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Transport and digestive alteration of uniformly ^{13}C-labeled diatoms in mudflat sediments

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ABSTRACT

Uniformly ^{13}C-labeled diatoms were used to elucidate the effect deposit feeders have on the distribution and composition of phytodetritus within the seabed. Mudflat infauna contained in microcosms reacted quickly to the emplacement of labeled diatoms onto the sediment-water interface by moving the phytodetritus nonlocally during feeding and hoeing activities. Although redistribution of tracer was rapid, not all infauna exposed to the tracer ingested it. Using four species of deposit-feeding annelids that had ingested the ^{13}C-labeled phytodetritus during the microcosm experiment, molecular-level, digestive alterations of ^{13}C-labeled diatoms were documented. Fecal material produced by the deposit feeders had ^{13}C amino acid signatures distinctly different from that of the diatom. Alterations in the amino acid composition of the diatom were correlated to the gut morphology and digestive physiology of the polychaete taxa.

1. Introduction

Radiotracer data have indicated that deep-sea deposit feeders may intercept and ingest most, if not all, particles newly deposited on the seabed (Lauerman *et al.,* 1997; Miller *et al.,* 2000). Additionally, techniques using uniformly ^{13}C labeled algae have been used to document the rapid response of deep-sea benthos to the delivery of phytodetritus (Blair *et al.,* 1996; Levin *et al.,* 1997; Blair *et al.,* 2001). During such experiments, deposit feeders quickly accessed freshly deposited, labeled phytodetritus and mixed it into the seabed within 24 hours; however, not all infauna exposed to the tracer fed upon it (Levin *et al.,* 1997).

Deposit feeders have a profound impact on the distribution, chemical composition and degradation rate of the organic material through activities such as feeding, irrigation, bacterial gardening/stimulation, and particle reworking (Hylleberg, 1975; Aller, 1982; Bianchi *et al.,* 1988; Sun *et al.,* 1993; Sun, 2000). Many studies have explored how deposit feeders change the composition of organic matter during digestion by following compound classes, such as protein, carbohydrates and lipids, through deposit feeder guts (Kofoed, 1975; Yingst, 1976; Kemp, 1986; Grémare, 1990; Olivier *et al.,* 1996). Fewer experiments have documented how deposit feeder ingestion and digestion changes the composition of

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detritus at the molecular level of resolution. Compound-level studies have indicated that specific lipids are not only added to gut contents during digestion (Bradshaw *et al.,* 1989), but also selectively ingested by some deposit feeders (Sun *et al.,* 1999).

The objectives of the experiments presented here were to determine (1) how shallow water infauna respond to the deposition of ^{13}C-labeled diatoms, (2) which infauna redistribute the labeled tracer through feeding activities and (3) how the deposit feeders which ingest tracer alter its biochemical make-up. To address objectives one and two, uniformly ^{13}C-labeled (U^{13}C) diatoms were added to intertidal mudflat sediments contained in closed microcosms. To address objective three, deposit-feeding polychaetes were fed U^{13}C-labeled diatoms and U^{13}C-labeled amino acids were tracked from the food diatom into the feces and worms. The polychaete species chosen for the feeding experiments ingested the tracer during the microcosm experiment. They also represented a range of gut architectures, which allowed us to test for correlations between worm morphology and patterns of amino acid digestion. Finally, an additional treatment of the feeding experiments was conducted with physically disrupted diatoms to examine the role of the silica frustule in controlling digestion of diatomaceous amino acids.

The amino acid fraction was the focus of the feeding experiments because (1) ^{13}C-labeled proteins have been used successfully to track amino acid incorporation in vertebrates (e.g., Berthold *et al.,* 1991), (2) amino acids are abundant in coastal sediments, comprising 5–15% of the particulate organic carbon and 20–70% of the total nitrogen (Henrichs *et al.,* 1984; Henrichs and Farrington, 1987; Burdige and Martens, 1988), and (3) measurable changes in the composition of the amino acid fraction were expected because nitrogen is frequently a limiting nutrient for benthic organisms (Phillips, 1984; Lopez and Levinton, 1987; Rice and Rhoads, 1989; Tenore, 1989; Taghon and Greene, 1990).

2. Methods

a. ^{13}C-labeled diatom production

^{13}C-labeled diatoms were grown as described previously in Levin *et al.* (1997). *Thalassiosira pseudonana* (CCMP 1335 clone, Provasoli-Guillard National Center for Culture of Marine Phytoplankton) was grown in closed 2 L bottles using synthetic seawater (Parsons *et al.,* 1984), f/2 media (Sigma Chemical Co.) and 98% ^{13}C bicarbonate (Cambridge Isotopes) as the sole carbon source. Cultures were grown under a 12 hr light/dark cycle. All batches of cells used in these experiments were inoculated with established ^{13}C-labeled cultures, so that cells could be harvested after any number of doublings and still be $\geq$95% ^{13}C. Cells were harvested by gentle filtration when the pH of the media rose to 9 (typically 7 days after inoculation). This ensured high cell densities with little senescence or carbon and nutrient limitation.

b. Sediment and animal collection site

Sediment and worms were collected from an intertidal mudflat at the University of North Carolina at Wilmington's research lease property near the mouth of Hewletts Creek

(34° 18′N, 77° 54′W). The mudflat environment is euryhaline with salinities generally ranging from 29 to 37‰ and water temperatures fluctuating between 9 and 32°C (Powell, 1994). Sediments are composed of fine sands, oyster substrate, and mud (porosities 0.5–0.7 $cm^3_{pw} cm^{-3}_{sed}$). The sediments used in these experiments were collected in intertidal areas that are subjected to semidiurnal tides. The mudflat ecosystem is fueled by detritus from the surrounding *Spartina alterniflora* marsh, planktonic inputs and patches of benthic diatoms and microbes (Powell, 1994, L.B. Cahoon and M. Posey, pers. comm.). Sands dilute organic matter in these sediments such that surface sediments are 0.2% organic carbon and 0.04% organic nitrogen by weight. Infauna densities range from 15,000 to 26,000 individuals m^{-2} during the summer months (Powell, 1994). Dominant fauna include polychaetes (spionids, capitellids, cirratulids and maldanids) and bivalves (Powell, 1994).

c. Microcosm experiments

Microcosms were cylindrical 14.5 cm i.d. × 25 cm (length) Lucite push cores (Blair *et al.,* 1996) filled with sediment collected at low tide during May 1996. Five microcosms were filled by hand by pushing the cores into the sediment approximately 12 cm and closing them at the bottom *in situ.* Overlying water in the microcosms was aerated during transport to N.C.S.U. The microcosms were maintained in a darkened environmentally controlled room at 19°C.

The algal tracer used in the microcosm experiment was prepared by freeze-drying U^{13}C-labeled diatoms onto kaolin (Levin *et al.,* 1997). Three grams of the diatom/kaolin tracer (5 mg dried diatom/g kaolin) was slurried with seawater and put in a 60 mL syringe. The tracer was added to the microcosms via syringe ensuring even coverage in a 1 mm layer over the sediment surface. The clay facilitated settling of the tracer and allowed for visual tracking of tracer movement as the experiment progressed.

The experimental incubations began with the addition of the tracer mixture into four of the microcosms. Two were processed immediately after tracer addition and served as time zero ($T = 0$) controls. One microcosm was processed without tracer addition for tracer-free chemical information ("background" core). The remaining two microcosms were incubated with tracer for approximately 3 days. The duration of the tracer incubations was chosen to: (1) insure enough of the diatom would be advected into the sediment and remineralized for determination of mixing mechanisms and tracer reactivity, (2) allow ample time for visual observation of animal behavior and (3) be of similar duration as the analogous deep-sea experiments presented in Blair *et al.* (1996). The lengthy processing time for each microcosm prevented exact duplication of incubation times. To avoid confusion, the microcosms will be referred to as the 61 hr and 89 hr incubation (reflecting the exact length of the incubations). The lack of true replicates was not considered a problem since the experimental objectives were to qualitatively observe the behavioral response of the infauna.

The incubations were ended by removing the overlying water and sectioning the

sediment in 1 cm intervals. The sediment was sectioned by spooning it into plastic containers where it was homogenized. Thirty mL of homogenate were centrifuged at 7500 rpm (5000 *g*) for 15 minutes in Teflon centrifuge tubes. The supernatant was removed and the remaining sediment pellet was frozen for POC analyses.

Surplus homogenate was fixed in plastic containers with 10% buffered formalin. After fixation, sediment was sieved through a 300 μm screen and the material caught on the seive was transferred to GC-MS grade isopropanol. Infauna were later sorted, identified to family or genus, and stored frozen in ashed borosilicate culture tubes (500°C for 4 hr) until analyses. Small individuals of the same genus from the same interval were pooled to insure large enough samples for isotope analyses. Macrofauna from the background and 61-hour mudflat incubations were analyzed for their tracer content. Macrofauna from the 89-hour incubation were not analyzed because of logistical constraints.

The concentration and isotopic composition of POC was determined on the centrifuged mud. 1–2 grams of sediment were acidified with 4N HCl and dried *in vacuo*. Organic carbon was combusted to CO_2 in a Carlo Erba 1108 CNS analyzer. Eluted CO_2 was collected cryogenically and analyzed for its $^{13}C/^{12}C$ content using a modified Finnigan MAT Delta E isotope ratio mass spectrometer (Hayes *et al.,* 1977). Macrofauna also were analyzed for their $^{13}C/^{12}C$ content in the same manner as the sediment (Blair *et al.,* 1996).

The concentration of labeled carbon (C_l) in the sediment samples was determined using the following expression (Blair *et al.,* 1996):

$$C_l = \frac{R_s {}^{12}C_b - {}^{13}C_b}{2F - 1}, \tag{1}$$

where:

$^{12}C_b$ and $^{13}C_b$ = background concentration of each species

R_s = $^{13}C/^{12}C$ ratio of the sample (background + tracer)

F = fractional abundance of ^{13}C in the tracer (0.95).

d. Feeding experiments

Worms for the feeding experiments were collected by shovelling surface sediment into buckets and adding raw seawater. At N.C.S.U. adult worms were picked and maintained in sieved (<500 μm) sediment from the site until use in experiments within 5 days. A spionid species (*Streblospio benedicti*), a maldanid species (*Axiothella mucosa*), and two species from the family Terebellidae (*Polycirrus* sp. and *Amaena* sp.) were used. Two species of terebellid were used because after the first of five collections, we could no longer find *Polycirrus* at the study site.

Before the start of the feeding experiments, a small amount of autoclaved sediment (3–4 g) and filter-sterilized seawater was placed in 9 petri dishes. 9–10 *Streblospio* were then added to each of 3 petri dishes, 3 terebellid individuals each were placed in another three petri dishes, and one *Axiothella* was placed in each of the remaining three dishes. The

number of individuals of each taxa placed in a dish was chosen to result in the same biomass in each treatment.

The experiments began when 3 mg of freshly harvested U^{13}C-labeled diatoms (dry wt, ~0.5 mg ^{13}C) were added to the dishes. *Streblospio* and *Axiothella* fecal pellets were removed immediately upon production and frozen. After 24–48 hours, when enough pellets (~30 mg salt corrected dry wt.) had been accumulated for amino acid analyses, the worms were moved to label-free sediment to insure clearance of labeled diatoms from their guts. The experiment was ended when all of the worms within one dish were pooled, frozen and henceforth considered a single sample. The entire experiment (9 dishes) was repeated three times.

The method had to be altered slightly in the terebellid treatments because the worms did not produce consolidated pellets and instead sprayed their fecal material into the water column. The diffuse ejecta was impossible to separate from the surrounding sediment, and more importantly, the tracer diatom. To insure that all the ^{13}C-labeled amino acids collected came from fecal material, the terebellids were allowed to fill their guts with the labeled diatom-sediment mixture and were then moved to label-free sediment. After they had voided their guts (visual observation) the worms were removed, and all of the sediment in the dish was frozen and analyzed. As a result of this modification, absolute concentrations of U^{13}C-labeled amino acids were lower in the terebellid fecal samples. However, comparison with the diatom and fecal pellets of the other two species was not hampered, because all subsequent data manipulation involved mole percentages of only U^{13}C-labeled amino acids (background sediment amino acids were ignored).

In order to compare the digestibility of the diatoms' cellular components with and without the complete protection of an intact cell wall, batches of U^{13}C-labeled diatoms that had been previously frozen and thawed were fed to all three worm families. Freezing and thawing the diatoms disrupted at least 80% of the cells (microscopic examination). The feeding experiment with previously frozen diatoms proceeded as described above for the experiment with fresh diatoms. Experiments with *Streblospio* were replicated a full three times (3 dishes × 3 times) allowing for statistical treatment of the data, while the low number of replicates with the *Amaena* (3 dishes × 1 time) and *Axiothella* (1 dish) only allowed for inferences from trends.

The amino acid compositions of worms, pellets, and diatoms from both treatments were determined as follows. Samples were lyophilized and weighed into glass ampoules. Degassed 6N HCl was added to the ampoules, the head space evacuated and the glass sealed. Samples were hydrolyzed for two hours at 150°C (modified from Cowie and Hedges, 1992). Hydrolysis conditions were optimized for the diatoms to give maximum yield and minimum sample-to-sample variability. Salts were separated from the hydrolysate using Dowex AG 50W-8x ion exchange resin. Hydrolyzed amino acids were converted to their n-Trifluoroacetyl methyl esters (Darbre and Islam, 1968) and analyzed by GC-MS (HP 5890 Series II) using a DB-5 capillary column (0.25 mm × 30 m) and helium as the carrier gas. The GC was equipped with an autosampler and cool on-column

injector. The oven and injector were set to ramp at 7 C° min^{-1} until reaching 150°C and then at 20 C° min^{-1} to 280°C where they held for 5 min. Peak identities were verified using the mass spectra of the derivatized standard amino acids. Mass spectra were obtained using continuous scan mode because separation time between peaks did not allow for effective use of ion-selective detection.

Two internal standards, norvaline and norleucine, were used to evaluate loss during hydrolysis, ion exchange, and derivatization. Norvaline was added to each sample prior to hydrolysis, and norleucine was added after ion exchange just prior to derivatization. The loss of either standard was $<5\%$. Concentrations of 11 amino acids in the samples were calculated using three point standard curves. Standard deviations of repeated analyses for diatom amino acids were $\leq 4\%$. $U^{13}C$-labeled amino acids are expressed as mole percentages of only $U^{13}C$ amino acids unless otherwise noted (i.e., no ^{12}C-containing amino acids were included).

Data from the feeding experiments was analyzed using SAS (Version 6.12). Amino acid data were visualized as a biplot (Gabriel, 1981). The biplots presented here coincidentally display the first two principle component scores of the mole percentages of $U^{13}C$-labeled amino acids (points) and the variation among the relative concentrations of amino acids (vectors). The length of the vectors are scaled to the magnitude of the standard deviation for the relative concentration for each amino acid quantified. Correlations between amino acids are the cosine of the angle between vectors.

3. Results and discussion

a. Objective 1: How do infauna react to the deposition of phytodetritus?

The transparent walls of the microcosms and the white coloration of the kaolin allowed for visual observations of infaunal responses to the tracer during incubations. Tracer was redistributed quickly in the microcosms through bulldozing, hoeing and feeding activities. For example, movement of a crab and clams across the sediment surface in the microcosms left tracks in the tracer layer, transporting the mixture horizontally over small distances (1–2 mm). In addition, tubes and burrows were visible to the bottom of the sediment in the microcosms, and animals could move tracer through them rapidly. Most notably, terebellid polychaetes were seen "hoeing" tracer along the walls of the microcosms into burrows ≥ 2 cm below the surface without ingestion.

Quantitatively, the visually striking movement of tracer within the microcosms created subsurface tracer inventories (>1 cm) of 0.39 and 0.67 μmoles tracer C cm^{-2} at the close of the 61 and 89 hour incubations, respectively accounting for 12 and 17% of the tracer added. Subsurface inventories were likely higher in the 89 hour microcosm, because of the additional 24 hours the animals had to subduct tracer. In comparison, less than 1% of the tracer applied was found below 1 cm in the time zero controls.

$PO^{13}C$ concentration and $\delta^{13}C$ profiles in the 61 and 89 hour microcosms exhibited well-defined subsurface peaks centered at 5, 7 and 9 cm (Fig. 1), suggesting nonlocal

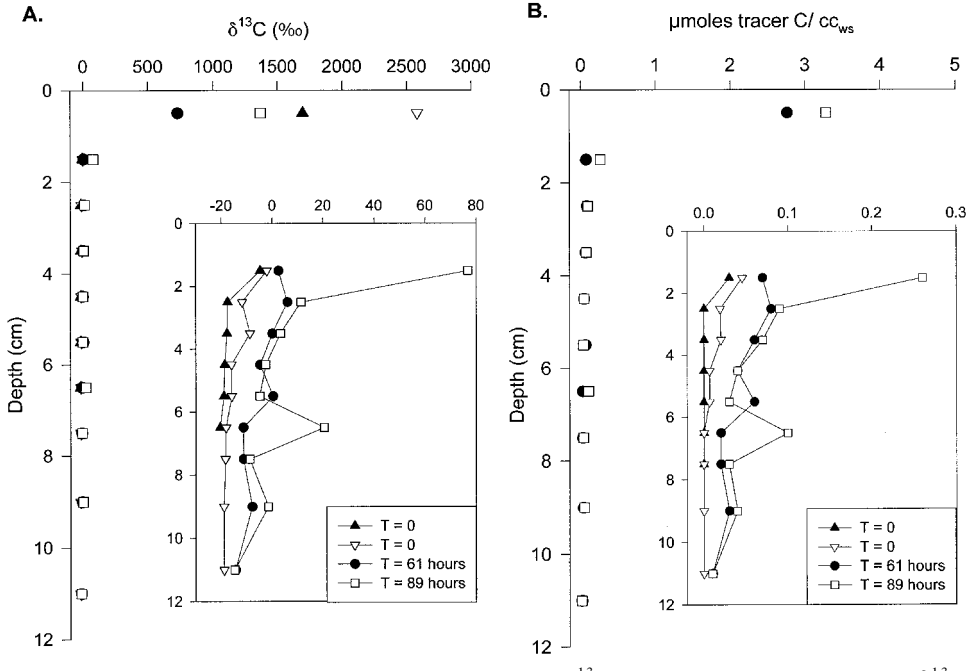

Figure 1. The distribution of solid phase diatom carbon ($PO^{13}C$) in microcosm incubations. (A) $\delta^{13}C$ of sediment in the microcosms. (B) The measured concentrations of tracer carbon within sediment intervals.

transport of the diatom. Although small in appearance, these subsurface peaks in tracer distribution are well outside the noise level. The $\delta^{13}C$ POC profile for sediment in the background core varied at most 0.4‰, yet the difference in the $\delta^{13}C$ of POC above and within a subsurface peak was as much as 14‰ (Fig. 1A). Similar to other experiments, the nonlocal movement of tracer seen here can be summarily attributed to two advective transport mechanisms: (1) tracer moved via ingestion, gut passage, and defecation by deposit feeders, and (2) tracer mechanically transported without gut passage by macrofauna (Rhoads, 1974; Hylleberg, 1975; Dobbs and Whitlatch, 1982; Moriarty *et al.,* 1985; Rice, 1986; Jumars *et al.,* 1990; Wheatcroft, 1992; Sun *et al.,* 1994; Blair *et al.,* 1996; Levin *et al.,* 1997).

The geochemical implications of the two generalized advective mechanisms could be vastly different. Where mixing is attributed to gut passage by macro- and megafauna, metazoans should be acting as reactors within the sediment and playing a direct role in diagenesis. Conversely, transport without ingestion, as during hoeing, should not alter the organics during transit. Therefore, we next need to understand which organisms are advecting the diatom via gut passage and how the coincident digestive reactions change the biochemical composition of the diatom.

b. Objective 2: Which animals redistributed the tracer by ingesting it?

Various animals including a crab, several clams, maldanid polychaetes, and juvenile holothurians were observed ingesting tracer at the sediment-water interface. Heavily labeled animals were recovered from all intervals of the microcosm sediment after incubation for 61 hours, although like the NC slope experiments all infauna were not labeled after 3 days (Table 1). Several *Glycera* (a carnivore), two capitellids and two maldanids were not labeled at the close of the 61-hour experiment. One crab and spionid and cirratulid polychaetes contained the most tracer. Other labeled animals included maldanid and paraonid polychaetes, mollusks, and holothurians. After ingesting sediment and tracer, some infauna deposited feces on the sediment surface. Pellet-, rod- and squiggly-shaped feces collected from the sediment surface at the close of the experiment were heavily labeled (Table 2).

c. Objective 3: How do deposit feeders change the biochemical composition of the tracer?

Gut passage affected the chemical make-up of the tracer diatom based on principle component analysis of the diatoms' and fecal material's U^{13}C-labeled amino acid concentrations (mole percentages). The amino acid data from the diatoms cluster tightly and separate from those of the pellets and the worm tissue in the biplot display (Fig. 2A). The first two principle components accounted for 93% of the variance in the amino acid data, and the differences between the amino acid signatures of the fecal material and the diatom are largely in the relative concentrations of U^{13}C-labeled PHE, LEU, and THR.

The worms used in the feeding experiments had different feeding styles and gut architectures (*sensu* Fauchald and Jumars, 1979 and Penry and Jumars, 1987) and could therefore be used to gauge whether different deposit feeders cause similar changes in the composition of the diatom. *Axiothella,* a head-down feeder, and *Streblospio,* a facultative-suspension, surface deposit feeder, both process particles within their gut in a manner analogous to plug flow reactors and package their fecal material into consolidated rods and coils. In general, plug flow digestors move food particles straight through a tubular gut (Penry and Jumars, 1987; Penry, 1989). The two terebellids, *Polycirrus* and *Amaena,* are tentaculate, surface deposit feeders and function more as mixed reactor-plug flow digestors, ejecting their fecal material less frequently into unconsolidated piles. In mixed-reactor guts, particles are mixed within a more complex digestive tract (Penry and Jumars, 1987; Penry, 1989) which may in turn result in selective retention of particles or hydrolysate (Mayer *et al.,* 1997). The differences between the two types of digestive systems should result in more internal particle sorting and mixing, larger digestive volumes, and a greater potential for gut symbionts within mixed reactor type guts; therefore, it was hypothesized that the terebellids would have different patterns of amino acid alteration and higher digestive efficiencies when compared to *Streblospio* and *Axiothella.*

In fact, the fates of amino acids during gut passage did differ among the three worm taxa. The U-^{13}C amino acid signatures of fecal pellets from *Axiothella* and *Streblospio* are

Table 1. $\delta^{13}C$ (‰) of infauna from the background and 61 hour incubations of mudflat sediment.

	61 hour microcosm					Background microcosm			
Taxa:	0–1 cm	1–2 cm	2–5 cm	5–10 cm	10–12 cm	0–1 cm	1–2 cm	2–5 cm	5–10 cm
Polychaetes:									
Capitellids	−1	−5	−13, −15, −17	−4, +4, +59		−17	−18	−18	−18
Cirratulids	+865	+1348	+1621, 1798	+497, 955, 2268	+1937	−19	−17	−17	−22
Glycerids			−20	−22				−16	
Magelonids					−8				
Maldanids			−15, −17	+69				−16	
Oweniids				+1112					
Paraonids				+46			−18, −18	−18	
Phyllodocids								−18	
Spionnids	+2614					−23, −18	−18	−19	
Syllids	+110		−24			−19			
Terebellids		−10, +40						−19	
Unidentified				+209					
Portunid	+4348								
Holothurian	+161		+37						
Mollusks:									
Gastropod	+12								
Bivalves	+85, 123								

Table 2. $\delta^{13}C$ (‰) of fecal material recovered at the close of the mudflat incubations. The probable source of the material was based solely on visual observations.

Microcosm	Description	Probable source	Interval (cm)	$\delta^{13}C$ (‰)
61 hours	small dark brown piles	Cirratulids	0–1	+380
	large coils in piles	Maldanids	0–1	+119
	small corkscrew shaped rods	Spionids	0–1	+3279
	ellipsoid pellets	Capitellids	2–3	−11
			8–10	−11
			10–12	−11
89 hours	piles of diffuse sediment next to burrow opening	Terebellids	0–1	+896
	very large sandy mound	Holothurians	0–1	+706
	small corkscrew shaped rods	Spionids	0–1	+2355
	ellipsoid pellets	Capitellids	0–1	+2556

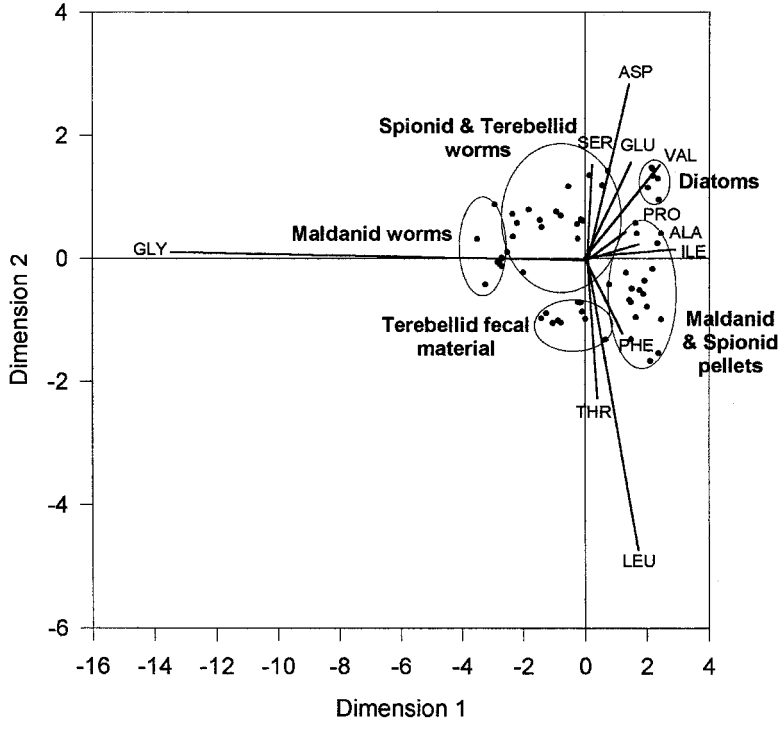

Figure 2. (A) Biplot from experiments with fresh diatoms. (B) Mole percentages of uniformly ^{13}C-labeled amino acids within the diatom, worms and fecal material from experiments with fresh diatoms. Error bars show one standard deviation.

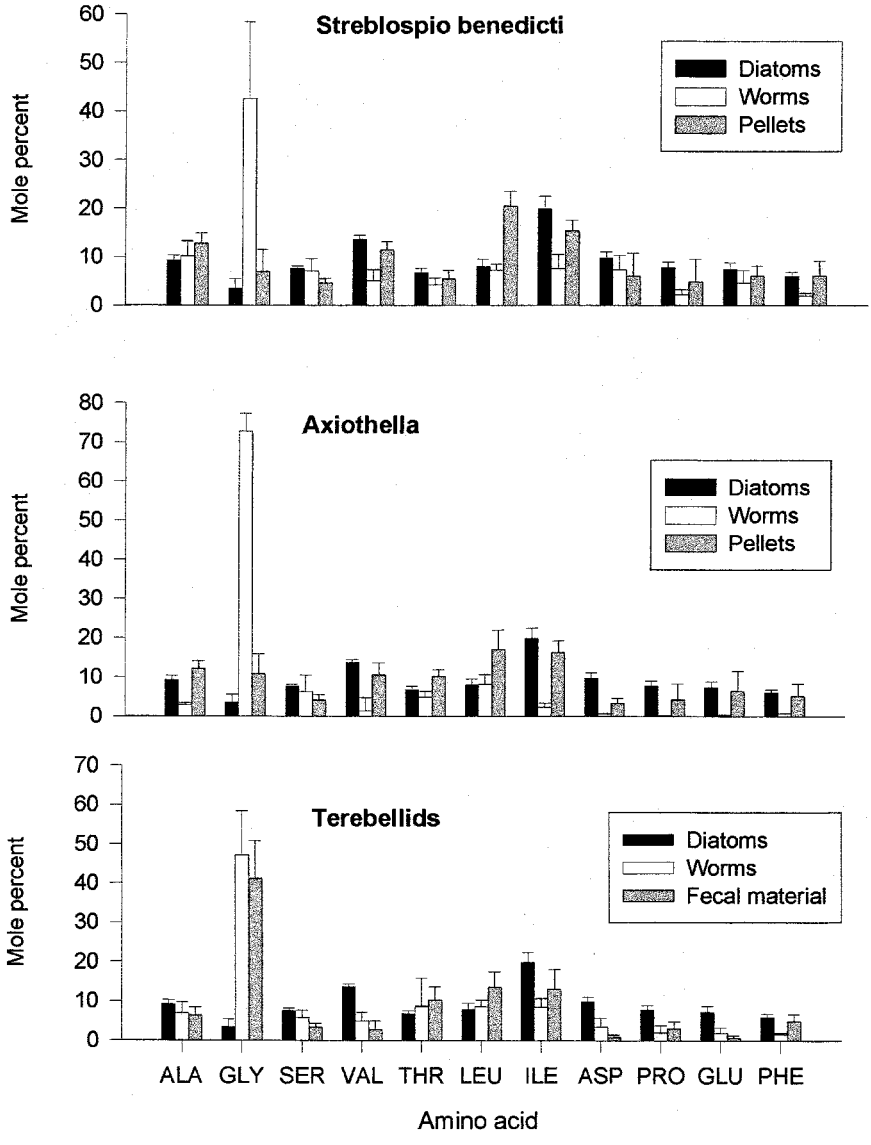

Figure 2. (Continued)

indistinguishable, and differ from the terebellids' fecal material as a result of their U^{13}C-labeled GLY and THR content (Fig. 2B; ANOVA, $n = 27$, $f_{GLY} = 0.0001$, $f_{THR} = 0.0004$). In addition to the U-^{13}C amino acids, the total amino acid compositions ($^{12}C + {}^{13}C$, per gram dry weight) of the fecal material from *Axiothella* and *Streblospio* were also statistically indistinguishable (data not shown). This suggests that the two species digest other amino acid-containing organics similarly, that the

animals are probably ingesting similar particles from the complex sediment mix, and/or a large fraction of ingested particles may pass undigested. The total ($^{12}C + {}^{13}C$) amino acid composition of the terebellids' fecal material could not be compared, because their feces were not separated from uningested sediment during experimentation.

As stated above, it was hypothesized that the differences in gut architecture would also result in higher digestive efficiencies in the terebellids than in the maldanid and spionid. The terebellids apparently did not absorb the labeled amino acids more efficiently. The terebellids and *Axiothella* absorbed 11 ± 8 and 10 ± 7 μmoles ^{13}C amino acids (mg worm)$^{-1}$ hr^{-1}, respectively, while *Streblospio* absorbed 17 ± 12 μmoles ^{13}C amino acids (mg worm)$^{-1}$ hr^{-1}. Estimates of assimilation rates were calculated using the concentration of U-^{13}C labeled amino acids in the worms and the elapsed time between the addition of diatoms and removal of the worms to label-free sediment. These rates are minima because they do not include amino acid turnover within the worm.

In addition to gut architecture, metabolic demands may have driven the selective assimilation of organics during digestion. *Axiothella*'s U-^{13}C amino acid signature differs from the other worms' signatures as the result of significantly higher relative concentrations of U^{13}C-labeled GLY within their tissues (Fig. 2; ANOVA, $N = 27, f \leq 0.0001$), and all three worm taxa contained U^{13}C-labeled GLY in amounts greater than expected if the worms merely absorbed amino acids in a ratio similar to their composition (Fig. 3). GLY absorption sites in polychaete guts may be more abundant than sites for other amino acids in order to meet high metabolic demands. For example, polychaetes are known to concentrate dissolved free glycine intra- and extra-cellularly for osmoregulation (Oglesby, 1978). Also, GLY is abundant in fibrous proteins contained in some marine invertebrates (Mayer *et al.,* 1995) thereby dictating high uptake rates of the amino acid during production of proteins for growth and tissue repair. It is important to note that U^{13}C-labeled glycine could have been synthesized by the worms only through the removal of the functional group from other U^{13}C-labeled amino acids.

Animal physiology and morphology may not be the only determinants of amino acid uptake patterns. Food matrix may also be a factor in the alteration of amino acid concentrations during gut passage. Diatoms use a protein template to lay down their porous silica frustules. Diatoms also exude organic material through the pores in the frustule creating a complex, multilayered cell wall. As a result of the template proteins, the relative distributions of amino acids found in the cell wall differs from that found in the cytoplasm (Hecky *et al.,* 1973). For example, SER, THR, and GLY are enriched in the cell wall of diatoms, and acidic and aromatic amino acids are more enriched in the cytoplasm.

It has been hypothesized that an apparent GLY + SER enrichment with depth in coastal marine sediments results from preferential preservation of organics associated with diatom frustule matrices relative to their intracellular counterparts (Burdige and Martens, 1988; Cowie and Hedges, 1991; 1992). Experiments with copepods have supported this reasoning. Cowie and Hedges (1996) fed ^{14}C-labeled *Thalassisora weissflogii* to *Calanus*

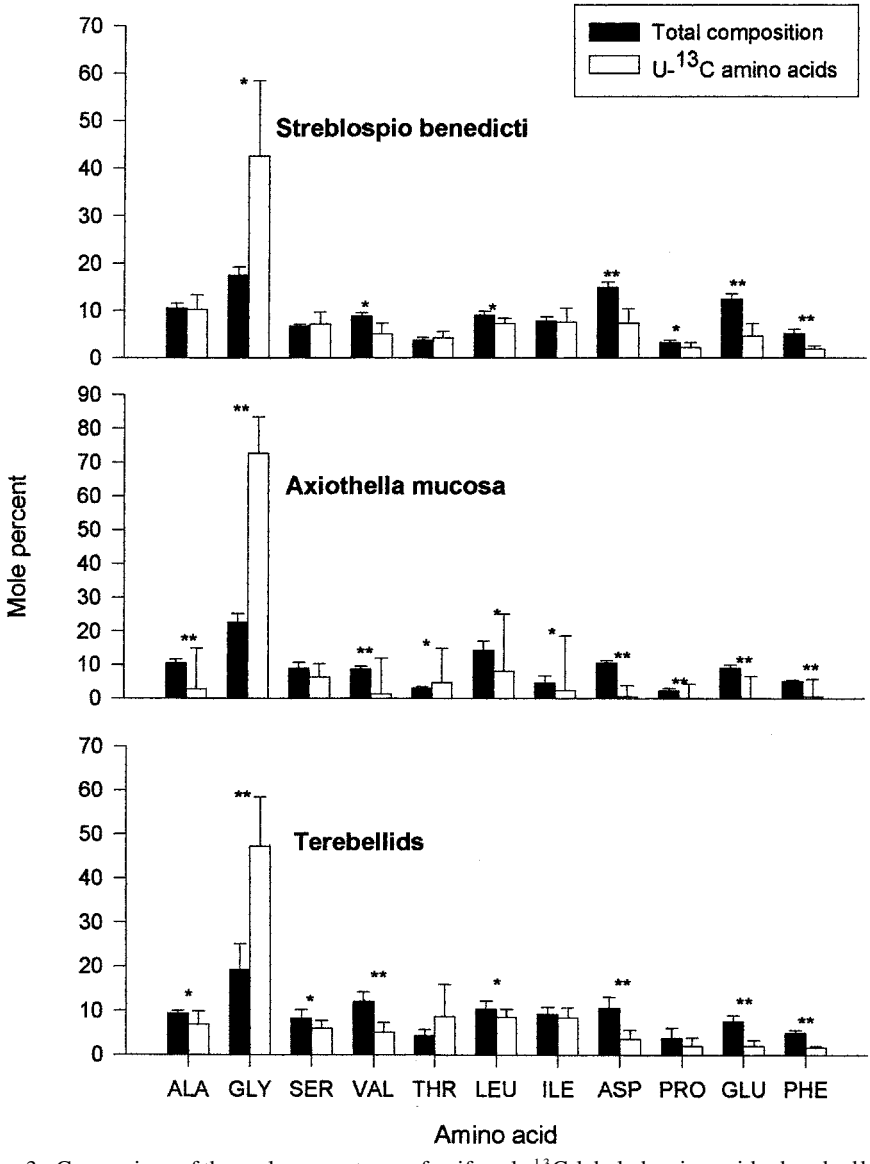

Figure 3. Comparison of the mole percentages of uniformly ^{13}C-labeled amino acids absorbed by the worm with the mole percentages of total amino acids (^{12}C + ^{13}C) contained in the worms' biomass. Error bars show one standard deviation. * indicates $p \leq 0.05$, and ** indicates $p \leq 0.001$ for an ANOVA.

pacificus and found large differences between the biochemical composition of the diatom and fecal pellets. Patterns in the selective degradation of both aldoses and amino acids pointed to digestion of intracellular materials and preservation of cell wall components.

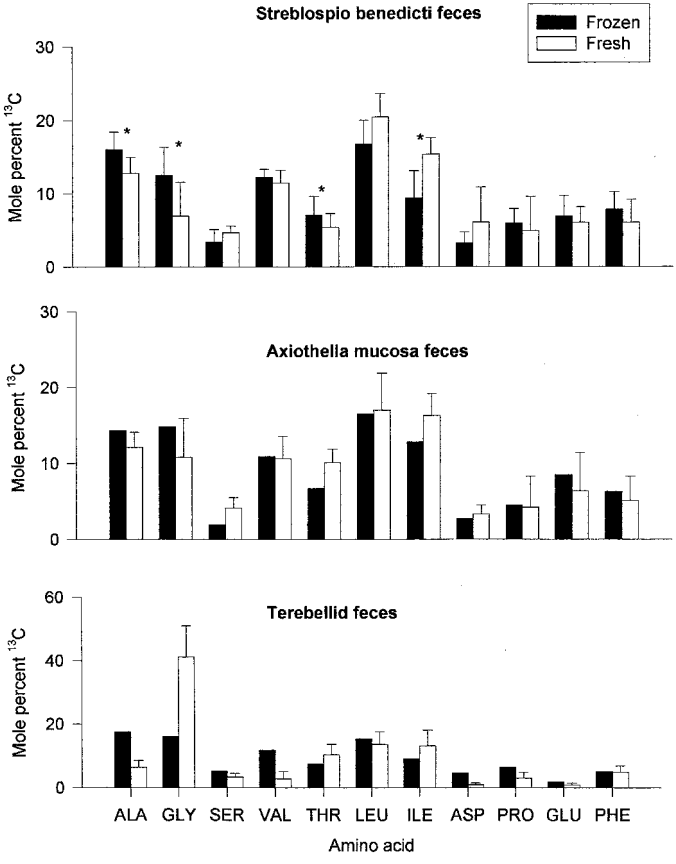

Figure 4. Comparison of the mole percentages of uniformly ^{13}C-labeled amino acids contained in pellets from worms fed fresh or previously frozen diatoms. * indicates $p \leq 0.05$ for an ANOVA.

Here, in the experiments with fresh diatoms the relative concentration of GLY in fecal material was enriched compared to the diatom, especially in the terebellid feces, but SER and THR were not (Fig. 2). Comparisons between the fresh and frozen diatom treatments were intended to provide a test of the hypothesis that freezing the diatom would eliminate protection of cellular components by the silica matrix and in turn would reduce any GLY enrichment in the fecal material by increasing the digestability of cell wall components. However, in experiments with *Streblospio,* freezing the diatom did not appear to increase the digestability of wall components relative to other cellular fractions. The relative percentages of U-^{13}C GLY and THR, which are concentrated in cell wall components, were significantly higher in pellets from the frozen treatment (Fig. 4). In addition, the relative amount of U^{13}-C labeled GLY recovered from worm tissue was not statistically different in the two treatments (Fig. 5). The amino acids ASP, GLU and PHE, which are enriched in intracellular regions, were taken up by the worms fed fresh diatoms in relative

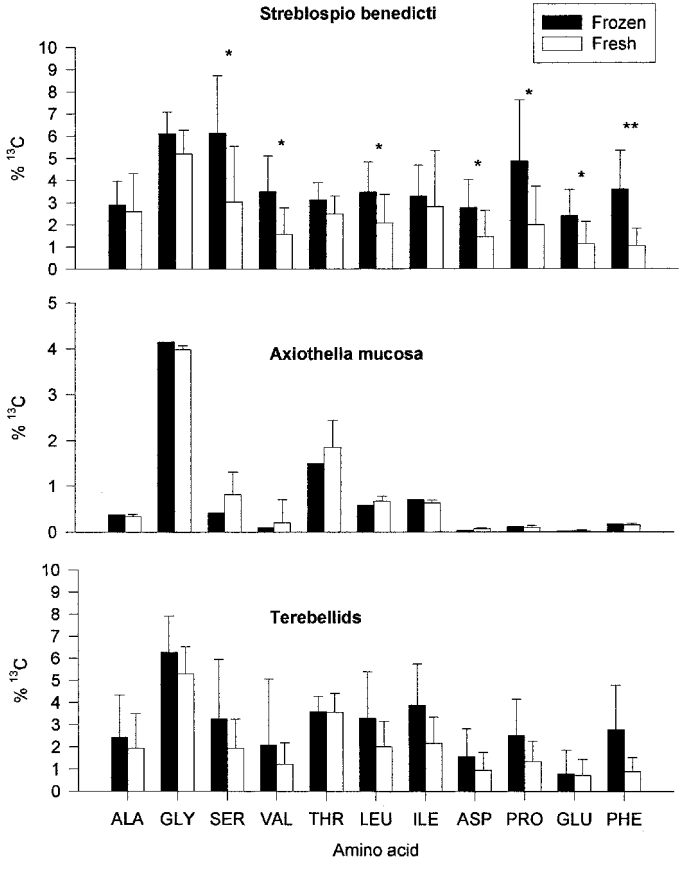

Figure 5. The percentages of each amino acid replaced with its ^{13}C-labeled analog during the experiments was higher when the worms were fed previously frozen diatoms. Error bars show one standard deviation. * indicates $p \leq 0.05$, and ** indicates $p \leq 0.001$ for an ANOVA.

concentrations significantly lower than those found in worm tissue (ANOVA, significance levels in figure) and were made more available by cell disruption (Fig. 5, ANOVA, significance levels in figure). These observations suggest that the deposit feeders used here are able to strip the organic coating from the outside of diatoms but may not sufficiently disrupt fresh, intact diatom cells during gut passage to gain access to intracellular carbon pools.

4. Conclusions

From these experiments it is concluded that deposit-feeding infauna from coastal sediments significantly affect the distribution and chemical composition of phytodetritus during feeding activities. Like numerous deep-sea observations, infauna reacted quickly to

the pulse of phytodetritus, advecting the tracer into the sediment column with and without ingesting it. Nonlocal transport of the tracer resulted in subsurface peaks in concentration profiles. Perhaps more importantly, deposit-feeding polychaetes that ingested the tracer diatom in the microcosm experiments were shown to significantly alter the amino acid composition of the diatom during digestion. Additionally, not all deposit feeders will affect the geochemical signature of phytodetritus in the same way. Worms with plug-flow type guts produced pellets with significantly different amino acid signatures than in fecal material produced by polychaetes with mixed-reactor type guts.

Correlation of subsurface peak composition with alterations seen during gut passage experiments similar to those presented here should help evaluate the efficacy of using molecular and isotopic signatures of fossil marine organics from bioturbated sediments as source and environmental indicators. At least one *in situ* study (Conte *et al.,* 1994; 1995) has compared the organic composition of a subsurface peak with that of incoming detritus and demonstrated the sensitivity of biomarker distribution to biological particle advection. Conte *et al.* (1994) suggest that reverse conveyor-belt deposit feeders are ingesting phytosterol-rich detritus at the sediment-water interface, selectively digesting C_{28} and C_{29} phytosterols, and defecating cholesterol-enriched, phytosterol-depleted pellets at depth. If material is ingested numerous times before burial in the geologic record, digestive alterations could hamper attempts to use reactive compounds such as amino acids and certain lipids as source indicators.

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