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Alteration of organic matter during infaunal polychaete gut passage and links to sediment organic geochemistry. Part II: Fatty acids and aldoses

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Abstract

The activities of sediment-dwelling fauna are known to influence the rates of and pathways through which organic matter is cycled in marine sediments, and thus to influence eventual organic carbon burial or decay. However, due to methodological constraints, the role of faunal gut passage in determining the subsequent composition and thus degradability of organic matter is relatively little studied. Previous studies of organic matter digestion by benthic fauna have been unable to detect uptake and retention of specific biochemicals in faunal tissues, and have been of durations too short to fit digestion into the context of longer-term sedimentary degradation processes.

Therefore this study aimed to investigate the aldose and fatty acid compositional alterations occurring to organic matter during gut passage by the abundant and ubiquitous polychaetes *Hediste diversicolor* and *Arenicola marina*, and to link these to longer-term changes typically observed during organic matter decay.

This aim was approached through microcosm experiments in which selected polychaetes were fed with ^{13}C-labelled algal detritus, and organisms, sediments, and faecal pellets were sampled at three timepoints over ~6 weeks. Samples were analysed for their ^{13}C-labelled aldose and fatty acid contents using GC-MS and GC-IRMS.

Compound-selective net accumulation of biochemicals in polychaete tissues was observed for both aldoses and fatty acids, and the patterns of this were taxon-specific. The dominant patterns included an overall loss of glucose and polyunsaturated fatty acids; and preferential preservation or production of arabinose, microbial compounds (rhamnose, fucose and microbial fatty acids), and

animal-synthesised fatty acids. These patterns may have been driven by fatty acid essentiality, preferential metabolism of glucose, and *Arenicola marina* grazing on bacteria.

Fatty acid suites in sediments from faunated microcosms showed greater proportions of saturated fatty acids and bacterial markers than those from afaunal controls. Aldose suite alterations were similar in faunated microcosms and afaunal controls, however the impact of faunal gut passage on sedimentary aldose compositions may be observable over longer timescales. Therefore this study provides direct evidence that polychaete gut passage influences OM composition both through taxon-specific selective assimilation and retention in polychaete tissues, and also through interactions with the microbial community.

1. Introduction

Marine sediments are an important location for burial of organic matter (OM), with an estimated 130 Mt C y^{-1} buried in deltaic and shelf settings (Berner, 1982; Burdige, 2007). At the same time, remineralisation of OM in marine sediments is a key mechanism for returning nutrients to the water column. Further, sedimentary OM dynamics exert a significant control over benthic ecology, with many infaunal organisms feeding on settled detritus and in situ microphytobenthos production.

The preservation of OM in sediments has been shown to be enhanced by rapid sediment accumulation rates, low oxygen exposure times, and sorptive protection on fine-grained sediment surfaces, factors which all minimise the extent of microbial degradation (e.g. Canfield, 1994; Burdige, 2007; Blair and Aller, 2012). The actions of macrofauna have also been shown to affect OM degradation and burial rates, through physical movement of particles during bioturbation, and by stimulating microbial activity during bioirrigation (e.g. Aller and Aller, 1998; Sun et al., 2002).

The least well studied mechanism through which fauna influence sedimentary OM dynamics is direct ingestion and digestion. Of the limited number of studies of OM digestion by marine fauna, many focus on pelagic species, and are dominantly concerned with questions regarding organism nutrition (e.g. Muller-Navarra, et al., 2000; Parrish et al., 2012). In some benthic settings, macrofauna may be abundant and active enough to 'process' (ingest and egest) most or all of the OM delivered to the system (Herman et al., 1999). For example, at a typical density of 40-80 individuals m^{-2}, the polychaete *Arenicola marina* can process the entire sediment surface to a depth of 17-40 cm every year (Kristensen, 2001). It is widely recognised that this gut passage must have an effect on the subsequent geochemistry and susceptibility to decay of sedimentary OM (e.g. Aller, 1994; Smallwood et al., 1999). However, studies that include a detailed characterisation of OM alteration following gut passage have been comparatively rare. Thus, this potentially key determinant of sedimentary OM composition and susceptibility to decay is not well understood. Developing this understanding is important for furthering our understanding of sedimentary C cycling, and may also provide insights into the source and formation of the uncharacterisable fraction of OM, which dominates that which is eventually buried.

Macrofaunal gut passage may alter OM composition and degradability in several ways. Bioturbation, for example, can transport OM between redox zones (Aller, 1994; Aller and Aller, 1998). Gut passage also leads to mixing and re-packaging of sediment, leaving fresh surfaces exposed to microbial attack. Further, macrofaunal guts feature a mix of enzymes, surfactants and acids, the purpose of

which is to extract nutrition from sedimentary OM (Mayer et al., 1997; Ahrens et al., 2001; Voparil and Mayer, 2004). In addition, they contain a gut flora, some of which may be egested with the sediment (Plante, 2010), and may also accelerate decay.

Many of the previous studies of geochemical changes occurring during polychaete gut passage come from contaminant literature, or have an ecological perspective. Thus, some work has focused on determining the bioavailability of heavy metals and organic pollutants in contaminated sediment (e.g. Wallace and Lopez, 1997). Other work has focused on measuring characteristics of organisms such as their feeding rates and the efficiency with which they assimilate the carbon in their diets (e.g. Amouroux et al., 1989; 1997). This dominantly ecological perspective means that, with notable exceptions (e.g. Bradshaw et al., 1990a; b; 1991; Sun et al., 1999; Grossi et al., 2006), links between studies of gut passage and wider sediment organic geochemistry are rarely made.

More recently, studies of digestion and gut passage have started to provide compositional detail on the alteration of OM during macrofaunal gut passage. For example, gut passage produces an increase in the concentration of the amino acid glycine in OM (Thomas and Blair, 2002; Woulds et al., 2012), although this change seems not to be specific to macrofaunal gut passage (Woulds et al., 2012). Other amino acids, such as methionine and glutamic acid, are rapidly lost during gut passage (Cowie and Hedges, 1996; Woulds et al., 2012).

Carbohydrates can account for 10-70% of algal biomass (Romankevich, 1984), were observed to account for 15-30% of C fixation by microphytobenthos in a labelling study (Oakes et al., 2010), and can contribute up to 85% of porewater dissolved organic carbon (Arnosti and Holmer, 1999; Burdige et al., 2000). Carbohydrates play varied, important roles in the functioning of coastal sediments, as extracellular polymeric substances (EPS) which contribute to sediment cohesion, as the first products of photosynthesis, a C store in algal cells, a C source for consumers and detritivores, and a long-term detrital C store (e.g. Boschker et al., 2008; Oakes et al., 2010). Further, estuarine and coastal sediments receive significant amounts of OM derived from macrophytes, in which carbohydrates serve important structural functions, particularly in the form of cellulose. Thus, their alteration during gut passage warrants further study. Previous studies of changes to aldose abundances during gut passage are less common than studies of amino acids and lipids. In a zooplankton feeding experiment, Cowie and Hedges (1996) found that faecal pellets showed depleted glucose and enriched mannose concentrations compared to the food source (diatoms), and concluded that this represented preferential assimilation or metabolism of cell contents, as opposed to cell-wall material. We are not aware of any studies of carbohydrate alteration during gut passage by benthic fauna, despite their importance as an energy source in the sediment.

Lipids constitute ~5-40 % of algal biomass (Romankevich, 1984), and thus form a significant fraction of the flux of C and energy to benthic ecosystems. Fatty acids in particular are assimilated, transformed and synthesised by organisms at all trophic levels, resulting in some which can be used as trophic markers for reconstructing food webs (Dalsgaard et al., 2003). Further, the concentration of specific fatty acids in primary producers have been shown to determine the efficiency of energy transfer from primary producers to primary consumers (Muller-Navarra et al., 2000), and a select group of such important fatty acids are essential in the diets of heterotrophs (e.g. Parrish et al., 2012). Specific lipid compounds are also used as biomarkers for OM sources, and the occurrence of a wide range of taxonomic groups and biogeochemical processes.

Studies of the alteration of lipid compositions during gut passage are only slightly more abundant than those for carbohydrates. Bradshaw et al. (1989; 1990a; b; 1991) established for several benthic invertebrates that fatty acids, and particularly mono- and poly-unsaturated fatty acids were preferentially assimilated, while odd-carbon-number 'bacterial' fatty acids were contributed to the faeces. In addition, certain sterols were assimilated, while others were contributed to the faeces. More recently, Sun et al. (1999) studied changes in sedimentary lipid compositions with and without the presence of the bivalve *Yoldia limatula*. The changes they measured were not necessarily due to gut passage (they analysed bulk sediment, not just faecal pellets), but they established that the presence of *Yoldia* accelerated the decay of algal lipids, and suppressed the accumulation of lipids generated during microbial degradation (Sun et al., 1999).

A notable shortcoming of many previous studies is that they have been limited to examining the compositional differences between food, faecal matter, and, sometimes, faunal tissues. This provides an incomplete picture of the fate of ingested biochemicals. For those compounds which are depleted in faecal matter, it is not clear whether they have been metabolised or assimilated, or, if assimilated, how long they may be retained in faunal tissues.

Isotope tracing techniques provide a way to quantify assimilation and retention of C. Initially, ^{14}C was used to determine feeding rates and assimilation efficiencies for bulk carbon (e.g. Amouroux et al., 1989; 1997; Charles et al., 1995). Further techniques have recently become available that allow stable isotopes, which are easier to work with, to be reliably detected both in bulk OM, and in individual biochemicals including fatty acids, amino acids, and aldoses (Sun, 2000; Boschker et al., 2008; Woulds et al., 2010).

Considering the methodological limitations of previous work, the aim of this study was to apply compound-specific stable isotope tracing techniques to study the uptake and losses of aldoses and

fatty acids during macrofaunal gut passage, and, for the first time, to link this to the organic geochemical changes observed during OM decay. Together with a previous publication reporting amino acid data from the same experiments (Woulds et al., 2012), this study represents the most complete description of OM alteration during gut passage that is available to date.

2. Methods

A feeding experiment was conducted in April-May 2007 at the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke.

2.1 Study sites and fauna

Two species of abundant and ubiquitous polychaete were chosen for use in a feeding experiment. These were the lugworm *Arenicola marina*, a sedentary, head-down conveyor belt feeder (Jones and Jago, 1993; Retraubun, 1996; Riisgard and Banta, 1998), and *Hediste* (formerly *Nereis*) *diversicolor*, a comparatively motile surface deposit feeder that also constructs gallery-type burrow systems (Evans, 1971; Ronn et al., 1988). These two species were chosen because they are abundant and ubiquitous in European estuaries, and thus have the potential to exert dominant controls over sediment biogeochemistry. They are also representative of temperate estuarine and coastal settings as a whole and represent valuable contrasts in feeding behaviour.

Surface (10 cm) sediment and fauna for the feeding experiment were collected from two intertidal sites in the Westerschelde estuary; a sandy, organic-matter-poor site at 51.487262 N, 4.058526 E was the source of *Arenicola* and associated sediment, and a muddy, more organic-rich site at 51.55384 N, 3.873247 E provided the same for *Hediste*.

2.2 Microcosm construction

Sediment collected from each site was homogenised and then frozen and thawed twice, to kill macro- and meiofauna. Despite the introduction of dead animal OM this causes, it was considered preferable to sieving the sediment as it produced more comprehensive defaunation (also removal of meiofauna), and at the same time avoided excessive disruption to sediment structure/texture. It was then used to construct replicate microcosms for each site, each one consisting of a column of sediment overlain by filtered (0.2 μm) estuarine water. *Hediste* microcosms were constructed in 10 cm i.d. acrylic tubes, sealed at the bottom with a bung, and covered with aluminium foil to exclude light. Sediment columns were ~15 cm deep. *Arenicola* microcosms were constructed in tubs with

980 cm^2 surface area, to allow the greater surface area and sediment depth (at least 20 cm) required by the species.

Six *Hediste* microcosms (2 per timepoint, see below), and three *Arenicola* microcosms (one per timepoint) were constructed. In addition, control microcosms were constructed using each type of sediment in 5.4-cm diameter acrylic tubes. They were smaller due to limitations on the quantity of ^{13}C labelled algae available. Control microcosms were fed as all other microcosms, but did not contain polychaetes. The number of microcosms used was limited by the availability of ^{13}C-labelled algae, which is expensive and labour intensive to grow. We favoured fewer, larger microcosms in order to better recreate natural conditions and to avoid wall effects. Replicate individual polychaetes were present in each microcosm (numbers given below). These individuals are actually pseudo-replicates, however a consideration of polychaete bulk isotopic signatures (Woulds et al., 2012) showed no significant difference between *Hediste* in replicate microcosms. Therefore most of the variability occurs between individual animals, and pseudo-replication provides useful information.

Specimens of *Hediste* and *Arenicola* were collected and stored for 2-3 days in aerated, filtered seawater in a controlled temperature laboratory. The following day, individuals that still appeared healthy were weighed and measured, before being introduced into the microcosms at a density of 1018 ind. m^{-2} for *Hediste*, and 71 ind. m^{-2} for *Arenicola,* which are within the natural observed density ranges for these taxa (Kristensen, 2001; Defew et al., 2002). Average wet weights were 1 g for *Hediste* and 6.7 g for *Arenicola*. The polychaetes were allowed to acclimatise in the microcosms for 2-3 days before the experiment began; thus the animals are not considered to have been starved before feeding.

2.3 Feeding experiment

The feeding experiment started with addition of dead, freeze-dried, ^{13}C-labelled (64.6 atom%) diatoms (*Skeletonema costatum*, grown at NIOZ, Yerseke) to each microcosm once per day for 8 consecutive days. Algae were added as a slurry to the overlying water, and allowed to settle onto the sediment surface. The total amount of food added was equal to 3.5 g C m^{-2} for *Hediste*, and 3.1 g C m^{-2} for *Arenicola*. Comparatively high doses were used to ensure that detectable label was present at the end of the experiment. The slight difference in carbon dose between *Hediste* and *Arenicola* microcosms was necessitated by the limited availability of ^{13}C-labelled algae.

Microcosms were ventilated by constant bubbling of air into the overlying water. The temperature was maintained at 15˚C, appropriate for the average temperature of the season, and lights were set

to an automatic 12-h light and dark cycle. To prevent accumulation of respiratory metabolites or salt (from evaporation), overlying water was exchanged every ~4 days.

Sub-sets of the microcosms were sacrificed and preserved as soon as possible after the last day of feeding (day 10 for *Hediste* and day 9 for *Arenicola*), and at 22 or 21 days (for *Hediste* and *Arenicola* respectively). At the 21/22 day timepoint, animals were carefully removed from the remaining, un-sacrificed microcosms and transferred into freshly constructed microcosms that had not been fed with ^{13}C-labelled algae, so that we could observe aldose and fatty acid retention in and loss from polychaete tissues. Animals remained in the unlabeled microcosms for a further ~15 days, with final sampling on days 38 and 37 for *Hediste* and *Arenicola* respectively. For *Hediste* the number of individual polychaetes recovered at the three successive timepoints (A and B replicates) were 15, 20 and 10, and for *Arenicola* the numbers were 9, 10 and 4. At regular intervals between sampling timepoints, samples of fresh *Arenicola* faecal matter were collected using a spatula and frozen, old faecal matter having first been cleared away the previous evening.

Microcosm sacrifice and sampling involved carefully extruding and sectioning sediment from *Hediste* microcosms. Animals were collected whole as they were discovered. All core sectioning was at 0.5 cm intervals to 2 cm depth, followed by 1 cm intervals to 10 cm depth. For *Arenicola* microcosms, samples of faecal matter were first collected from the sediment surface using a spatula. Triplicate sub-cores were then collected and sectioned in 60-ml syringe barrels with the closed ends cut off. The remaining sediment was then carefully dug out, and animals were collected whole as they were found.

Sediment samples were frozen. Animals were placed in petri dishes containing only water. They were allowed to egest their gut contents for 2-5 h, before they and their gut contents were frozen separately in pre-combusted glass vials. Gut clearance was not complete after this time (~80% complete, visual estimation). Complete gut clearance can take weeks in starvation conditions, and a longer wait was avoided in order to prevent changes in aldose and fatty acid metabolism. Further, physical extraction of gut contents before analysis would have caused loss of tissue and fluids which would have introduced a greater artefact than allowing a small amount of relatively OM poor sediment to remain in the sample. Data from gut contents are not presented here, but, as samples were too small to analyse, this gap in the data is unlikely to have impacted on quantitative aldose budgets (see below). When interpreting data from sediment samples it should be remembered that they were likely to contain fresh algal OM, as well as that which had been subject to gut passage.

2.4 Analytical

231 Sediment and faecal matter samples were freeze-dried and homogenised by gently disaggregating
232 the dried sample by hand. Frozen animals were thawed and homogenised using a potter tube,
233 before being freeze dried.

234 *Aldoses*. Samples were analysed for ^{13}C-labelled aldoses as their alditol acetate derivatives following
235 the protocol of Woulds et al. (2010). Briefly, dried samples were hydrolysed in concentrated H_2SO_4 at
236 room temperature for 30 mins, followed by a 9x dilution, and continuation of hydrolysis at 100˚C for
237 3 h. Myo-inositol internal standard was added, and samples were neutralised to pH 5.5-6.5 through
238 addition of $BaOH_2$. The solution was separated from the precipitate by centrifugation, and aldoses
239 were reduced to alditols overnight following addition of KBH_4. Samples were cleaned up by passage
240 through cation (Dowex 50WX8 Hydrogen form 50-100 mesh) and anion (Dowex 1X8 Chloride form
241 100-200 mesh) exchange resin, and dried. Alditols were acylated in the presence of methyl imidazole
242 and acetic anhydride at 110˚C for 10 mins. The reaction was quenched with Milli-Q, and alditol
243 acetates were extracted with dichloromethane (DCM). Derivatised samples were analysed on an
244 Agilent 6890 gas chromatograph (GC) with an HP 5973 mass spectrometer detector (MS) using a 60-
245 m, 0.32-mm i.d. SP2330 (Supelco) column with a 0.2 μm film thickness. The oven programme started
246 at 90˚C and ramped at 20˚min^{-1} to 190˚C, and then at 4˚Cmin^{-1} to 250˚C where it was held for 11
247 mins. The splitless injector was at 280˚C, and the He carrier gas flowed at 2 ml min^{-1}. The interface
248 between GC and MS was held at 280˚C, and the quadrupole was at 106˚C. Chemical ionisation was
249 used, with methane at 20% as the reagent gas, and a relatively low source temperature of 154˚C.
250 This yielded pseudo-molecular ions that were detected using selective ion monitoring, and used for
251 quantification (Woulds et al., 2010). Calibration of naturally present, unlabeled aldoses was
252 conducted using the internal standard and a single external standard included in each sample batch.
253 Commercially available standards were not suitable for calibration of ^{13}C-labelled aldoses, as they do
254 not contain highly ^{13}C-labelled quantifier ions. Therefore 'isotope'calibration curves (Woulds et al.,
255 2010) were constructed using volumetric mixes of independently characterised aldose derivatives
256 from labelled algae and unlabeled sediment. Thus the amounts of algae-derived and natural
257 sediment-derived aldoses in each mix was known, and this was then related to the responses of the
258 natural and ^{13}C-labelled quantifier ion for each compound. For further details see Woulds et al.
259 (2010). Relative standard deviations on concentrations of ^{13}C-labelled aldoses using this method are
260 ~10% for faunal samples, and ~25% for the less homogenous sediment samples (Woulds et al.,
261 2010). Even with this degree of variability, statistically significant trends in the data were found.

262 *Fatty acids*. Lipids were extracted using a modified Bligh-Dyer extraction. Freeze-dried samples were
263 shaken for 2 hours at room temperature in a 2:1:1 volumetric mix of methanol:chloroform:water.

Layers were separated following addition of further chloroform and water, and the chloroform layer was collected quantitatively. Total lipid extracts were loaded onto activated silicic acid columns, and eluted with chloroform, followed by acetone and then methanol. The methanol fraction was collected and dried. C12:0 and C19:0 fatty acid methyl ester (FAME) internal standard were added, and they were methylated in the presence of methanolic NaOH at room temperature for 15 minutes, after which the reaction was quenched with acetic acid and water. The hexane layer was removed and twice washed and dried before being taken up in hexane. Samples were analysed by GC-IRMS using a 30-m, 0.25-mm i.d., 25μm film thickness BPX70 column. The injector and IRMS interface were held at 240°C and 260°C respectively. The initial oven temperature of 80°C was held for 2 min, ramped to 120°C at 20°C per min, and then to 240°C at 3°C per min. On leaving the GC column, compounds were combusted in a Thermo type II interface, and the isotopic composition of the resultant CO_2 was determined on a Thermo Delta plus IRMS (Middelburg et al., 2000). Fatty acids were identified based on retention times in comparison with a known standard mixture, and some peak identities were checked using mass spectra from separate GC-MS analysis. Analysis of replicate animals (i.e. from the same microcosm) gave a mean RSD of 33% for fatty acid concentrations, and some of this variability will have been inherent in the samples. The mean RSD for isotope ratios was 10%.

2.5 Data treatment

^{13}C-labelled aldose concentrations are reported in several ways. Firstly, labelled aldose compositions are reported as weight percentages of the total amount of labelled aldoses in a given sample, calculated as shown in equation 1:

$$Wt\%glu = \frac{[glu]}{\sum[al]} \; x\,100$$

Equation 1

Where [glu] is the concentration of a chosen labelled aldose (in this case exemplified by glucose), and ∑[al] is the sum of the concentrations of all labelled aldoses in the same sample.

In addition, quantitative budgets were constructed for each aldose, and these are reported in terms of the percentage of each aldose added in the algae that was recovered (in sediments plus faunal tissues) at the 9/10-d and 21/22-d timepoints (Equ. 2).

$$\%\ Recovered = \frac{([al]worm\ x\ wt\ worm) + ([al]sed\ x\ wt\ sed)}{[al]algae\ x\ wt\ algae} \; x\,100$$

Equation 2.

Where [al] is the concentration of a chosen labelled aldose in either polychaete (worm), sediment (sed), or algae (algae), and wt is the dry weight of each present in or added to the microcosms (surface 2 cm in the case of sediment).

Quantitative budgets could not be constructed for the 37/38-d timepoint, as by then animals had been removed to fresh, unlabelled sediment, and a large proportion of the added aldoses would have been lost in the discarded sediment.

Fatty acid concentrations and isotopic signatures were used to calculate the amount of ^{13}C found in each compound. Data for this 'labelled fatty acid suite' is presented by considering the proportion of the total ^{13}C found in fatty acids represented by each individual compound (Equ. 3).

$$Proportion\ of\ labelled\ fatty\ acids = \frac{[fai]x\frac{13Cfai}{13Calgae}}{\sum\left([fa]x\frac{13Cfa}{13Calgae}\right)}$$

Equation 3.

Where [fai] is the concentration of a given fatty acid, and [fa] is the concentrations of all fatty acids (which are summed). 13Cfai is the atom % abundance of ^{13}C in the fatty acid in question, and 13Cfa and 13Calgae are those for the other fatty acids and the bulk algae, respectively.

In the case of polychaete tissue data this can be considered as the 'proportion of total uptake', and is equivalent to the weight percentage presentation of the aldose data. The majority of this 'proportion of total uptake' data is for labelled fatty acid suites only, but in some cases, for comparison, a similar treatment has been applied to unlabeled fatty acid data from natural sediment or fauna samples.

In addition, the specific labelling of individual fatty acids is considered through the calculation of $\Delta\delta$ values, which show the change in isotopic composition of each fatty acid that occurred during the experiment (Equ. 4).

$$\mathbf{\Delta}\delta fa = \delta\mathbf{13}Cfa\,\mathbf{exp} - \delta\mathbf{13}Cfa\ nat$$

Equation 4.

Where d13Cfa exp is the $\delta^{13}C$ signature of the fatty acid in question in the tissue of an experimental polychaete, and d13Cfa nat is that in the tissue of a polychaete which has not been fed labelled algae.

In most cases the compositional data presented in figures are means from the replicate individual animals collected at each time point. Sediment and faecal matter data are also means of all samples from each time point. In the case of sediment data this means that samples from different sediment depths have been averaged. However, all sediment samples analysed were from the surface 2-cm of the sediment, and the lack of downcore trends was established before the averaging took place.

Statistical analysis of results was conducted using Minitab 16. The Anderson-Darling test for normality followed by Mann-Whitney and t-tests were used to test for differences between groups of samples. Principal component analyses were also conducted on aldose weight percentages and on PLFA 'proportion of total PFA' data, in order to elucidate compositional differences. $$Wt\%glu = \frac{[glu]}{\sum[al]}\ x\ 100$$

3. Results

3.1 Aldoses

Labelled aldose weight percentage compositions differed markedly among labelled algae, polychaete tissues, and microcosm sediments (both with and without fauna, Fig. 1). Polychaete tissues tended to be depleted in glucose and arabinose, and enriched in all other aldoses compared to the source algae, and this pattern was more marked for *Hediste* than for *Arenicola*. Sediment and faecal matter were also depleted in glucose, as well as galactose compared to the source algae (Fig. 1b). However, they tended to show enrichments in arabinose, and also to some extent in rhamnose and mannose. Further, sediments from *Hediste* microcosms show a trend in composition over time, with progressive reductions in the weight percentages of ribose, mannose, galactose and glucose, and an increase in that of arabinose (Fig. 1b). In absolute terms, comparatively high concentrations of ^{13}C labelled arabinose and mannose were seen in *Hediste* tissues, and of glucose in *Arenicola* tissues (Fig. 1c, d).

Compositional differences are further revealed by principal component analysis (PCA) results. A PCA that included data from all sample types showed polychaete tissues and the algal diet to have generally more positive PC1 scores than faecal material and sediment samples, driven by lower arabinose, and higher galactose, fucose and glucose weight percentages in polychaete tissues (Fig.

2). Further, *Hediste* and *Arenicola* tissues were separated on the PC2 axis, with the former showing higher rhamnose, xylose and mannose, and the latter higher glucose and ribose weight percentages (Fig. 2).

In addition to being different from the suite of aldoses present in the source algae, the suite of labelled aldoses measured in polychaete tissues differs from the natural composition of those tissues (Fig. 3). Labelled glucose was present in the tissues in a lower weight percentage than that in which it was naturally present, and this was statistically significant for both *Hediste* and *Arenicola* (Mann-Whitney U, $P < 0.001$, and t-test, $P < 0.001$, respectively). Most other labelled aldoses were present in polychaete tissues in higher weight percentages than their natural counterparts, particularly rhamnose, ribose, arabinose and mannose. All differences were significant (Mann-Whitney and t tests, $P < 0.001$), except for fucose, arabinose, and galactose in *Arenicola* (Fig. 3).

Quantitative budgets show relatively high recoveries (ranging from 26-111%) for all aldoses (Fig. 4), with glucose, galactose, and to some extent ribose showing generally lower recoveries (ranging from 3-39%) than the other compounds. Occasionally a recovery in excess of 100% was calculated, and this is most likely a result of multiplication of analytical errors during calculation. In the case of *Hediste*, recoveries tended to decrease between the 10-d and 22-d timepoints, as might be expected. However these patterns are unlikely to be statistically significant (n is too small for statistical testing). Further, *Arenicola* microcosms frequently showed the opposite trend, with higher recoveries after 21d than after 9d. Therefore it is most likely that recoveries did not change significantly over time. Similarly, recoveries in microcosms with fauna were similar to those in the afaunal controls (Fig. 4).

3.2 Fatty Acids

A broad look at the composition of unlabeled polychaetes compared to the composition of labelled fatty acids in their tissue shows that they contained approximately the same proportions of saturated fatty acids, and this was similar to the proportion of saturated fatty acids in algae (Fig. 5). In contrast, monounsaturated fatty acids (MUFAs) were present in polychaete tissues (both labelled and natural suites) in greater proportions than those in which they were present in algae. Further, polyunsaturated fatty acids (PUFAs) represented a considerably lower proportion of the total fatty acids (for both labelled and natural suites) in polychaete tissues than in algae (Fig. 5). Thus, on a broad scale, polychaetes showed a suite of ^{13}C-labelled fatty acids that was similar to their own natural composition.

379 The suites of labelled fatty acids quantified in polychaete tissue and sediment samples from
380 different timepoints are shown in figure 6. The large number of fatty acids quantified in each sample
381 meant that PCA was an appropriate way to look for patterns in labelled fatty acid compositions. A
382 PCA of the proportion of total uptake data for all samples showed compositional differences
383 between polychaete and sediment samples, with polychaete samples showing more positive PC1
384 scores (Fig. 7). An examination of factor coefficients alongside figure 6 showed that sediment
385 samples tended to be comparatively rich in the bacterial fatty acids i-C15:0 and ai-C15:0 (Bellinger et
386 al., 2009). *Hediste* sediments were also relatively enriched in the algal marker C16:1w7 (Graeve et
387 al., 2005) and the algal or bacterial compound C18:1w7 (Bellinger et al., 2009); and *Arenicola*
388 sediments in the diatom marker C16:2w4 (Bellinger et al., 2009), compared to polychaete tissues.
389 Conversely, polychaete tissues were comparatively enriched in C14:0, the algal C22:6w3 (Graeve et
390 al., 2005), and the animal-synthesised C20:1w9 (Graeve et al., 2005; Mayor et al., 2011). *Hediste*
391 tissues were further enriched in the algal marked C20:5w3 (Graeve et al., 2005; Mayor et al., 2011)
392 and the animal-synthesised C22:1w11 (Graeve et al., 2005; Mayor et al., 2011). The fact that *Hediste*
393 tissue showed a greater number and degree of compositional differences from sediments is
394 consistent with the fact that they showed the most positive PC1 scores.

395 Further, *Arenicola* tissues showed more positive PC2 scores than *Hediste* tissues and all sediments
396 (Fig. 7). A similar examination of PCA factor coefficients and figure 6 shows that *Arenicola* tissues
397 were comparatively enriched in the algal C16:1w7, the algal or bacterial C18:1w7, and C14:0 than
398 *Hediste* tissues.

399 It is also apparent that certain fatty acids showed their maximal proportions of the ^{13}C PLFA suite at
400 the first experimental timepoint, and thereafter decreased with time (Fig. 6). These compounds also
401 tended to constitute a greater proportion of the total ^{13}C-labelled PLFA suite than the natural version
402 did in unlabeled tissues. The most significant of this type of compound were C14:0, and the algal
403 marker C16:1w7c, though some C18 PLFAs in *Arenicola*, and some C15, 17 and 18 PLFAs in *Hediste,*
404 showed a similar trend. Conversely, other fatty acids showed the opposite trend, with their share of
405 the ^{13}C-labelled PLFA suite increasing over time, and their maximal proportion of the PLFA suite
406 occurring in natural polychaete tissues. Compounds that showed this pattern in both polychaete
407 species included the animal or bacterial marker C18:1w9c, and also C22:4w6, C22:5w3, C20:2, C18:0,
408 C19:1, C20:1, and a C17 compound that was either C17:1w7c or cy C17:0 (Fig. 6). Other relatively
409 minor compounds also showed this trend for *Arenicola*.

410 A targeted PCA of only sediment revealed that differences existed in sedimentary labelled fatty acid
411 suites in faunated microcosm sediments over time, and between faunated microcosm sediments

412 and afaunal controls (Fig. 8). Faunated sediments from the 9- and 10-d timepoints, together with
413 afaunal control sediments, showed more negative PC1 scores, and PC1 scores tended to become
414 more positive with experiment duration for faunated microcosm sediments (Fig. 8). Cross
415 referencing factor coefficients with figure 6 showed that more negative PC1 scores were associated
416 with C14:0, algal C16:1w7c, and algal or bacterial C18:1w7c. More positive PC1 scores were
417 principally driven by higher relative abundances of the bacterial PLFAs i15:0 and ai15:0.

418 In addition to looking at which PLFAs contained the greatest abundance of ^{13}C, it is also instructive to
419 consider which compounds showed the greatest change in $\delta^{13}C$ from their natural signature, as this
420 indicates which compounds were turned over most rapidly in polychaete tissues, even though they
421 may have had comparatively low concentrations (Fig. 9). The compounds with the highest delta-
422 delta values (2000-6000 ‰) were C14:0, C18:2, the algal marker C16:1w7c, and the bacterial
423 markers i-C17:0 and i-C18:0. Compounds with values between 100 and 2000 ‰ included the
424 bacterial marker i-C15:0, and C18:2w6t and C22:6w3, which are markers for cyanobacteria and
425 diatoms respectively. Fatty acids thought to only be synthesised by animals, such as C18:1w9c,
426 C22:1w11c and C20:1w9c, showed comparatively low delta-delta values of up to 1771‰ (Fig. 9).It is
427 worth noting, however, that as an indicator of compound incorporation and synthesis, delta-delta
428 has its limitations, as it cannot be calculated for compounds that did not occur in the unlabeled
429 polychaete samples.

430 # 4. Discussion

431 ## 4.1 Bulk C Uptake and Remineralisation

432 Bulk carbon isotopic signatures of sediments and polychaete tissues from these experiments showed
433 that polychaetes assimilated C from the added algae and became highly labelled during the 8 day
434 feeding period, and that the assimilated C remained in their tissues for several weeks after they
435 were removed from the food supply (Woulds et al., 2012). Consistent with their feeding behaviours,
436 surface-deposit feeding *Hediste* individuals assimilated label more rapidly than the sub-surface
437 feeding *Arenicola*. It is recognised however that a potentially large proportion of the algal diet and
438 the biochemicals it contained will have been metabolised during the experiment. This is suggested
439 by the fact that surface sediment $\delta^{13}C$ values returned to near-background levels 15-20 days into the
440 experiment (Woulds et al., 2012). Further, fatty acids and aldoses were most likely metabolised
441 more rapidly than bulk C, as although polychaete tissue $\delta^{13}C$ values remained relatively constant
442 after the end of the feeding period (Woulds et al., 2012), absolute concentrations of labelled aldoses
443 and fatty acids decreased over time (Fig. 1c and d, absolute data not shown for fatty acids). This is

consistent with previous similar experiments, where as much as 30-85% of added or freshly produced OM has been observed to decay over 20 days (Andersen and Kristensen, 1992; Oakes et al., 2010). Thus it is noted that the following discussion of the behaviour of different compounds during gut passage relates in part to the fractions of aldoses and fatty acids that were not metabolised.

4.2 Individual Biochemicals

Variations in the suites of ^{13}C-labelled biochemicals were observed between the algae, polychaete tissues and sediments, and between the two polychaete species and different timepoints. A range of processes could have been responsible for these changes, including selective assimilation in the polychaete gut, synthesis in polychaete tissues, microbial production in the sediment or polychaete gut, and preference for, or protection from, metabolism. The fact that sediment samples are likely to have contained fresh labelled algae should also be considered during data interpretation.

4.2.1 Aldoses

4.2.1.1. Preferential loss: Glucose

In weight percentage terms, all sample types were depleted in glucose relative to the source algae (Fig. 1), and quantitative aldose budgets showed lowest percentage recoveries for glucose (Fig. 4). This suggests that, compared to other aldoses, glucose was preferentially lost through metabolism, and this is consistent with one of the key functions of carbohydrates, and of the glucose polymer glucan in particular, as an energy store and source in diatom cells (Goes et al., 1996; Smith ad Underwood, 1998). The rapid loss of glucose is also consistent with previous feeding and decay experiments, in which it has been observed to be most rapidly lost (e.g. Oakes et al., 2010; see below). For example, in situ labelling experiments of intertidal sediments and biofilms have shown glucose to be the most rapidly produced, and then most rapidly lost, amongst the aldoses (Bellinger et al., 2009; Oakes et al., 2010). In addition, polychaete tissues showed lower weight percentages of glucose in their labelled aldose suites than in their naturally present unlabeled aldose suites (Fig. 3). This further suggests that freshly assimilated glucose was used to supply the animals' energy requirements in preference to any pre-existing stores, and instead of being incorporated into biomass. Whether this preferential metabolism of glucose occurred in polychaete cells, or in the gut through gut bacteria is not clear. An in situ labelling experiment by Boschker et al. (2008) observed particularly rapid production of labelled glucose in an intertidal sediment, further suggesting that glucose tends to be rapidly turned over.

4.2.1.2. Net accumulation

Many aldoses showed preferential accumulation in polychaete tissues, having greater weight percentages there compared to in the source algae (all except glucose and arabinose, Fig. 1), and greater weight percentages in labelled suites than in natural polychaete tissues (all aldoses except glucose, although differences were not significant for fucose and arabinose in *Arenicola*, Fig. 3).

The relative enrichment of polychaete tissues in most/all labelled aldoses except for glucose may be the result of more rapid metabolism of freshly assimilated glucose than of any other sugar. The marked decrease in weight percentage of the relatively dominant glucose caused by its rapid metabolism may have caused an increase in weight percentage of all other aldoses in polychaete tissues and sediment compared to the source algae, without any active selection in their favour during gut passage, or protection from degradation. As rapid glucose metabolism is the clearest pattern to emerge from previous feeding and metabolism studies this seems a likely explanation (Cowie and Hedges, 1996; Bellinger et al., 2009; Oakes et al., 2010).

Further, the preferential uptake and/or retention of aldoses showing net accumulation does not appear to be driven by a scarcity in the sediment. This is shown by a sediment deficiency index (Table 1), calculated using equation 5 after a similar calculation made for amino acid deficiency by Dauwe and Middelburg (1998).

$$\text{Sed. Deficiency index} = (S_{wt\%} - P_{wt\%})/P_{wt\%} \times 100 \quad \text{Equation 5.}$$

Where $S_{wt\%}$ and $P_{wt\%}$ are the weight percentages of naturally present (not labelled) aldoses in the sediment and polychaete tissues respectively. Negative deficiency index values indicate a compound that is present in the sediment in a lower weight percentage than in polychaete tissues, and which is thus in limited supply. While we concede that the composition of polychaete tissues and sediments are not necessarily rigorous measures of an organism's dietry requirements, nor of the bioavailability of OM (Dell'Anno et al., 2000), this index has contributed previously to explaining selective uptake and retention of amino acids (Dauwe and Middelburg, 1998; Woulds et al., 2012). All aldoses, except for glucose, and, in the case of *Arenicola*, arabinose and xylose, showed positive deficiency values (Table 1). Thus, comparative scarcity in the sediment may only be linked to accumulation in tissues in the case of two compounds, so limited availablity is not a clear explanation for patterns of selective aldose accumulation in polychaete tissues.

4.2.1.3. Arabinose

Arabinose showed the most marked net accumulation pattern. It exhibited a depletion in polychaete tissues compared to the labelled algae, and an enrichment in the sediments (Figs. 1 and 2), suggesting either that it was selected against during gut passage, or that the polymers in which it occurs in algal cells are particularly resistant to polychaete digestion and/or microbial degradation. Arabinose is not often discussed in detail during carbohydrate digestion and degradation studies, perhaps because it is not resolved by all analytical techniques (Boschker et al., 2008; Oakes et al., 2010), does not always or consistently show either selective preservation or degradation, and is not dominantly associated with just one type of carbohydrate in the algal cell (structural or cell contents, see below). However, the apparent selection against arabinose assimilation during polychaete gut passage, and its accumulation in the sediment is consistent with the observation that arabinose had the highest export ratio of all aldoses sinking below the photic zone in Sagami Bay (Hamanaka et al., 2002), that it was unreactive compared to other aldoses during sinking through the water column of Dabob Bay (Hedges et al., 1988), and that it is preferentially preserved during fungal decay of buried wood (Hedges, 1990). However, full explanations for this have not been provided, with Hedges et al. (1988) conceding that the reason was unknown. Arnosti and Repeta (1994) noted that a disaccharide composed of glucose and arabinose showed a slower microbial degradation rate even when compared to long-chain glucose polysaccharides. They suggest that the combination of a pentose and a hexose made microbial attack difficult, and that degradation did not proceed until an appropriate microbial community had time to multiply. Thus, selective preservation is likely to be the explanation for arabinose accumulation in sediments.

4.2.1.4. Comparison with previous digestion studies

We are not aware of any published studies of the alteration of aldose suites following polychaete gut passage, nor of studies of carbohydrate alteration by marine benthic organisms. Therefore, the patterns observed here are compared more broadly with alteration during invertebrate gut passage in soils and marine pelagic settings.

The patterns of aldose selective loss or retention discussed above are consistent with those found by Cowie and Hedges (1996) for copepods grazing on diatoms. They also observed a depletion of glucose in faecal pellets, and enrichment with mannose, giving digestion efficiencies for those two aldoses of 87% and 19%, respectively. They point out that aldoses that were enriched in faecal pellets were usually the same as those that dominated cell wall compositions (xylose, mannose, galactose). Thus they conclude that cell contents were more easily digested than cell wall components. Their digestion efficiencies thus show the same pattern as the mean recoveries of 6%

and 55% for glucose and mannose, respectively (note that digestion efficiency is the % lost during gut passage, whereas recoveries measured here are % retained in the whole microcosm).

Digestion of soil organic matter by the pill millipede produced a less marked alteration of the aldose suite in faecal pellets, leading the authors to state that the monosaccharide suite was unchanged (Rawlins et al., 2006). However, there was a slight decrease in the xylose to mannose ratio (from 3.9 to 1.4 following gut passage), which was attributed to preferential assimilation of plant as opposed to bacterial aldoses (Rawlins et al., 2006). No consistent change in this ratio was observed in this study. The lack of the rapid loss of glucose observed in this study may be explained by the fact that Rawlins et al. (2006) collected oak leaf litter from the forest floor to use as their food source. Thus it would have already undergone initial degradation, which could have involved rapid glucose metabolism (possibly even before the leaves fell from the trees).

4.2.1.5 Links with sedimentary decay patterns

Some aspects of the aldose loss patterns observed here are consistent with those observed during decay of marine organic matter. The most consistently observed patterns of selective aldose loss are mainly associated with early decay of fresh organic matter, rather than with late-stage decay of sedimentary organic matter (e.g. Cowie et al., 1995; Hernes et al., 1996; Veuger et al., 2012).

The cell contents of marine algae tend to be dominated by glucose, with arabinose, xylose, galactose, and fucose sometimes being similarly important (Biersmith and Benner, 1998). Cell walls also tend to be dominated by glucose, with mannose and galactose playing important roles (Biersmith and Benner, 1998, Cowie and Hedges, 1996). Aldoses in the interiors of cells are thought to be more accessible to microbial attack.For example, this was the explanation given for greater loses of ribose than of arabinose during export of POC from the euphotic zone of Sagami Bay (Hamanaka et al., 2002). Similarly, across the sediment-water interface in Saanich Inlet preferential loss of glucose, ribose and arabinose compared to mannose, galactose, fucose and rhamnose has been observed (Hamilton and Hedges, 1988; Cowie et al., 1992). Also, Ittekkot et al. (1984a) attribute the relative lack of glucose in sinking particles in the Sargasso Sea to preferential loss of cell contents. Ittekkot and Degens (1982) observed two stages of aldose decay in a North Sea plankton bloom. The first stage was characterised by loss of cell contents represented by glucose, and the second by loss of structural sugars represented by galactose. In perhaps one of the most extensive studies of aldose decay patterns, a survey of settling particles and sediments at several sites in the equatorial Pacific led Hernes et al. (1996) to suggest three fractions of aldoses, comprising rapidly degraded cell contents dominated by glucose and ribose, relatively refractory structural components

including rhamnose, xylose, fucose and mannose, and an intermediate fraction containing galactose and arabinose. This is further consistent with aldose decay rates observed during an in situ labelling study in intertidal sediments, in which freshly produced glucose was observed to degrade most rapidly, and mannose least rapidly (Oakes et al., 2010). It should be noted that, while relative patterns of aldose loss during decay tend to be being broadly similar amongst different studies, there is not always exact agreement. Thus, the low recoveries of glucose, galactose and ribose, and comparatively high recoveries of rhamnose, fucose, arabinose and mannose observed here (Fig. 4) are consistent with previous studies, and with the preferential loss/metabolism of aldoses from the cell contents. This consistency with both feeding and decay studies suggest that aldose suite alterations are similar during gut passage and microbial decay.

In contrast, however, Skoog and Benner (1997) observed an accumulation of glucose with depth in the water column in the equatorial Pacific, and attributed it to geopolymerisation. Further, preferential decay of carbohydrates relative to bulk OM is not always observed (e.g. Vichkovitten and Holmer, 2005, Cowie and Hedges, 1984, Cowie et al., 1995, Hedges et al., 1999), and in other cases preferential preservation of carbohydrates relative to bulk OM has been suggested (Hernes et al., 1996, Danovaro et al., 2001). Observations of aldose suite alterations tend to be associated with the earlier stages of decay (Hamilton and Hedges, 1988, Ittekkot et al., 1984 b, Hernes et al., 1996, Unger et al., 2005, Wakeham et al., 1997); thus it seems likely that different fractions of carbohydrates, with different labilities, exist. However, some studies have shown similar loss rates amongst the different aldoses even in relatively fresh OM, and have concluded that selective degradation only occurs during decay of polysaccharides with a carbon or energy storage role, and not for those with a structural role (Hedges et al., 1988; Veuger et al., 2012). Organic matter sourced directly from primary producers is likely to have a relatively high storage polymer content, while bacterial secondary production results in a greater dominance of structural polymers (Veuger et al., 2012), and will account for a greater proportion of total OM in previously degraded sediments. Thus we suggest that degradation of algal OM is likely to exhibit compound selectivity amongst the aldoses, while degradation of bacterial OM may not (Veuger et al., 2012).

4.2.2. Fatty Acids

As with the aldoses, PCA results clearly showed that polychaete ingestion and gut passage produced different suites of labelled fatty acids in polychaete tissues and microcosm sediments. Sediments, where bacterial degradation occurs, were relatively enriched in bacterial and some algal fatty acids, which mostly fell into the saturated and MUFA groups (Figs. 6 and 7). In contrast, polychaete tissues were relatively enriched in fatty acids that included algal markers, and these were mostly MUFAs

603 and PUFAs (Figs. 6 and 7). Fatty acids thought to be synthesised by animals (Graeve et al., 2005)
604 occurred only in polychaete tissues. In the following discussion fatty acids will be grouped and
605 discussed in relation to this range of processes.

606 #### *4.2.2.1. PUFAs*

607 Of the various broad groups of fatty acids, PUFAs showed the clearest patterns. Several of the
608 compounds that showed enrichment in polychaete tissues and thus separated them from sediment
609 samples during PCA were PUFAs, and several individual PUFAs were observed to accumulate in
610 polychaete tissues over time (Fig. 6). This is consistent with previous observations that both
611 copepods and *Hediste* have high assimilation efficiencies for PUFAs (>90% PUFA assimilation
612 efficiency by *Nereis*, Bradshaw et al., 1990b; fatty acid assimilation efficiency 25-97% for *Calanus*
613 spp., Mayor et al., 2011). However, overall, PUFAs constituted a smaller proportion of the total
614 labelled fatty acid suite in both polychaete tissues and (to an even greater extent) microcosm
615 sediments than they did in the source algae. This cannot be explained by either preferential
616 assimilation or egestion of PUFAs, but instead indicates a disproportionate loss of PUFAs from the
617 microcosms through metabolism. PUFAs have been observed previously to be preferentially lost
618 from sediments as a result of microbial activity (Bradshaw et al., 1990b). It has been suggested that
619 foraminiferal metabolism can also produce this effect (Nomaki et al., 2009), and that efficient
620 assimilation during polychaete and copepod digestion strips PUFAs from sediments, with
621 implications for their availability to other organisms (Graeve et al., 2005; Mayor et al., 2011). In this
622 study there was evidence for both accumulation of some PUFAs in polychaete tissues, and overall
623 metabolic loss of PUFAs; thus it seems likely that polychaete metabolism also contributes to rapid
624 loss of PUFAs. Nomaki et al. (2009) went on to note that uptake and retention of PUFAs by
625 foraminiferans will increase the turnover time of PUFAs in the sediment environment, and this
626 suggestion can also be extended to polychaetes.

627 #### *4.2.2.2. Selective uptake and essentiality*

628 Some algal marker fatty acids were enriched in polychaete tissues while others were not, suggesting
629 preferential uptake and/or retention of certain fatty acids, and preferential egestion or metabolism
630 of others. On a broad scale, the labelled fatty acid suites found in polychaete tissues were similar to
631 those found in their natural tissues (Fig. 5). This suggests that uptake and retention were driven by
632 polychaete requirements as denoted by tissue composition, rather than by availability in the algae.
633 This is further suggested by the enrichment of polychaete tissues with C22:6w3 and C20:5w3
634 (docosapentaenoic acid and eicosapentaenoic acid, respectively) which are essential fatty acids, and

must be acquired by animals from the diet (Parrish et al., 2012). Further, C22:4w6, which is also essential, was observed to increase its proportion of the labelled fatty acid suite in polychaete tissue over time (Fig. 6). These findings are consistent with previous feeding studies, which have shown preferential retention of these fatty acids in copepods (Parrish et al., 2012), and have also linked strong growth and egg production in zooplankton to higher concentrations of C20:5w3 in their food (Muller-Navarra et al., 2000).

4.2.2.3. Temporal trends

Several fatty acids, including C14:0 and the algal marker C16:1w7c, were initially assimilated by polychaetes in greater proportions than those in which they were present in polychaete tissues, and those proportions declined over time. Thus, these fatty acids seemed to be relatively rapidly assimilated or produced from the diet, before being either metabolised or used in the synthesis of other biochemicals. Rapid assimilation of algal fatty acids is consistent with observations during copepod feeding experiments (Graeve et al., 2005). However, the rapid assimilation of C14:0 marks a difference between copepods and the polychaetes in this study.

Other fatty acids showed the opposite pattern, with their proportion of the labelled fatty acid suite increasing steadily through time towards the proportion they represented in the natural polychaete fatty acid suite. Compounds that showed this pattern in both polychaete species included the animal or bacterial marker C18:1w9c, which could have been due to cumulative synthesis by the animals. However, the delta-delta values for fatty acids thought to be synthesised by the polychaetes were not large, indicating relatively slow biosynthesis compared to uptake of dietary fatty acids, and this is consistent with patterns in previous studies (Graeve et al., 2005).

4.2.3. Synthesis

4.2.3.1. Comparison of biochemical classes during gut passage and decay

Studies of organic matter alteration during both faunal gut passage and degradation in the sediment typically have concluded that particular groups are assimilated more efficiently and metabolised more rapidly than others.

During decay of sedimentary organic matter, lipids typically are turned over most rapidly, followed by amino acids, then carbohydrates, and all are lost more rapidly than bulk organic carbon (e.g. Cowie and Hedges, 1984; Hamilton and Hedges, 1988; Dell-Anno et al., 2000; Amaro et al., 2010 Veuger et al., 2012). During decay of microbial biomass in sediments from the Westerschelde

estuary, the same general order of reactivity (phospholipid fatty acids > total fatty acids > hydrolysable amino acids > monosaccharides) was found (Veuger et al., 2012).

A similar pattern was also shown in holothurian feeding experiments, in which amino acids showed the greatest assimilation efficiency (67%) followed by lipids (50%), and then carbohydrates (23%, Amaro et al., 2010). Linking this to the locations of different enzymes in the holothurian gut, they concluded that the organisms preferentially digested and assimilated N-rich compounds (preferring them even over lipids). Similarly, Dell-Anno et al. (2000) found that enzymatically hydrolysable carbohydrates were more conservative with depth in deep Atlantic sediments than enzymatically hydrolysable amino acids, concluding that their lower N content led them to be in lower demand as a food source. *Hediste* has also been shown to have a very high assimilation efficiency (~90%) for fatty acids (Bradshaw et al., 1990b).

Unfortunately the experimental design used here did not allow the calculation of assimilation efficiencies, but the percentages recovered in quantitative budgets for amino acids (Woulds et al., 2012) and aldoses showed a similar pattern to previous work (they could not be produced for fatty acids due to a lack of high-quality data on the fatty acid composition of the algae). Amino acid recoveries (considering all compounds and microcosms) showed a mean of 11%, while the value for aldoses was 33%, and there was a significant difference between aldose and amino acid recoveries (Mann-Whitney, $P < 0.001$). Thus, in these experiments amino acids appear to have been preferentially metabolised compared to aldoses. This preferential use of amino acids is consistent with the holothurian feeding experiment described above, where amino acids were preferentially assimilated (a precursor to metabolism by the holothurians), most likely due to their high N contents (Amaro et al., 2010). It has further been suggested that carbohydrates may instead play a greater role in supporting sub-surface feeders (Dell Anno et al., 2000; Amaro et al., 2010).

4.2.3.2. Interactions between gut passage and microbial decay

Certain features of the labelled biochemical suites observed in the experimental microcosms were indicative of microbial decay rather than polychaete gut passage.

In the case of aldoses, rhamnose, fucose and galactose in marine sediments have been attributed to microbial biomass and/or to preferential preservation during decay (Hamilton and Hedges, 1988; Hedges et al., 1994; Opsahl and Benner, 1999). Slow degradation and/or production of rhamnose and fucose has also been observed in extracellular polymeric substances, a biofilm, and in intertidal studies (Giroldo et al., 2003; Bellinger et al., 2009; Oakes et al., 2010). Ribose is also found to be typically enriched in microbial biomass (Cowie and Hedges, 1984; Hedges et al., 1994). In line with

this, relatively high recoveries of labelled rhamnose and fucose were observed in this study (Fig. 4), which may be partially accounted for by growth of microbial biomass following feeding on labelled algae. However, selective preservation of rhamnose and fucose, or preferential loss of other aldoses, remain possible explanations. Rhamnose and fucose recovery in afaunal control microcosms was intermediate between the 10-d and 22-d timepoints for faunated *Hediste* microcosms (Fig. 4); therefore it was not possible to determine whether the presence of fauna had influenced their accumulation. In contrast, amongst *Arenicola* microcosms, recovery of rhamnose and fucose was generally greater in the absence of fauna (Fig. 4); thus, polychaete activity may have suppressed the accumulation of bacterial aldoses, either through grazing or through their direct consumption.

The fatty acids C15:0, i-C15:0, ai-C15:0, and equivalent C17 PLFAs, along with C18:1w7c and C18:w9 are usually attributed to bacterial production (e.g. Bellinger et al., 2009). Further, C18:1w9 and C18:1w11, and branched C15 and C17 fatty acids are thought to be contributed to the sediment, as a result of gut bacterial activity (Bradshaw et al., 1990; Grossi et al., 2006). While the activities of burrowing macrofauna are often thought to stimulate sediment microbial activity (e.g. Aller and Aller, 1998), their relationship with microbial lipids is not necessarily straightforward. It has been suggested that *Arenicola* strips bacterial fatty acids out of the sediment that it ingests, and Sun et al. (1999) observed that net microbial lipid accumulation was suppressed in the presence of *Yoldia limatula*.

In this study, the bacterial markers i- and ai-C15:0 only constituted a substantive part of the labelled fatty acid suite in sediment samples (not in faunal tissues, Fig. 6). Further, along with C18:1w7c, the most abundant bacterial marker, their contributions to the labelled fatty acid suite increased over time (Fig. 6), which suggests that they were produced during the experiments through degradation of algae by sediment bacteria. This is in line with observations made during previous algal degradation experiments in the presence of macrofauna (Sun et al., 1999), but differences in net microbial lipid production rates between faunated microcosms and afaunal controls could not be discerned. In *Hediste* microcosms, C18:1w7c and the branched C15 FAs were more important in the sediment than in the polychaete tissues. However, in *Arenicola* microcosms C18:1w7c was equally important in both polychaete tissues and sediments (Fig. 6). We suggest that this fatty acid was produced in the sediment through bacterial degradation of algae, and that *Arenicola* then assimilated the labelled bacterial FA, in line with suggestions that *Arenicola* strips bacterial FAs from the sediment (Boon and Haverkamp, 1979; Bradshaw et al., 1990). This could be further investigated by analysing faecal matter for labelled fatty acids, however this has not been possible due to the small sample sizes available.

4.2.3.3. Linking faunal gut passage to long-term decay

The impact of polychaete gut passage on OM composition and cycling pathways should be distinguishable from changes occurring due to microbial degradation through differences between the labelled biochemical compositions of sediments in faunated microcosms and afuanal controls.

In the case of aldoses, however, there was a general lack of systematic difference in aldose suites between faunated microcosm sediments and those in afaunal controls (Figs. 1, 5); thus it has not been possible to distinguish between polychaete gut passage and microbial metabolism in terms of their impact on the composition of sedimentary OM. This is consistent with a previous study of digestion by *Arenicola* in which polychaete gut passage resulted in similar OM compositional alteration as for aerobic degradation without polychaetes present (anaerobic degradation produced a different pattern of change, Boon and Haverkamp, 1979). The compound selectivity of polychaete aldose accumulation makes it slightly surprising that they do not impose a characteristic aldose composition on the sediment; however, bacterial degradation of carbohydrates can also be compound selective (Arnosti and Repeta, 1994). However, the relatively low nutritional value, low lability, and the less marked suite alterations amongst the labelled aldoses compared to fatty acids and amino acids (Amaro et al., 2010, see above), perhaps suggests that they are the biochemical class least likely to display a clear impact of polychaete gut passage on sediment composition.

One possible reason for the difficulty in distinguishing polychaete-induced and microbial changes to aldose composition is that natural microbial communities were present in the microcosm sediments. Microbial activity can account for large proportions of total sediment community respiration (e.g. Moodley et al., 2005), thus this activity may have been sufficient to mask polychaete-specific effects. Alternately, in the case of amino acids, it was concluded that microbial and polychaete induced changes to suites were very similar, and difficult to de-convolve for that reason (Woulds et al., 2012), and this could also be the case for aldoses. Further, polychaete guts contain enteric bacteria, therefore an unknown portion of the compositional changes which occurred during gut passage may have been due to the actions of gut bacteria. Enteric bacteria are presumably integral to polychaete functioning, therefore it can be argued that their activity should simply be considered as one of the mechanisms through which polychaete gut passage effects compositional change on OM.

Differences between faunated and afaunal microcosms may be revealed by extracting and quantifying only the enzymatically hydrolysable (bioavailable) fraction of the carbohydrates, which have been shown to represent only 13% of total carbohydrates in Porcupine Abyssal Plain surface

761 sediments (Dell Anno et al., 2000). Further, in the case of amino acids it was suggested that longer
762 experiments may have eventually produced a measurable difference (Woulds et al., 2012).

763 In the case of fatty acids, greater proportions of saturated fatty acids would be expected in faunated
764 microcosm sediments than those from afaunal controls, as *Hediste* is particularly efficient at
765 assimilating PUFAs, and thus removes them from the sediment (Bradshaw et al., 1990b). In fact
766 *Hediste* control sediments did show slightly lower proportions of saturated fatty acids and slightly
767 higher proportions of PUFAs compared to faunated microcosms, but the difference was within the
768 error of the measurements (Fig. 5). In addition, a PCA of labelled fatty acid suites in just sediment
769 samples showed a difference in PC1 scores between afaunal control sediments and faunated
770 sediments from the 21/22-d and 37/38-d timepoints (Fig. 8). PC1 scores for control sediments and
771 those from the earliest timepoint (9/10-d) in faunated microcosms were driven by greater
772 proportions of two algal markers, while those for faunated microcosm sediments from later
773 timepoints were driven by greater proportions of bacterial markers (Fig. 8). Thus, in line with
774 previous studies, the presence of fauna accelerated the loss of algal fatty acids (Sun et al., 1999), and
775 facilitated the accumulation of bacterial products. Therefore, the mechanisms through which fauna
776 influence fatty acid degradation must include increasing microbial activity, through hosting enteric
777 bacteria, and stimulation of free-living bacterial activity. This is consistent with the findings of Grossi
778 et al. (2006), who noted that gut passage imparted a bacterial fatty acid signature on egested OM,
779 which they ascribed to enteric microbial activity. This has potential implications for the use of
780 bacterial fatty acids as trophic markers, as some of those used to indicate a bacterial food source or
781 bacterial degradation in the sediment (Dalsgaard et al., 2003) may in fact be partly derived from
782 invertebrate gut passage.

783 #### *4.2.3.4. Taxon-specific effects*

784 In the cases of both aldoses and fatty acids, compositional differences were observed between the
785 labelled suites present in *Hediste* and *Arenicola* tissues (Figs. 1, 6 and 7). This suggests not only that
786 one or several of the processes to which ingested OM is subjected are compound-specific but also
787 that the patterns of dependence are taxon-specific. This lends support to our hypothesis that
788 distinctive OM alteration through a combination of compound-selective assimilation, retention,
789 metabolism and egestion during polychaete gut passage must play a role in determining the organic
790 composition of marine sediments. Moreover, the pattern of that influence will be determined to
791 some extent by the macrofaunal species present.

5. Summary and Conclusions

Together with Woulds et al. (2012), this study provides the most comprehensive characterisation yet of OM alteration during gut passage by intertidal polychaetes, in addition to making links between gut passage and longer-term sedimentary decay. The main findings of this study were:

- The net accumulation of biochemicals in polychaete tissues was observed to be compound specific for both aldoses and fatty acids.
- Compound selectivity during uptake into polychaete tissues was observed to be taxon specific.
- The dominant patterns of OM alteration included an overall loss of glucose and PUFAs, with indications that polychaetes were at least partly responsible, through glucose metabolism and PUFA assimilation.
- Aldose suite alterations were linked to the location of different sugars in algal cells, with preferential assimilation and metabolism of cell contents as opposed to cell wall components.
- Arabinose showed marked selective preservation, which could be attributable to polymers containing arabinose being less susceptible to enzymatic attack, and selection against during gut passage.
- Essential fatty acids were preferentially accumulated in polychaete tissues.
- As a class, aldoses were less prone to loss from microcosms through total metabolism than amino acids (Woulds et al., 2012), and this could be linked to their lower nutritional value.
- Aldose and fatty acid suites both showed evidence of microbial OM degradation in microcosm sediments. Evidence suggested that *Arenicola* grazed on bacteria and suppressed accumulation of bacterial fatty acids.
- Fatty acid suites in sediments from faunated microcosms showed greater proportions of saturated fatty acids and bacterial markers than those from afaunal controls. Aldose suite alterations were similar in faunated microcosms and afaunal controls, however the impact of faunal gut passage on sedimentary aldose compositions may be observable over longer timescales.
- Therefore this study provides direct evidence that polychaete gut passage influences OM composition both through taxon specific selective assimilation and retention in polychaete tissues, and also through interactions with the microbial community.

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Table 1. Sediment deficiency index for aldoses, based on mean natural aldose composition data derived from all sediment and polychaete tissue analyses. Negative deficiency indeces indicate a compound which is present in the sediment in a lower weight percentage than that in which it is present in polychaete tissues.

Aldose	Deficiency Index *Hediste*	Deficiency Index *Arenicola*
Rhamnose	1106	14
Fucose	183	36
Ribose	28	13
Arabinose	2997	-14
Xylose	487	-11
Mannose	156	12
Galactose	367	6
Glucose	-73	-6

Figure 1. Average labelled aldose compositions of A: polychaete tissues (and diet algae), and B: controls, sediment and faecal pellet samples (and diet algae), C: in absolute terms in *Hediste* tissues, and D in absolute terms in *Arenicola* tissues.

Figure 2. Results of a PCA of labelled aldose weight percentages for all samples. A) Sample scores B) Factor coefficients.

Figure 3. Mean labelled and natural aldose compositions of polychaete tissues for A) Hediste, and B) Arenicola. Means include samples from all timepoints. Error bars are ± 1 standard deviation. The * symbol indicates a significant difference in weight percentage between labelled and natural suites.

Figure 4. Quantitative aldose budgets for A) *Hediste*, and B) *Arenicola* microcosms. Error bars are plotted for *Hediste* 10-d and 22-d, as duplicate microcosms were run. Error bars are ± 1 standard deviation. 'Cont.' indicates sediment from an afuanal control microcosm. 'A' controls were sacrificed on days 9 and 10, and 'B' controls on days 37 and 38 for *Arenicola* and *Hediste* respectively.

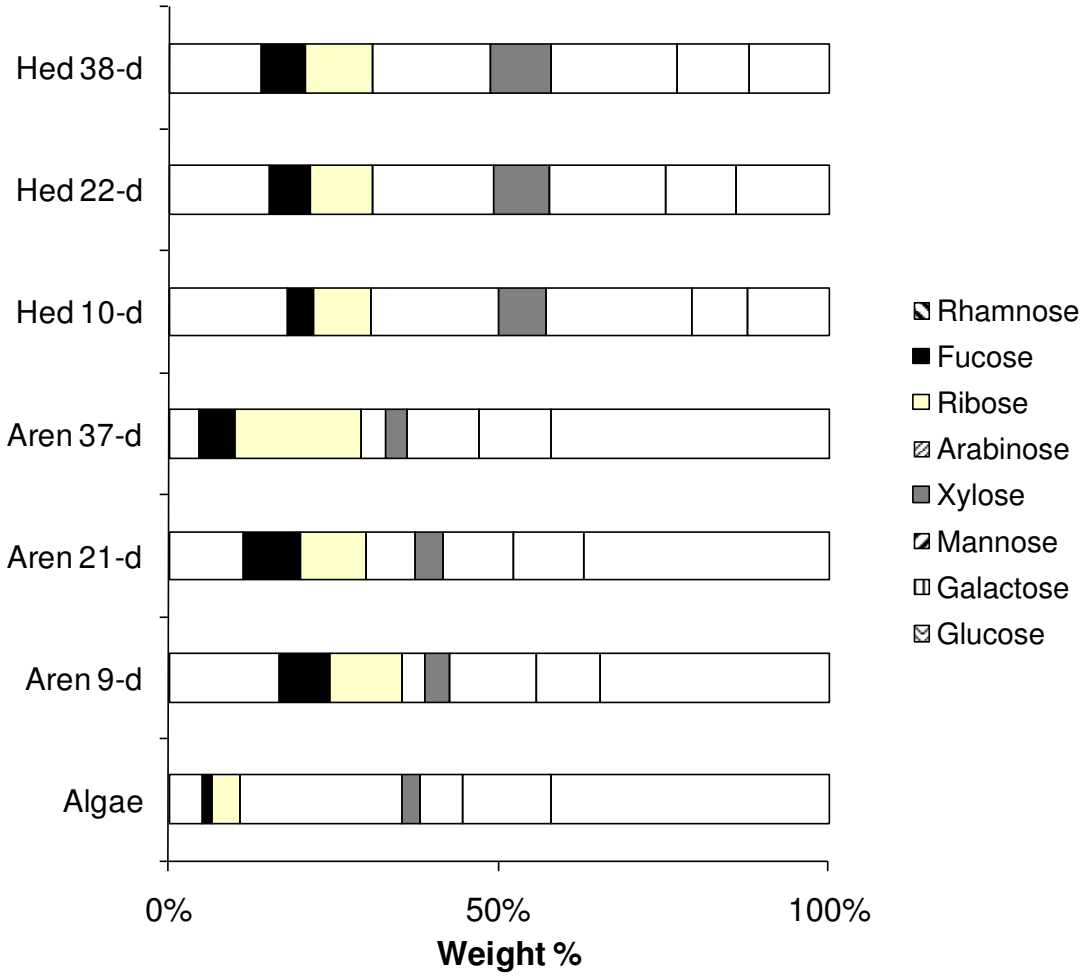
Figure 5. The ^{13}C-labelled fatty acid suites in experimental samples, and natural fatty acid suites in natural polychaete tissues and algae in A) *Hediste* tissues, B) *Arenicola* tissues, C) *Hediste* sediments, and D) *Arenicola* sediments. Values are means of replicates, and error bars are ± 1 standard deviation.

Figure 6. ^{13}C-labelled and natural fatty acid suites for A) *Hediste* and B) *Arenicola* microcosms. Abundances are plotted as proportion of total ^{13}C-labelled fatty acid in each sample type (except for natural suites). Values are means of replicate individuals (n=2-4), and error bars are ± 1 standard deviation.

Figure 7. PCA results for fatty acid suite data for all samples. Sample scores are given in A, and factor coefficients of particularly abundant and influential fatty acids are given in panel B (for clarity, other compounds included in the PCA are not plotted).

Figure 8. PCA results for fatty acid suite data for sediment samples only. Sample scores are given in panel A, and factor coefficients of particularly abundant and influential fatty acids are given in panel B (for clarity, other compounds included in the PCA are not plotted).

Figure 9. The ^{13}C enrichment of selected fatty acids compared to their signatures in natural samples ($\Delta\delta = \delta^{13}$C in labelled sample-$\delta^{13}$C in natural sample). Values are means of replicates, and error bars are ± 1 standard deviation. The plot is shown in two sections for clarity.


Hed 38-d
Hed 22-d
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Aren 21-d
Aren 9-d
Algae
0%
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Weight %
Rhamnose
Fucose
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose

1125

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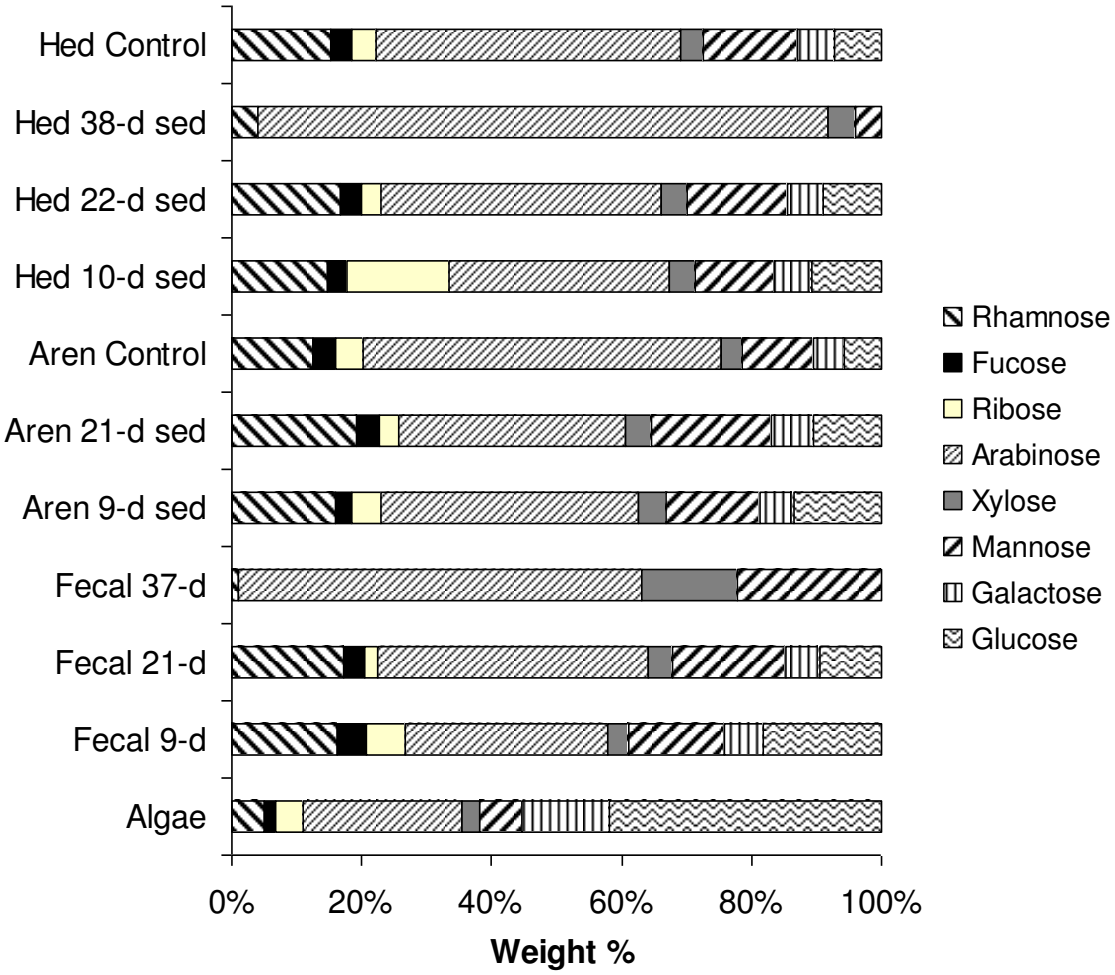
Hed Control
Hed 38-d sed
Hed 22-d sed
Hed 10-d sed
Aren Control
Aren 21-d sed
Aren 9-d sed
Fecal 37-d
Fecal 21-d
Fecal 9-d
Algae
0%
20%
40%
60%
80%
100%
Weight %
Rhamnose
Fucose
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose

1127

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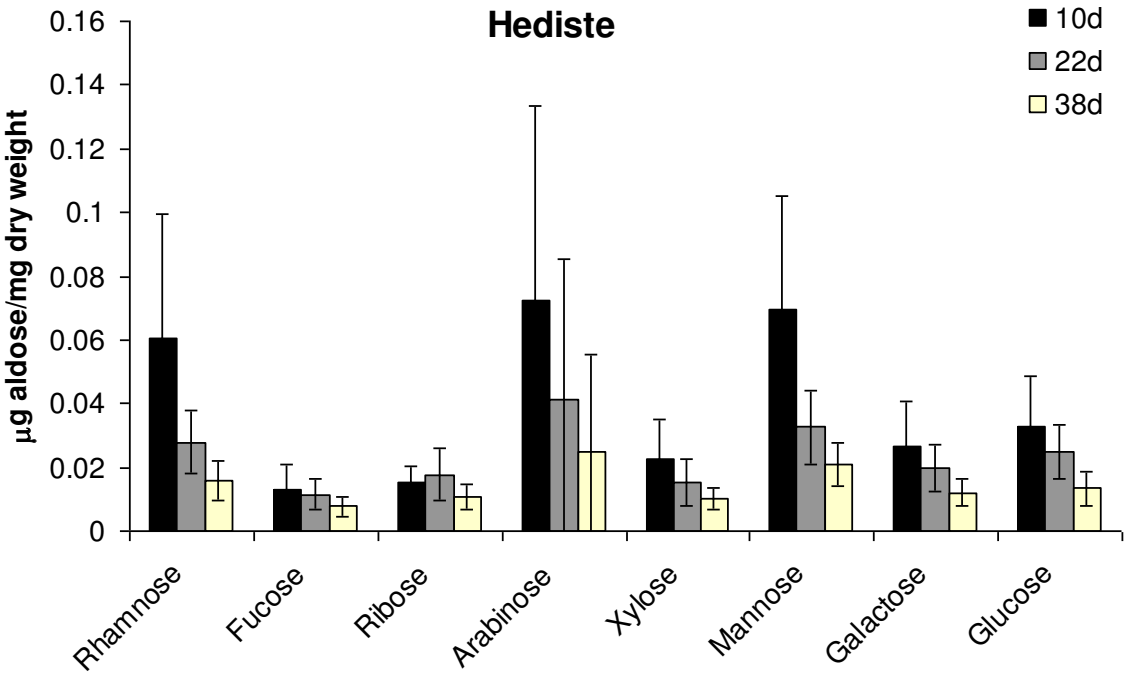
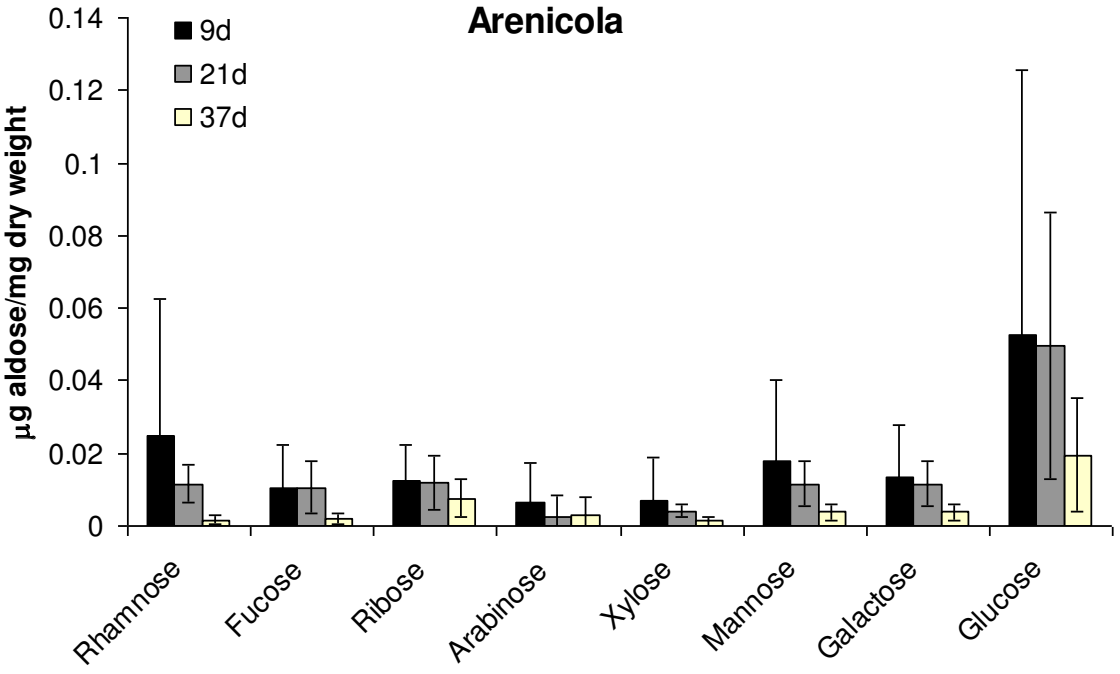


1129

1130 C

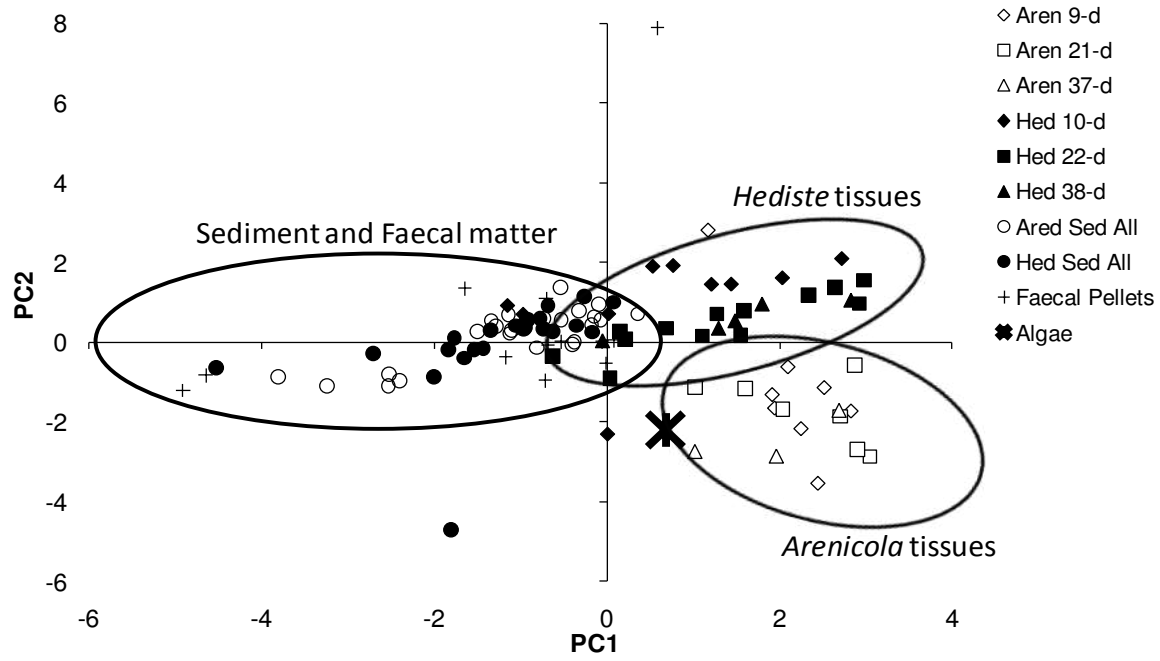


1131

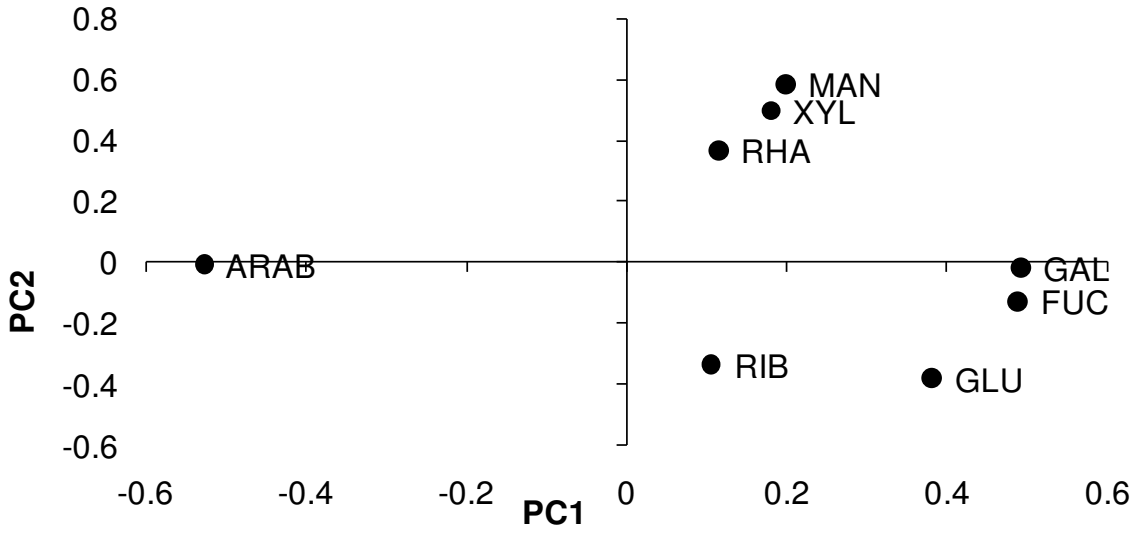
1132 D

1133 Figure 1.

1134

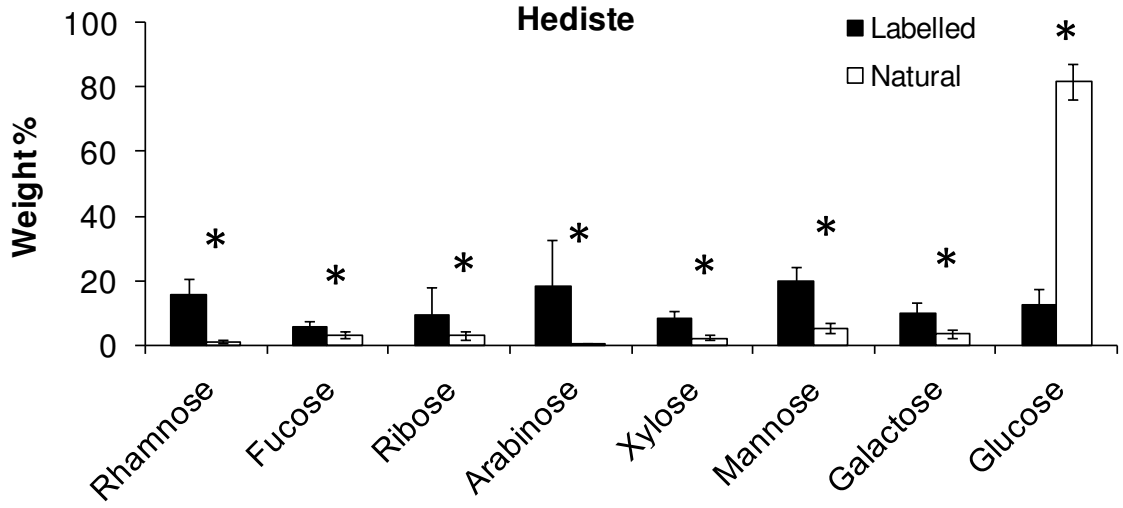

Aren 9-d
Aren 21-d
Aren 37-d
Hed 10-d
Hed 22-d
Hed 38-d
Ared Sed All
Hed Sed All
Faecal Pellets
Algae
Hediste tissues
Sediment and Faecal matter
Arenicola tissues
PC1
PC2

A

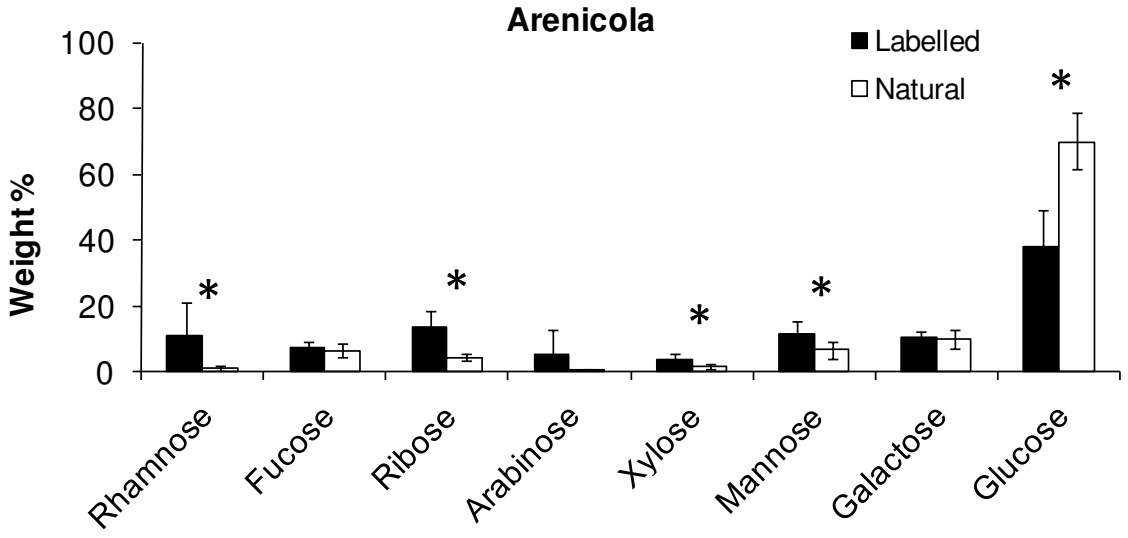

MAN
XYL
RHA
ARAB
GAL
FUC
RIB
GLU
PC1
PC2

B

Figure 2.

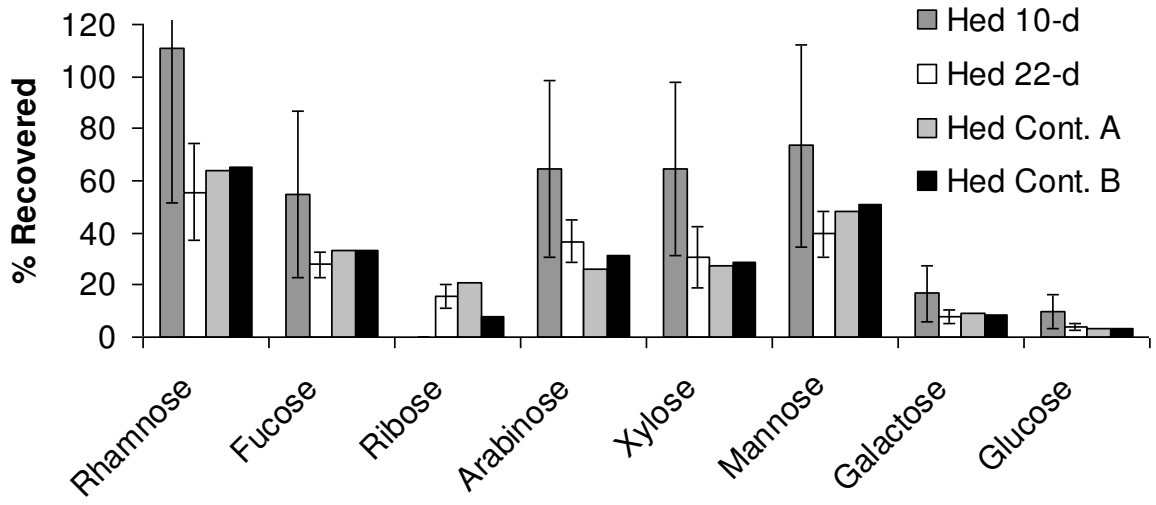

Hediste
Labelled
Natural
Weight %
100
80
60
40
20
0
Rhamnose
Fucose
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose

A


Arenicola
Labelled
Natural
Weight %
100
80
60
40
20
0
Rhamnose
Fucose
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose

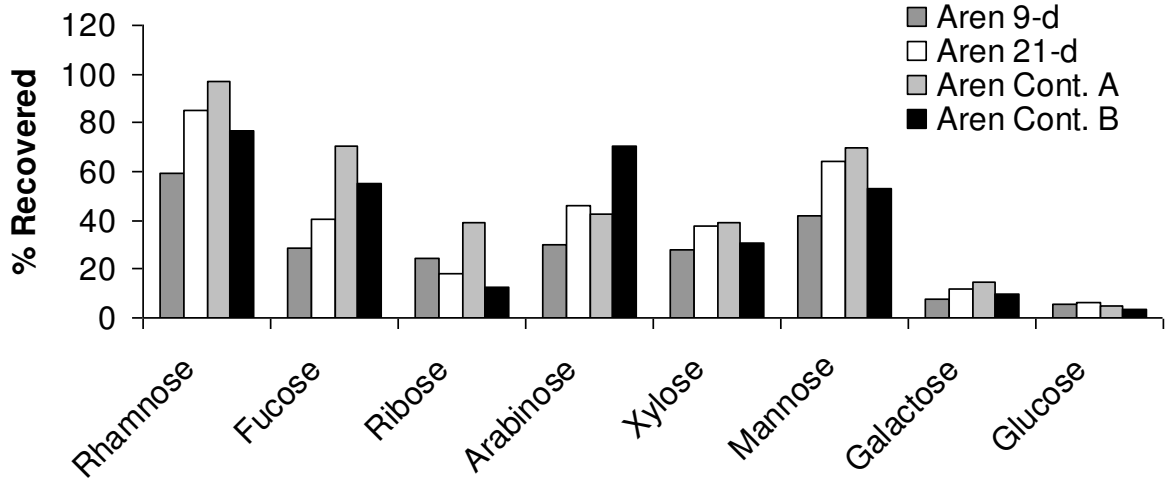
B

Figure 3.



1149

1150 A



1151

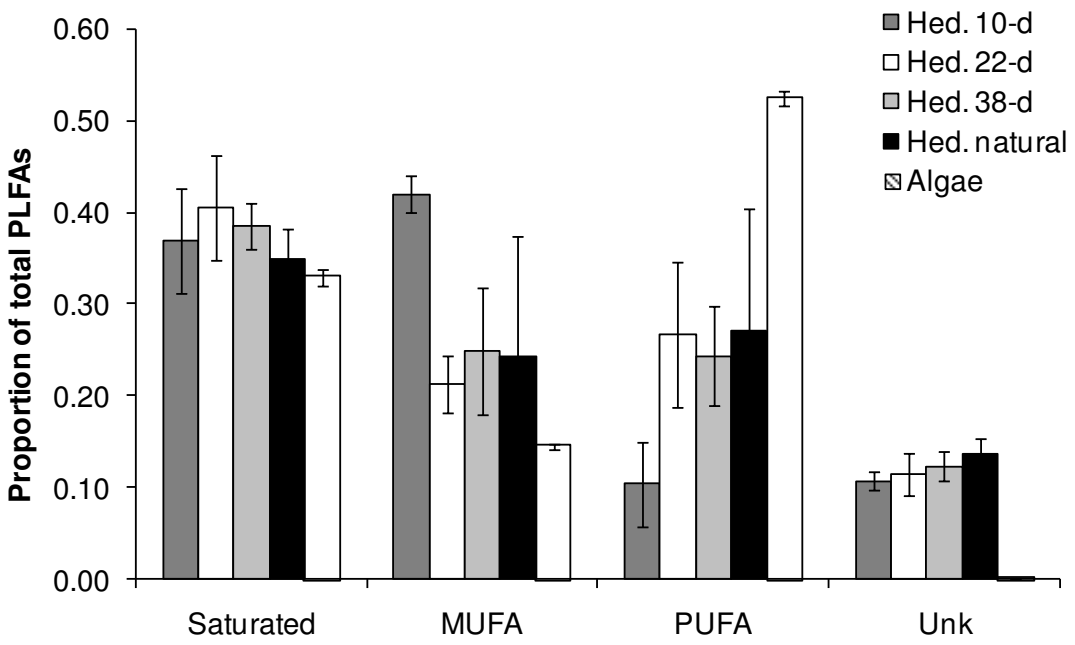
1152 B

1153 Figure 4.

1154

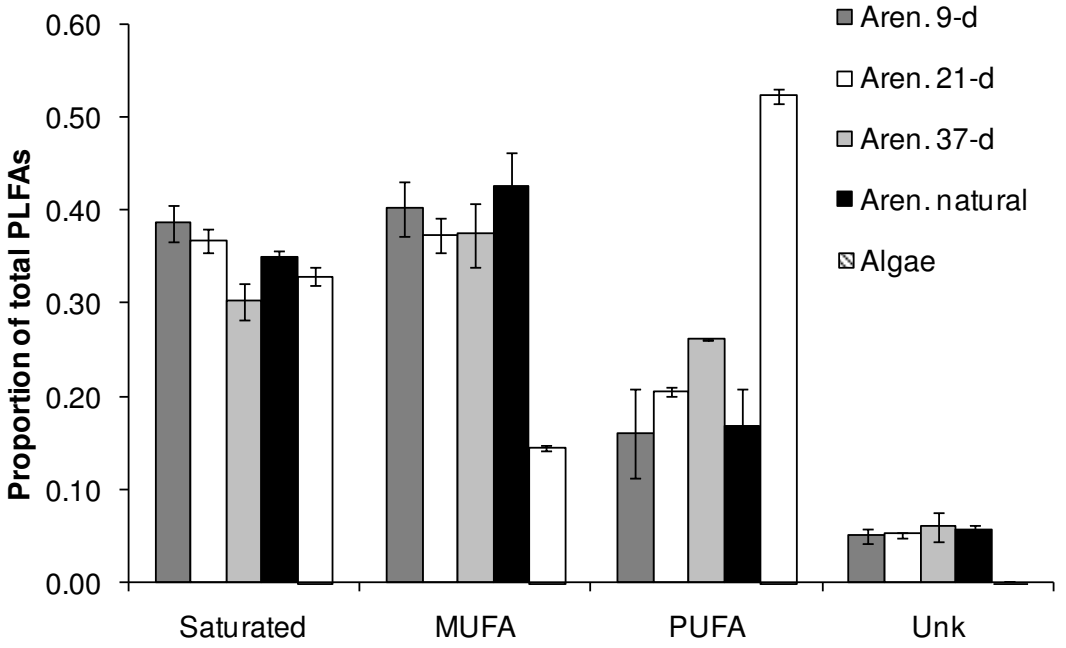
1155

1156



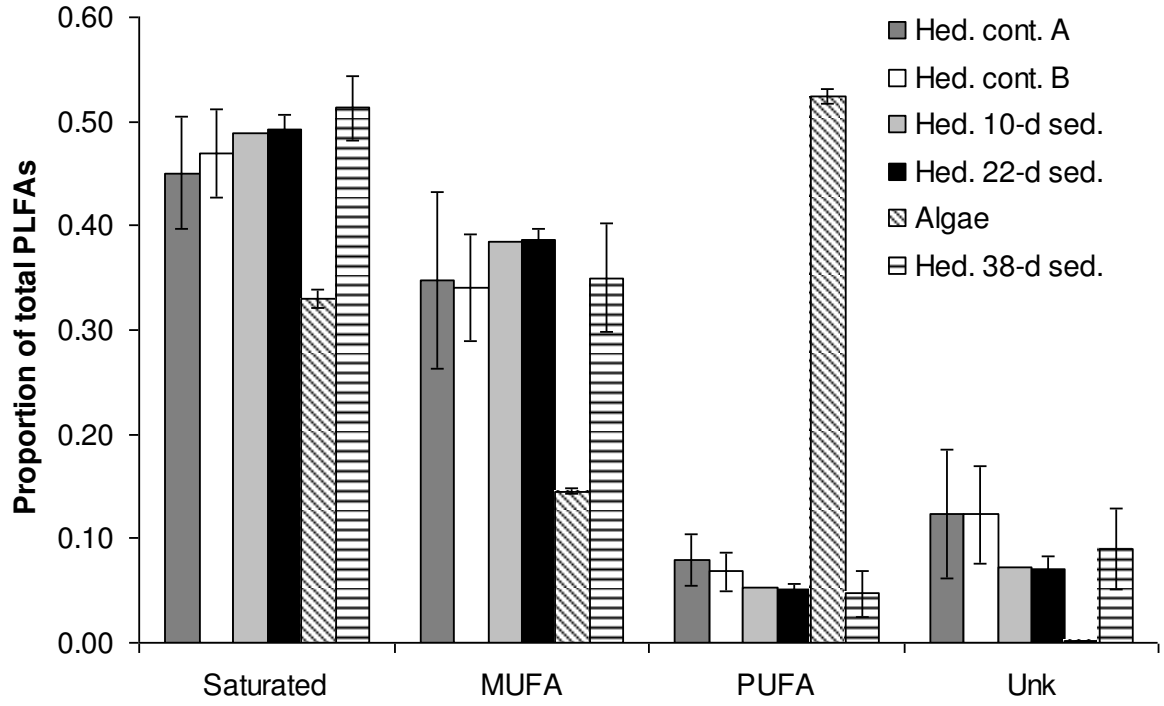
1157

1158 A



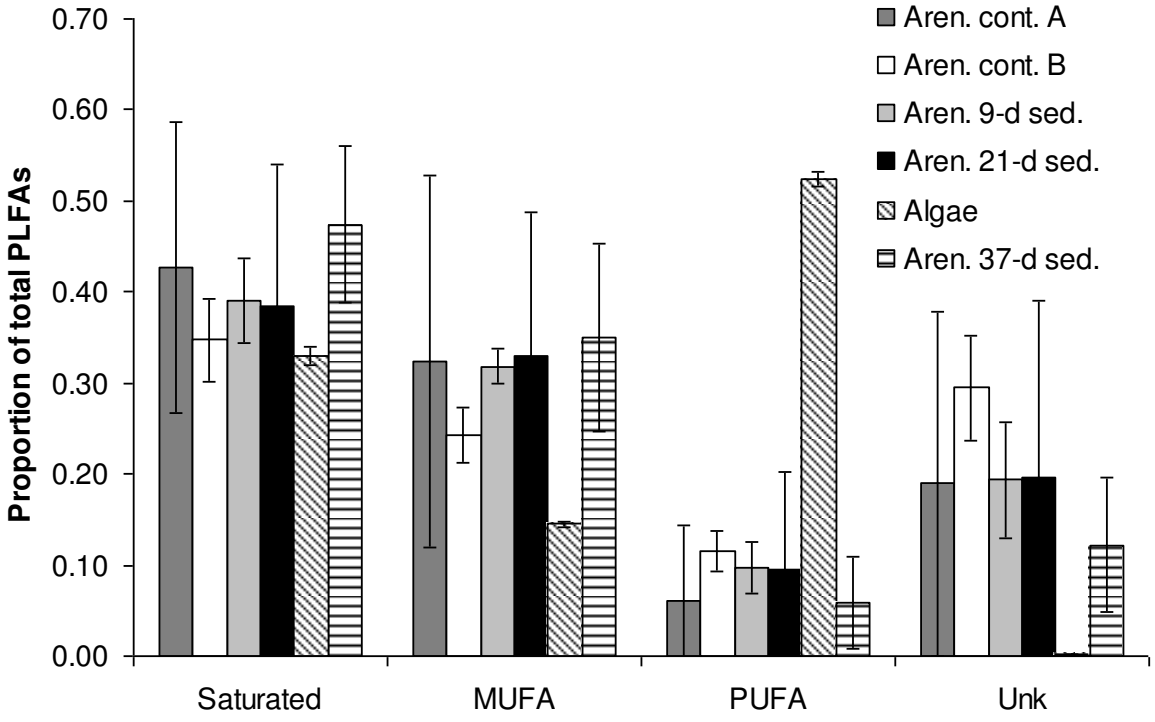
1159

1160 B



1161

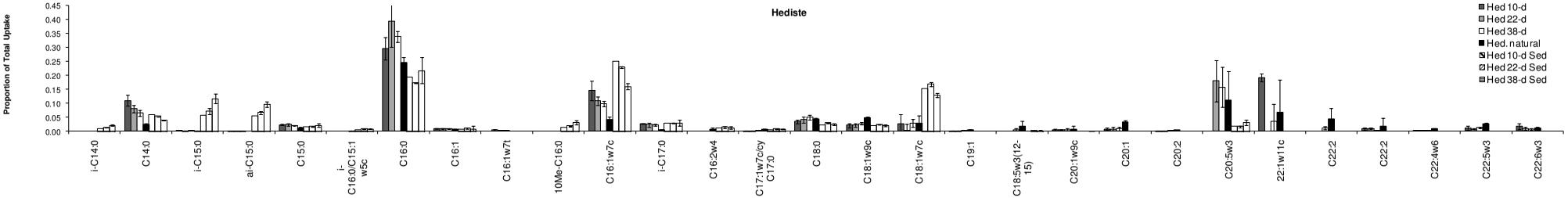
1162 C



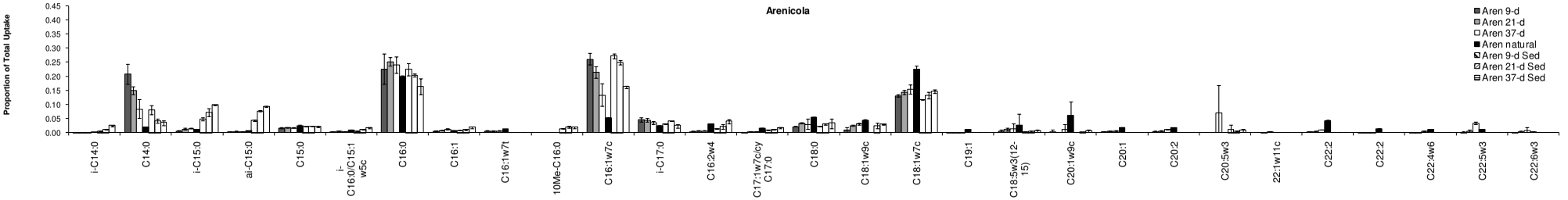
1163

1164 D

1165 Figure 5.

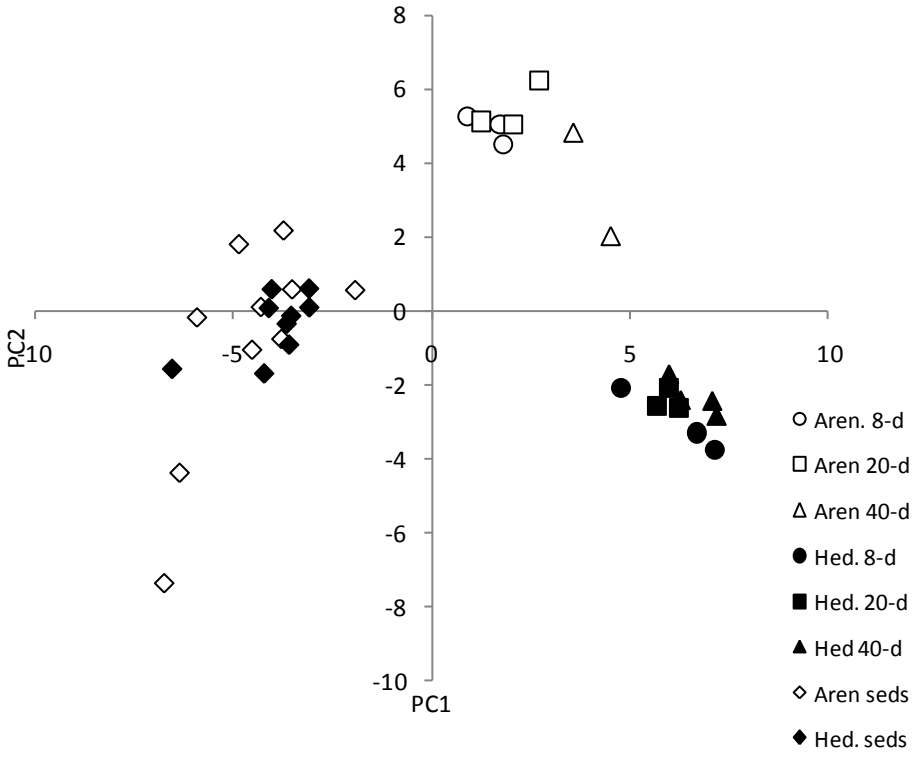

Hediste
Proportion of Total Uptake
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Hed 10-d
Hed 22-d
Hed 38-d
Hed. natural
Hed 10-d Sed
Hed 22-d Sed
Hed 38-d Sed
i-C14:0
C14:0
i-C15:0
ai-C15:0
C15:0
i-C16:0/C15:1 w5c
C16:0
C16:1
C16:1w7t
10Me-C16:0
C16:1w7c
i-C17:0
C16:2w4
C17:1w7c/cy C17:0
C18:0
C18:1w9c
C18:1w7c
C19:1
C18:5w3(12-15)
C20:1w9c
C20:1
C20:2
C20:5w3
22:1w11c
C22:2
C22:2
C22:4w6
C22:5w3
C22:6w3

A

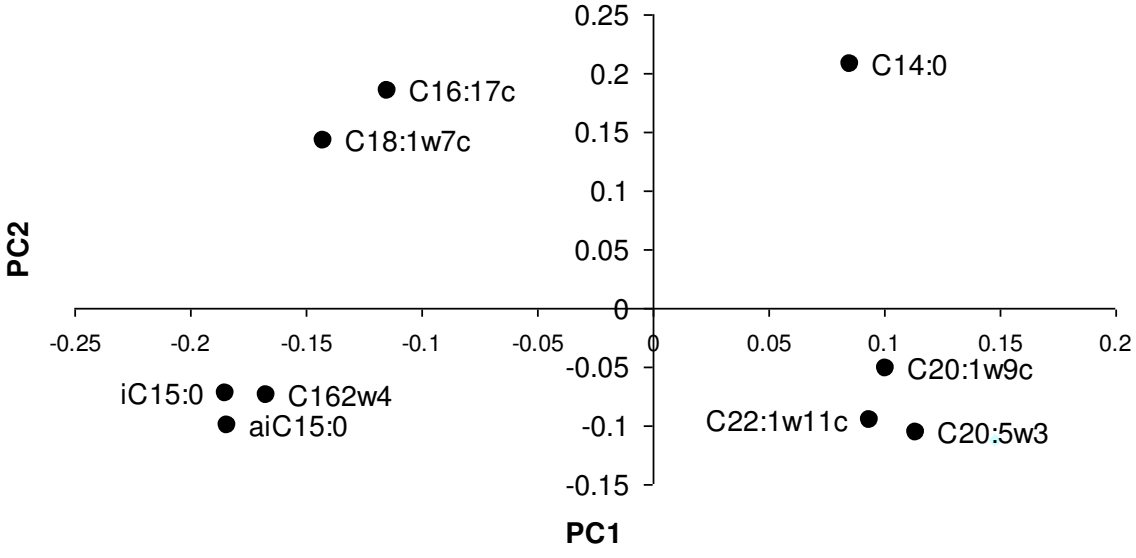

Arenicola
Proportion of Total Uptake
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Aren 9-d
Aren 21-d
Aren 37-d
Aren natural
Aren 9-d Sed
Aren 21-d Sed
Aren 37-d Sed
i-C14:0
C14:0
i-C15:0
ai-C15:0
C15:0
i-C16:0/C15:1 w5c
C16:0
C16:1
C16:1w7t
10Me-C16:0
C16:1w7c
i-C17:0
C16:2w4
C17:1w7c/cy C17:0
C18:0
C18:1w9c
C18:1w7c
C19:1
C18:5w3(12-15)
C20:1w9c
C20:1
C20:2
C20:5w3
22:1w11c
C22:2
C22:2
C22:4w6
C22:5w3
C22:6w3

B

Figure 6.

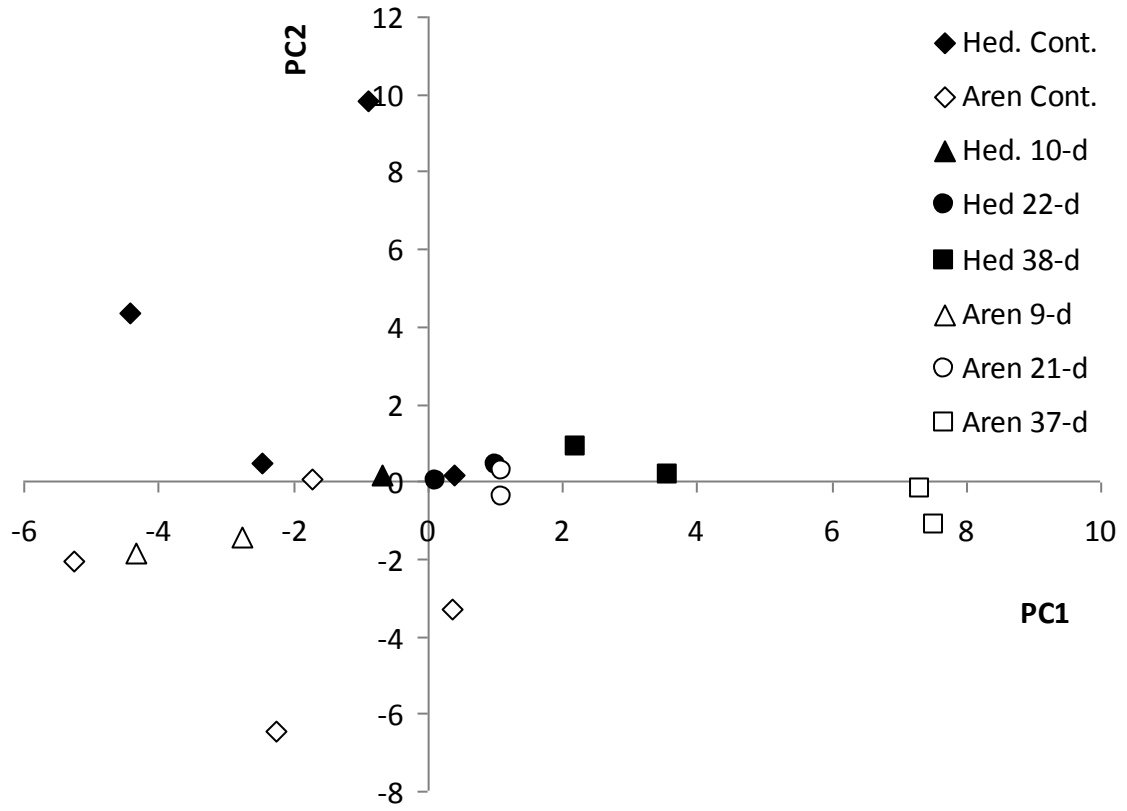

8
6
4
2
0
-2
-4
-6
-8
-10
PC2
-10
-5
0
5
10
PC1
Aren. 8-d
Aren 20-d
Aren 40-d
Hed. 8-d
Hed. 20-d
Hed 40-d
Aren seds
Hed. seds

A

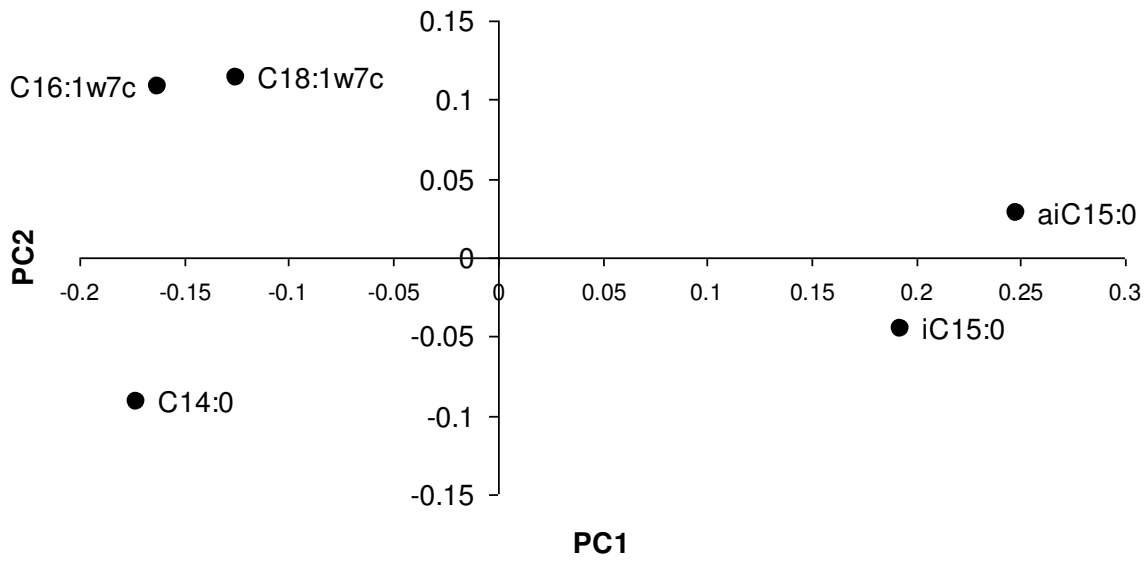

0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.25
-0.2
-0.15
-0.1
-0.05
0
0.05
0.1
0.15
0.2
PC2
PC1
C16:17c
C18:1w7c
C14:0
iC15:0
C162w4
aiC15:0
C20:1w9c
C22:1w11c
C20:5w3

B

Figure 7.


PC2
PC1
Hed. Cont.
Aren Cont.
Hed. 10-d
Hed 22-d
Hed 38-d
Aren 9-d
Aren 21-d
Aren 37-d

A


C16:1w7c
C18:1w7c
aiC15:0
iC15:0
C14:0
PC2
PC1

B

Figure 8.

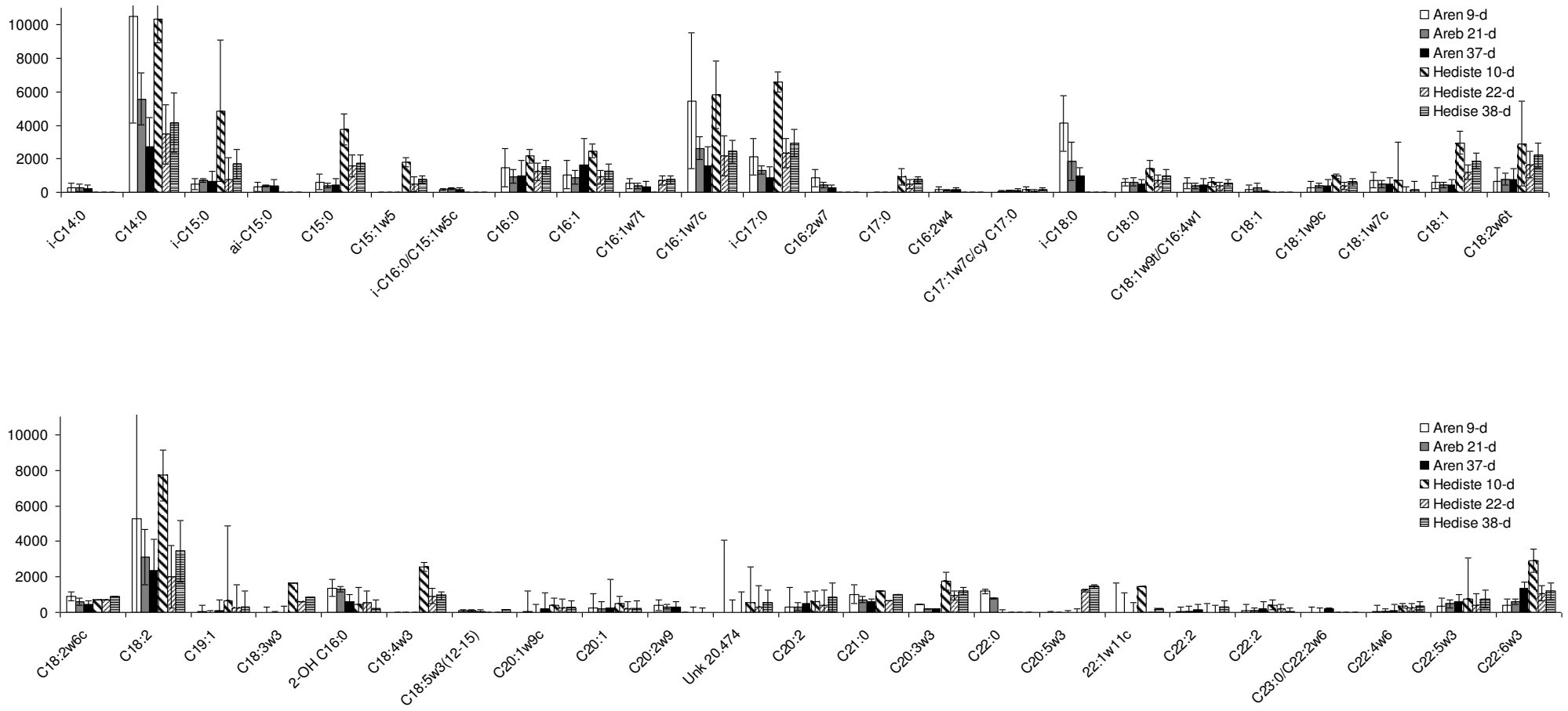

10000
8000
6000
4000
2000
0
Aren 9-d
Areb 21-d
Aren 37-d
Hediste 10-d
Hediste 22-d
Hedise 38-d
i-C14:0
C14:0
i-C15:0
ai-C15:0
C15:0
C15:1w5
i-C16:0/C15:1w5c
C16:0
C16:1
C16:1w7t
C16:1w7c
i-C17:0
C16:2w7
C17:0
C16:2w4
C17:1w7c/cy C17:0
i-C18:0
C18:0
C18:1w9t/C16:4w1
C18:1
C18:1w9c
C18:1w7c
C18:1
C18:2w6t
10000
8000
6000
4000
2000
0
Aren 9-d
Areb 21-d
Aren 37-d
Hediste 10-d
Hediste 22-d
Hedise 38-d
C18:2w6c
C18:2
C19:1
C18:3w3
2-OH C16:0
C18:4w3
C18:5w3(12-15)
C20:1w9c
C20:1
C20:2w9
Unk 20.474
C20:2
C21:0
C20:3w3
C22:0
C20:5w3
22:1w11c
C22:2
C22:2
C23:0/C22:2w6
C22:4w6
C22:5w3
C22:6w3

Figure 9.