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Fate of microbial nitrogen, carbon, hydrolysable amino acids, monosaccharides, and fatty acids in sediment

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Abstract

The fate of microbial carbon, nitrogen, hydrolysable amino acids (HAAs), monosaccharides, and fatty acids in sediment was investigated experimentally. The microbial community of a tidal flat sediment was labeled with ^{13}C-enriched glucose and ^{15}N-enriched ammonium, and sediment was incubated for up to 371 days. Analysis of total concentrations and ^{13}C- and ^{15}N content of bulk sediment, hydrolysable amino acids (including D-alanine), monosaccharides, total fatty acids (TFAs), and phospholipid-derived fatty acids (PLFAs) allowed us to trace the fate of microbial biomass and -detritus and the major biochemical groups therein (proteins, carbohydrates, and lipids) over intermediate time scales (weeks–months). Moreover, the unidentified fraction of the labeled material (i.e. not analyzed as HAA, FA, or carbohydrate) provided information on the formation and fate of molecularly uncharacterizable organic matter. Loss of ^{13}C and ^{15}N from the sediment was slow (half live of 433 days) which may have been due to the permanently anoxic conditions in the experiment. Loss rates for the different biochemical groups were also low with the following order of loss rate constants: PLFA > TFA > HAA > monosaccharides. The unidentified ^{13}C-pool was rapidly formed (within days) and then decreased relatively slowly, resulting in a gradual relative accumulation of this pool over time. Degradation and microbial reworking of the labeled material resulted in subtle, yet consistent, diagenetic changes within the different biochemical groups. In the HAA pool, glycine, lysine, and proline were lost relatively slowly (i.e. best preserved) while there was no accumulation of D-alanine relative to L-alanine, indicating no relative accumulation of bacterial macromolecules rich in D-alanine. In the fatty acid pool, there was very little difference between PLFAs and TFAs, indicating a very similar lability of these pools. Differences between individual fatty acids included a relatively slow loss of i15:0, ai15:0 and 18:1ω7c which likely resulted from production of these bacterial fatty acids during bacterial reworking of the organic matter. Differences between loss rate constants for individual monosaccharides were not significant. An exception was ribose that was produced and lost relatively rapidly, which may be related to ribose being an important component of RNA. Losses of bulk ^{13}C and ^{15}N were closely coupled despite partly being present in different biochemicals and partly being derived from different microbial sources, indicating no selective preservation of either C or N during organic matter diagenesis.

1. INTRODUCTION

Microorganisms are the primary mediators of biogeochemical processes in sediments, including the processing of organic matter (degradation, remineralization, and reas-

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similation). Even though microbial biomass is only a small fraction of total organic matter in sediments, the continuous processing of organic matter by benthic microbes combined with turnover of microbial biomass results in a continuous flux of microbial detritus into the sediment organic matter pool. In this way, microbial reworking of organic matter can result in a substantial microbial contribution to the total organic matter pool and may also alter its composition and thereby change its long-term fate (Keil and Fogel, 2001; Burdige, 2007; Kaiser and Benner, 2008). Despite this strong potential impact on the quality and quantity of the sediment organic matter pool, microbial reworking and secondary production has been underappreciated in geochemical studies (Zonneveld et al., 2010). The short term fate of benthic bacterial biomass, in particular grazing by benthic fauna, has been investigated in a number of studies (e.g. Van Oevelen et al., 2006; Pascal et al., 2009) while the fate of bacterial biomass and detritus in natural sediments over longer time scales has remained largely unstudied.

The major biochemical components of microbial biomass and sediment detritus are proteins, carbohydrates, and lipids. Proteins are structural components consisting of amino acids. Hydrolysable amino acids (HAAs) are a major fraction of total proteinaceous material and account for 50–80% of total nitrogen in microbial biomass (Cowie and Hedges, 1992) and 10–20% of total organic matter in marine sediments (Burdige, 2007). The relative composition of the HAA pool can provide a good indication of the degradation state of organic matter (e.g. Dauwe and Middelburg, 1998; Keil et al., 2000 and references therein). A particularly interesting group of HAAs are the D-enantiomers, which are considered to be specific for bacterial macromolecules (Madigan et al., 2000; Kaiser and Benner, 2008).

Carbohydrates consist of monosaccharides and can serve both structural (e.g. cellulose) and energy storage functions (e.g. amylose). Total carbohydrates make up 20–40% of fresh marine organic matter and account for 5–20% of the sediment organic matter pool and sediment carbon remineralization (Burdige, 2006). The relative composition of the total carbohydrate pool can provide information about sources of organic matter in sediments (e.g. Cowie and Hedges, 1984) and can also show diagenetic changes (Jensen et al., 2005; Burdige, 2006). Carbohydrate diagenesis has not been investigated as extensively as that of amino acids. The recent introduction of a method for analysis of the ^{13}C content of individual monosaccharides by HPLC-IRMS provides a powerful new tool for investigation of carbohydrates in stable isotope (^{13}C) tracer studies (Boschker et al., 2008).

Lipids are complex structures with both structural functions and a role as energy source. Lipids make up 5–30% of fresh marine organic matter and <1% to 8% of the sediment organic matter pool (Burdige, 2007). Fatty acids are a major component of lipids and can be divided into various sub-fractions including phospholipid-derived fatty acids (PLFAs). PLFAs are derived from cell membranes and are amongst the most labile compounds in microbial biomass.

Proteins, carbohydrates, and lipids together with some minor biochemicals like amino sugars and nucleic acids, make up the identifiable fraction of microbial biomass and sediment organic matter. This identifiable fraction typically makes up 30–40% of total organic matter in surficial marine sediments, meaning that 60–70% is not identifiable with conventional analytical techniques (Hedges et al., 2000; Burdige, 2007). This so-called molecularly uncharacterized organic matter consists of compounds that are resistant to common extraction and separation techniques (Burdige, 2007). The formation, composition, and fate of molecularly uncharacterized organic matter is an intensively studied, but poorly understood, topic in sediment biogeochemistry.

The fate of microbial biomass and detritus and the associated changes in biochemical composition have been studied in two different ways that reflect distinctly different time scales. One approach involves analysis of the biochemical composition of fresh organic matter, usually algal-derived, as it gets degraded in laboratory experiments or in the field using a (stable) isotope tracer approach (Harvey et al., 1995; Sun et al., 2002; Oakes et al., 2010). These studies generally involve time scales of hours to weeks and results therefore reflect the earliest stages of organic matter degradation. The other approach involves investigation of the biochemical composition of organic matter along a given diagenetic gradient in the field (for example a sediment depth profile). This provides information about the alteration of organic matter, including microbial reworking, on the long term (months to geological time scales) (e.g. Wakeham et al., 1997a; Dauwe and Middelburg, 1998; Keil and Fogel, 2001). For both these approaches, studies usually focus on a specific biochemical group. Only a few studies have simultaneously investigated the fate of different biochemical groups from a given source in sediments (e.g. Henrichs and Doyle, 1986; Harvey et al., 1995), sinking particles (Wakeham et al., 2009) and soil (Kindler et al., 2009; Miltner et al., 2009). Moreover, most studies focus on the fate of algal biomass (primary producers) while the fate of organic matter derived from secondary producers, and thereby their potential effect on organic matter degradation in general, has been investigated only scarcely.

In the current study we investigate the fate of ^{13}C- and ^{15}N-labeled microbial biomass and detritus in sediment over a 1-year period. Alongside ^{13}C and ^{15}N, we also traced the specific fate of ^{13}C and ^{15}N in HAAs and ^{13}C in monosaccharides, total fatty acids, and PLFAs, as well as concentrations of these different compounds. Sediment was homogenized, sieved, and incubated *in vitro* in order to exclude macrobiological loss processes (e.g. bioturbation, grazing) and physical loss processes (e.g. resuspension by currents and waves) typically encountered in the field. This unique approach allowed us to investigate the longer-term fate of C and N as well as proteins, carbohydrates, and lipids from a defined pool of microbial biomass in natural sediment. This study aims to bridge the gap between short-term experimental studies on degradation of defined pools of organic matter and observational studies assessing the net results of diagenesis on natural organic matter over long time scales.

2. MATERIALS AND METHODS

2.1. Sediment collection and incubation

Sediment was collected from a tidal flat (Paulinapolder) in the mid region of the turbid, nutrient-rich, and heterotrophic Scheldt Estuary (The Netherlands). The sampling site is characteristic of tidal flats in this part of the estuary. Sediment at the sampling site was characterized as muddy with a silt content of 42% and a median grain size of 72 μm. On 14 June, 2006, 20 small sediment cores (2.5 cm i.d., 10 cm deep) were collected from the tidal flat at low tide. Sediment from the cores was transported to the laboratory where it was suspended in water from the sampling site and sieved (1 mm mesh) to remove macrofauna. Sieved sediment was allowed to settle overnight in the dark. The following morning, excess water was removed, the remaining sediment slurry was homogenized and 40-ml subsamples were transferred to 100-ml glass bottles. Incubations were started by addition of 0.38 ml of label solution containing 21 mM ^{13}C-glucose (Cambridge Isotope laboratories, U–^{13}C6, 99% ^{13}C) and 27 mM ^{15}N-ammonium (added as $(^{15}NH_4)_2SO_4$, Isotech, >98% ^{15}N) dissolved in Milli-Q water freshly mixed with 0.2 μm-filtered seawater (yielding a salinity similar to that of the water at the sampling site). Corresponding concentrations of ^{13}C and ^{15}N in the slurries were 0.81 μmol ^{13}C ml^{-1} and 0.17 μmol ^{15}N ml^{-1}. After label addition, 20 ml of water from the sampling site was added to each bottle, after which bottles were shaken and closed with open-top screw caps containing a silicone liner (allowing gas exchange through the caps without loss of water). The sediment quickly settled to form a 25–30 mm thick layer with a similar volume of water on top. Labeled sediments were incubated in the dark at 17 °C and sampled after 1, 4, 7, 18, 30, 69, 133, 250, and 371 days of incubation. Two bottles were sacrificed per sampling event. An additional set of unlabeled sediments was processed directly to determine the natural background abundances of ^{15}N and ^{13}C. Sampling involved shaking the bottles and freezing the resulting sediment slurry. Frozen sediment was freeze-dried and analyzed for abundance and stable isotope composition of bulk organic carbon (OC) and nitrogen, hydrolysable amino acids (HAAs), monosaccharides, and fatty acids (see below). For the same sediment we also analyzed concentrations of photosynthetic pigments and survival of diatoms. These results are presented in a separate paper (Veuger and van Oevelen, 2011)

2.2. Analyses

For analysis of concentrations OC and N and relative abundance of ^{13}C and ^{15}N in bulk sediment, freeze-dried sediment was ground, acidified to remove inorganic carbon, and analyzed with an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Thermo Delta V). For analysis of concentrations and relative abundance of ^{13}C and ^{15}N in HAAs, including the bacterial biomarker D-alanine (D-Ala), sediment was processed as described in Veuger et al. (2005). Briefly, samples (~1 g) of freeze-dried sediment were washed with 2 M HCl and Milli-Q water (removing dissolved and dissolvable amino acids) and subsequently hydrolyzed in 6 M HCl at 110 °C for 20 h. After purification by cation exchange chromatography, amino acids were derivatized with isopropanol and pentafluoropropionic anhydride and samples were further purified by solvent extraction. Concentrations and ^{15}N- and ^{13}C-enrichment of the derivatized D- and L-amino acids were analyzed by gas-chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) on a HP 6890 GC with a Thermo type III combustion interface and Thermo Delta Plus IRMS. Amino acids included in the analyses are D-alanine (D-Ala), L-alanine (L-Ala), threonine (Thr), valine (Val), glycine (Gly), isoleucine (Ile), leucine (Leu), proline (Pro), aspartic acid (Asp), L-glutamic acid (L-Glu), phenylalanine (Phe), tyrosine (Tyr) and lysine (Lys). Thr and Val are presented as one combined peak because their peaks could not always be clearly separated. Since glutamine (Gln) is converted to Glu and aspargine (Asn) is converted to Asp during sample hydrolysis, peaks for L-Glu and Asp included Gln and Asn, respectively. For Ala and Glu, D- and L-enantiomers could be separated. However, only D-Ala peaks were high and pure enough for proper quantification and stable isotope analysis. The sum of all amino acids analyzed will be referred to as total hydrolysable amino acids (THAAs). The effect of washing the sediment before hydrolysis on the HAA pool size and composition was tested by processing a selection of samples from the present experiment with- and without washing. Results revealed only ~10% reduction of THAA concentrations due to washing with very little compositional effect, meaning that qualitative and quantitative effect of washing on the total HAA pool was negligible.

Monosaccharides were extracted from freeze-dried sediment by hydrolysis in 11 M H_2SO_4 for 1 h at room temperature. Subsequently, samples were diluted 10 times with Milli-Q water and heated in an oven at 120 °C for 1 h. Samples were neutralized to pH 5.5–6.0 by adding $BaCO_3$ and the $BaSO_4$ precipitate was removed by centrifugation. Concentrations and carbon isotope composition of the monosaccharides were analyzed by high performance liquid chromatography (HPLC) coupled to IRMS as described in Boschker et al. (2008). Rhamnose and arabinose could not be separated sufficiently and are therefore presented together.

Fatty acids were extracted from freeze-dried sediment in chloroform–methanol–water using a modified Bligh and Dyer method. For total fatty acids (TFA) the whole chloroform fraction was used while for isolation of phospholipid-derived fatty acids (PLFAs), the total extract was fractionated on silica gel into different classes by polarity, with the most polar fraction containing the PLFAs. Both the TFA and PLFA fractions were derivatized by mild methanolysis yielding fatty acid methyl esters (FAMEs) that were analyzed for concentrations and ^{13}C-enrichment GC-c-IRMS. Further details on extractions and analyses can be found in Boschker (2004). Only compounds that were detected in all samples will be presented. Compounds 20:5ω3 and 20:4ω6 were present in the samples but were found in strongly reduced yields in freeze-dried samples compared to fresh material (data not shown). Therefore, these two compounds are not included in this paper.

2.3. Data treatment

Stable isotope data will be presented as concentrations of ^{15}N or ^{13}C per gram dry sediment, which was calculated as: concentration $X = [(\text{at\%}X_{sample} - \text{at\%}X_{control})/100] \times$ [concentration N or C in sample] with X representing ^{15}N or ^{13}C. At%X was calculated from δX as: at% $X = [100 \times R_{standard} \times (\delta X_{sample}/1000) + 1]/[1 + R_{standard} \times (\delta X_{sample}/1000) + 1]$ and δX was calculated as: δX (‰) $= [(R_{sample}/R_{standard)} - 1] \times 1000$. For ^{15}N, $R = {}^{15}N/{}^{14}N$ and $R_{standard} = 0.003677$. For ^{13}C, $R = {}^{13}C/{}^{12}C$ and $R_{standard} = 0.011180$. Unlabeled sediment was used as control sample (to correct for ambient ^{15}N and ^{13}C). The reproducibility error of concentration and isotope analyses was much lower than variation between replicate incubations.

$\delta^{13}C$ values for the HAAs were corrected for addition of extra C during derivatization as described in Veuger et al. (2006). Total concentrations of the bacterial biomarker D-Ala and concentrations of ^{13}C and ^{15}N in D-Ala will be presented in direct comparison with that in L-Ala as the D/L-Ala ratio which is calculated as D-Ala/L-Ala. D-Ala data are not corrected for hydrolysis-induced racemization (the formation of D-Ala from L-Ala during sample hydrolysis). However, a racemization background of ~0.017 (see Kaiser and Benner, 2005; Veuger et al., 2007b) will be taken into account in the relevant discussion and calculation of the bacterial contribution of total label incorporation (see Section 4).

Loss-rate constants (k) for biochemical groups and individual compounds were determined by fitting individual data points with an exponential decay model by minimizing the sum of squared model-data deviations in R (R development core team, 2010) using the function *nls*. This function returns the optimal parameter values and corresponding standard errors. Half lives were calculated as $(\ln 2)/k$.

3. RESULTS

Results comprise two distinct periods (Fig. 1). The period between day 1 and day 69 will hereafter be referred to as the ‘labeling phase’ while the period between day 69 and day 371 will be referred to as the ‘loss phase’. These two phases will be presented and discussed separately with the main focus on the loss phase as this was the most relevant phase with respect to the objectives of this study. The causes for the occurrence of these two phases and rationale for presenting the two phases separately will be addressed in Section 4.

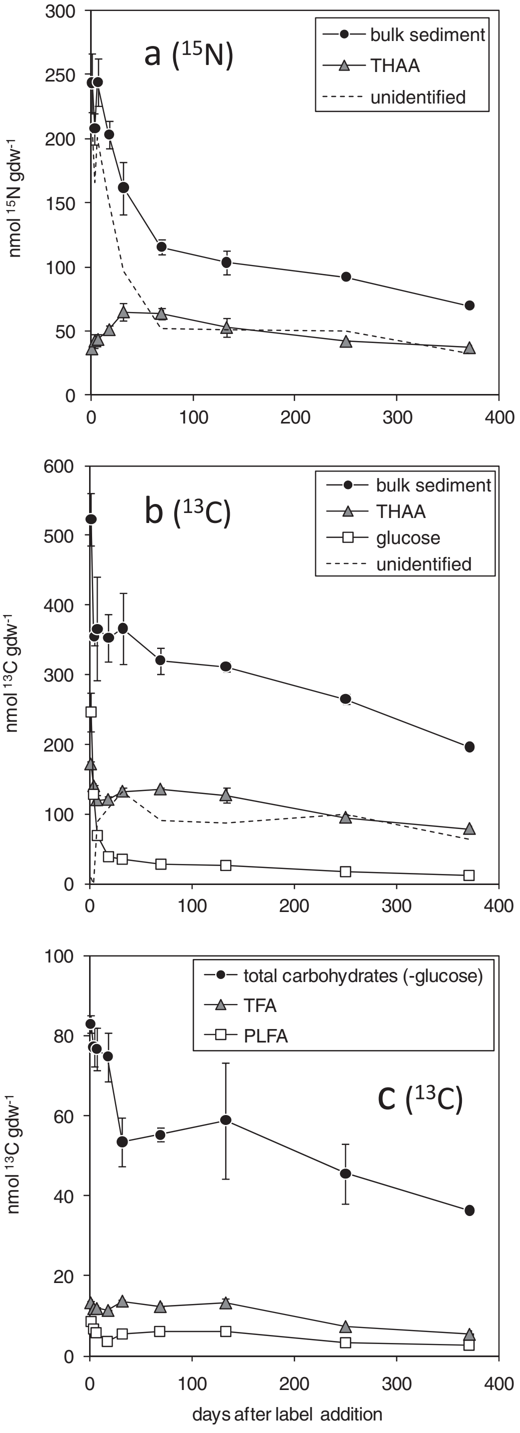

Fig. 1. Concentrations ^{15}N (a) and ^{13}C (b + c) in bulk sediment, various biochemical groups, and the unidentified fraction versus time. Error bars indicate data range (min–max) for replicate bottles and are in some cases too small to be visible.

3.1. Bulk sediment

Concentrations of total organic carbon and nitrogen in the sediment remained at a steady level during the experiment with values around 400 μmol-C gdw^{-1} (range 350–497) and 40 μmol-N gdw^{-1} (range 34–48) (Table 1). Addition of the labeled substrates resulted in clear ^{13}C- and ^{15}N-enrichment of bulk sediment with maximum $\delta^{13}C$ and $\delta^{15}N$ values of 127‰ and 2058‰, respectively. Concentrations of ^{13}C and ^{15}N in the bulk sediment showed highest values 1 day after labeling and represent 41% and 90% of the total amounts of added ^{13}C (as ^{13}C-glucose) and ^{15}N (as ^{15}N-ammonium), respectively. Concentrations of ^{13}C in the bulk sediment decreased gradually during the following year while ^{15}N concentrations showed a rela-

Table 1

Mean concentrations of total C, total N, ^{13}C, and ^{15}N in bulk sediment, various biochemical groups and unidentified pools, including ^{13}C/^{15}N ratios and loss rate constants (*k*) for loss phase. When no *k*-value is given, there was no significant loss.

Days	0	1	4	7	18	32	69	133	250	371	*k* (day^{-1})[a]	0	1	4	7	18	32	69	133	250	371
	Concentrations total C (μmol-C gdw^{-1})											Contribution to bulk OC (%)									
Bulk OC	423	377	350	401	393	452	410	391	399	354											
THAA	50	47	45	42	44	50	53	49	38	40	0.0012 ± 0.0004	12	13	13	11	11	11	13	12	10	11
TCH	65	65	71	65	67	56	57	56	54	48	0.0006 ± 0.0004	15	17	20	16	17	12	14	14	14	13
TFA	0.66	0.67	0.66	0.70	0.65	0.75	0.75	0.79	0.67	0.60	0.0007 ± 0.0002	0.16	0.18	0.19	0.17	0.16	0.17	0.18	0.20	0.17	0.17
PLFA	0.27	0.28	0.27	0.26	0.20	0.27	0.35	0.33	0.29	0.24	0.0012 ± 0.0001	0.07	0.07	0.08	0.06	0.05	0.06	0.08	0.08	0.07	0.07
Unidentified[b]	307	264	234	292	281	346	299	285	306	265		73	70	67	73	72	76	73	73	77	75
	Concentrations total N (μmol-N gdw^{-1})											Contribution to bulk N (%)									
Bulk N	41	37	35	41	41	44	38	41	41	40											
THAA	11	11	10	9	10	11	12	11	9	9	0.0012 ± 0.0004	27	28	29	22	23	25	30	27	21	24
Unidentified[c]	30	27	25	32	32	33	27	30	32	30		73	72	71	78	77	75	70	73	79	76
	Concentrations ^{13}C (nmol ^{13}C gdw^{-1})											Contribution bulk ^{13}C (%)									
Bulk		524	355	366	352	366	321	312	250	196	0.0016 ± 0.0003										
THAA		172	139	120	120	134	137	127	95	78	0.0019 ± 0.0003		33	39	33	34	37	43	41	38	40
TCH (-glucose)		83	77	77	75	53	55	59	46	36	0.0015 ± 0.0007		16	22	21	21	15	17	19	18	19
TFA		13	12	12	11	14	13	13	7	5	0.0027 ± 0.0006		2.5	3.4	3.3	3.2	3.8	3.9	4.3	3.0	2.7
PLFA		8.8	6.8	5.8	4.0	5.6	6.2	6.1	3.4	2.6	0.0030 ± 0.0005		1.7	1.9	1.6	1.1	1.5	1.9	2.0	1.4	1.3
Glucose		247	128	70	38	35	27	26	18	12	0.0027 ± 0.0005		47	36	19	11	10	9	8	7	6
Unidentified[b]		9	0	88	108	129	89	87	85	64	0.0009 ± 0.0004		2	0	24	31	35	28	28	34	33
	Concentrations ^{15}N (nmol ^{15}N gdw^{-1})											Contribution to bulk ^{15}N (%)									
Bulk		244	208	244	203	162	115	103	88	70	0.0016 ± 0.0003										
THAA		36	42	43	51	65	64	53	42	37	0.0020 ± 0.0004		15	20	18	25	40	55	51	48	53
Unidentified[c]		208	165	200	152	96	51	50	46	33	0.0013 ± 0.0004		85	80	82	75	60	45	49	52	47
	Excess ^{13}C/^{15}N ratios																				
Bulk		2.1	1.7	1.5	1.7	2.3	2.8	3.0	2.8	2.8											
THAA		4.8	3.3	2.8	2.4	2.1	2.1	2.4	2.3	2.1											

[a] Loss rate constant for day 69–371.

[b] Unidentified = bulk C − (THAA + TCH + TFA).

[c] Unidentified = bulk N − THAA.

tively rapid decrease during the first two months (Fig. 1). During the loss phase, both ^{13}C and ^{15}N in the bulk sediment decreased exponentially with a loss rate constant (*k*) of 0.0016 d^{-1}, which corresponds to a half life of 433 days. The ^{13}C:^{15}N ratio for the bulk sediment was 1.5–2.1 directly after labeling, increased to 2.8 at day 69 and then remained at that level for the rest of the experiment (Table 1).

3.2. Hydrolysable amino acids

THAA concentrations were stable during the labeling phase (42–53 μmol-C gdw^{-1}) and decreased during the loss phase with a corresponding loss rate constant of 0.0012 d^{-1} (Table 1). HAAs were clearly enriched in ^{13}C and ^{15}N with highest $\delta^{13}C$ values of 169–542‰ and $\delta^{15}N$ values of 843–2626‰ for individual HAAs. The ^{13}C concentration in THAAs remained at a steady level during the first two months after label addition while ^{15}N concentrations gradually increased during the first month (Fig. 1). During the loss phase, both ^{13}C and ^{15}N in THAAs decreased exponentially with similar loss rate constants of 0.0019 d^{-1} and 0.0020 d^{-1}, respectively (corresponding to half lives of 364 and 347 days, respectively). The ^{13}C:^{15}N ratio for the THAAs was relatively high (4.8) during the first days of the

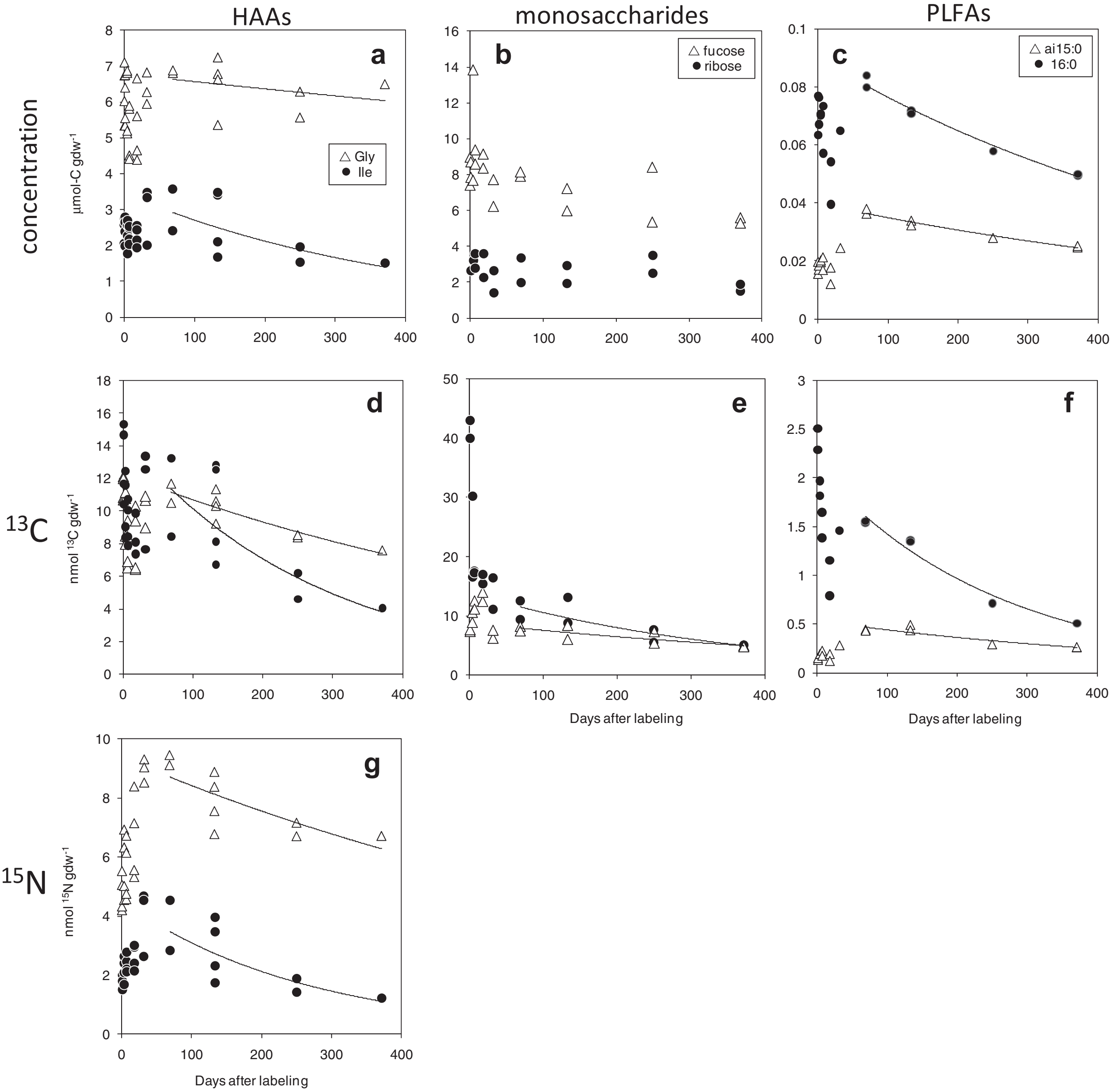

Fig. 2. Concentrations of total C (a, b, c), ^{13}C (d, e, f), and ^{15}N (g) in a selection of individual HAAs (a, d, g), monosaccharides (b, e) and PLFAs (c, f). Selected compounds are representative for those with highest and lowest loss rate constants within each compound group. Lines indicate exponential fits for loss phase (day 69–371) used to determined loss rate constants (*k*). Fits for concentrations of monosaccharides (b) are not shown as these were not significant.

experiment and rapidly decreased during the following month (due to the increase in ^{15}N concentration) and then stabilized at ~2.2 (Table 1). The relative contribution (%) of ^{13}C in THAAs to that in the bulk sediment increased slightly during the first weeks and then leveled off at ~40% for the rest of the experiment while the relative contribution of ^{15}N in THAAs to that in the bulk sediment showed a much stronger increase during the first two months (from 15% to 55%) after which values leveled off at ~50% (Table 1).

Time trends for individual HAAs are illustrated by those for Gly and Ile with Gly being one of the HAAs that were lost relatively slowly while Ile was lost most rapidly (Fig. 2). See Table 2 for other HAAs. Corresponding loss rate constants (k) for individual HAAs ranged between 0 and 0.0041 d^{-1} with a common pattern for concentrations, ^{13}C, and ^{15}N (Fig. 3). Loss rate constants were lowest for Lys and Pro, for which there was no net loss in concentrations at all. Differences in loss rate constants between ^{13}C- and ^{15}N-labeled pools were generally very small, except for Asp, and L-Glu for which loss rates for ^{15}N were somewhat higher than those for ^{13}C (Fig. 3). Loss rate constants for concentrations of individual HAAs were somewhat lower than those for the ^{13}C- and ^{15}N-labeled pools.

Table 2
Total concentrations and concentrations of ^{13}C and ^{15}N in individual HAAs, including corresponding D/L-Ala ratios. D-Ala concentrations are not corrected for racemization.

Days	0	1	4	7	18	32	69	133	250	371
	Total concentrations (μmol-AA gdw^{-1})									
D-Ala	0.08	0.08	0.07	0.07	0.07	0.08	0.09	0.08	0.06	0.06
L-Ala	1.45	1.34	1.23	1.12	1.11	1.33	1.45	1.29	0.96	1.02
Thr + Val	1.15	1.05	0.98	0.94	0.99	1.15	1.36	1.02	0.77	0.52
Gly	3.15	3.00	3.00	2.57	2.66	3.17	3.28	3.25	2.58	3.25
Ile	0.42	0.41	0.39	0.38	0.38	0.49	0.56	0.44	0.31	0.25
Leu	0.67	0.64	0.63	0.61	0.62	0.61	0.64	0.63	0.50	0.52
Pro	0.94	0.88	0.92	0.80	0.85	0.89	0.86	0.95	0.79	1.11
Asp	1.27	1.16	1.16	0.98	1.08	1.23	1.33	1.12	0.97	0.84
L-Glu	0.97	0.90	0.82	0.74	0.81	1.07	1.05	0.94	0.71	0.64
Phe	0.33	0.33	0.29	0.33	0.33	0.37	0.36	0.38	0.28	0.34
Tyr	0.12	0.13	0.09	0.12	0.12	0.13	0.14	0.13	0.10	0.11
Lys	0.62	0.63	0.58	0.60	0.61	0.57	0.55	0.64	0.55	0.84
D/L-Ala	0.058	0.056	0.057	0.059	0.060	0.060	0.063	0.060	0.063	0.059
	Concentration ^{13}C (nmol ^{13}C gdw^{-1})									
D-Ala		0.66	0.59	0.57	0.58	0.72	0.75	0.69	0.44	0.46
L-Ala		19.0	14.5	12.5	12.3	16.1	15.4	14.6	9.0	8.2
Thr + Val		25.6	20.5	18.0	18.2	20.0	21.5	18.7	11.9	9.6
Gly		11.4	9.6	7.8	8.2	10.2	11.1	10.4	8.5	7.6
Ile		13.0	10.4	9.3	8.8	11.2	10.8	10.0	5.4	4.0
Leu		23.3	19.0	16.8	15.9	16.6	16.6	15.2	11.3	8.4
Pro		9.9	9.6	8.1	8.7	10.0	10.9	10.4	9.7	9.1
Asp		14.1	12.3	10.0	11.1	11.7	12.3	11.2	8.0	6.5
L-Glu		22.0	16.9	13.4	14.6	15.2	15.1	13.7	9.9	7.2
Phe		11.7	9.4	8.3	7.9	8.6	8.5	8.5	6.8	5.4
Tyr		3.6	3.0	2.7	2.6	3.0	2.6	2.4	2.2	1.5
Lys		17.3	13.5	12.2	11.6	11.0	11.2	11.2	11.5	10.5
D/L-Ala		0.035	0.041	0.046	0.047	0.045	0.049	0.047	0.048	0.056
	Concentration ^{15}N (nmol ^{15}N gdw^{-1})									
D-Ala		0.17	0.21	0.22	0.25	0.35	0.36	0.30	0.17	0.17
L-Ala		5.1	5.9	6.0	6.7	9.5	8.8	7.3	4.9	4.4
Thr + Val		4.9	5.6	5.8	6.9	8.2	8.8	6.8	5.1	4.6
Gly		4.8	5.7	5.5	6.6	8.9	9.3	7.9	6.9	6.7
Ile		1.8	2.2	2.4	2.6	3.9	3.7	2.9	1.7	1.2
Leu		3.2	4.1	4.3	4.9	5.8	5.6	4.6	3.7	2.9
Pro		1.9	2.5	2.5	3.0	3.7	4.0	3.5	3.3	3.2
Asp		3.3	4.3	4.1	5.1	6.7	6.4	5.0	3.5	2.9
L-Glu		4.2	4.6	4.6	5.7	8.1	7.2	5.7	3.6	2.8
Phe		1.3	1.4	1.8	2.1	2.5	2.4	2.1	1.8	1.4
Tyr		0.4	0.5	0.6	0.7	0.8	0.7	0.6	0.5	0.4
Lys		5.0	5.3	5.7	6.5	7.0	6.9	6.4	6.9	6.3
D/L-Ala		0.033	0.035	0.036	0.036	0.038	0.040	0.041	0.035	0.039

D/L-Ala ratios for ^{13}C- and ^{15}N were well above the racemization background of 0.017 (reflecting formation of D-Ala from L-Ala during sample hydrolysis, see Veuger et al., 2007b) and showed little to no change over time, in particular during the loss phase (Table 2). Only for ^{13}C, there was a moderate increase from 0.048 to 0.056 between days 250 and 371.

3.3. Monosaccharides

Concentrations of total monosaccharides showed very little change over time with only a very small decrease during the loss phase ($k = 0.0006\ \mathrm{d}^{-1}$) (Table 1). Differences between loss rate constants for individual monosaccharides could not be assessed because fits were not significant and differences between individual monosaccharides were very small (Table 3). The ^{13}C-labeled total monosaccharide pool was initially dominated by glucose because of the addition of ^{13}C-glucose as ^{13}C-substrate. ^{13}C concentrations in glucose rapidly decreased during the first week ($k = 0.27\ \mathrm{d}^{-1}$) (Fig. 1 and Table 3) and decreased much slower during the rest of the experiment ($k = 0.0027\ \mathrm{d}^{-1}$). ^{13}C concentrations in total monosaccharides (not including glucose) decreased very slowly during the whole experiment (Fig. 1) with a loss rate constant of $0.0015\ \mathrm{d}^{-1}$ for the loss phase (Table 1). Loss of ^{13}C from individual monosaccharides was very similar for galactose, mannose, xylose, rhamnose + arabinose, and fucose (Fig. 2) with corresponding loss rate constants (k) around 0.0010–0.0015 d^{-1}. However, fits were not significant and loss rate constants are therefore not shown. An exception to the general trend was ^{13}C-ribose which was labeled relatively rapidly and showed a relative high (and significant) loss rate constant of $0.0027\ \mathrm{d}^{-1}$ (Fig. 2 and Table 3).

3.4. Fatty acids

Total concentrations of TFAs and PLFAs showed similar trends, with PLFAs comprising on average 40 ± 4% of TFAs (Table 1). Concentrations of TFAs and PLFAs increased ~1.2× and ~1.3× during the first months of the experiment with highest concentrations on day 133 (Table 4) and subsequently decreased with loss rate constants of $0.0007\ \mathrm{d}^{-1}$ and $0.0012\ \mathrm{d}^{-1}$ for TFAs and PLFAs, respectively, for the loss phase (Table 1). The relative compositions of the PLFA and TFA pools were very similar with 16:0 and 16:1ω7c being the most abundant fatty acids (Table 4). Trends in concentrations of individual TFAs over time are represented by those for PLFAs 16:0 and ai15:0 in Fig. 2c. Over the labeling phase, concentrations of most individual PLFAs and TFAs remained at a rather steady level while some (e.g. ai15:0) even showed a substantial increase (Fig. 2 and Table 4). Loss rate constants (k) for concentrations during the loss phase ranged from 0.0005 to $0.0019\ \mathrm{d}^{-1}$ for TFAs and 0.0010 to $0.0023\ \mathrm{d}^{-1}$ for PLFAs (Fig. 3). Exceptions are 18:1ω7c and 18:0 which showed no net decrease in concentrations over time (Fig. 3 and Table 4).

^{13}C concentrations in summed PLFAs showed relatively complex dynamics over time. Values were highest directly after labeling (8.8 nmol ^{13}C gdw^{-1}), then rapidly decreased to their lowest values at day 18 (4.0 nmol ^{13}C gdw^{-1}),

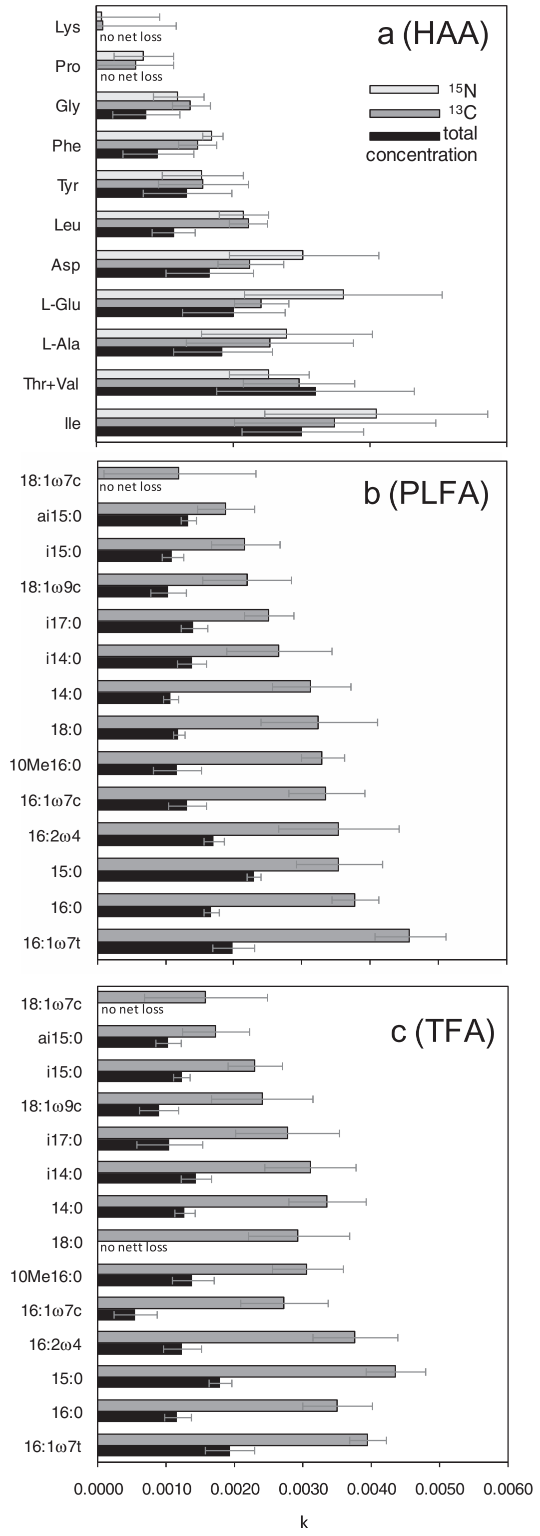

Fig. 3. Loss rate constants (k) for total concentrations and concentrations of ^{13}C and ^{15}N in individual HAAs (a), PLFAs (b) and TFAs (c) for loss phase (day 69–371). Error bars show standard error.

Table 3
Concentrations of total C and ^{13}C in individual monosaccharides.

Days	1	4	7	18	32	69	133	250	371
Total concentration (μmol-C gdw^{-1})									
Glucose	7.7	7.9	7.5	7.0	5.3	5.1	5.9	5.6	4.7
Fucose	8.3	10.8	9.0	8.7	7.0	8.0	6.6	6.9	5.4
Rhamnose + arabinose	15.8	17.0	16.2	17.1	14.4	15.1	15.3	14.0	12.4
Galactose	11.2	11.9	10.8	11.2	10.5	9.7	9.4	9.7	8.9
Xylose	15.5	16.0	14.9	15.7	13.5	13.3	13.0	12.8	11.6
Mannose	4.0	4.3	4.2	4.1	3.3	3.0	3.5	3.4	2.7
Ribose	2.9	3.0	2.9	3.1	1.7	3.1	2.7	2.0	2.0
Concentration ^{13}C (nmol ^{13}C gdw^{-1})									
Glucose	247	128	70	38	35	27	26	18	12
Fucose	7.5	9.7	11.8	13.2	6.8	7.8	7.2	6.4	4.9
Rhamnose + arabinose	12.0	16.6	18.0	20.2	14.7	15.3	17.8	13.7	10.8
Galactose	6.1	6.6	8.6	8.8	7.3	7.8	8.2	7.0	6.0
Xylose	8.1	11.7	13.5	11.7	6.7	8.5	9.2	7.4	6.1
Mannose	7.7	9.2	7.5	4.6	4.3	4.9	5.5	4.5	3.5
Ribose	41.5	23.4	17.4	16.2	13.7	11.0	10.9	6.6	5.1

subsequently increased to ~6.1 nmol ^{13}C gdw^{-1} on days 69 and 133 and then decreased exponentially with a corresponding loss rate constant of 0.0030 d^{-1} for the loss phase (Table 1). ^{13}C concentrations in summed TFAs showed a less complex trend, with steady values up to day 133 followed by an exponential decrease, with loss rate constant of 0.0027 d^{-1} for the loss phase (Table 1). The ^{13}C-labeled TFA and PLFA pools were very similar in composition with 16:0 and 16:1ω7c being the most abundant FAs (Table 4). Time trends for ^{13}C in individual TFAs and PLFAs (represented by those for PLFAs 16:0 and ai15:0 in Fig. 2) were generally similar to those for ^{13}C in summed TFAs in Fig. 1, with no net loss during the labeling phase and a relatively rapid loss during the loss phase. ^{13}C concentrations in a number of individual PLFAs and TFAs (e.g. ai15:0) even increased during the first months (Fig. 2 and Table 4). Loss rate constants (k) for the loss phase ranged from 0.0012 to 0.0046 d^{-1} (Fig. 3) with very similar values for each compound in the PLFA and TFA pools.

3.5. Relative composition of total organic matter and unidentified fractions

Since concentrations of all the biochemical groups changed only little over time, the relative compositions of the total OC and N pools also showed little change (Table 1). The total OC pool comprised 10–13% THAAs, 12–20% carbohydrates, and 0.16–0.20% TFA, meaning that 67–76% of the bulk OC remained unidentified (Table 1). The decrease of this pool was too small to allow curve fitting to obtain a significant loss rate constant. The total N-pool comprised 21–29% THAA-N which leaves an unidentified fraction (bulk-THAA) of 71–79% that did not decrease during the loss phase (Table 1). For the ^{13}C- and ^{15}N-labeled pools, loss rates for the different compound groups were also very similar, which resulted in only small changes in the relative composition of the total ^{13}C- and ^{15}N-pools (Table 1). The bulk ^{13}C pool comprised 33–43% THAAs, 15–22% carbohydrates and 2.5–4.3% TFA, which leaves an unidentified fraction of 0–35% (Table 1). The bulk ^{15}N pool comprised 15–55% THAAs which leaves 45–85% unidentified (Table 1). Loss rate constants (k) for the unidentified ^{13}C and ^{15}N pools over the loss phase were 0.0009 d^{-1} and 0.0013 d^{-1}, respectively (Table 1).

4. DISCUSSION

4.1. Methodological aspects and general setting

The chosen setup of glass bottles closed with a silicone liner proved to be easy and effective. The silicone liner prevented loss of water from the bottles while allowing gas exchange between the headspace in the bottles and the surrounding air. This means that the overlying water could remain oxygenated, which was confirmed by a 61% O_2 saturation in the overlying water measured after two months of incubation. However, despite the overlying water being oxygenated, conditions in the 25–30 mm thick sediment column must have been predominantly anoxic given that the O_2 penetration depth in organic-rich, muddy sediment is typically only a few millimeters. The anoxic conditions may have been enhanced by the strongly reduced faunal biomass and activity due to sieving of the sediment before the start of the incubations, thus ensuring no oxidation of deeper sediment layers due to bioturbation of bioirrigation.

The recovery of 41% of added ^{13}C after 1 day indicates that a large fraction of the added ^{13}C-glucose was remineralized to $^{13}CO_2$ which was likely lost from the bottles. This is consistent with the rapid decrease of the ^{13}C-glucose pool during the first few days of the experiment (Fig. 1b). In contrast, the strong recovery of ^{15}N after 1 day (90%) means that almost all ^{15}N remained in the sediment (as free or bound ^{15}N-ammonium or assimilated into microbial biomass), indicating that potential loss of $^{15}N_2$ and/or $^{15}N_2O$ through nitrification–denitrification and possibly anammox was small.

Addition of ^{13}C-glucose and ^{15}N-ammonium to the sediment was chosen as method to label the microbial commu-

Table 4
Concentrations of total C and ^{13}C for individual PLFAs and TFAs.

Days	Total concentration (nmol-C gdw^{-1})										Concentration ^{13}C (nmol-^{13}C gdw^{-1})								
	0	1	4	7	18	32	69	133	250	371	1	4	7	18	32	69	133	250	371
TFA																			
i14:0	11	11	11	13	11	13	12	11	9	8	0.12	0.14	0.14	0.14	0.14	0.14	0.15	0.08	0.06
14:0	57	54	53	55	49	56	51	51	41	36	0.32	0.40	0.39	0.40	0.51	0.50	0.51	0.26	0.20
i15:0	35	35	39	44	39	46	47	43	36	33	0.26	0.37	0.44	0.42	0.53	0.51	0.54	0.34	0.27
ai15:0	36	37	39	46	42	55	67	67	55	50	0.24	0.31	0.38	0.39	0.54	0.71	0.83	0.53	0.46
15:0	24	25	26	28	25	29	28	26	21	16	0.28	0.69	0.91	1.10	1.42	1.31	1.09	0.54	0.40
16:0	195	192	186	192	174	198	189	186	151	136	3.50	2.92	2.72	2.33	2.91	2.58	2.53	1.32	0.93
16:1ω7t	8	10	9	9	9	10	12	12	9	7	0.71	0.68	0.61	0.47	0.47	0.35	0.29	0.17	0.10
10Me16:0	12	12	12	11	12	16	13	14	10	9	0.19	0.22	0.23	0.25	0.27	0.22	0.22	0.12	0.09
16:1ω7c	111	123	120	122	122	137	142	163	145	126	4.96	3.82	3.59	3.33	4.09	3.35	3.76	2.09	1.54
i17:0	9	9	9	9	10	11	12	12	9	9	0.11	0.14	0.15	0.17	0.20	0.19	0.21	0.11	0.09
16:2ω4	8	13	13	21	19	22	21	21	16	15	0.11	0.27	0.43	0.56	0.77	0.68	0.61	0.30	0.25
16:3ω4	12	12	11	11	10	10	10	11	11		0.11	0.14	0.15	0.19	0.23	0.22	0.28		
18:0	42	40	39	40	36	41	37	37	32	50	0.22	0.22	0.24	0.25	0.33	0.31	0.36	0.17	0.14
18:1ω9c	35	38	35	35	34	43	39	42	36	31	0.39	0.38	0.39	0.41	0.41	0.37	0.44	0.29	0.20
18:1ω7c	60	59	56	59	55	65	68	90	86	74	1.83	1.20	1.18	1.05	1.12	1.13	1.48	1.13	0.68
PLFA																			
i14:0	4.1	4.0	4.3	4.3	3.3	4.5	5.6	5.3	3.9	3.9	0.06	0.07	0.07	0.05	0.06	0.07	0.08	0.04	0.04
14:0	19	19	19	18	13	16	21	20	16	15	0.16	0.19	0.16	0.12	0.18	0.24	0.24	0.11	0.10
i15:0	12	13	15	14	11	17	22	21	16	16	0.13	0.19	0.20	0.15	0.23	0.28	0.30	0.17	0.16
ai15:0	18	18	20	19	15	24	37	33	28	25	0.14	0.19	0.20	0.16	0.28	0.44	0.46	0.29	0.26
15:0	7.7	7.1	7.7	7.2	5.2	7.4	10.1	8.9	7.0	5.0	0.10	0.15	0.15	0.12	0.20	0.25	0.25	0.13	0.08
16:0	70	72	70	65	47	65	82	71	58	50	2.40	1.89	1.51	0.97	1.46	1.55	1.35	0.71	0.51
16:1ω7t	3.5	4.0	4.4	4.0	3.7	4.1	5.5	4.3	4.0	2.9	0.46	0.41	0.32	0.21	0.20	0.17	0.11	0.07	0.04
10Me16:0	4.0	4.8	4.3	4.3	4.0	4.3	5.3	4.5	4.1	3.7	0.12	0.11	0.10	0.08	0.09	0.09	0.08	0.05	0.04
16:1ω7c	52	55	52	49	36	53	72	66	51	50	3.30	2.29	1.98	1.35	1.90	1.87	1.76	0.85	0.73
i17:0	4.2	4.2	4.5	4.1	3.6	4.4	6.2	5.5	4.8	4.0	0.08	0.09	0.08	0.07	0.08	0.12	0.11	0.08	0.05
16:2ω4	6.8	6.7	6.5	6.5	8.3	9.7	10.0	9.2	7.1	6.1	0.05	0.08	0.08	0.08	0.14	0.17	0.17	0.06	0.06
16:3ω4	5.7	5.2	4.3	4.3	3.3	3.5	4.1	4.2	3.1		0.08	0.09	0.09	0.08	0.10	0.11	0.14		
18:0	17	17	15	14	11	13	13	12	11	9	0.14	0.14	0.13	0.11	0.17	0.17	0.18	0.08	0.06
18:1ω9c	14	15	14	12	10	12	15	15	13	11	0.20	0.16	0.11	0.08	0.11	0.13	0.15	0.09	0.07
18:1ω7c	37	38	34	32	24	27	37	48	58	40	1.34	0.75	0.61	0.38	0.42	0.54	0.76	0.67	0.37

nity in the sediment as an alternative to addition of a defined pool of organic matter from a laboratory culture. This approach has the advantage of labeling the natural sediment microbial community. The disadvantage of this approach is the presence of added ^{13}C-glucose and ^{15}N-ammonium in the sediment which resulted in continued assimilation of label into microbial biomass during the first weeks of the experiment. This is clearly illustrated by the continued net incorporation of ^{15}N into THAAs (Fig. 1a) and the corresponding decrease of ^{15}N in the unidentified pool. During the labeling phase, the latter consisted of ^{15}N-ammonium and therefore the rapid decrease of this pool indicates depletion of the ^{15}N-ammonium after 69 days. Moreover, the increase in concentrations of some PLFAs and TFAs, particularly those specific for bacteria (e.g. i15:0 and ai15:0), during the first two months (Table 4) suggests that there was net production of microbial biomass during this period. Altogether, by the end of the labeling phase, both labeled substrates were depleted and microbial biomass had stabilized. This means that results for the loss phase reflect net loss of total and labeled compounds from microbial biomass and detritus. For consistency, loss rate constants (k) were determined by fitting data for the loss phase only.

4.2. Composition of the labeled microbial community

The D/L-Ala ratios for ^{13}C and ^{15}N for the labeling phase (Table 2) provide information about the composition of the microbial community that incorporated the ^{13}C and ^{15}N. Briefly, D-Ala is specific for bacteria while L-Ala is present in all organisms. Therefore, the ratio between label incorporated into these two stereo isomers provides a direct indication of the bacterial contribution to total microbial label incorporation (see Veuger et al., 2007b for details). Measured ^{13}C-D/L-Ala ratios of up to 0.047 are within the range of values reported for various natural bacterial communities (0.04–0.10, see Veuger et al., 2007b). Combined with the ^{13}C-labeling of the PLFAs, which showed little to no ^{13}C-enrichment of algal PLFAs, in particular 20:5ω3 (data not shown), this indicates that ^{13}C had been incorporated predominantly by bacteria. In contrast, the lower D/L-Ala ratios for ^{15}N (Table 2) indicate a non-bacterial contribution to total incorporation of ^{15}N. Assuming that the maximum ^{13}C-D/L-Ala ratio for the labeling phase of 0.047 represented a 100% contribution by bacteria and assuming a racemization background of 0.017 (Kaiser and Benner, 2005; Veuger et al., 2007b) the average ^{15}N-D/L-Ala ratio of 0.035 for ^{15}N indicates a bacterial contribution of ~60% ((0.035 − 0.017)/(0.047 − 0.017) × 100) and hence a non-bacterial contribution of ~40%. This non-bacterial contribution most likely involved uptake of ^{15}N-ammonium by benthic microalgae, more specifically diatoms, as these were present and active in the sediment (Veuger and van Oevelen, 2011). Algal N assimilation is not as light dependent as algal C fixation and diatoms are known to assimilate ammonium in the dark. In addition, label uptake may also have included a contribution by Archaea, the third domain of life whose bio(geo)chemical functions in sediments such as these are unclear (e.g. Nicol and Schleper, 2006). Archaea are present in sediments of the Scheldt Estuary (Sahan and Muyser, 2008) and have been reported to contain D-Ala (Nagata et al., 1999). However, D-Ala concentrations in Archaea appear to be much lower than in bacteria (Nagata et al., 1999) and the potential archaeal contribution to label incorporation into D-Ala was therefore likely negligible. This means that label incorporation by Archaea would be included in the "non bacterial" contribution to total label incorporation (as explained above). Given that the ^{13}C D/L-Ala ratios are typical values for bacteria, the contribution of Archaea to total ^{13}C uptake must have been negligible. However, we cannot exclude an Archaeal contribution to non-bacterial ^{15}N uptake. Therefore, in this paper, the term "microbes" covers bacteria, benthic microalgae, and Archaea.

4.3. The loss phase: general setting

During the loss phase (day 69–371), loss of label from the bulk sediment and THAAs was no longer masked by continued incorporation of label from added ^{13}C-glucose and ^{15}N-ammonium as these pools had now been depleted (see Section 4.1). The labeled organic matter in the sediment during the loss phase comprised a mixture of living microbial biomass and microbial detritus that was formed by death and degradation of microbial biomass. The formation of a labeled detritus pool in the present study is evident from the presence of differences in loss rates for the various compounds since no difference would have occurred in case of full retention of label in living microbial biomass only. This is consistent with the measured loss rate constants being much lower than typical turnover rates for bacterial biomass in surface sediments of 1–100% d^{-1} (k-values of 0.01–1) (Sander and Kalff, 1993; Alongi, 1994; Luna et al., 2002) indicating that measured loss rates reflect degradation of microbial detritus rather than turnover (death and degradation) of living microbial biomass. The low degradation rates may be related to the permanently anoxic conditions in the sediment since organic matter degradation rates can be strongly reduced under anoxic conditions (Sun et al., 2002; Burdige, 2007 and references therein). A process that may have contributed to the low loss rates in the present study is recycling in the form of reassimilation of (labeled) degradation products by the benthic microbial community, which slows down the net loss of these degradation products from the sediment.

4.4. Hydrolysable amino acids

Observed differences in loss rates for the individual HAAs (Fig. 3) are consistent with general trends in studies on the dynamics of individual HAAs during diagenesis (see Dauwe et al., 1999; Keil et al., 2000 and references therein). In these studies, Gly is the HAA that shows the most consistent diagenetic behavior. The increase in its relative abundance during diagenesis is often attributed to a relatively high abundance of Gly in refractory diatom cell walls (Hecky et al., 1973). In the present study, this mechanism may have been relevant only for ^{15}N since algae assimilated ^{15}N but no ^{13}C (see Section 4.2). However, considering the similar loss rate constants for ^{13}C-Gly and ^{15}N-Gly (Fig. 3a), the importance of accumulation of diatom cell walls in the diagenetic behavior of Gly seems to be negligible.

A similar mechanism that may explain the relatively refractory character of Gly is accumulation of the refractory bacterial cell wall component peptidoglycan, which is relatively rich in Gly (Madigan et al., 2000). However, as will be discussed below (Section 4.5), the accumulation of peptidoglycan appears to have been negligible. Consequently, the relative accumulation of Gly in the present study cannot be attributed to accumulation of specific macromolecules. Potential mechanisms for the relative accumulation of Gly include (1) accumulation of Gly-rich transformation- or degradation products, (2) selective association with complex macromolecular structures (‘humic matter’) (Burdige, 2007) and (3) preferential retention of Gly or Gly-rich material during microbial recycling (Vandewiele et al., 2009).

Pro and Lys also displayed a relatively conservative behavior (Fig. 3). Unlike Gly, Pro is usually not included in studies on individual HAAs during diagenesis as these studies generally are based on HPLC analysis of amino acid concentrations which does not include Pro. This means that relatively little is known about the diagenetic behavior of Pro. Comparison of the abundance of Pro in the total sediment THAA pool with that in ^{13}C- and ^{15}N-labeled THAA pools in various stable isotope labeling studies similar to the present study (e.g. Veuger et al., 2005, 2007a; Gribsholt et al., 2009) consistently shows a higher relative abundance of Pro in the total sediment (a mixture of fresh and degraded material) than in the labeled pools (fresh microbial biomass), which confirms the relatively conservative character of Pro. Pro also showed a refractory behavior similar to that of Gly during *in situ* degradation of ^{13}C-labeled bacterial biomass (Veuger et al., 2006). The refractory character of Pro may be related to the relatively complex molecular structure of this secondary amino acid which includes a cyclic side group. Unlike Gly and Pro, Lys is not typically refractory in behavior and showed a neutral or labile character in other studies (e.g. Keil et al., 2000; Lee et al., 2000; Veuger et al., 2006). One possible explanation for the relative accumulation of Lys may be relatively strong binding of Lys to the sediment matrix (Henrichs and Sugai, 1993). In that case, differences between studies may be related to differences in amino acid binding properties of the sediments.

Altogether, the diagenetic changes in the HAA pools indicate that the overall “degradation state” of the labeled organic material increased over time. The degradation state of a given HAA pool can be quantified in the form of a degradation index (Dauwe et al., 1999). However, this is not straightforward for the present study as the HAA pool analyzed in this study (GC-based) only partially overlaps with those analyzed in most other (HPLC-based) studies. Alternatively, here we use the relative contribution of Gly to the THAA pool (%Gly) as a measure of the degradation state for comparison with other studies since Gly shows a consistent diagenetic behavior and is included in all studies. The magnitude of increase in %Gly between the average value for the labeling phase (day 1–69) and the end of the experiment (day 371) in the labeled HAA pools in this study was ×.36 for ^{13}C (from 7.1% to 9.7%), ×.35 for ^{15}N (from 13.4% to 18.1%) (Table 2). For comparison, in other studies, %Gly increased about × along a gradient from fresh organic matter (10–12% Gly) to sediments with a high degradation index (20–25% Gly) with strongest changes occurring during the early stages of diagenesis (Dauwe and Middelburg, 1998; Keil et al., 2000). In this context, the changes in %Gly in the current study were moderate, meaning that the material remaining after 1 year was still relatively “fresh”. This is consistent with the labeled HAA pools representing a mixture of living microbial biomass and recently produced microbial detritus.

In addition to the *labeled* HAA pools, the individual amino acids in the *total* HAA pool (i.e. labeled + unlabeled) showed trends and loss rate constants similar to those for the labeled pools (Fig. 3 and Table 2). The magnitude of the changes in the total THAA pool is surprisingly high, especially when taking into account that THAA concentrations remained at a steady level (Table 1) which means that losses of specific HAAs due to degradation of detritus must have been compensated for by production of other HAAs in the form of new microbial biomass (and subsequent transformation to detritus). This continuous production of new (unlabeled) HAAs reduced the magnitude of the compositional changes resulting from degradation of the detritus and also explains why absolute concentrations of Pro and Lys even *increased* over time (Table 2). The similarity in magnitude of the diagenetic changes in the labeled and total THAA pools suggests that the lability of the labeled microbial detritus was comparable with that of the total proteinaceous material.

4.5. D/L-Ala ratio

For the loss phase, the ratio between label in D-Ala versus that in L-Ala can no longer be used as an indication of the bacterial contribution to total label incorporation because this now also includes D-Ala and L-Ala in labeled detritus. However, the D/L-Ala ratio does provide information about potential relative accumulation of compounds rich in D-Ala relative to total proteinaceous material (represented by L-Ala). Generally, accumulation of D-Ala has been attributed to accumulation of the bacterial cell wall component peptidoglycan in sediments (Pedersen et al., 2001; Grutters et al., 2002; Lomstein et al., 2006; Veuger et al., 2006; Vandewiele et al., 2009). Recent work by Kaiser and Benner (2008) revealed that D-Ala has also been reported to be present in other bacterial macromolecules and that relative accumulation of D-AAs does not exclusively reflect accumulation of *intact* peptidoglycan or other bacterial macromolecules but may also reflect accumulation of remnants of these macromolecules. However, the primary result from the present experiment is that D/L-Ala ratio remained stable during the loss phase, meaning that there was no relative accumulation D-Ala. These results suggest that relative accumulation of D-Ala occurs over longer time scales than the measured compositional changes in the “common” HAA pool discussed above.

4.6. Carbohydrates

Out of the different biochemical groups analyzed, carbohydrates showed lowest loss rates. This refractory character of carbohydrates is consistent with results from other studies. The most comparable study is that by Jensen et al. (2005) in which sediment samples of various depth layers from different locations in the Baltic-North Sea transition were incubated anoxically for 480 days. Results for these incubations showed little to no change in concentrations and composition of the carbohydrate pools in the majority of samples. In general, the lability of carbohydrates seems to be highly dependent on the type of source material: carbohydrates that function as storage of energy and carbon, primarily for algae, are highly labile and rapidly degraded (Harvey and Macko, 1997; Oakes et al., 2010), whereas carbohydrates that are part of structural biomass components (such as cell walls) are far more refractory and can therefore persist in sediment over long periods of time (Jensen et al., 2005; Oakes et al., 2010).

The carbohydrate pools (total and labeled) investigated in the present study must have been predominantly structural carbohydrates from bacterial biomass because the present experiment was conducted in darkness, meaning that algal photosynthetic activity (and associated production of labile storage carbohydrates) must have been low. This is consistent with the very low algal contribution to ^{13}C incorporation during the labeling phase (see Section 3.2).

Differences between individual monosaccharides were small and loss rate constants could not be determined for most monosaccharides, except for glucose and ribose. The rapid loss of ^{13}C-glucose during the uptake phase can be considered a methodological artifact since this mainly reflects loss of the free ^{13}C-glucose that was added to the sediment. The latter likely also caused the relatively high loss rate constant ($0.0027\ d^{-1}$) for ^{13}C-glucose during the loss phase. Ribose showed relatively high ^{13}C-labeling during the first days of the experiment and a loss rate constant for the ^{13}C-labeled pool that was the same as for ^{13}C-glucose ($0.0027\ d^{-1}$). This coupling with glucose may be related to ribose being an important component of RNA, a cell component that can be rapidly synthesized (hence rapid ^{13}C-labeling) and rapidly degraded upon cell death (hence relatively high loss rate constant). This relatively labile character of ribose is also reflected in the relatively rapid loss of total ribose (i.e. concentrations) (Table 3).

4.7. Fatty acids

Out of the different biochemical groups investigated, lipids (represented by TFAs and PLFAs) were lost most rapidly. This relative lability is consistent with results from other studies in which lipids were found to be preferentially remineralized relative to bulk OC (Wakeham et al., 1997a,b; Burdige, 2006). Both PLFAs and TFAs were analyzed in our study as PLFAs are considered to be amongst the most labile components of microbial biomass whereas TFAs comprise a more diverse pool including both the PLFAs and more refractory FAs. This difference in lability is reflected in the higher loss rate constants for concentrations of PLFAs versus TFAs ($k = 0.0012$ and $0.0007\ d^{-1}$, respectively) (Table 1). A similar difference between TFAs and PLFAs was reported by Kindler et al. (2009) for ^{13}C-labeled bacterial FAs in a soil system. In contrast, there was no such difference between the ^{13}C-labeled PLFAs versus TFAs, indicating that the lability of the non-PL fraction of the ^{13}C-labeled TFA pool was very similar to that of the PLFAs. A point to take into account here is that the TFA pool includes the PLFAs and only involved the easily extractable fraction of the total sediment lipid pool (i.e. readily extractable with organic solvents). This means that a less well extractable, and likely more refractory, pool of lipids remained in the sediment and hence that the TFA pool probably represented a relatively labile fraction of the total lipid pool.

Even though differences in loss rates for the individual ^{13}C-labeled TFAs and PLFAs over time were small, there is a consistent order in loss rate constants (Fig. 3). The FAs with lowest loss rate constants for the loss phase are also those that increased in total concentration and ^{13}C concentration during the labeling phase (Table 4). The three most refractory FAs within this group were 18:1ω7c, ai15:0, and i15:0. The relatively refractory character of these bacteria-specific FAs is consistent with findings by Wakeham et al. (1997a,b) who also found 18:1ω7c and ai15:0 to be the most refractory FAs within a wider range of FAs along a diagenetic gradient from marine plankton via sediment trap material to the sediment. Similar observations were made in marine sediments by Canuel and Martens (1996) and Sun et al. (1997). ^{13}C may have been retained in these compounds relatively efficiently as a result of recycling of ^{13}C-labeled degradation products by a part of the bacterial community that was rich in these FAs. This is consistent with the net production (i.e. increase in concentrations) of these compounds during the uptake phase. The production of i15:0, ai15:0 and 18:1ω7c has also been observed during degradation of algal material in sediment (Middelburg et al., 2000; Ding and Sun, 2005). Unfortunately, the taxonomic resolution of the PLFA profile is insufficient for further identification of this part of the bacterial community.

4.8. Comparison of biochemical groups and the unidentified fraction

The relative composition of the total sediment organic matter pool (Table 1) is comparable with that reported for other sediments (Burdige, 2007 and references therein) while the relative composition of the total ^{13}C-labeled pool (Table 1) is consistent with the general composition of microbial biomass (Cowie and Hedges, 1992; Burdige, 2007; Kaiser and Benner, 2008). Only few studies investigated a similar wide range of biochemicals from a given organic matter pool during degradation. Wakeham et al. (1997a) investigated the fate of amino acids, carbohydrates and lipids as well as pigments in organic matter along a diagenetic gradient from fresh marine plankton to the sediment. Resulting relative differences in reactivity between compound-groups are comparable to those for the ^{13}C-labeled biochemicals in the present study with lipids being the most labile biochemical group while carbohydrates were most refractory. In contrast, Miltner et al. (2009) and Kindler et al. (2009) found a much stronger loss of fatty acids (76%) than that of HAAs (<5%) in their study on the fate of ^{13}C-labeled HAAs and fatty acids from ^{13}C-labeled *Escherichia coli* in soil over a time scale comparable to that of the present study (224 days).

The quantitative analysis of the three main biochemical groups from microbial biomass and organic matter also allows us to investigate the unidentified fraction of the total organic matter pool. The unidentifiable pool in the present study made up 67–79% of total OC and N, which is similar to values from previous studies (Wakeham et al., 1997a; Burdige, 2007 and references therein). The unidentifiable fraction of the *labeled* pools during the loss phase was much smaller (28–35% of bulk ^{13}C, 45–52% of bulk ^{15}N). These values are similar to those reported for fresh plankton and sinking particles in the ocean (Burdige, 2007 and references therein). The unidentified fraction in the present study likely included some amino sugars, nucleic acids and lipids extractable with more rigorous extraction methods. However, these minor compound groups typically make up only a small fraction of total organic matter. The unidentified fraction may also have included some dissolved and dissolvable (H)AAs as these are included in the bulk sediment isotope analyses but not in the HAAs analyses as sediment was washed before HAA extraction (see Section 2.2). However, this pool was also found to be only a small fraction of the total HAA pool (see Section 2.2). Finally, during the labeling phase, the unidentified ^{15}N pool also included added ^{15}N-ammonium. Altogether, for total concentrations and ^{13}C, the majority of the unidentified fraction appears to be truly unidentifiable at the molecular level by conventional analytical techniques. This so-called molecularly uncharacterized organic matter (Hedges et al., 2000) comprises complex macromolecular structures containing for example non-hydrolysable amino acids (i.e. proteinaceous material not susceptible to acid hydrolysis) and bound-lipids (Hwang and Druffel, 2003; Burdige, 2007). This material is formed by abiotic condensation- and polymerization reactions (geopolymerization or humification), by interactions of organic matter with organic and inorganic matrices (physical protection from extraction and degradation), and by formation of refractory macromolecules by microorganisms (see Burdige, 2007 and references therein). During the loss phase, the unidentified fractions of OC and ^{13}C generally followed the same trends as the HAAs, carbohydrates and lipids with only a slightly slower loss (Fig. 1 and Table 1). The corresponding loss rate constants for the ^{13}C-labeled and total unidentified pools were 2–4 times lower than those for the identified pools, which is consistent with a gradual relative accumulation of molecularly uncharacterized organic matter. An interesting observation is the rapid formation of the unidentified ^{13}C pool during the first week of the experiment (Fig. 1 and Table 1). However, this pool did not build up gradually from day 1 onwards but rather showed a sudden increase from ~0–10 nmol ^{13}C gdw^{-1} to ~100 nmol ^{13}C gdw^{-1} between day 4 and day 7 (Table 1). This delay in the transfer of ^{13}C from ^{13}C-glucose to the unidentified pool suggests that this transfer was mediated by microorganisms.

4.9. C–N coupling

The stable ^{13}C/^{15}N ratios for the bulk sediment and the THAAs during the loss phase (Table 1) implies that retention of ^{13}C and ^{15}N was strongly coupled, and hence that loss of ^{13}C and ^{15}N was also closely coupled. For HAAs, this strongly coupled loss can be explained by AAs being either fully degraded and remineralized (i.e. full loss of AA-N and AA-C) or being recycled as complete molecules (i.e. recycling both AA-N and AA-C). For the non-HAA fraction (bulk–THAAs) there must have been a similarly strong coupling (given the stable ^{13}C/^{15}N ratios for both the bulk sediment and the THAAs). This is somewhat surprising given the differences in composition of the non-HAA ^{13}C versus ^{15}N pools (see Section 4.8) based on which one would expect different loss rates for various compounds to results in different loss rates for ^{13}C versus ^{15}N (i.e. decoupling). The absence of such decoupling indicates that the rate-limiting step in degradation was degradation of intact microbial detritus (i.e. initial stages of degradation) rather than degradation of different compound groups upon release from detritus.

Moreover, as well as partly being present in different compounds, ^{13}C- and ^{15}N-labeled compounds were also partly derived from different types of microbial biomass since ^{13}C was primarily present in bacterial biomass while ^{15}N had also been incorporated by benthic microalgae and possibly Archaea (see Section 4.2). Surprisingly, this difference did also not cause any decoupling of ^{13}C and ^{15}N, suggesting that algal N was lost at the same rate as bacterial N and C.

Altogether, results indicate that loss of microbial C and N was not directly dependent on the type of compounds it was present in, nor on the source organism. The strong coupling in retention of ^{13}C and ^{15}N suggest that there was little or no selective preservation of neither C nor N during microbial reworking of the labeled material in the present study. This is somewhat surprising because degradation of fresh organic matter normally results in preferential loss of N over C. The latter is reflected for example by comparison of C/N ratios for fresh microbial biomass (C/N ~ 4–6) versus that on sinking particles (C/N ~ 8) versus sediment (C/N ~ 10) in Dabob Bay (Cowie and Hedges, 1992) and by the negative correlation between C/N ratio and the HAA-based degradation index for North Sea sediments (Dauwe and Middelburg, 1998). The absence of the expected decoupling between C and N in the present study may have been due to a lower lability of the labeled microbial detritus versus that of fresh phytodetritus and sinking material in the water column. This may have been the direct result of a relatively refractory character of the labeled benthic microbial detritus. Another relevant factor concerns the absence of faunal activity in the present study. Physical activity of benthic macrofauna (both bioturbation and bioirrigation) can cause redox oscillations in the sediment that can result in enhanced organic matter degradation (e.g. Kristensen, 1988; Na et al., 2008). Moreover, faunal activity can enhance loss of ammonium from the sediment through nitrification–denitrification as well as by release of ammonium to the overlying water (Na et al., 2008). Therefore, the absence of faunal activity can result in strong retention of ammonium in anoxic sediments and enhance the reassimilation of N into benthic microbial biomass, resulting in little or no selective loss of N (versus C) during diagenesis. The effect of the absence of faunal activity in the present study is also reflected by the low loss rate constants in general and the small differences in loss rates between individual compounds. As such, these results illustrate the importance of faunal activity on the biogeochemistry of marine sediments.

4.10. Synthesis

Despite intensive previous work on organic matter diagenesis, the intermediate time scale (weeks–years) has been covered by few studies (e.g. Canuel and Martens, 1996; Wakeham et al., 1997a,b; Kindler et al., 2009; Miltner et al., 2009). The present study successfully investigated the comparative fate of HAAs, monosaccharides and fatty acids from the in situ microbial community of a tidal flat sediment on this intermediate time scale. Despite low loss rates and relatively small differences between biochemical groups and individual compounds within these biochemical groups, consistent diagenetic patterns emerged that are generally consistent with results from other studies. Results illustrate the potential effect of reworking of organic matter (i.e. secondary production) on the composition and fate of organic matter in sediments. Secondary production is a major topic in microbial and community ecology and our results confirm that it should receive more attention in organic geochemical studies.

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