Alteration of organic matter during infaunal polychaete gut passage and links to sediment organic geochemistry. Part I: Amino acids

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Abstract

Of the factors which control the quantity and composition of organic matter (OM) buried in marine sediments, the links between infaunal ingestion and gut passage and sediment geochemistry have received relatively little attention. This study aimed to use feeding experiments and novel isotope tracing techniques to quantify amino acid net accumulation and loss during polychaete gut passage, and to link this to patterns of selective preservation and decay in sediments. Microcosms containing either Arenicola marina or Hediste (formerly Nereis) diversicolor were constructed from defaunated sediment and filtered estuarine water, and maintained under natural temperature and light conditions. They were fed with \textsuperscript{13}C-labelled diatoms daily for 8 days, and animals were transferred into fresh, un-labelled sediment after \textsuperscript{13}C\textsubscript{20} days. Samples of fauna, microcosm sediment and faecal matter were collected after 8, \textsuperscript{13}C\textsubscript{24} and \textsuperscript{13}C\textsubscript{40} days, and analysed for their bulk isotopic signatures and \textsuperscript{13}C-labelled amino acid compositions. Bulk isotopic data showed that, consistent with their feeding modes, Hediste assimilated added \textsuperscript{13}C more quickly, and attained a higher labelling level than Arenicola. Both species retained the added \textsuperscript{13}C in their biomass even after removal from the food. A principal component analysis of \textsuperscript{13}C-labelled amino acid mole percentages showed clear differences in composition between the algae, faunal tissues, and sediment plus faecal matter. Further, the two species of polychaete showed different compositions in their tissues. The amino acids phenylalanine, valine, leucine, iso-leucine, threonine and proline showed net accumulation in polychaete tissues. Serine, methionine, lysine, aspartic and glutamic acids and tyrosine were rapidly lost through metabolism, consistent with their presence in easily digestible cell components (as opposed to cell walls which offer physical protection). All sample types (polychaete tissues, sediments and faecal matter) were enriched in labelled glycine. Possible mechanisms for this enrichment include accumulation through inclusion in tissues with long residence times, preferential preservation (i.e. selection against) during metabolism, production from other labelled amino acids during varied metabolic processes, and accumulation in refractory by-products of secondary bacterial production. Overall, similarities were observed between amino-acid decay patterns in faunated microcosms, afaunal controls, and those previously reported in marine sediments. Thus, while polychaete gut passage did produce compound-selective accumulation and losses of certain amino acids in polychaete tissues and faecal matter, the impact of polychaete gut passage on sediment organic geochemistry was difficult to deconvolve from microbial decay. Despite processing large volumes of organic matter, polychaetes may not have distinctive influence on sediment compositions, possibly because metabolic processes concerning amino acids may be broadly similar across a wide range of organisms.

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1. INTRODUCTION

The deposition, degradation and burial of organic carbon in marine sediments are key features of the global carbon cycle, and represent a mechanism for long-term C sequestration. In addition, the deposition of organic matter (OM) is a significant source of energy and elemental raw materials for most benthic communities, and thus sedimentary OM dynamics exert a strong control over marine benthic biodiversity.

A significant amount of previous study has been devoted to understanding the factors and processes which control the abundance and composition of deposited and preserved/buried sedimentary OM (Hedges and Keil, 1995; Burdige, 2005, 2007). Factors controlling OM deposition include water column primary productivity, water depth and sedimentation rate, and these combine with factors such as OM source and reactivity, dissolved oxygen concentration and sediment grain size to control bulk sediment OM contents and preservation (e.g. Canfield, 1994; Hedges and Keil, 1995; Hartnett et al., 1998). The organic geochemical composition of sedimentary OM is also controlled by these factors. Most notably however, OM composition has been shown to relate to OM degradation state. A range of studies have shown that the contributions of amino acids and carbohydrates to total organic matter, and the relative concentrations of biochemicals within particular classes (e.g. pigments, amino acids or carbohydrates) vary systematically with progressive degradation, and these compositional trends are replicated in multiple locations (e.g. Lee and Cronin, 1982; Cowie et al., 1992, 1995; Cowie and Hedges, 1994; Dauwe and Middelburg, 1998; Hedges et al., 1999; Wouds and Cowie, 2009).

Of the processes which control sedimentary OM composition, one has received relatively little attention. Most geochemical studies consider OM degradation as a dominantly microbial process, and thus the role of invertebrate ingestion and gut passage in transforming OM compositions has been overlooked. However, benthic invertebrates have been shown to be extremely efficient at rapidly searching out and ingesting freshly deposited, highly bioavailable OM (e.g. Miller et al., 2000), and in some settings almost all sedimentary OM may pass through the guts of fauna before being subjected to further degradation processes (e.g. Lauerman et al., 1997). Moreover, the digestive tracts of benthic invertebrates contain bacteria, enzymes, acids and surfactants (Longbottom, 1970; Mayer et al., 1997; Ahrens et al., 2001a; Voparil and Mayer, 2004), and are thus adapted to break down complex polymers, releasing simple biochemicals for assimilation. The rate at which these gut fluids act is very rapid, with Ahrens et al. (2001a) reporting the majority of contaminant desorption to occur in the first minute of contact with sediment. Further, gut passage has been shown to move OM between redox zones, causing physical alteration of sedimentary OM, repackaging it into faecal pellets, and exposing new surfaces (Aller, 1994).

Thus, gut passage causes significant biochemical alteration of OM. However, while many studies have examined faunal digestion and assimilation of sediment constituents from an ecological standpoint, conclusions have only rarely been drawn regarding the impact of faunal activity on sediment organic geochemistry.

Early studies of the polychaete gut processes were mostly concerned with morphology, histology, and the details of peristaltic movements, although a few studies also looked at iron absorption and pigment assimilation (Fox, 1950; Dales, 1955, and references therein). A considerable amount of research into infaunal invertebrate gut passage has focused on determining the bioavailability and assimilation efficiency of heavy metal and organic pollutants (e.g. Wallace and Lopez, 1997; Chen and Mayer, 1999; Ahrens et al., 2001a; Casado-Martinez et al., 2010; Janssen et al., 2010). These and other studies have identified a wide range of factors that control benthic invertebrate ingestion rates and assimilation efficiencies. These factors include food source (e.g. macroalgal species), and composition (Hargrave, 1970; Amouroux et al., 1989, 1997; Charles, 1993; Charles et al., 1995, 1996), surfactant micelle concentration in gut fluids (Voparil and Mayer, 2000; Ahrens et al., 2001a), and the sediment to fluid ratio in the gut (e.g. Chen and Mayer, 1999; Ahrens et al., 2001a). Further, several studies suggest that animal taxon and physiology are important controls on faunal feeding and digestive processes (Mayer et al., 1996; Wang et al., 2002). Different taxa have been shown to ingest different particle sizes, and to assimilate compounds from different food sources with varying efficiency (Gremare et al., 1989). Ahrens et al. (2001a) report that Nereis succinea showed significantly higher gut surfactant concentrations than Pectinaria godilii, and Chen and Mayer (1999) found that Arenicola gut fluids released more Cu from sediment than two holothurians (Chen and Mayer, 1998).

In addition, previous research supports the suggestion that invertebrate gut passage will influence OM composition. For example, Thomas and Blair (2002) showed that polychaete faecal pellets were enriched in amino acids including glycine and leucine compared to the food material, and Cowie and Hedges (1996) studying zooplankton observed particularly efficient removal during gut passage (i.e. absence from faecal pellets) of compounds including lysine, glutamic acid and glucose. Bradshaw et al. (1989, 1991) have shown that marine invertebrates preferentially assimilate mono- and poly-unsaturated fatty acids over saturated forms, and that certain sterols are not absorbed at all.

A number of methodological constraints have limited the questions addressed by previous studies. Firstly, many previous studies were conducted without the use of isotopic labels (e.g. Bradshaw et al., 1991). This limits findings to compositional differences between food, faecal matter, and organism tissue. An improvement was offered in the form of 14C-labelled food sources, either as algae (Hargrave, 1970), or as formaldehyde sorbed onto organic matter (Charles et al., 1995). When combined with modelling or a conservative Si tracer, this was capable of distinguishing bulk carbon uptake (Amouroux et al., 1989, 1997), or overall losses of individual amino acids (Cowie and Hedges, 1996). However, it did not allow assessment of assimilation or retention of compounds in the faunal biomass, and it cannot fully resolve production or transformation of bio-
chemicals within animal tissues or by gut bacteria (e.g. Bradshaw et al., 1990). Compound-specific isotopic analysis has recently become widely available, which means that these limitations can now be overcome.

Secondly, experiments used to quantify absorption efficiency during gut passage have usually involved feeding for very short periods (hours) in artificial environments (e.g. in tubes), where the sediment ingested can be tightly controlled and faecal matter can be easily collected (e.g. Ahrens et al., 2001a). Thus, such experiments do not allow study of animals in their natural state, or of long-term, simultaneous compositional changes in the ambient sediment. This focus on organisms in previous studies, has therefore led to a comparative lack of links being made between gut passage and more widely observed compositional changes during OM decay.

The amino acids are an important biochemical class in the marine environment, which can contribute as much as 37% of the C and 81% of the N in sinking organic matter, and 10% of the C and 37% of the N in OM deposited in coastal sediments (Cowie and Hedges, 1992a). Further, they have been shown to constitute as much as ~30% of the most labile fraction of settling organic C, and thus represent an important food source for benthic communities (Cowie et al., 1992). In addition, amino acid suites have been shown to change in a systematic way during OM decay (Cowie and Hedges, 1994; Dauwe and Middelburg, 1998), allowing principal component analysis to be used as an indicator of OM degradation state and bioavailability (Dauwe and Middelburg, 1998; Dauwe et al., 1999; Vandewiele et al., 2009). Given this pre-existing link between amino acid composition and OM degradation, the amino acids provide a logical focus for the consideration of the links between faunal gut passage and OM decay.

Considering the limitations of previous work, the objectives of this study were: (1) to characterise the pattern of net amino acid accumulation in faunal tissues; (2) to characterise the pattern of amino acid loss through metabolism; (3) to link net accumulation and metabolism by fauna to patterns of OM decay. Our overarching hypothesis was that compound selective faunal gut passage and accumulation processes (encompassing digestion, assimilation, anabolism, transformation and metabolism) play a role in determining the long-term compositional patterns observed in particulate OM degradation. This hypothesis was tested through long-term (40-d) feeding experiments using a $^{13}\text{C}$-labelled food source, conducted in microcosms that mimicked the natural environment.

2. METHODS

2.1. Study species and sites

Experimental work was conducted at the Netherlands Institute for Ecology, Centre for Estuarine and Marine Ecology (CEME), in Yerseke, The Netherlands.

Two polychaete taxa were chosen for study, the lugworm Arenicola marina, and the ragworm Hediste (formerly Nereis) diversicolor. They will hereafter be referred to as Arenicola and Hediste, respectively. These species were chosen because they are both common and ubiquitous in European estuaries, and play a significant role in mediating sediment biogeochemistry (Jones and Jago, 1993; Kristensen, 2001 and references therein; Nielsen et al., 2003). At a typical density of 40–80 ind. m$^{-2}$, Arenicola reworks a sediment column of 17–40 cm in a year. For Hediste at a density of 1000 ind. m$^{-2}$ the length of sediment column reworked in a year is a still considerable 0.7 cm (Kristensen, 2001). Further, the two species represent different lifestyles and feeding modes, and so produce an interesting contrast. Arenicola is a head-down conveyor feeder, ingesting detritus nonselectively at depth and excreting at the surface, and is relatively sedentary once it has constructed a burrow (Jones and Jago, 1993; Retraubun et al., 1996; Riisgard and Banta, 1998). Arenicola also has a marked impact on sediment biogeochemistry through its intense bioirrigation activities (e.g. Hylleberg, 1975; Rasmussen et al., 1998; Volkenborn et al., 2010). Hediste, by contrast, has been classified as a surface deposit feeder, a carnivore and a scavenger (Evans, 1971; Ronn et al., 1988), and has also been shown to filter feed in appropriate conditions (Riisgard, 1991). Hediste exhibits considerable motility around a ‘gallery’ of burrows (Francois et al., 2001), and will also emerge onto the sediment surface to feed (personal obs.). Thus, the diet of Hediste includes fresh detritus, suspended particulate organic matter and small invertebrates, while that of Arenicola is dominated by buried OM and possibly ‘gardened’ bacteria (Hylleberg, 1975). It is therefore reasonable to suspect that they will exhibit different patterns of OM alteration during gut passage.

Sediment and polychaete specimens were collected from two intertidal sites in the Scheldt estuary in the south of the Netherlands. Specimens of Hediste, plus surface sediment, were collected from a location (51.55384N, 3.873247E) exhibiting relatively fine grained, muddy sediments, with an organic carbon content (% C$_{org}$) of 0.56%. A separate intertidal site (51.487262N, 4.058526E), showing coarser, less organic rich (% C$_{org} = 0.19\%$), sandy sediments was used for the collection of Arenicola individuals and accompanying surface sediment.

2.2. Microcosm construction

Microcosm construction and experiments were conducted in a controlled temperature laboratory which was set to the seasonal average temperature (15 ºC), and with the lights set to a natural light/dark cycle (typical day length 15–16 h in May at the site latitude).

Surface (top 10 cm) sediment was defaunated before use in microcosms by being twice frozen and thawed to kill macro- and meiofauna. After homogenisation it was then used to partly fill microcosm containers, which were topped up with filtered estuarine water (water column at least 10 cm). In the case of Hediste, microcosm containers consisted of 10 cm i.d. acrylic tubes with rubber stoppers at the base, and sediment columns of ~15 cm. For Arenicola, which require more space per individual, rectangular plastic tanks with a surface area of 980 cm$^2$ were used, and the sediment column was always longer than 20 cm. Microcosms...
were allowed to stand overnight before animals were introduced.

Polychaete specimens (all of a similar size within each species, to avoid variation in feeding and growth rates, Ahrens et al., 2001b) were kept in filtered seawater for 3–5 days before introduction into microcosms, and were acclimated in microcosms for 2–3 days before feeding experiments began. *Hediste* were added to microcosms at a density of 1018 ind. m⁻². This is below the ‘typical’ densities of 3000–4000 ind. m⁻² reported by Kristensen (2001), however it is within the natural range, being higher than the 357 ind. m⁻² found on an Eden estuary mudflat (Defew et al., 2002). Individuals had an average wet weight of 1 g. The density of *Arenicola* in mesocosms was 71 ind. m⁻², in line with typical reported densities of 40–80 ind. m⁻² (Kristensen, 2001). Average individual wet weight was 6.7 g, equivalent to ~13,400 mg ash-free dry weight per m², and within the range of 1.2–22.5 g ash-free dry weight per m² at the nearby Molenplaat (Herman et al., 2000).

Microcosms were kept aerated by bubbling air into the surface water. This aeration also served to keep the water in the microcosms mixed. In order to avoid the build-up of metabolites and increasing salinity due to evaporation, the overlying water in each microcosm was replaced with new filtered estuarine water once every ~4 days. The microcosms were open to the atmosphere for aeration and ¹³C-CO₂ generated could thus escape from the system, preventing determination of closed C budgets.

Polychaete survival was good up to the 21/22-d timepoint (see below), with one individual recovered dead from some microcosms. At the 37/38-d timepoint survival was not as high, with 3–4 individuals missing from some microcosms. Missing animals are presumed to have decayed in the microcosms, as escape would not have been possible.

### 2.3. Feeding experiments

Polychaetes were fed by addition of ¹³C-labelled (64.6 atom%) diatom detritus (*Skeletonema costatum*), grown at the Netherlands Institute of Ecology, Yerseke. Algal slurry (freeze-dried algae suspended in filtered estuarine water) was introduced into the water column of each microcosm once per day on 8 consecutive days, after which no additional feeding took place. The total feeding dose after all 8 feeding days was 3.1 g C m⁻² for *Arenicola*, and 3.5 g C m⁻² for *Hediste* microcosms. The high doses used were to ensure detectable label at the end of the experiments (see below), and the slight discrepancy between species was due to the practical constraint of ¹³C-labelled algae availability. As soon after the end of feeding as possible (on day 9 for *Arenicola*, day 10 for *Hediste*) a sub-set of the microcosms was sacrificed and samples were preserved. The next timepoint occurred on day 21 for *Arenicola* and day 22 for *Hediste*. At this timepoint another sub-set of microcosms was sacrificed. In addition, animals from the remaining microcosms were removed with great care, and transferred to newly-constructed equivalents that did not contain any ¹³C-labelled algae. This was in order to observe the retention or loss of amino acids from their tissues. Animal and sediment samples from these new microcosms were collected on day 37 for *Arenicola*, and on day 38 for *Hediste*.

For *Arenicola* (due to the amount of algae required), there was one microcosm per timepoint, each containing multiple animals. For *Hediste*, two replicate microcosms were sacrificed at each timepoint. It is recognised that multiple animals within one microcosm are pseudoreplicates, rather than true replicates. Additional replicate microcosms could not be run due to the expense, and thus limited availability, of labelled algae, as well as the labour-intensive nature of amino acid analyses. We chose not to construct a larger number of small microcosms, as we were attempting to replicate natural conditions, and wished to minimise wall effects. Examination of polychaete bulk isotope signatures (see below) from *Hediste* replicate microcosms shows no significant difference at either 10 or 22 day timepoints (*t*-test, *p* = 0.869 and 0.402, respectively), and this lack of variation between microcosms suggests that variation between individual animals is the primary source of spread in the data.

In addition to the faunated microcosms, two ‘control’ microcosms, to which no animals were added, were constructed (in 54 mm diameter acrylic tubes) using defaunated sediment from each site. These were fed as for the faunated microcosms. One core from each pair of controls was sectioned on day 9 or 10, and the other on day 37 or 38 (for *Arenicola* and *Hediste* controls, respectively). These microcosms were smaller than the microcosms with fauna due to the limited availability of labelled algae. This is unlikely to have affected the data, as the absence of fauna means that the dominant processes will have been microbial, and thus not affected by the small-scale patchiness of macrofaunal communities. Similarly, limited availability of algae is the reason why control cores were not sacrificed at the 21/22-d timepoint.

For *Arenicola* the process of sacrificing a microcosm entailed extruding and sectioning (at 0.5 cm intervals to 2 cm depth, followed by 1 cm intervals to 10 cm depth) three subcores (two taken with a 50-ml syringe with the end removed, and one with a 54 mm i.d. core tube) from the microcosm. Overlying water was then siphoned off, and all visible faecal matter was collected and preserved. The remaining sediment was then dug out with great care so as not to damage the polychaetes. In the case of *Hediste* microcosms, overlying water was siphoned off and sediments were extruded and sectioned. Care was taken not to damage the polychaetes, which were extracted by hand as they were found. All sediment samples were placed in bags and frozen. Polychaetes were transferred to large petri dishes containing filtered estuarine water and left for 2–5 h to egest their gut contents. Polychaetes and gut contents were then transferred to pre-combusted glass vials, and frozen. It should be noted that gut evacuation was not complete (~80% complete, visual estimation), as this could have taken days-weeks in the starvation conditions of the petri dish. Such a delay could have produced changes in amino acid metabolism. Data from gut contents are not presented, as the samples were too small to analyse. Therefore, the lack of this data is unlikely to have impacted significantly on quantitative budgets (see Section 3.2.3).
2.4. Analytical techniques

Polychaete samples were defrosted and homogenised using a Potter tube tissue grinder prior to being freeze dried. Freeze-dried *Hediste* sediments were gently disaggregated using a pestle and mortar to aid homogenisation. This was not necessary for * Arenicola* sediments, which were easily disaggregated by manually squeezing sample bags.

Sub-samples of faunal tissues, sediments and faecal matter was prepared for bulk C isotopic analysis by weighing into ultraclean silver capsules, and decarbonation with 6 N (sediment and faecal matter) or 0.1 N (faunal tissues) HCl. Samples were analysed on a Europa Scientific (Crew, UK) Tracermass isotope ratio mass spectrometer (IRMS) with a Roboprep Dumas combustion sample converter at the Scottish Crop Research Institute. Carbon contents were determined from IRMS peak areas and calibrated against standards of acetic acid. This method gave an average relative standard deviation of 4.6% for organic carbon quantity, and 0.7 per mil for $\delta^{13}C$ ($n = 27$, Wouds et al., 2007).

Quantification of $^{13}C$-labelled amino acids in sediment, fauna and faecal matter was conducted as described by Wouds et al. (2010). Briefly, samples were hydrolysed in oxygen-free 6 N HCl for 70 min at 150 $^\circC$. Charge-matched internal standards (Cowie and Hedges, 1992b) were added, and samples were dried. This was followed by ion exchange cleanup using Dowex 50W8 resin, from which amino acids were eluted in NH$_4$OH and again dried. Derivatisation to trifluoroacetic isopropyl esters was initiated by dissolving the samples in acidified propanol and heating to 110 $^\circC$ for 70 min. Samples were then dried under a stream of N$_2$, and twice washed and dried with dichloromethane. Samples were then acetylated by dissolving in trifluoroacetic anhydride and dichloromethane, and heating to 110 $^\circC$ for 10 min. Samples were finally twice washed and dried with dichloromethane.

Analysis was achieved using a GC–MS equipped with a 30-m, 0.32 mm diameter, 0.25 $\mu$m film thickness Equity 5 column (Supelco). The initial temperature of 35 $^\circC$ was held for 1.5 min before ramping to 100 $^\circC$ at 5.5 $^\circC$ min$^{-1}$. This was followed by a ramp at 4 $^\circC$ min$^{-1}$ to 190 $^\circC$, and then to 280 $^\circC$ at 70 $^\circC$ min$^{-1}$ with a 5 min hold to bake the column. The injector temperature was 290 $^\circC$, and the He carrier gas flow rate was 1.5 ml min$^{-1}$.

Positive ion chemical ionisation with CH$_4$ at 20% and selective ion monitoring was used. Quantification was based on a characteristic, pseudomolecular ion for each of the natural (unlabelled) and added ($^{13}C$-labelled) versions of each amino acid. Preservation of these large ions required the use of a relatively low ion source temperature (154 $^\circC$). For details see Wouds et al. (2010).

Naturally present (unlabelled) amino acids in each sample were quantified using the internal standards and a single external standard containing known amounts of all compounds. Since unlabelled standards were not suitable for quantifying $^{13}C$-labelled amino acids (as they did not yield the appropriate quantifier mass fragments), isotope calibration curves were constructed for this purpose. Known volumetric mixes of derivatives of $^{13}C$-labelled algae and natural, unlabelled sediment were made and analysed by GC–MS. The amino acid concentrations in each derivative were independently quantified beforehand using GC-FID. Therefore, the amounts of algae-derived and sediment-derived amino acids in each mix was known, and calibration curves were constructed which related this to the ratio of the MS responses for the labelled and unlabelled quantifier ions of each amino acid. These calibration curves were used to quantify $^{13}C$-labelled amino acids in samples, based on the quantity of the unlabelled version, and the ratio in MS response between the labelled and unlabelled quantifier ions. For further discussion see Wouds et al. (2010).

2.5. Data treatment

Results are presented as the average ± standard deviation of replicate/pseudoreplicate microcosms and animals. Error bars consequently include analytical uncertainty (small) and experimental variability (larger component). Normality testing, Mann–Whitney U and Kruskal–Wallis tests, and principal component analyses were applied to the data (see below), using Minitab 15.

3. RESULTS

3.1. Bulk $^{13}C$ uptake

Bulk isotopic signatures show that *Hediste* rapidly assimilated the added diatom detritus, with some individuals becoming highly $^{13}C$-enriched (Fig. 1a). *Arenicola* individuals also assimilated added algal material (Fig. 1b), but after 9 days were still not as $^{13}C$-enriched as the surface 0.5 cm of sediment. In addition, *Hediste* (max. $\delta^{13}C = 2454^{\circ}_{\%}$) attained higher maximal labelling levels than *Arenicola* (max. $\delta^{13}C = 1273^{\circ}_{\%}$). These trends are unsurprising, since, as a head-down feeder, *Arenicola* will not have had access to the labelled algae until it penetrated to the depth of the burrow bottoms. In contrast, *Hediste* individuals were observed to emerge from their burrows ~5 min after feeding, presumably to actively collect the fresh food. In general, faunal tissues showed their maximal isotopic enrichments at the end of feeding (9 or 10 days). However, there were no significant decreases in tissue isotopic signatures with time (Kruskall–Wallis $H = 0.72$, 0.18; $P = 0.697$, 0.912; DF = 2 for *Hediste* and *Arenicola*, respectively; Fig. 1). Thus, the assimilated C appears to be efficiently retained in the biomass, even after the $^{13}C$-labelled food source was removed (i.e. at day 21 or 22).

3.2. Amino acids

3.2.1. Amino acid suites

The suite (expressed as mole percentage of all amino acids detected) of $^{13}C$-labelled amino acids present varied considerably between faunal tissue, faecal matter, sediment, and the source algae (Fig. 2), indicating that net accumulation and loss processes were compound-selective. The visually evident trends in Fig. 2 include an enrichment of faunal tissues in glycine at the expense of other amino acids such as alanine in comparison to the $^{13}C$-labelled algae.
Amongst sediment samples there were enrichments in alanine, glycine and leucine and depletions in aspartic and glutamic acids compared to the source algae (Fig. 2b).

In order to conduct a rigorous analysis of compositional difference between samples, a principal component analysis (PCA) was carried out on 13C-labelled amino acid mole percentage data (Fig. 3). Principal component 1 (PC1) explained 32% of the variation, and principal component 2 (PC2) a further 17% (totalling 49% for PC1 and PC2 together). The PCA revealed clear differences in composition between the 13C-labelled algae and all other sample types (fauna, sediments and faecal matter), which all showed lower principal component 1 scores than the algae. In addition, faunal tissues were separated from algae on the PC2 axis, showing lower scores. Factor coefficients (Fig. 3b) indicate that these differences in scores result from the algae being relatively enriched in aspartic acid, glutamic acid, serine and methionine compared to samples taken from the microcosms, suggesting that these compounds are preferentially metabolised. Conversely, polychaete tissues, faecal matter and sediment samples were relatively enriched in glycine, leucine and proline. Polychaete tissues were separated from sediment and faecal matter samples by their lower PC2 scores (t-test, \( P < 0.001 \); Fig. 3a), indicating that they tend to have greater mole percentages of proline, phenylalanine, valine and lysine (Fig. 3b).

In addition, the two species of polychaete showed different labelled amino acid compositions, denoted by their separation from each other along the PC1 axis (t-test \( P < 0.001 \); Fig. 3a). This is largely linked to greater mole percentages of glycine in \textit{Arenicola} tissues compared to those of \textit{Hediste} (Fig. 3b). Thus, amino-acid-selective accumulation and transformation/loss processes are, to some extent, taxon-specific.

### 3.2.2. Selective net accumulation

The use of mole percentages (relative concentrations) and PCA to elucidate selective accumulation or loss of various amino acids has its limitations, as a marked shift in the abundance of one major amino acid (e.g. glycine) can either force or mask smaller changes in the mole percentages of less abundant compounds. In order to avoid this pitfall, amino acid suites for faunal tissues were also compared on an absolute basis. Variations in concentrations due to different levels of bulk 13C uptake by fauna were normalised by expressing 13C-labelled amino acid concentrations as micro moles of compound per mg of assimilated 13C. These

![Fig. 1. Isotopic compositions of polychaete tissues (experimental and natural background) and microcosm surface (0.5 cm) sediments with time through the experiments.](image-url)
were then used to derive an ‘enrichment factor’, by subtracting the $^{13}$C-normalised concentration found in the source algae from the $^{13}$C-normalised concentration found in each polychaete sample (Fig. 4). In fact, enrichment factors derived from $^{13}$C-normalised absolute concentrations showed similar patterns (although not precisely the same) to those calculated in the same way using the data in mole percentage form (Fig. 4b). Enrichment factors show that faunal tissues consistently contained certain amino acids in lower concentrations/proportions than those in which they were present in the source algae. These amino acids were alanine, serine, methionine, aspartic acid, glutamic acid, tyrosine and lysine (mole percentages in polychaete tissues statistically significantly lower than in algae for all except lysine, Mann–Whitney U, $P \leq 0.0067$). It is likely that these compounds were either selected against during solubilisation and absorption in the gut, or rapidly metabolised in polychaete guts and tissues. The possibility remains that they were rapidly metabolised before ingestion. However, this seems unlikely, as the low concentrations were present in polychaete tissues even at the first sampling timepoint, which was only one day after the final dose of algae had been added to the microcosms. Further, visual observations suggest that Hediste individuals tended to leave their burrows and feed on the surface within minutes of algae being added; thus they were very likely selectively ingesting fresh algae, at least for the first 8 days of the experiment.

Fig. 2. Mole percentage compositions of $^{13}$C-labelled amino acids in faunal tissues, faecal matter (a), and microcosm sediments (surface and downcore, b). Faunal and faecal matter data are averages. Surface sediment data are for the top 0.5 cm, and downcore data are for the 1.5–2 cm horizon. $n = 1$ for all samples in panel B, as there is no justifiable basis for averaging across different depth horizons. ALA = alanine, GLY = glycine, THR = threonine, BALA = β-alanine, SER = serine, VAL = valine, LEU = leucine, ISO-LEU = iso-leucine, MET = methionine, PRO = proline, PHE = phenylalanine, GABA = γ-aminobutyric acid, ASP = aspartic acid, GLU = glutamic acid, TYR = tyrosine, LYS = lysine, ORN = ornithine.

(a)
In contrast to the above, several amino acids were often present in faunal tissues in greater concentrations/proportions than those in which they occurred in the source algae. The most notable example was glycine (mole percentages in polychaete tissues statistically significantly higher than in algae, Mann–Whitney U, \( P = 0.0044 \)), but threonine, valine, leucine, proline, and phenylalanine also showed this tendency (Fig. 4). This net accumulation in tissues could have been the result of selective assimilation, retention and/or production in faunal tissues.

Enrichment factors could not be calculated for sediment and faecal matter samples due to a mismatch in the samples analysed for bulk isotopes and amino acids. Therefore differences in mole percentage compositions between sediments/faecal matter and algae have to be considered instead, and these are open to interfering effects between different amino acids. Despite this limitation, mole percentage differences showed similar patterns to faunal tissue enrichment factors. These included marked losses of aspartic and glutamic acids, as well as lysine, methionine, valine, serine, and threonine, and enrichments in glycine and leucine (Fig. 4c and d). Once again, these could be ascribed to particularly rapid metabolism of certain compounds either by polychaetes or sediment/gut bacteria. Enrichments (or accumulation) of certain amino acids in the sediment during decay have previously also been ascribed to sorptive protection from degradation afforded by the diatom silica test, and this mechanism remains as a possible explanation for the glycine enrichment observed here (e.g. Nguyen and Harvey, 1997).

3.2.3. Quantitative budgets

Labelled amino acid concentrations from sediments and polychaetes were combined with sediment porosity data to produce quantitative budgets for each amino acid at the 9–10 and 21–22 d timepoints (Fig. 5). Meaningful amino acid budgets for the 37–38 d timepoint could not be constructed because animals were transferred to unlabelled sediment for...
the latter half of the experiment. When all faunated microcosms were considered, there were significant differences amongst amino acids in the percentage recovered (the percentage of the total amount of each compound that was added in the form of algae to be recovered from faunal tissues and sediment) (Kruskal–Wallis DF = 16, $P < 0.001$).

Amino acids such as glycine, and in the case of *Hediste* microcosms, leucine, valine and β-alanine, showed relatively high recovery, and thus were either actively produced, retained, or were resistant to degradation or protected by sorptive association with diatom tests (e.g. *Nguyen and Harvey, 1997; Thomas and Blair, 2002*). In contrast, methionine, and to a lesser extent aspartic acid, glutamic acid, tyrosine, lysine and serine showed relatively low recovery, and thus were preferentially metabolised by macrofauna or microbes.

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**Fig. 4.** Enrichment of samples in each amino acid compared to the source algae. Note, in a and b the y axis is truncated. Values for glycine were up to 3.5 (panel a) and 50 (panel b). Plot A compares the $^{13}$C normalised concentrations of $^{13}$C-labelled amino acids, and shows similar trends to plot b, in which differences in mole percentages between polychaete tissues and source algae are plotted. Due to the similarity, only the latter approach was used in panels c and d, which show the differences between sediment sample and algal compositions for *Hediste* and *Arenicola* microcosms, respectively. Error bars are ±1 standard deviation.
In general there was no difference in the percentage of amino acids recovered from microcosms between faunated microcosms and afaunal controls (Mann–Whitney U, $P = 0.051–0.999$). The only systematic trend was that controls which ran until the end of the experiment (37–38 d) showed generally lower recovery than all other microcosms (significantly for methionine, phenylalanine, glutamic acid and lysine, Mann–Whitney U, $P = 0.0413$), and this was
most likely a function of the longer time available for metabolism/ degradation. Thus, differences in recovery between faunated microcosms and afaunal controls may have emerged if the experiment had run for longer before the polychaetes were moved into unlabelled sediment. In addition, there was variation between *Hediste* and *Arenicola* microcosms in the average percentage recoveries (Fig. 5), and these differences were significant in the cases of glycine, tyrosine, and y-aminobutyric acid (Mann–Whitney U, \( P = 0.0304 \)).

4. DISCUSSION

This discussion will focus on the processes which produced the observed bulk $^{13}$C and amino acid distributions. It is worth noting that the observations are the net result of a complex set of simultaneous processes, therefore it is not always possible to draw firm conclusions on the occurrence/significance of individual mechanisms. It should also be noted that the experiments were conducted under food replete conditions, which were judged most relevant for springtime, when microphytobenthos and phytodetritus are readily available. Had starvation been allowed it may have affected polychaete feeding behaviour, and caused a decrease in growth and respiration rates, possibly leading to a different fate for the labelled algae (Nielsen et al., 1995).

4.1. Bulk C uptake and remineralisation

The $\delta^{13}$C values attained by *Hediste* were greater than those attained by *Arenicola* (Fig. 1), and this is consistent with the attributes of the taxa. *Hediste* is a motile, surface feeder (Evans, 1971; Ronn et al., 1988), and will have had
more rapid access to the algae than the sedentary, head-down feeder *Arenicola*. In addition, *Hediste* is a smaller organism, and can be expected to have a faster growth rate, consistent with its greater labelling levels. Further, by the 9/10 day timepoint, *Hediste* individuals showed up to ~1.5 at.% greater labelling level than the surface sediments, implying that they were feeding selectively on the added algae. In contrast, *Arenicola* individuals exhibited a lower labelling level than the surface sediment at 9/10 d, and were only 13C-enriched compared to surface sediments (by ~0.9 at.%) later, when sediment enrichment had returned to near-background (Fig. 1). Thus there is no evidence for *Arenicola* feeding selectivity. The difference in C uptake rates and feeding behaviours between the taxa is further highlighted by the fact that, after feeding, 6.7% of the total C present in the uppermost 1 cm of *Arenicola* sediments was derived from the added algae, whereas this value was only 4.6% in *Hediste* sediments. In spite of this, *Hediste* attained greater δ13C values. Finally, the data show for both species that 13C is retained in faunal tissues for longer than in the sediment (see later for further discussion).

Before a discussion of the mechanisms driving amino acid suite variations, the importance of bulk algae, and amino acid remineralisation should be considered. Bulk OM remineralisation has previously been shown to consume as much as 30–40% of added OM over 20 days in similar experiments to those presented here (Andersen and Kristensen, 1992). Further, Langenbuch and Portner (2002) used O2 consumption/ammonia production ratios in annelid tissues to suggest that most or all of its cellular energy demand was being supplied by amino acid catabolism. In addition, carnivorous fish may cover >40% of the total energy need with amino acids (Conceicao et al., 2002).

Quantitative budgets for bulk 13C are not available here, but the fact that surface sediment isotopic signatures approached natural background levels after 15–20 days (Fig. 1) suggests that substantial mineralisation of bulk algae occurred. Amino acid quantitative budgets show that, across all microcosms, 81–94% of the bulk amino acid pool, and 39–100% of individual amino acids were lost. Further, it is likely that amino acids were preferentially lost compared to bulk organic carbon as, despite sustained bulk 13C enrichments, the concentrations of labelled amino acids in faunal tissues decreased between timepoints (Fig. 5). Thus, compound-selective mineralisation of OM must be considered as a major driver of the amino acid composition patterns discussed below.

4.2. Amino acids

4.2.1. Amino acid behaviour patterns

For the following discussion amino acids have been grouped, based on similar behaviour.

4.2.1.1. Selective accumulation. Phenylalanine, valine, leucine, iso-leucine, threonine and proline (and glycine, see later) showed varying degrees of net accumulation in polychaete tissues compared to the source algae (Figs. 2–4), and it is therefore suggested that they were selectively assimilated and/or preferentially retained. Heterotrophic organisms must obtain certain essential amino acids from their diets, and studies of flour beetles, carpet beetles and honey bees have shown similar amino acid essentiality to rats, dogs, chicks, mice and humans (Meister, 1965). It is therefore likely that the same pattern of essentiality follows for marine polychaetes. Thus, the observed accumulation of phenylalanine, valine, leucine, iso-leucine, and threonine is consistent with the fact that they are all essential (Meister, 1965; Phillips, 1984). Selective uptake and/or preferential retention of essential amino acids is consistent with previous findings that invertebrate growth is not always limited during low availability of essential amino acids, possibly due to compensatory mechanisms which limit their degradation (Phillips, 1984, and references therein).

The relative concentrations in which organisms require different amino acids is often taken to be indicated by the proportions in which they are present in faunal tissues. For example, in fish, protein synthesis and growth are maximised when the ratios of amino acids supplied closely matches those in their tissues (e.g. Saavedra et al., 2010). Thus selective assimilation and/or preferential retention is also indicated by the fact that *Hediste* contained valine, leucine and iso-leucine in greater proportions than those naturally present in its tissues (Fig. 6).
Selective assimilation or preferential retention are also suggested when polychaete amino acid requirements are compared with amino acid availability in the sediment. Dauwe and Middelburg (1998), did this by comparing the natural amino acid compositions of sediment and polychaete tissues, and calculated a sediment deficiency index for each compound, using Eq. (1).

$$\text{Sed Deficiency index} = \frac{(S_{\text{Mol\%}} - P_{\text{Mol\%}})}{P_{\text{Mol\%}}} \times 100$$ (1)

$S_{\text{Mol\%}}$ and $P_{\text{Mol\%}}$ are the mole percentages of each (naturally present, not $^{13}$C-labelled) amino acid in the sediment and the polychaete tissues, respectively. Negative values denote amino acids which are ‘deficient’ in the sediment. This index is limited, as it does not account for the fact that only a small proportion of total sedimentary organic matter is typically labile, and available to organisms (Mayer et al., 1995; Purinton et al., 2008), nor for variations in bioavailability between compounds. Nevertheless, it provides an interesting indication of potential sediment deficiency.

The sediment deficiency index showed that most of the amino acids that accumulated in polychaete tissues were deficient in the sediments (Fig. 7, although valine, isoleucine and proline were only deficient for Hediste). It follows that selective assimilation of these essential amino acids by abundant polychaetes could be partly responsible for their deficiency in the sediment.

Selective uptake or retention of individual amino acids may also be linked to their relative importance in metabolic processes. For example, phenylalanine is an essential precursor to the semi-essential tyrosine, which in turn is the precursor for thyroxine, required for metabolism and growth, as well as of adrenaline, and of hormones that regulate the nervous system and response to stress (Sauvedra et al., 2010). A complete review of amino acid metabolic rates and pathways is beyond the scope of this article, and the reader is directed instead to Caspi et al. (2010).

In summary, the pattern of net accumulation is consistent with amino acid essentiality and natural availability, and may constitute a direct link between faunal gut passage and sediment geochemistry.

4.2.1.2. Rapid loss. A group including serine, methionine, lysine, aspartic and glutamic acids and tyrosine showed depleted mole percentage concentrations in polychaete tissues, faecal matter and sediment samples (Fig. 4) compared to the source algae (especially for serine, aspartic acid and tyrosine, Fig. 2). They also showed particularly large losses in absolute terms (Fig. 5). Thus, these amino acids were subject to particularly rapid degradation or metabolism, which may have occurred in polychaete tissues, guts and faecal matter, as well as in the sediment, through gut or ambient microbial activity.

Studies of amino acid catabolism find that non-essential amino acids are metabolised to a greater extent than essential ones (e.g. Concejiao et al., 2002; Applebaum and Ronnestad, 2004). Further, studies in rats have shown that alanine, glutamate and glutamine are preferentially catabolised in preference to other non-essential and all essential amino acids (Tanaka et al., 1995; Concejiao et al., 2002; Applebaum and Ronnestad, 2004). Apart from aspartic acid and tyrosine, all the amino acids in the ‘rapid loss’ group were essential (Phillips, 1984) and also deficient in the sediment (Fig. 7). Thus, it seems unlikely that their rapid metabolism occurred in polychaete tissues, as essential amino acids and those in short supply have been observed to be actively retained or protected in invertebrates (Phillips, 1984, and references therein).

The observed rapid loss of glutamic acid is potentially consistent with knowledge of the catabolism of glutamate, which is converted to glutamic acid during the analytical procedure used here. Applebaum and Ronnestad (2004) noted less marked absorption of glutamate than of other amino acids by Atlantic halibut larvae, and attributed it to masking of absorption by very rapid metabolism. Glutamate metabolism has been observed to exceed that of alanine, potentially because it occupies a pivotal position in catabolic pathways, where several other amino acids (glutamine, histidine, proline and arginine) are converted to glutamate before catabolism (Applebaum and Ronnestad, 2004). Further, our observation of rapid loss of lysine is consistent with the suggestion by Gomez-Requeni et al. (2004), that lysine is rapidly used in protein synthesis or catabolism by young gilthead sea bream.

Other mechanisms for the rapid loss of certain amino acids include sloppy feeding and dissolution (Cowie and Hedges, 1996). In addition, freeze-dried (lysed) cells were used in this study; thus, the cell contents were easily accessible to digestive and microbial processes. Thomas and Blair (2002) found that lysing cells increased the uptake of the cell interior amino acids aspartic acid, glutamic acid and phenylalanine by polychaetes, and Cowie and Hedges (1996) explained the loss of aspartic acid, glutamic acid and lysine from zooplankton faecal pellets by their location in the easily digested cell interior. Thus, this explanation may also apply to the rapid losses of aspartic acid, glutamic acid and lysine, observed here.

4.2.1.3. Glycine. Glycine has numerous potential functions within polychaete tissues, such as being a precursor in the synthesis of many important biochemicals, including porphyrins, (precursors to pigments and haemoglobin), and...
creatinine (essential in muscles and nerves), and purine nucleotides that form part of the backbone of DNA (Lehninger, 1982). Further, it functions as a solute in extracellular fluids used for osmoregulation (Oglesby, 1978), and is concentrated in the fibrous tissues of polychaetes (Mayer et al., 1995). However, it has also been suggested that glycine has a relatively low nutritional value (Dauwe and Middelburg, 1998), and a low energy yield during metabolism.

The most notable alteration observed in amino acid suites was an enrichment of polychaete tissues, faecal matter and sediments with glycine (Figs. 2 and 4). It also accumulated in polychaete tissues in greater proportions than those in which it was naturally present (Fig. 6), despite being neither essential (it can be synthesised from serine), nor deficient in the sediment (Fig. 7). Further, glycine showed the highest percentage recovery in quantitative amino acid budgets (Fig. 5).

This enrichment of all sample types with 13C-labelled glycine is consistent with previous experiments where polychaete tissues (Thomas and Blair, 2002), and polychaete and zooplankton faecal pellets (Cowie and Hedges, 1996; Thomas and Blair, 2002) have shown high glycine concentrations in relation to food, and natural faunal tissue.

One explanation for glycine accumulation in faunal tissues is its selective assimilation during gut passage. However, simultaneous glycine enrichments in faecal matter and sediments, together with its potentially low nutritional value (Dauwe and Middelburg, 1998) suggest this is unlikely. Other possibilities include glycine being more resistant to decay than other amino acids. Such resistance could result from protection from enzyme attack due to its close resemblance to other amino acids, and the functional R group from other amino acids, and the numerous metabolic pathways in which glycine is involved (Caspi et al., 2010) provide multiple opportunities for this to occur. Glycine production may thus create a dynamic pool of 13C-labelled glycine, although it is unclear why the labelled dynamic pool is larger than that which is naturally present.

The enrichment of faecal matter and sediments with glycine suggests that, in addition to production by polychaete digestive, metabolic, and/or gut-microbial processes, it is also produced during sediment microbial activity. This leads to a further mechanism for glycine accumulation in faunal tissues, whereby bacterial glycine production (possibly the result of gardening) is followed by selective ingestion of bacteria by fauna (Hylleberg, 1975). Veuger et al. (2006) proposed such faunal uptake and retention to explain the longer persistence of glycine in sediments than the more refractory peptidoglycan.

Finally, glycine enrichment in sediments and faecal matter may also result from secondary microbial production, and preservation of the persistent, glycine-bearing cell membrane compound peptidoglycan (Pedersen et al., 2001). Veuger et al. (2006) conducted in situ labelling of an intertidal microbial community and observed an accumulation of labelled glycine in the sediment. However, they note that this may not have been in the form of peptidoglycan, but rather as simple amino acids within refractory degradation products (Pedersen et al., 2001; Veuger et al., 2006).

It is not clear which of the above mechanisms dominates, however the occurrence of strong glycine enrichments in faunal tissues and faecal matter suggest that faunal tissues and digestive tracts are at least important locations for the production and/or retention of glycine.

4.2.1.4. Non-protein amino acids. The non-protein amino acids β-alanine (BALA) and γ-aminobutyric (GABA) acid are the decay products of aspartic and glutamic acids (respectively). They originate from microbial activity (Whealan, 1977; Vandewiele et al., 2009), and their mole percentage contribution to the total amino acid pool is an indicator of advanced OM degradation state (Cowie et al., 1992; Cowie and Hedges, 1994).

In this study, BALA and GABA were present in low concentrations in the source algae, due to slight degradation in the non-axenic cultures before harvesting. They did not become enriched in faunal tissues compared to the source algae (Fig. 4a and b), but did become enriched in some *Arenicola* microcosm sediments and faecal matter (Fig. 4c and d). Further, *Arenicola* sediments and faecal matter showed slight (although not statistically significant, Mann–Whitney U) increases in BALA and GABA between the 9 d and 21 d timepoints (Fig. 4).

As BALA and GABA are produced during microbial degradation, they might have been expected to show high recoveries in quantitative amino acid budgets. However, recoveries of both were similar to many protein amino acids in *Hediste* microcosms (Fig. 5), and only GABA showed relatively high recovery in *Arenicola* microcosms. Thus, microbial production of non-protein amino acids was relatively subdued, however this is consistent with their normal association with more advanced stages of OM decay than.
were observed here (Cowie and Hedges, 1994; Dauwe et al., 1999).

The relatively high concentrations of GABA in Arenicola microcosms suggest that Arenicola sediments, or the animals themselves, harbour or stimulate particularly active microbial communities. The notable presence of GABA in Arenicola faecal matter (Figs. 2 and 4) suggests that a significant proportion of the microbial activity may occur within the Arenicola gut, and/or on freshly egested material, which is consistent with findings that enteric microbes contribute particular fatty acids to the faeces of several marine invertebrates (Bradshaw et al., 1989), and that gut passage alters the bacterial species composition of ingested material (Plante and Mayer, 1994; Plante, 2010). The fact that faunated microcosms generally showed higher GABA recoveries than the equivalent afaunal controls (Fig. 5, not statistically significant, Mann–Whitney U) also supports previous suggestions that fauna stimulate sediment microbial activity (Aller and Aller, 1998).

4.2.2. Taxon-specific effects

In several cases Hediste and Arenicola samples displayed different distributions of labelled amino acids. Hediste tissues contained greater mole percentages of labelled valine, lysine, leucine, iso-leucine, methionine, proline, aspartic and glutamic acids, and threonine than Arenicola tissues, and lower mole percentages of glycine (Figs. 2 and 3, Mann–Whitney U, $P \leq 0.001–0.007$). In addition, Hediste showed enrichment in valine, leucine, iso-leucine and proline compared to the source algae that Arenicola did not (Fig. 4b). Further, Hediste accumulated valine, leucine and iso-leucine in greater proportions than those naturally present in its tissues, and Arenicola did not show this effect (Fig. 6). Their natural amino acids suites were also different, with Arenicola showing a greater glycine content, and Hediste slightly more proline (Fig. 6).

Thomas and Blair (2002) also found different OM alteration patterns amongst polychaete taxa as a result of gut passage, with terebellid faecal pellets showing higher proportions of threonine and glycine than those of maldanids and spionids. Further, maldanid tissues showed greater labelled glycine mole percentages compared to spionids and terebellids. These differences were attributed to a difference in gut architecture between plug flow reactors (maldanids and spionids) and mixed reactor plug flow digestors (terebellids). In addition they suggested that differences could
further arise from differences in feeding behaviour and particle selection, as well as metabolic processes. Similarly, *Hediste* and *Arenicola* exhibit contrasting feeding behaviours. *Hediste* is a particle-selective surface deposit feeder, scavenger/carnivore and filter feeder, while *Arenicola* is a head-down deposit feeder with limited particle selectivity (Evans, 1971; Ronn et al., 1988; Rüsgard, 1991; Jones and Jago, 1993; Rüsgard and Banta, 1998). They may also have different anabolism/catabolism ratios, therefore, it is not surprising that they exhibited different patterns of net amino acid accumulation and loss.

### 4.3. Impact of faunal gut passage on sedimentary organic geochemistry

The sustained isotopic enrichment of bulk fauna tissue (Fig. 1) observed here shows that benthic polychaetes are a short-term reservoir for freshly deposited C. Also, the differences in labelled amino acid compositions between faunal tissues, food, and faecal matter suggest that faunal ingestion and gut passage of OM leads to net accumulation of certain compounds in the biomass. This pathway for organic C through the marine sedimentary system is under-acknowledged by geochemists, but is the prime interest of benthic ecologists studying secondary production (Deming and Baross, 1993; Cartes et al., 2011).

It was expected that the impact of gut passage on the pattern of OM decay would show as a difference in sediment $^{13}$C-labelled amino acid suites between faunated microcosms and afaunal controls. Thus, PCAs of faunated and control sediments, plus faecal matter data were conducted, one for each taxon (Fig. 8). For *Hediste* data, PC1 explained 49% of the variation, and PC2 explained 17%, and for *Arenicola* data the values were 54% and 15%, respectively. The distinction in PC scores between faunated and afaunal sediments was greater for *Hediste* data, in which the two types of sediment grouped in separate clusters (Fig. 8). It is difficult to determine which amino acids drove the difference in scores, as each cluster covers a large area, however factor coefficients suggest that faunated sediments showed higher concentrations of alanine, methionine, aspartic and glutamic acids and tyrosine, and lower concentrations of glycine. Faunated and afaunal *Arenicola* sediments did not show clear differences in PC scores, except that some 21-d faunated sediments showed higher PCI scores than the controls (Fig. 8). These scores are attributable to higher GABA and proline concentrations, which are associated with microbial activity (Veugelie et al., 2006; Vandewiele et al., 2009). Therefore, *Arenicola* may have influenced sediment labelled amino acids suites through stimulation of microbial activity. Thus, the evidence weakly suggests that the presence of fauna influenced the suite of $^{13}$C-labelled amino acids remaining in the sediment. However, longer experiments are required to identify firm compositional differences.

Aspects of the amino acid loss patterns observed here are consistent with previous observations of OM decay. Most notably, glycine generally becomes a more dominant constituent of the amino acid suite as degradation progresses (e.g. Dauwe and Middelburg, 1998; Lee et al., 2000; Veugel et al., in press), possibly due to its association with the diatom test (Cowie et al., 1992). Here, a range of mechanisms for the enrichment of sediments, faunal tissues and faecal matter with glycine have been discussed, which suggest that faunal gut passage and gut bacterial processes may also contribute towards glycine accumulation during decay. In addition, as here, proline was observed to accumulate during a recent year-long decay experiment (Veugel et al., in press).

In addition, methionine, glutamic acid, tyrosine, phenylalanine, leucine and iso-leucine are often observed to degrade relatively rapidly in sediments (Cowie et al., 1992; Dauwe and Middelburg, 1998; Lee et al., 2000). For glutamic acid, tyrosine, methionine and lysine, this is consistent with the preferential losses observed in this study (Figs. 4 and 5). In contrast, phenylalanine was not observed to be rapidly lost, while leucine, and sometimes iso-leucine, were enriched in faunal tissues and sediments (Fig. 4). Further, serine and aspartic acid have previously been observed to accumulate during OM degradation (Cowie et al., 1992; Dauwe and Middelburg, 1998; Lee et al., 2000), but in this study they were rapidly lost (Figs. 4 and 5).

These inconsistencies with previous observations may partially result from variations in study site, sediment type and geochemistry, time scales, OM source, and biological community. They also reflect variations in findings amongst previous amino acid decay studies. For example, in Saanich Inlet, Cowie et al. (1992) found that glutamic acid was not particularly reactive, in contrast to Dauwe and Middelburg’s (1998) observations in North Sea sediments. Other studies have found a lack of change in amino acid suites during marked OM degradation in both coastal and deep-sea settings (Henrichs and Farrington, 1987; Cowie et al., 1995; Horsfall and Wolff, 1997), and Suthhof et al. (2000) found that only tyrosine was particularly labile during early decay. Moreover, the data presented here focus on very early decay of fresh phytodetritus, and thus decay patterns vary from sedimentary environments receiving OM which has been degraded for days to months during sinking. Thus, there is still a gap in our understanding of amino acid suite alteration over the whole range of decay timescales. Closing this gap requires further experiments lasting months to years, such as those recently conducted by Veugel et al. (in press).

### 5. CONCLUSIONS

In summary, bulk remineralisation of algal detritus was an important process, which was probably a major driver of OM compositional changes. Despite this, polychaetes retained $^{13}$C in their tissues, and thus faunal biomass represents an understudied route for OM through the sedimentary system. Polychaete tissues displayed net accumulation and losses of certain amino acids, the patterns of which tended to align with amino acid essentiality and deficiency in the sediment. The most notable alteration of the labelled amino acid suite was an enrichment of all sample types with glycine. Potential mechanisms for this include selective accumulation through inclusion in tissues with long residence times, selection against during
metabolism, production from other labelled amino acids during metabolic processes, and accumulation in refractory by-products of secondary bacterial production.

Overall, however, although Arenicola and Hediste are large, active and abundant polychaetes, it was difficult to characterise their impact on the decay pathways of fresh OM as distinct from decay during microbial activity only. It is therefore suggested that, while the finer details of net amino acid accumulation and loss in polychaete tissues appear to be taxon-specific, there is an overall broad similarity in the metabolism of amino acids by polychaetes and bacteria.

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Amino acid alteration during polychaete gut passage


