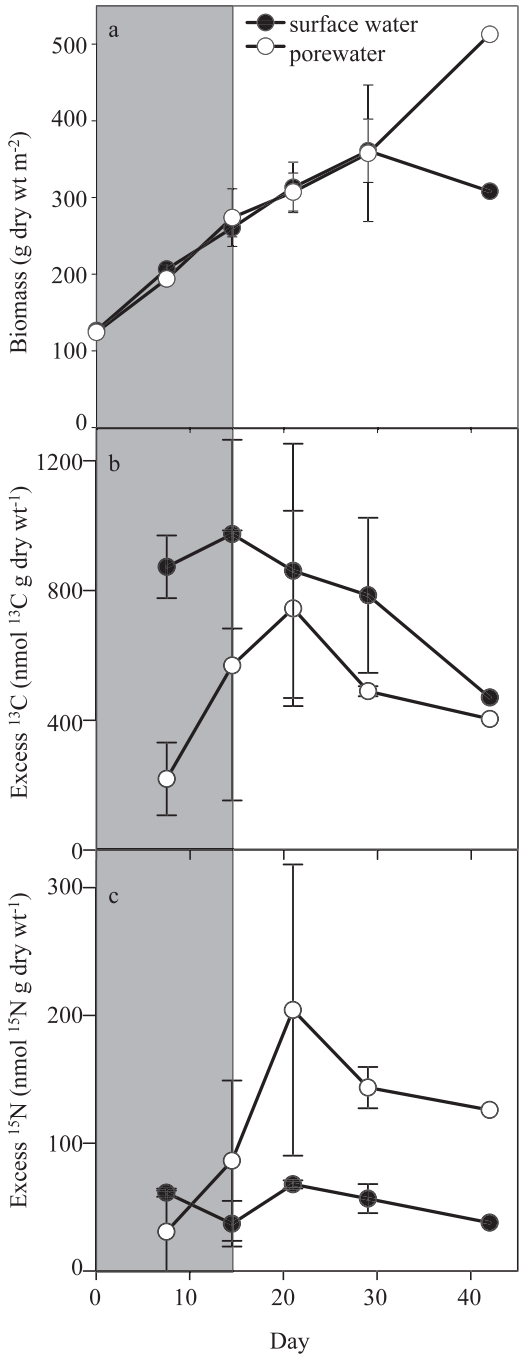

Fig. 3. (a) Macroalgal biomass and (b) excess ^{13}C and (c) ^{15}N in surface water (filled symbols) and pore water (open symbols) treatments. The grey shaded area indicates the isotope addition period. Values are mean ± SE ($n = 2$).

excess ^{15}N in both SW and PW treatments: light treatments were more enriched than dark treatments, and treatments with macroalgae were less enriched than treatments without macroalgae (Fig. 5a,b; Table 1). For ^{13}C and ^{15}N, we calculated uptake rates (nmol X [g dry wt]$^{-1}$ d^{-1}) during the labeling period as the slopes of excess label (X = ^{13}C or ^{15}N) from days 1 through 21, when the highest enrichments were measured. Similarly, we calculated loss rates (nmol X [g dry wt]$^{-1}$ d^{-1}) during the postlabeling period as the slopes of excess label from days 21 through 42 (Table 2). Uptake rates were higher for light treatments than dark. Within a treatment, rates of ^{13}C and ^{15}N uptake into bulk sediments during the labeling period generally exceeded loss rates, which were often small or not significantly different from zero ($p > 0.05$). Uptake rates were highest for light treatments without macroalgae.

PLFA—Across all sampling days, PLFA made up a constant fraction of TOC: 1.1% ± 0.3% and 0.3% ± 0.1% of TOC across light and dark treatments, respectively (n = 20 treatment means for light; $n = 10$ for dark). Excess ^{13}C in total PLFA followed patterns similar to bulk sediments (Fig. 6g,h). In both SW and PW treatments, ambient light treatments were more enriched than the dark (Table 1). Among the light treatments, excess ^{13}C in mesocosms with macroalgae was significantly lower than treatments without macroalgae (Table 1). As with the bulk sediments, we calculated uptake and loss rates of ^{13}C–PLFA (Table 2). Most uptake and loss rates for PLFA were not significantly different from zero ($p > 0.05$) because of high variability between replicates.

Excess ^{13}C in specific groups of fatty acids provided insight into the sediment microbial groups responsible for the label incorporation. Excess ^{13}C in PUFA, which represented BMA uptake, showed patterns similar to total PLFA, displaying both light and macroalga effects (Fig. 6i,j; Table 1). Excess ^{13}C in BrFA, which represented bacterial uptake, also showed patterns similar to total PLFA (Fig. 6k,l). In both SW and PW treatments, light treatments were more enriched than dark treatments (Table 1). There was no significant macroalga difference; however, treatments without macroalgae were generally higher than those with macroalgae, following the same trend as total PLFA and PUFA (Table 1). To compare the relative uptake between bacteria and BMA in the light treatments, we used the BAR (Fig. 7a,b). For both SW and PW, BAR increased throughout the experiment. There were no significant differences in BAR between light treatments with and without macroalgae (Table 1).

THAA—Across all sampling days, THAA made up 33% ± 6% and 26% ± 6% of TN and 14% ± 2% and 10% ± 1% of TOC in light and dark treatments, respectively (SE; n = 20 treatment means for light; $n = 10$ for dark). Excess ^{13}C and ^{15}N in THAA showed the same general patterns as bulk sediment and PLFA, displaying both light and macroalga effects (Figs. 5c,d, 6c,d; Table 1). Uptake and loss rates were calculated for THAA as described above for bulk sediments (Table 2). Uptake rates were higher for light treatments than dark, and within a treatment, rates of ^{13}C and ^{15}N uptake exceeded loss rates, which were often small or not significantly different from zero ($p > 0.05$). As with bulk sediments, uptake rates were highest for light treatments without macroalgae.

Excess ^{13}C and ^{15}N in D-Ala, a bacterial biomarker, showed the same general patterns as THAA (Figs. 5e,f, 6e,f). There was a significant light effect for both ^{13}C and ^{15}N (Table 1), although, among the light treatments, there

Table 1. Results of two-factor repeated measures ANOVA used to test for differences in isotope delivery source, macroalgae, and light over time for isotopic enrichments (^{13}C or ^{15}N) of various sediment pools. Significant p values (< 0.05) are indicated in bold. Bulk, bulk sediments; Macro, macroalgae.

		Isotope delivery			Macroalgae			Light			Day		
Parameter	Isotope	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Bulk	^{15}N	6	23.9	**0.0027**	6	20.2	**0.0042**	6	63.6	**0.0002**	54	19.1	**<0.0001**
	^{13}C	6	2.86	0.1416	6	14.8	**0.0085**	6	77.5	**0.0001**	54	22.6	**<0.0001**
THAA	^{15}N	5	10.5	**0.0231**	5	14.3	**0.0128**	5	37.9	**0.0016**	32	29.3	**<0.0001**
	^{13}C	5	2.03	0.2135	5	10.2	**0.0242**	5	52.3	**0.0008**	33	50.2	**<0.0001**
D-Ala	^{15}N	5	9.98	**0.0251**	5	9.13	**0.0293**	5	25.1	**0.0041**	32	26.0	**<0.0001**
	^{13}C	5	0.73	0.4331	5	5.36	0.0684	5	41.3	**0.0014**	33	46.0	**<0.0001**
D : L-Ala	^{15}N	5	0.66	0.4543	5	0.47	0.5243	5	183.7	**<0.0001**	31	25.7	**<0.0001**
	^{13}C	5	14.6	**0.0124**	5	0.64	0.4593	—	—	—	27	28.1	**<0.0001**
Macro	^{15}N	2	3.63	0.1972							6	21.4	**0.0011**
	^{13}C	2	3.57	0.1993							6	0.97	0.4861
PLFA	^{13}C	6	2.45	0.1684	6	8.34	**0.0278**	6	15.1	**0.0081**	30	30.7	**<0.0001**
BrFA	^{13}C	6	3.30	0.1190	6	4.50	0.0782	6	13.5	**0.0105**	30	19.1	**<0.0001**
PUFA	^{13}C	6	0.95	0.3679	6	23.0	**0.0030**	6	54.7	**0.0003**	30	63.1	**<0.0001**
BAR	^{13}C	6	0.16	0.7014	6	0.56	0.4829	6	67.0	**0.0002**	30	29.6	**<0.0001**
Chl *a*	—	6	3.06	0.1306	6	2.00	0.2070	6	42.5	**0.0006**	55	5.74	**<0.0001**

was a significant macroalgae effect for ^{15}N but not ^{13}C (Table 1). However, treatments without macroalgae showed a trend of being generally higher in ^{15}N and ^{13}C than in those with macroalgae for both SW and PW additions, following the same trend as observed in bulk sediments, PLFA, and THAA. To compare the relative uptake between bacteria and BMA in the light treatments, we used the ratio of excess ^{13}C or ^{15}N in D-Ala to L-Ala (D : L-Ala; Fig. 7c–f). For ^{13}C and ^{15}N, in both SW and PW, D : L-Ala increased throughout the experiment. We estimated bacterial contribution to total label incorporation according to Eq. 5. For ^{13}C, there was an increase over the course of the experiment from 0% to 22% bacterial uptake for SW and 3% to 36% bacterial uptake for PW (Fig. 7c,d, right axes). For ^{15}N, this represented an increase from 10% to 34% bacterial uptake for SW and 9% to 57% bacterial uptake for PW (Fig. 7e,f). There were no significant differences between light treatments with and without macroalgae (Table 1).

Discussion

Macroalgal nutrient uptake—Macroalgal growth rates of ~ 5–6% d^{-1} in the mesocosms were within the range of rates reported for *Gracilaria* spp. in temperate systems similar to HIB (Raikar et al. 2001; Marinho-Soriano et al.

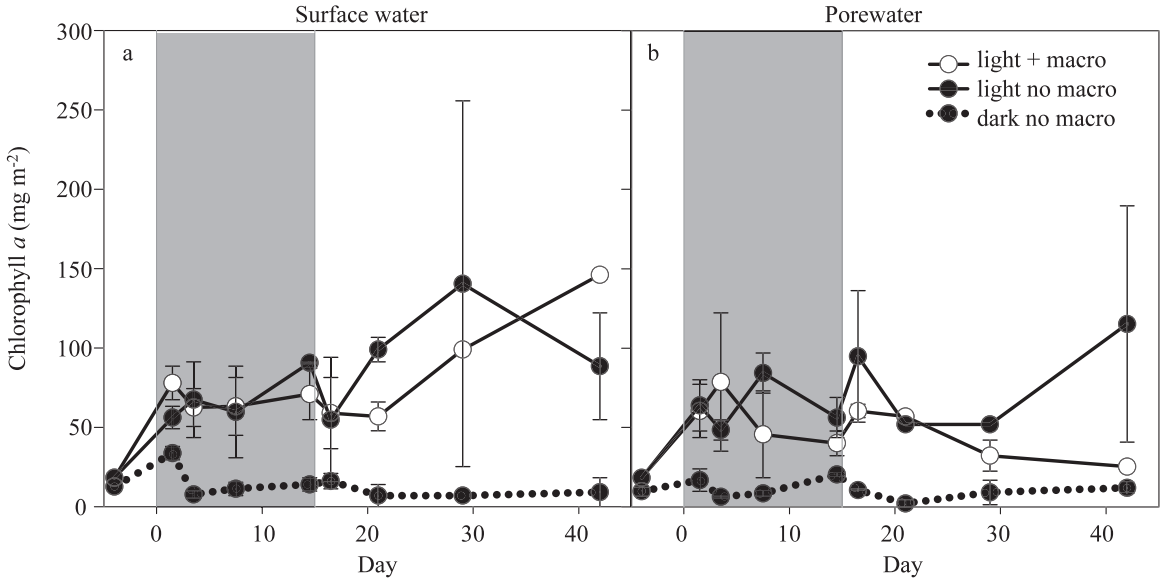

Fig. 4. Benthic Chl *a* concentrations in (a) surface water and (b) pore water treatments. Treatments shown are light with macroalgae (solid lines, open symbols), light without macroalgae (solid lines, filled symbols), and dark without macroalgae (dotted lines, filled symbols). Baseline samples were taken 4 d before adding the nutrients and macroalgae. Values are mean ± SE ($n = 2$).

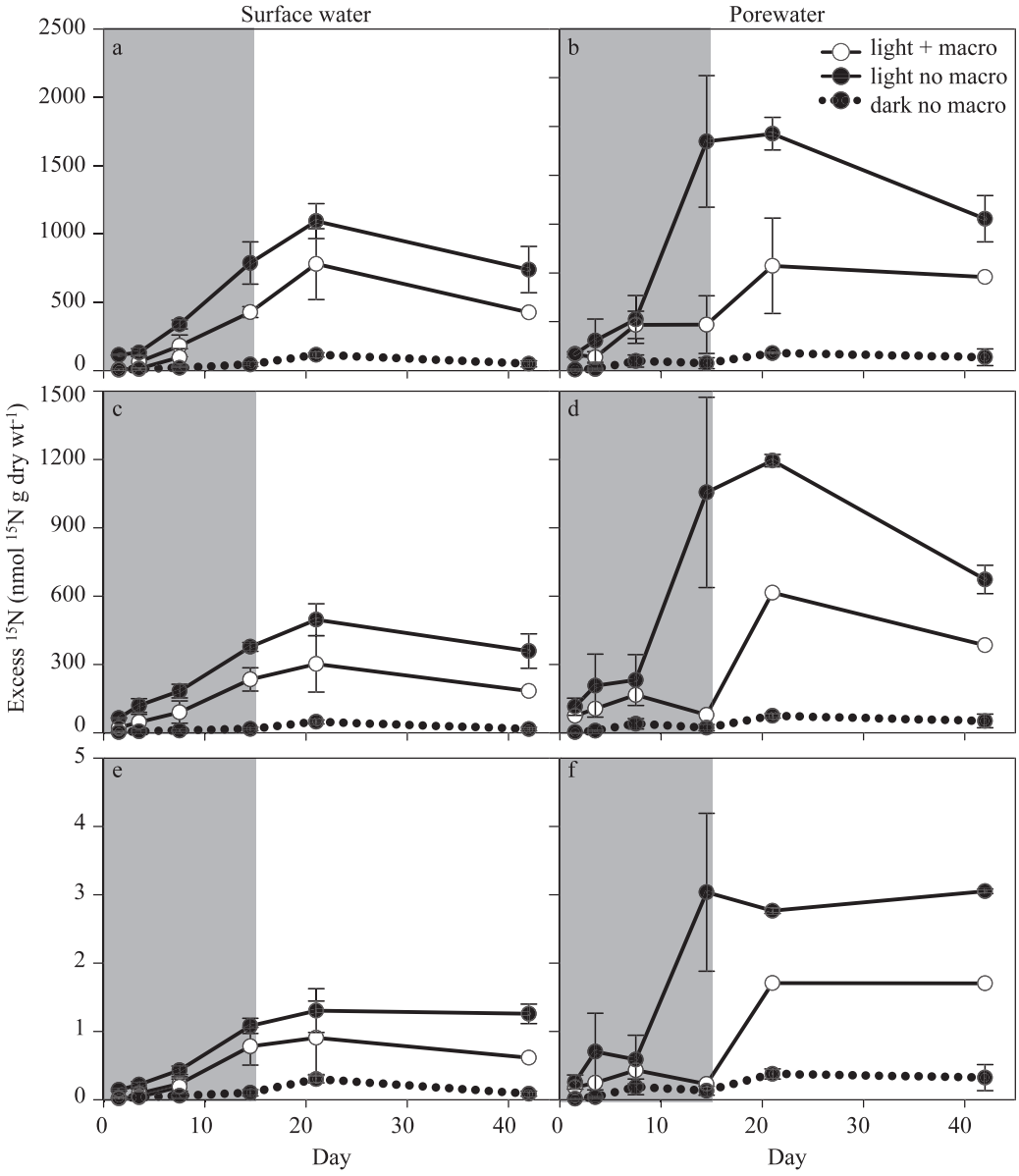

Fig. 5. Excess ^{15}N in (a, b) bulk sediments, (c, d) THAA, and (e, f) D-alanine in (a, c, e) surface water and (b, d, f) pore water treatments. Values are mean ± SE (n = 2).

2006). Growth was constant through the labeling and postlabeling periods because nutrients were continuously added throughout the experiment. Addition of isotopically labeled nutrients allowed us to track ^{13}C and ^{15}N into macroalgal biomass, which provided insight into macroalgal nutrient uptake patterns that we could not have learned by monitoring growth rates alone. For example, regardless of whether isotopes were delivered via SW or PW, macroalgae took up ^{13}C and ^{15}N, suggesting that macroalgae used C and N from both sources, which is consistent with previous studies (McGlathery et al. 1997; Tyler et al. 2001). Continued isotopic enrichments of macroalgal tissue following the end of the isotope addition period provides additional insight into nutrient cycling dynamics within a macroalgal mat. ^{13}C and ^{15}N enrichments in macroalgae peaked on day 21, 1 week after the isotope addition ceased. Since the flushing rates of the SW and PW were ~ 2 d, isotopes in the surface water or released from the sediments were available for macroalgal uptake for a couple of days before being flushed out. However, continued enrichment of macroalgal tissue for a week or more after the end of the isotope addition may also have reflected recycling of or use of reserved ^{13}C and ^{15}N, or both, within the mat, as observed in previous studies (Krause-Jensen et al. 1999). Thybo-Christesen and Blackburn (1993) measured large and frequent changes in nutrients, oxygen, pH, and temperature within the layers of a mat, which, they suggested, behave almost as a closed system. At day 21, ^{13}C and ^{15}N content in macroalgal tissue decreased, likely reflecting dilution by unlabeled C and N

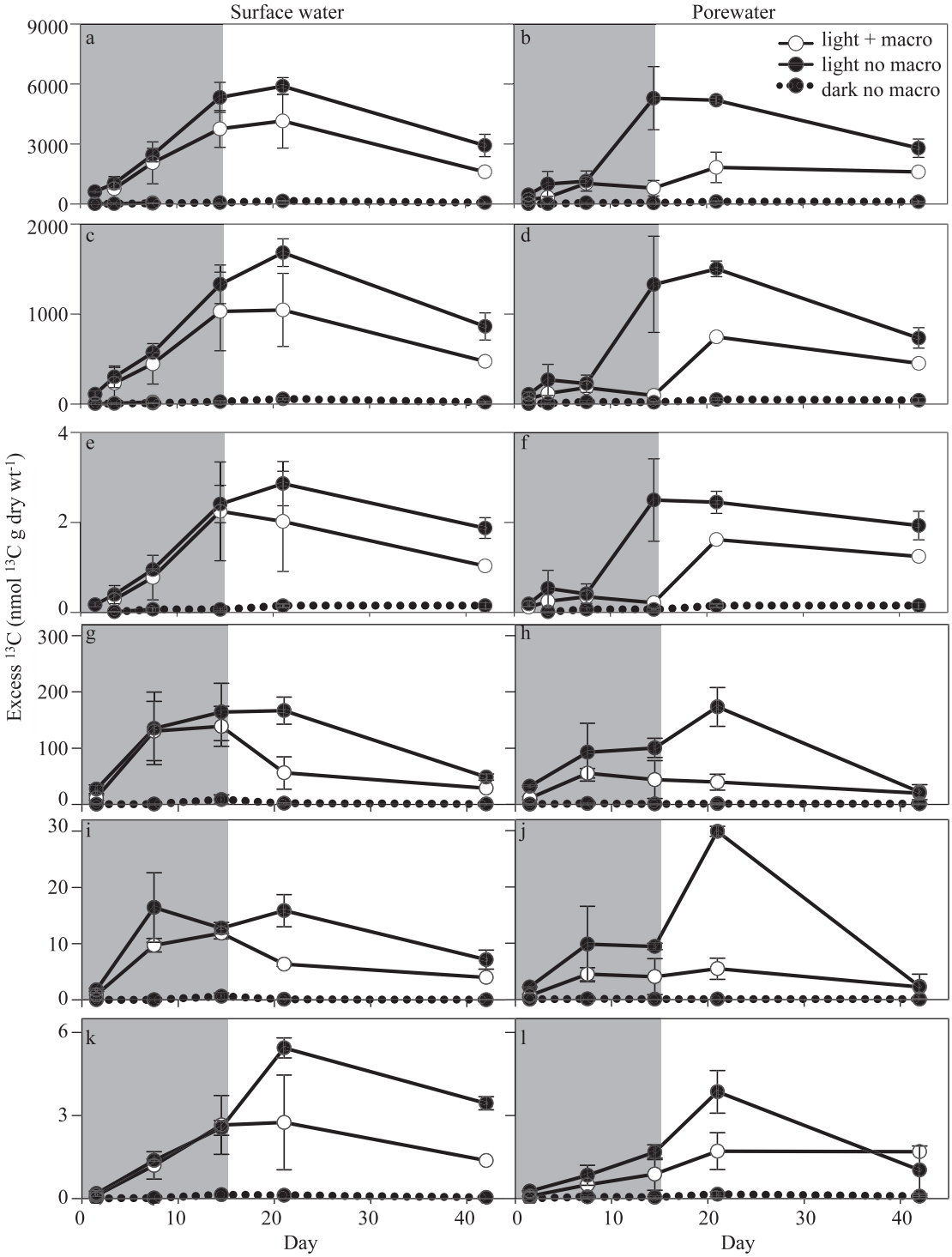

Fig. 6. Excess ^{13}C in (a, b) bulk sediments, (c, d) THAA, (e, f) D-alanine, (g, h) PLFA, (i, j) PUFA, and (k, l) BrFA in (a, c, e, g, i, k) surface water and (b, d, f, h, j, l) pore water treatments. Values are mean ± SE (n = 2).

as macroalgae continued to grow and take up nutrients. By day 42, the isotopic content of the macroalgae had not yet returned to background levels, indicating storage of label as biomass and suggesting that macroalgae act as a temporary C and N sink (for at least 4 weeks), which is in agreement with other studies (Thybo-Christesen and Blackburn 1993; McGlathery et al. 1997).

Macroalgae were a sink for C and N during our experiment, as in field studies, where blooms have grown to > 500 g dry wt m^{-2} (Sfriso et al. 1992; Hauxwell et al.

Table 2. Uptake and loss rates for label into bulk, THAA, and PLFA. Values are mean (SE), $n = 2$. Uptake rates (nmol X [g dry wt]$^{-1}$ d^{-1}) were calculated as the slope of excess label ($X = {}^{13}C$ or ^{15}N) from days 1 to 21. Loss rates (nmol X [g dry wt]$^{-1}$ d^{-1}) were calculated as the slope of excess label from days 21 and 42. Uptake and loss rates with significant p values (< 0.05) are indicated in bold.

		Bulk				THAA				PLFA	
		^{13}C		^{15}N		^{13}C		^{15}N		^{13}C	
Treatment	Rate	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
Pore water: dark	Uptake	**6.35 (1.41)**	0.67	**6.04 (1.92)**	0.50	**2.09 (0.58)**	0.62	**3.05 (0.90)**	0.59	0.033 (0.031)	0.17
	Loss	−0.23 (2.72)	0.00	−1.63 (2.23)	0.12	−0.405 (1.26)	0.05	−1.05 (1.48)	0.20	−0.0105 (0.023)	0.10
Light+macro	Uptake	**104 (32.4)**	0.51	**50.7 (13.2)**	0.60	28.4 (12.3)	0.64	22.2 (10.3)	0.61	1.40 (1.74)	0.25
	Loss	−10.1 (56.0)	0.01	−12.0 (22.9)	0.08	−14.0	—	−10.9	—	−1.56	—
Light, no macro	Uptake	**296 (60)**	0.71	**109 (21)**	0.74	**79.1 (15.5)**	0.77	**61.7 (11.9)**	0.77	**6.53 (1.96)**	0.65
	Loss	**−119 (32)**	0.78	**−37.1 (10.9)**	0.74	**−36.6 (6.9)**	0.93	**−24.8 (3.2)**	0.97	−7.01 (1.77)	0.89
Surface water: dark	Uptake	**6.74 (1.31)**	0.73	**3.66 (0.82)**	0.67	**2.65 (0.40)**	0.84	**2.13 (0.44)**	0.77	0.203 (0.280)	0.08
	Loss	−3.81 (2.16)	0.44	−2.66 (1.28)	0.52	−1.79 (0.50)	0.87	−1.52 (0.57)	0.78	−0.0712 (0.0334)	0.82
Light+macro	Uptake	**221 (43)**	0.73	**26.8 (7.56)**	0.56	**53.2 (17.8)**	0.56	**15.2 (3.8)**	0.70	2.02 (3.42)	0.06
	Loss	−138 (78)	0.52	−13.1 (11.8)	0.29	−27.2 (33.4)	0.40	−5.65 (10.2)	0.24	−1.29 (2.38)	0.23
Light, no macro	Uptake	**305 (36)**	0.88	**43.3 (3.5)**	0.94	**83.3 (7.2)**	0.94	**22.5 (1.9)**	0.94	6.40 (2.88)	0.45
	Loss	−143 (68)	0.52	−14.0 (13.2)	0.22	−39.1 (10.3)	0.88	−6.57 (4.89)	0.47	**−5.24 (1.18)**	0.91

2001). Our experiment was conducted during the peak growing season for macroalgae in Hog Island Bay (McGlathery et al. 2001; Tyler et al. 2001), where the macroalgal population often displays a precipitous decline in mid- to late summer, similar to other coastal lagoons (Sfriso et al. 1992; Valiela et al. 1992). Thus, in nature, macroalgae only store C and N temporarily. Once the bloom begins to decline, dissolved organic matter and inorganic nutrients are released to the water column or incorporated into the sediments, fueling bacterial and microalgal production (McGlathery et al. 2001; Hardison et al. 2010). In systems that experience the most extreme die-offs, hypoxic or anoxic conditions can develop in the water and sediments, further disrupting nutrient cycling and organic matter decomposition (Sfriso et al. 1992; Hauxwell et al. 2001).

Macroalgal–BMA interactions—In shallow systems where light reaches the sediment surface, BMA have been shown to play a central role in regulating nutrient cycling at the sediment–water interface (McGlathery et al. 2004; Anderson et al. 2010); we measured several parameters that suggest that they were active in our mesocosms as well: (1) Benthic Chl *a* concentrations and label enrichments in bulk sediments in light treatments were significantly higher than in the dark, indicating that $H^{13}CO_3^-$ and $^{15}NH_4^+$ uptake into bulk sediments in the light was dominated by BMA. (2) Excess ^{13}C and ^{15}N in THAA and excess ^{13}C in total PLFA showed a strong dependence on light. Label enrichment in these pools represents uptake by the microbial community, including both autotrophic and heterotrophic organisms. The light dependence of ^{13}C and ^{15}N uptake into these pools indicates the importance of autotrophic (BMA) uptake or recycling by heterotrophic organisms, or both, of autotrophic production. (3) Elevated excess ^{13}C in PUFA provided the most direct evidence that BMA were fixing ^{13}C. (4) The ratios of excess ^{13}C in BAR and excess ^{13}C and ^{15}N in D : L-Ala during the labeling period were low, suggesting that total label incorporation into surface sediments was dominated by BMA rather than bacteria in this study.

Excess ^{13}C and ^{15}N in bulk sediments, THAA, and total PLFA were lower in treatments with macroalgae, suggesting that macroalgae limited BMA C and N uptake. The most specific biomarkers for BMA were the PUFA, which showed less ^{13}C enrichment in the treatments with macroalgae. Although there was no significant effect of macroalgae on benthic Chl *a* concentrations in the surface sediments, benthic Chl *a* concentrations do not necessarily indicate BMA productivity because pigment levels can vary depending on light availability, nutrient concentration, and algal species (Agusti et al. 1994). Macroalgae growing above the sediment surface have the capacity to compete with BMA for nutrients, reduce the amount of light available to microalgae, or both (Sundback and McGlathery 2005). Because we supplied nutrients simultaneously via the SW and PW, neither C nor N was likely limiting in our treatments. Furthermore, in the treatments with macroalgae, we observed labeling of both macroalgae and BMA regardless of isotope source. Thus, macroalgae were not sequestering all of the label in the SW treatments, thereby

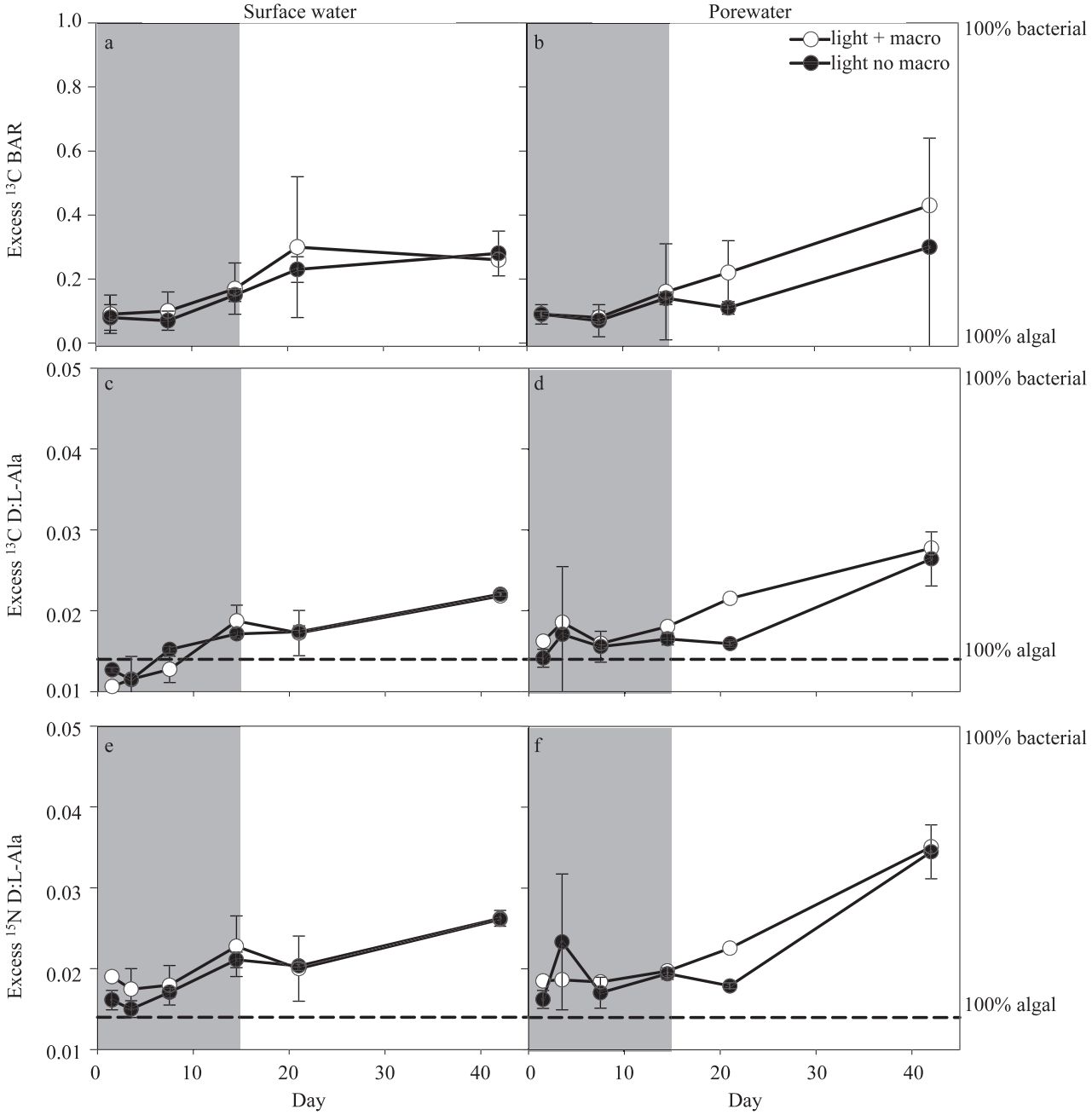

Fig. 7. The bacteria-to-algae ratio (BAR): the ratio of excess ^{13}C in branched odd fatty acids (BrFA) to the sum of BrFA and polyunsaturated fatty acids (BrFA + PUFA) for light mesocosms in (a) surface-water and (b) pore water treatments. The ratio of excess (c, d) ^{13}C and (e, f) ^{15}N in D-alanine : L-alanine (D : L-Ala) for light mesocosms in (c, e) pore water and (d, f) surface water treatments. (c–f) The dashed horizontal lines represent the racemization background (0.014). Values on the right *y*-axis correspond to estimates of bacterial and algal contribution to total label incorporation. Values are mean ± SE ($n = 2$).

preventing BMA uptake of that label, and BMA did not intercept all of the labeled nutrients in the PW treatments. As a result, we believe macroalgae limited BMA productivity primarily through shading.

Macroalgal mats are often sufficiently dense to self-shade the layers of the mat nearest the sediment surface (McGlathery et al. 1997; Brush and Nixon 2003); thus, they must limit the light reaching BMA. Krause-Jensen et al. (1996) estimated complete shading of BMA to occur at macroalgal densities $>$ 300 g dry wt m^{-2}. In our experiment, macroalgae reached that density by day 14, suggesting that BMA productivity may have been diminished during the first 2 weeks of the experiment and reduced for the remainder of the experiment as macroalgae continued to grow through day 42. Our results are consistent with those of Tyler et al. (2003), who found sediments underlying macroalgal mats to be net heterotrophic. On average, macroalgal densities in Hog Island Bay

Table 3. Total label (^{15}N or ^{13}C) in macroalgal bloom and surface sediments (0–1 cm) of entire mesocosm (0.29 m^2) for surface water and pore water treatments across 4 d. % reduction refers to the decrease in total label in surface sediments for treatments with macroalgae vs. without macroalgae. Values are mean (SE), $n = 2$.

		^{15}N (mmol ^{15}N mesocosm^{-1})				^{13}C (mmol ^{13}C mesocosm^{-1})			
			Sediment		%		Sediment		%
	Day	Macro	No macro	+macro	reduction	Macro	No macro	+macro	reduction
Surface water	7	3.67 (0.21)	1.43 (0.14)	0.77 (0.34)	46	52.3 (5.2)	10.4 (1.4)	8.72 (4.4)	16
	14	2.75 (0.86)	2.99 (0.31)	1.82 (0.17)	39	73.0 (13.2)	24.0 (4.6)	15.9 (3.9)	29
	21	6.14 (0.25)	4.03 (0.52)	2.73 (1.02)	32	76.3 (19.4)	27.6 (2.0)	19.6 (6.23)	30
	42	3.37 (1.27)	2.72 (0.62)	1.56	43	42.0 (18.7)	13.7 (2.6)	7.51	45
Pore water	7	1.68 (1.23)	2.22 (1.04)	1.98 (0.59)	11	12.2 (4.1)	4.82 (2.16)	4.36 (0.35)	10
	14	6.50 (2.86)	8.29 (2.60)	1.99 (1.25)	76	42.9 (19.0)	24.8 (7.3)	3.34 (1.62)	85
	21	17.8 (6.1)	9.12 (0.42)	4.33 (1.22)	53	65.2 (15.2)	24.1 (1.0)	8.76 (3.41)	65
	42	18.7 (2.1)	5.67 (0.74)	3.17	44	60.0 (5.4)	13.0 (2.1)	7.60	43

are < 300 g dry wt m^{-2}; however, localized blooms > 300 g dry wt m^{-2} have been observed (McGlathery et al. 2001; Hardison et al. 2010). Moreover, the densities attained during this experiment are within the range of those observed in more eutrophic systems (Sfriso et al. 1992; Hauxwell et al. 2001). Whether through nutrient or light competition, macroalgae reduced BMA productivity, thereby diminishing retention of C and N as BMA biomass.

Algal–bacterial interactions—Our results further suggest coupling between sediment bacterial and algal production. The negative influence of macroalgae on BMA production likely translated to diminished bacterial production as well. As with BMA biomarkers, ^{13}C and ^{15}N label incorporation into bacterial biomarkers (D-Ala and BrFA) were light dependent and diminished in the presence of macroalgae. Excess ^{13}C in PUFA and bacterial biomarkers were linearly related (BrFA: $r^2 = 0.60$, $p < 0.0001$; D-Ala: $r^2 = 0.52$, $p < 0.0001$), demonstrating that labeling of BMA and bacteria tracked one another, which supports observations from numerous studies that bacteria may rely on BMA production as an energy or nutrient source, or both (Middelburg et al. 2000; Cook et al. 2007). Although it has been difficult to demonstrate experimentally, BMA and bacterial production are thought to be coupled in at least three ways: (1) Because BMA turnover is on the order of days (Sundback et al. 1996; Middelburg et al. 2000), bacteria can directly recycle BMA biomass, which would transfer BMA ^{13}C and ^{15}N to bacteria. (2) BMA have been shown to exude $> 50\%$ of C fixed as extrapolymeric substances (EPS), which may be a substrate for bacterial production (Smith and Underwood 2000). Because EPS is N-poor, bacteria would likely have to take up $^{15}NH_4^+$ directly to meet their metabolic needs (Cook et al. 2007), which, together, would result in ^{13}C and ^{15}N labeling of bacteria. (3) Bacterial remineralization of ^{13}C- and ^{15}N-labeled BMA releases inorganic ^{13}C and ^{15}N that can be subsequently taken up by BMA.

To further illustrate the coupling between bacteria and BMA in this system, we analyzed the ratios of excess ^{13}C in BAR and excess ^{13}C and ^{15}N in D:L-Ala in the light treatments. Changes in these ratios over time illustrated changes in the relative contributions of BMA and bacteria to total label uptake. The ratios were initially low, indicating dominance by BMA, began to increase by day 21, and reached their highest levels on day 42. This increase corresponded to relatively more label uptake into bacterial biomass, suggesting that both ^{13}C and ^{15}N first passed through BMA before being taken up by bacteria. This is corroborated by findings of Middelburg et al. (2000) and Evrard et al. (2008), suggesting rapid and direct transfer of ^{13}C from BMA to bacteria in intertidal and subtidal sediments, respectively. Although macroalgae affected absolute label uptake into the microbial pools, they did not affect either BAR or the D:L-Ala ratios, suggesting that the relative contribution to total uptake from bacteria and BMA remained unchanged in the presence of macroalgae. The shuttling of C and N back and forth between BMA and bacteria likely increased retention in the sediments and accounted for the slower rates of isotope loss in bulk sediments and THAA, compared with the rates of uptake during the labeling period (Table 2). These results further suggest that macroalgae may reduce overall retention of C and N in sediments by reduction of BMA production, which, in turn, reduced bacterial production.

Nutrient retention and eutrophication—Our experiments corroborate previous work showing that macroalgae are a sink for C and N in shallow coastal systems (McGlathery et al. 2004; Pedersen et al. 2004). We also demonstrated that the sediments served as a sink for C and N because isotopic labels persisted in the bulk sediments for at least 4 weeks after the isotope additions ended. The leveling off of the ^{13}C and ^{15}N isotope trajectories in the bulk sediments suggests that our system had approached complete turnover near day 21. Turnover of the entire sediment C or N inventory would take much longer (~ 100 d in the light treatments, according to our estimations), which suggests that there is a small pool within the sediments that is actively cycling and turning C and N over more rapidly. This, along with our biomarker data, suggests that the sediment microbial community facilitates the retention of C and N.

To determine the relative sizes of the macroalga and sediment sinks, we compared the total label (^{13}C or ^{15}N) sequestered by macroalgae with that of the sediment surface (0–1 cm; 0.29 m^{-2}) of each mesocosm (Table 3). Label "storage" in macroalgae was always higher than in the underlying sediments. Furthermore, the macroalgal sink was often larger than the sediment sink in treatments without macroalgae, so macroalgae represented a large, albeit temporary, C and N sink in these systems. The size of the sediment sink in the presence of overlying macroalgae was diminished by ~ 40% relative to treatments without macroalgae, which clearly has important ecological consequences.

Retention within sediment microbes would be expected to be a more stable sink than retention as ephemeral macroalgal biomass. Macroalgae efficiently take up nutrients from the sediments or the water column and can accumulate in large blooms. However, once macroalgae die, their nutrients are re-released to the water column, where they can support phytoplankton, including harmful algal blooms, and bacterial metabolism (Sfriso et al. 1992; McGlathery et al. 2001; Tyler et al. 2003). In contrast, sequestration of nutrients by sediment microbes may remove nutrients from the water column, and the close coupling between BMA and bacteria may effectively retain those nutrients within the sediments during times of the year that are favorable for phytoplankton blooms. Additionally, the uptake and retention of N in sediments may provide a link to benthic nitrification–denitrification, which is a primary pathway for permanent removal of excess N from the water column. Thus, shunting nutrients through macroalgae rather than BMA will likely provide a positive feedback to eutrophication, whereas the sediment microbial community may play an important role in buffering the effects of increased nutrient loading. This role is likely diminished in the presence of macroalgae.

Nutrient retention within the sediments of our experimental system may have been more pronounced than in a natural, open system. In a related study, we deployed perfusionators in situ to track ^{13}C and ^{15}N retention in sediments in an energetic setting (Hardison et al. 2011), and we observed patterns similar to those described in our mesocosms, although enrichments and retention were generally lower than in the current study. C and N retention in natural sediments will depend on numerous site-specific factors, including sediment type and vulnerability to advective flow and resuspension, and macrofaunal activity. While we attempted to have the mesocosms reflect natural conditions to the extent possible (e.g., temperature and light regime, natural sediments, etc.), our goal was not to use the mesocosms to replicate in situ conditions but, rather, to use the mesocosms as a tool for understanding the complex mechanisms underlying the biogeochemical processes in subtidal sediments. The mesocosms provided a unique opportunity to examine processes that are difficult, if not impossible, to study under field conditions.

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