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Microbial contributions to N-immobilization and organic matter preservation in decaying plant detritus

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Abstract

Microbial contributions to the detritus of two vascular plant tissues, smooth cordgrass (*Spartina alterniflora*) and black mangrove leaves (*Avicennia germinans*), were estimated over a 4-year decomposition period under subaqueous marine conditions. During this period, 93–97% of the initial plant tissues was decomposed. Bulk elemental and isotopic compositions of the detritus were measured along with hydrolyzable amino sugars (AS) and amino acids (AA), including the bacterial biomarkers muramic acid and the D-enantiomers of AA. A major enrichment in N relative to C occurred during decomposition. Net increases of AS, AA, and bacterial biomarkers in decaying detritus were observed. Three independent approaches indicated that on average 60–75% of the N and 20–40% of the C in highly decomposed detritus were not from the original plant tissues but were mostly from heterotrophic bacteria. During decomposition hydrolyzable AS + AA yields (~54% of total N) were strongly correlated with total N in both types of detritus. The uncharacterized N appeared to have the same origin and dynamics as AA, suggesting the contribution of other bacterial biomolecules not measured here. There was little indication of humification or abiotic processes. Instead, N-immobilization appeared primarily bacterially mediated. Although varying dynamics were observed among individual molecules, bacterial detritus exhibited an average reactivity similar to plant detritus. Only a minor fraction of the bacterial detritus escaped rapid biodegradation and the relationship between bacterial activity and N-immobilization is consistent with an enzymatically mediated preservation mechanism. Bacteria and their remains are ubiquitous in all ecosystems and thus could comprise a major fraction of the preserved and uncharacterized organic matter in the environment.
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1. Introduction

Vascular plants represent the largest component of living biomass on Earth, and plant detritus is the dominant component of reactive organic matter in terrestrial and aquatic environments. During decomposition, the molecular composition of the original plant material is highly altered, resulting in over half of the organic matter in soils and sediments being unidentified at the molecular level (Hedges et al., 2000 and references therein). The mechanisms of these alterations and of organic matter preservation are uncertain. Therefore, studies of the diagenesis of plant material are critical for gaining a fundamental understanding of these important aspects of organic biogeochemistry.

During the decomposition of plant tissues, a short leaching phase is followed by a longer and more extensive decomposition phase attributed to microbial degradation (e.g., Benner et al., 1990, 1991; Blum and Mills, 1991; Rice and Tenore, 1981; Valiela et al., 1985). Changes in the nitrogen (N) content of plant detritus are particularly noticeable. Following net losses of N during leaching, the incorporation of exogenous N, termed N-immobilization, is commonly observed during the phase of microbial degradation (Rice, 1982; White and Howes, 1994; and references above). The process of N-immobilization is of great ecological importance given the role of N as a limiting nutrient in many ecosystems. Microorganisms are thought to be important in this process and are suspected to be a source of immobilized N (Melillo et al., 1984; Rice and Tenore, 1981). However, relatively little is known about the origins

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and mechanisms of N-immobilization (Hicks et al., 1991; White and Howes, 1994). Several studies have suggested that the interaction of immobilized N with phenolic components of plant tissues, such as lignins and tannins, produces molecules that are resistant to further decomposition (Buchsbaum et al., 1991; Hernes et al., 2001; Rice, 1982; Suberkropp et al., 1976). If microorganisms contribute to the detrital organic matter, their contribution of biomolecules that are not found in plant tissues should become increasingly apparent as decomposition progresses. In addition, microbial activity might play a central role in the production of uncharacterized molecules and in organic matter preservation (Ogawa et al., 2001).

Specific amino sugars (AS) and amino acids (AA) can be used to trace the presence and abundance of microbially derived organic matter (Benner and Kaiser, 2003; Dittmar et al., 2001; McCarthy et al., 1998). For instance, the AS muramic acid is only found in the bacterial cell wall polymer, peptidoglycan (Brock et al., 1994). Microorganisms are rich in other AS, such as galactosamine and glucosamine which are trace or minor components of vascular plant tissues, respectively (Glaser et al., 2004; Sharon, 1965). The D-enantiomers of AA (D-AA) are only prevalent in bacterial cell walls and have not been found in higher plants (Asano and Lübbehüsen, 2000; Brock et al., 1994).

In this study, the incorporation of microbial N and C into plant detritus was investigated during a long-term (4 year) subaqueous decomposition study. Two well-characterized plant tissues, smooth cordgrass (*Spartina alterniflora*) and black mangrove leaves (*Avicennia germinans*), were chosen to represent contrasting sources of detrital material in estuarine and coastal environments. AS and D/L AA were analyzed to trace the microbial and bacterial contributions of C and N to the detritus. The dynamics of these molecules provide new insights into N-immobilization and organic matter transformation and preservation during diagenesis.

2. Materials and methods

2.1. Plant collections and litter bag experiment

Details of these long-term decomposition experiments are given elsewhere (Opsahl and Benner, 1995). Briefly, senescent aboveground portions of the smooth cordgrass, *S. alterniflora*, and yellow leaves from mangrove trees, *A. germinans*, were picked from plants in the vicinity of Port Aransas, Texas, USA. All tissues were air-dried at ~25 °C and weighed prior to placement in 60 μm mesh litter bags. Bags were suspended in 750 L tanks that were covered to prevent algae from growing inside the tanks and photochemical transformations. Estuarine water from Aransas Channel was continuously pumped through the tanks. Salinity ranged from 18 to 35, temperature ranged from 14 to 30 °C, and dissolved oxygen concentration ranged from 125 to 250 μM. Average nutrient concentrations were 11.5 μM NO_3^-, 9.8 μM NH_4^+, and 1.1 μM PO_4^{3+}. Duplicate litter bags were collected at each time point over a 4-year period. Each litter bag was rinsed in distilled water to remove salts and the contents were then dried at 45 °C for 3 days and weighed. Samples were ground in a Wiley mill to pass a 40-mesh sieve. All analyses were conducted with material from each replicate sample.

2.2. Ash, elemental, and isotopic analyses

Ash determinations were made by combusting duplicate subsamples from each litter bag at 550 °C for 6 h. Elemental C and N contents were measured using a Carlo-Erba EA 1108 CHN analyzer. Several samples selected from the time series were acidified prior to CHN analysis and carbonates were found to be insignificant in all samples. The average mean deviations for ash determinations and elemental analyses were <2%. The stable N isotopic composition was determined on selected samples by Coastal Science Labs, Austin, TX. Values are reported as the relative proportion, in δ values (‰), of the heavy ^{15}N isotope with reference to atmospheric N_2. The average mean deviation of these measurements was 0.2‰.

2.3. Lignin analyses

The lignin content of these samples was presented in a previous study (Opsahl and Benner, 1995). Samples were analyzed for lignin phenols using the CuO oxidation method and gas chromatography. Lignin contents are reported as the sum of the carbon-normalized yields of syringyl and vanillyl phenol, or Λ_6 (mg lignin phenols/100 mg organic C).

2.4. Amino sugar analyses

Details of sample preparation and chromatography are described in Kaiser and Benner (2000). About 5 mg of dried plant detritus was hydrolyzed with 3 M HCl at 100 °C for 5 h in a sealed ampoule. Tests have shown that hydrolysis with 6 M HCl usually gives slightly lower yields of glucosamine (GlcN) and mannosamine, and slightly higher yields for galactosamine (GalN) compared with the 3 M HCl hydrolysis. During strong acid hydrolysis, acetyl groups are removed from AS. Calculations of %C as AS in samples were based on the deacetylated form. Therefore, the values presented herein could underestimate %C as AS by as much as 25%. Likewise, no corrections for hydrolysis efficiency were used in the present study (Benner and Kaiser, 2003).

Hydrolyzed samples were centrifuged, and the supernatant was neutralized with the self-absorbed AG11 A8 resin (Bio-Rad) and split into two aliquots. One aliquot was used for analysis of GlcN, GalN, and mannosamine, and the other was frozen for muramic acid (Mur) analysis. After desalting using the cation-exchanger AG50 X8 resin (Na^+ form, Bio-Rad), GlcN, GalN, and mannosamine were separated and measured using high-performance anion

exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). A Dionex 500 ion chromatography system with a CarboPac PA20 column (3 × 150 mm) with guard column (3 × 30 mm) and PAD detector were used for all analyses. Separation was performed under isocratic conditions with a 2 mM NaOH mobile phase. This separation relies on the ionization of one or more AS hydroxyl groups.

Mur was measured using reversed-phase high-performance liquid chromatography coupled with a fluorescence detector. The protocol used is similar to that employed for AA analyses (see Section 2.5.) but with important modifications. Neutralized hydrolysates containing Mur were derivatized with *o*-phthaldialdehyde (OPA) and *N*-isobutyl-L-cysteine in a borate buffer (pH 9.5). Separation and detection were performed on an Agilent 1100 system with a C-18 Superspher 100 RP-18 column (4 × 125 mm, 4 μm beads) and a guard column (LiCrospher 100 RP-18, 4 × 4 mm, 5 μm). The column temperature was maintained at 20 °C and the mobile phase flow rate was 0.8 mL/min. A mobile phase linear gradient was applied, from 100% 29 mM sodium acetate (pH 6.2) to 18% methanol after 37 min and 60% methanol after 42 min. The detector excitation and emission wavelengths were set to 330 and 450 nm, respectively. Quantification was done using external Mur standards with glucosamininc acid (2-amino-2-deoxy-gluconic acid) as an internal standard. A complete procedure blank, with the internal standard added before derivatization, was also analyzed.

2.5. Amino acid analyses

Details of the D/L-AA analysis are described elsewhere (Kaiser and Benner, 2005). Briefly, ~5 mg of selected dried plant detritus was hydrolyzed with 6 M HCl at 110 °C for 20 h in a sealed ampoule containing 0.12 μmol/mL ascorbic acid. HCl was removed from the hydolysate by repeated drying under nitrogen and water dissolutions. Automated precolumn derivatization with OPA together with *N*-isobutyryl-L-cysteine or *N*-isobutyryl-D-cysteine was performed in a borate buffer (pH 9.5). Derivatization of AA with the L-reagent results in the formation of the diastereomeric pairs D–L and L–L, which are separable on an achiral stationary phase. A second run after derivatization with the D-reagent gives the D–D and L–D pairs leading to a reversal of the elution order of D- and L-AA (Brückner et al., 1994). These two analyses provide verification for the occurrence and quantification of D- and L-AA. The presence of ammonium would also produce a signal but at the same characteristic elution time in both runs.

Separation and detection were performed on an Agilent 1100 system with a C-18 LiCrospher 100 RP-18 column (4 × 250 mm, 5 μm beads) with guard column (4 × 4 mm, 5 μm). The flow rate was 0.8 mL/min and the elution temperature was 20 °C. A mobile phase linear gradient was applied, from 100% 40 mM KH_2PO_4 (pH 6.2) to 39, 54, and 60% methanol/acetonitrile (13:1 v/v) after 50, 72, and 80 min, respectively. The detector excitation and emission wavelengths were set to 330 and 450 nm, respectively. The quantification was done using two external AA standard solutions containing a mix of glycine (Gly), 14 L-AA, 14 D-AA, and L-glutamic acid methyl ester as internal standard. The 14 AA were: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), threonine (Thr), alanine (Ala), arginine (Arg), tyrosine (Tyr), methionine (Met), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), and lysine (Lys). A complete procedure blank, with internal standards added before derivatization, was also analyzed.

D/L-AA measurements were corrected for chemical racemization occurring during hydrolysis. Racemization rates for free and protein AA were measured (Kaiser and Benner, 2005), and the average of these rates was used for corrections. Racemization correction had a negligible effect on L-AA concentrations (i.e., <2%), but the effects on D-AA concentrations were substantial. Peaks of D-AA leading to zero or negative values after average racemization correction were considered hydrolysis artifacts and thus rejected. Also rejected were the D-AA peaks showing more than 15% variability between the L- and D-reagent runs. Such variability indicated coelution problems.

2.6. Biomarker yields in source microorganisms

Microbial biomarker yields measured in detritus were converted into bulk microbial C and N contents using C- and N-normalized biomarker yields measured in microorganisms. Considering that these litter bags were submerged in seawater in the dark, yields were calculated for a mixture of 95% Gram-negative and 5% Gram-positive heterotrophic bacteria (Giovannoni and Rappé, 2000) based on culture and coastal natural populations (Benner and Kaiser, 2003; Kaiser and Benner, unpublished data). The biomarker yields in these bacteria were: 21.0 nmol/mgC and 107.2 nmol/mgN for Mur, 24.6 nmol/mgC and 125.2 nmol/mgN for GalN, and 38.0 nmol/mgC and 193.6 nmol/mgN for D-Ala.

3. Results

3.1. Bulk parameters

Decomposition is herein defined as any loss of organic matter from the litter bags. Early weight losses during the first 42 days of decomposition (Table 1) are mostly attributed to leaching. Cordgrass and mangrove leaves lost 10.8 and 31.3%, respectively, of their ash-free dry weight during the leaching phase (i.e., 0.26 and 0.75% day^{-1}). During the decomposer phase, rates of weight loss decreased to 0.15% day^{-1} in both detritus during the remainder of the first year and continued to decrease to 0.016 and 0.003% day^{-1} in the last year for cordgrass and mangrove leaf detritus, respectively.

Table 1
Percent remaining, C and N contents, and stable N isotopic composition of plant detritus during 4 years of decomposition

Sample	% Remaining (AFDW)	C (wt %)	N (wt %)	C/N (atom)	δ^{15}N (‰)
Smooth cordgrass					
Senescent	100	42.1	0.79	62.1	1.2
42 days	89.2 (1.8)	42.9 (0.2)	0.58 (0.02)	87.0 (1.9)	nd
189 days	77.7 (0.3)	41.1 (0.2)	0.63 (0.01)	76.2 (1.5)	3.8
1.0 years	41.4 (5.9)	40.5 (0.6)	0.97 (0.07)	49.1 (4.3)	4.0
1.8 years	28.2 (2.5)	39.9 (0.5)	1.14 (0.09)	40.9 (4.4)	4.9
3.0 years	12.4 (1.2)	44.1 (0.9)	1.98 (0.54)	28.0 (7.1)	5.8
4.0 years	6.7 (0.2)	35.6 (0.9)	2.48 (0.03)	16.8 (0.2)	6.1
Mangrove leaves					
Senescent	100	46.3	0.89	60.6	6.4
42 days	68.7 (0.7)	48.7 (0.2)	1.05 (0.06)	54.0 (3.0)	nd
189 days	37.9 (1.4)	50.5 (0.2)	1.74 (0.02)	33.9 (0.3)	5.7
1.0 years	19.0 (0.8)	47.5 (0.4)	2.61 (0.10)	21.3 (1.0)	6.1
1.8 years	9.1 (1.0)	37.7 (4.1)	2.80 (0.30)	15.7 (0.1)	7.4
3.0 years	3.7 (0.4)	27.9 (3.4)	2.40 (0.33)	13.6 (0.3)	8.8
4.0 years	2.6 (0.8)	22.2 (0.4)	1.86 (0.04)	14.0 (0.1)	8.7

Values in parentheses represent the percent mean deviation from duplicate litter bags. *Abbreviations used:* AFDW, ash-free dry weight; wt, weight; nd, not determined.

After 4 year, only 6.7 and 2.6% of the initial ash-free dry weights remained. The 4-year detritus had a brown-black appearance, similar to humic acids, in stark contrast to the yellow-beige senescent tissues.

Fluctuations in the organic C content (weight %, Table 1) were mostly related to changes in ash content (Opsahl and Benner, 1995). As a result, the ash-corrected weight %C values were less variable with average values of 47% (±3%) for cordgrass and 52% (±6%) for mangrove leaves (data not shown). The behavior of N was very different from that of C. The ash-free dry weight %N increased ~4-fold in both detritus during the 4-year experiment, resulting in a large decrease in C/N atomic ratios (Table 1). One exception to this trend was the increase of the C/N atomic ratio, from 62 to 87 during the leaching phase in cordgrass detritus. After this phase when net N-losses were important, the quantity of N in the litter bags remained fairly constant between 42 and 189 days, a period when bulk C- and lignin-losses were extensive, especially in mangrove leaf detritus (Figs. 1A and C). This finding suggests that N-losses were counterbalanced by N-immobilization during this period of intense microbial degradation. Following this period, N-losses offset N-immobilization in both detritus, but the resulting net N-losses occurred at a rate lower than those of net C- and lignin-losses.

Stable N isotopic compositions (δ^{15}N) exhibited important changes during decomposition (Table 1). In cordgrass, the δ^{15}N value was 1.2‰ in senescent tissue and steadily increased to 6.1‰ after 4 years. The δ^{15}N value of mangrove leaves was affected by leaching, which resulted in a 0.7‰ decrease along with major weight losses. However, after 189 days of submersion, δ^{15}N values increased, like in cordgrass detritus, from 5.7‰ to a maximum of 8.8‰.

3.2. Amino sugars, amino acids, and biomarkers

The percentage of C as total hydrolyzable amino sugars (THAS or AS) and amino acids (THAA or AA) increased by up to 30-fold and 5-fold, respectively, during the decomposer phase (Table 2). Fig. 2 illustrates this trend and indicates that AA + AS represented between 9.7 and 12.9% of the C in the 4-year detritus. During the leaching phase, selective losses (vs. bulk C) of AS in mangrove leaves and AA in cordgrass were observed (Table 2). The absolute amount of AA (Figs. 1A and C) and AS (Figs. 1B and D) also generally decreased during the leaching phase. Then, between 42 and 189 days, a net immobilization of AA in cordgrass detritus and a stabilization of AA amount in mangrove leaf detritus were observed. After 189 days, net losses of AA, similar to bulk N, were measured. Net immobilization of AS was also observed in the first year of submersion, but to a much greater extent (Figs. 1B and D). After 1 year, the amount of hydrolyzable AS exceeded the amount present in the initial senescent tissues (up to 250% in cordgrass). To produce such net increases, these inputs of biomolecules were quite large considering the important weight losses during decomposition.

The mol % galactosamine (GalN) increased dramatically in both detritus during decomposition, whereas the mol % glucosamine (GlcN) declined (Table 2). The mol % muramic acid (Mur) increased during the first 189 days of decomposition, during the peak of microbial activity, and then declined (Table 2). Mannosamine data were not included in Table 2, but its minor contribution to total AS (~1–11%) can be calculated by difference. The maximum amount of Mur was measured before the maximum of total AS in both detritus (Figs. 1B and D) and could be a consequence of the relatively labile nature of Mur.

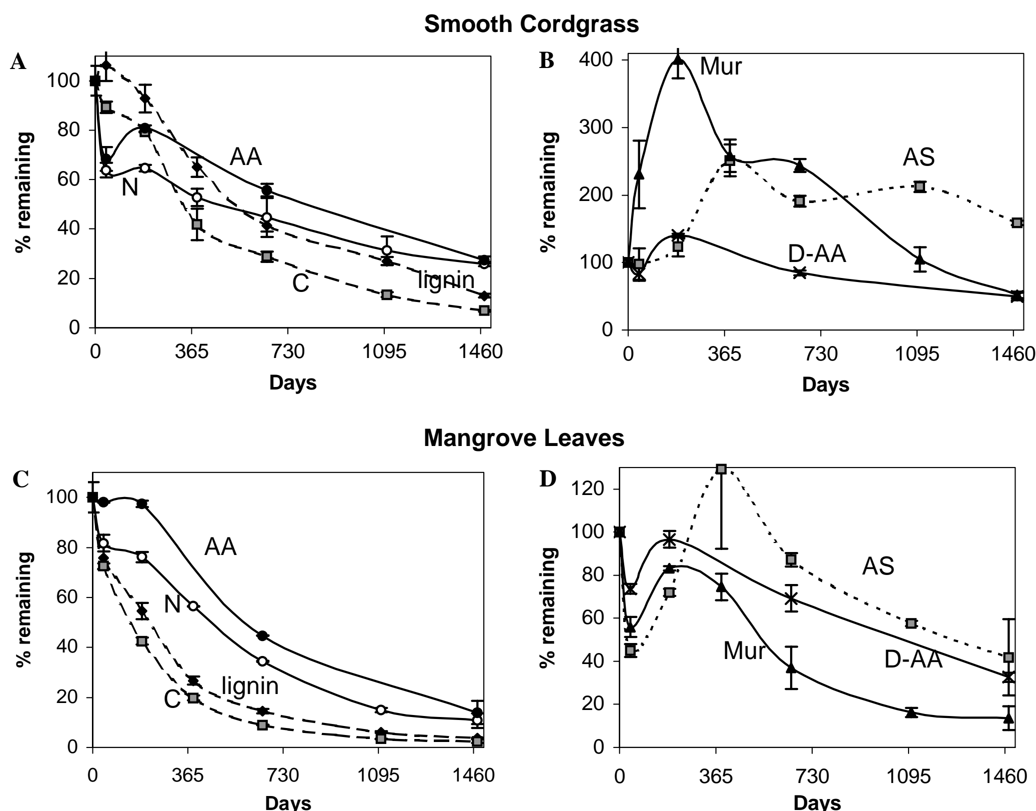

Fig. 1. Percentages of initial carbon, nitrogen, hydrolyzable amino sugars (AS), muramic acid (Mur), total and D-amino acids (AA, D-AA), and lignin phenols (from Opsahl and Benner, 1995) remaining in smooth cordgrass and mangrove leaf detritus during decomposition. Error bars represent percent mean deviations from duplicate samples.

Table 2
Yields and molar compositions of hydrolyzable amino sugars and total L and D amino acid enantiomers in plant detritus over the decomposition time series

Sample	%C as THAS	%N as THAS	GlcN (mol %)[a]	GalN (mol %)[a]	Mur (mol %)[a]	%C as THAA	%N as THAA	D-AA (mol %)[b]
Smooth cordgrass								
Senescent	0.04	0.39	70.1	14.2	3.8	2.26	41.5	1.2
42 days	0.04 (0.01)	0.58 (0.11)	67.3 (4.2)	18.2 (1.5)	9.1 (1.6)	1.73 (0.19)	43.9 (4.9)	1.4 (0.1)
189 days	0.06 (0.01)	0.72 (0.09)	52.6 (3.4)	28.2 (0.9)	12.8 (1.1)	2.30 (0.04)	51.1 (0.2)	2.0 (0.05)
1.0 years	0.24 (0.06)	1.88 (0.30)	70.3 (4.6)	23.5 (4.0)	4.0 (0.9)	nd	nd	nd
1.8 years	0.26 (0.01)	1.71 (0.23)	51.0 (0.7)	42.1 (2.2)	4.9 (0.1)	4.35 (0.10)	52.6 (6.6)	1.8 (0.05)
3.0 years	0.61 (0.06)	2.76 (0.41)	65.3 (1.8)	31.5 (3.0)	1.9 (0.3)	nd	nd	nd
4.0 years	0.88 (0.02)	2.43 (0.03)	53.6 (0.5)	43.7 (2.1)	1.2 (0.2)	8.86 (0.07)	44.5 (1.0)	2.1 (0.2)
Mangrove leaves								
Senescent	0.05	0.53	87.0	6.1	5.5	2.19	40.8	1.3
42 days	0.03 (0.01)	0.29 (0.01)	65.2 (0.7)	12.4 (0.2)	6.7 (0.6)	2.96 (0.05)	48.1 (2.2)	1.0 (0.01)
189 days	0.09 (0.01)	0.50 (0.01)	59.3 (0.3)	33.3 (1.1)	6.3 (0.2)	5.02 (0.10)	50.5 (0.6)	1.2 (0.04)
1.0 years	0.35 (0.08)	1.23 (0.35)	58.1 (7.3)	37.6 (0.9)	3.1 (0.2)	nd	nd	nd
1.8 years	0.53 (0.02)	1.37 (0.06)	42.5 (0.6)	52.2 (1.2)	2.2 (0.5)	10.9 (0.1)	52.2 (0.7)	2.1 (0.2)
3.0 years	0.92 (0.02)	2.08 (0.08)	50.6 (0.1)	44.6 (1.2)	1.5 (0.2)	nd	nd	nd
4.0 years	0.87 (0.15)	2.01 (0.34)	38.0 (5.6)	56.3 (2.3)	1.8 (0.2)	12.0 (0.7)	52.3 (3.4)	2.5 (0.3)

Values in parentheses represent the percent mean deviation from duplicate litter bags. *Abbreviations used:* THAS, total hydrolyzable amino sugars, GlcN, glucosamine; GalN, galactosamine; Mur, muramic acid; THAA, total hydrolyzable amino acids; nd, not determined.
[a] Includes mannosamine.
[b] Total D-AA × 100/total THAA.

The mol % of D-AA increased gradually during the course of the experiments (Table 2). A net immobilization of Mur (up to 400% of initial quantity) and D-AA in detritus was observed in the first 189 days (Figs. 1B and D). These bacterial biomarkers contain N and their contribution to bulk N increased as bulk δ^{15}N values increased.

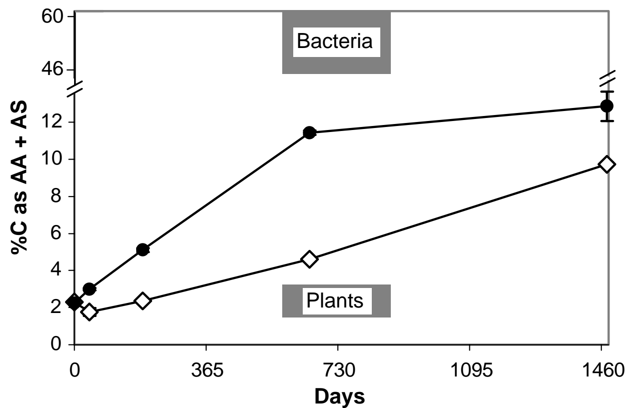

Fig. 2. Percentages of total C as hydrolyzable amino acids (AA) and amino sugars (AS) in decaying smooth cordgrass (◇) and mangrove leaves (●). Values for plants are from this study and values for bacteria are from Cowie and Hedges (1992) and Benner and Kaiser (2003). Error bars represent mean deviations from duplicate samples.

The percentage of total N in AS increased during decomposition and reached a maximum (~2–3%) after 3 years (Table 2). AA already accounted for a high percentage of total N in senescent tissues (~41%) and this percentage increased minimally during decomposition. Such values are expected in vascular plant tissues, other than wood, and in bacteria (Cowie and Hedges, 1992). There was a strong linear regression between the N content in AA + AS and bulk N content in detritus from both plants ($R^2 = 0.992$, $p < 0.001$) (Fig. 3). Therefore, changes in the AA + AS content in detritus reflected changes in total N, including the molecularly unidentified N (~50% of N). In other words, the unidentified N fraction followed AA + AS content. There was no indication of slow (i.e., rate significantly lower than immobilization) transformations of AA and AS into other N-containing molecules or into hydrolysis resistant AA and AS. The slope of this line indicates that, on average, AA and AS accounted for 54% (±2%) of total N in both decaying plant detritus. AA alone accounted for 52% of total N (linear regression not shown, $R^2 = 0.990$, $p < 0.001$).

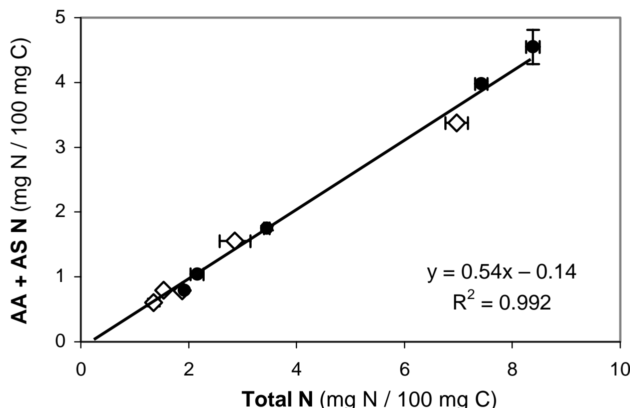

Fig. 3. Sum of nitrogen yields from hydrolyzable amino acids (AA) and amino sugars (AS) versus total nitrogen yields in smooth cordgrass (◇) and mangrove leaf (●) detritus. A linear regression of the data is shown. Error bars represent mean deviations from duplicate samples.

The mol % of individual AA was relatively constant throughout the experiment (Fig. 4) despite the large increases in AA concentrations and microbial contributions. Similar AA compositions in diverse organisms have been observed (Cowie and Hedges, 1992). Minor decreases in the mol % Lys and Glu, and increases in the mol % Thr and Gly were measured. Such trends have been reported during sedimentary organic matter diagenesis (Keil et al., 2001). Non-protein AA (e.g., β-Ala) increased minimally during decomposition (data not shown).

The D-enantiomers of Ser, Leu, Glu, Ala, and Asp were measured at all time points (Fig. 5). These molecules have been reported in bacterial peptidoglycan and in some bacterial antibiotics (Schleifer and Kandler, 1972; Yang et al., 2003). Other D-AA (e.g., D-Arg or D-Val) were detected but could not be reliably quantified because of very low concentrations or coelution problems (see Section 2). Overall, the percentage D-AA increased in both detritus during decomposition. However, there were differences between the two detritus in the dynamics of D-AA during the leaching phase. During this period the percentage D-enantiomer increased for most AA in cordgrass, whereas the percentage D-enantiomer decreased for most AA in mangrove leaves.

Senescent cordgrass and mangrove leaves had GlcN/GalN ratios of ~5 and ~14, respectively, but these ratios decreased rapidly during decomposition and stabilized, between 0.7 and 3, after 189 days (Fig. 6). The GlcN/GalN ratio appears to be a reliable AS source marker. Values be-

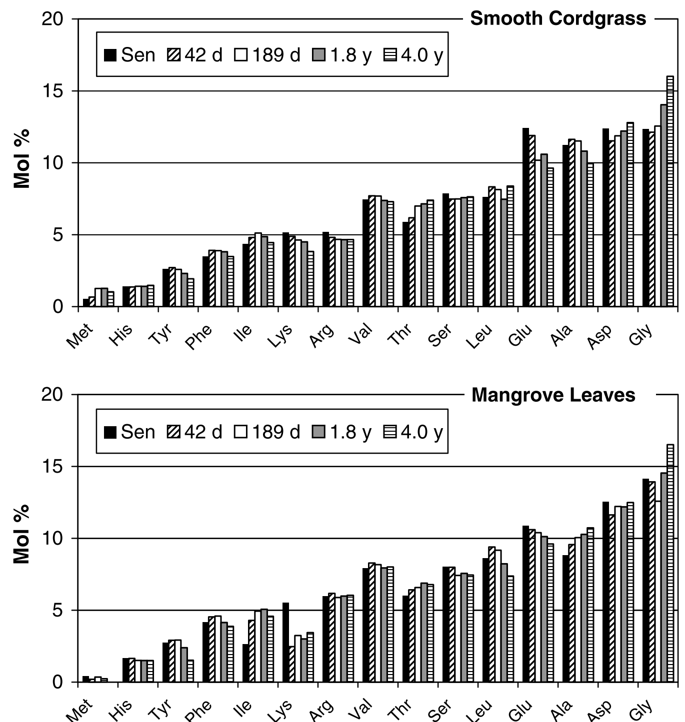

Fig. 4. Mole percent compositions of hydrolyzable amino acids during the decomposition of plant detritus. Sen, senescent tissues; see Section 2 for the other abbreviations.

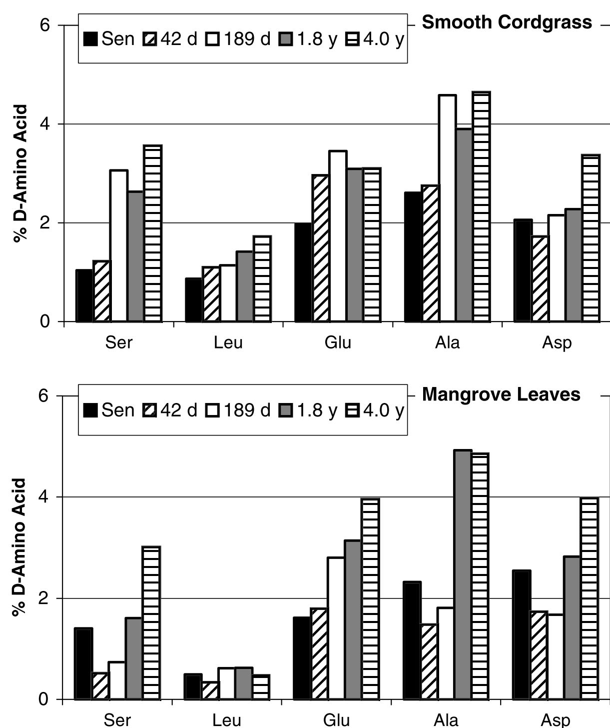

Fig. 5. Molar percentages of D-enantiomers (D × 100/(D + L)) for specific amino acids during the decomposition of plant detritus. Sen, senescent tissues; see Section 2 for the other abbreviations.

tween 4 and 20 are typical of plants, fungi, and zooplankton (Benner and Kaiser, 2003; Glaser et al., 2004; Sharon, 1965). In contrast, soil bacteria (Glaser et al., 2004; Solomon et al., 2001; Zhang and Amelung, 1996) and marine Gram-negative bacteria (Benner and Kaiser, 2003) have GlcN/GalN ratios lower than ~3. Similarly low values were measured in marine particulate and dissolved organic matter from different regions (Benner and Kaiser, 2003; Gupta and Kawahata, 2003; Liebezeit, 1993; Niggemann, 2005), indicating that this compositional uniformity is rapidly imprinted and diagenetically insensitive. GlcN/GalN ratios lower than 3 are thus consistent with AS mostly of prokaryotic origin.

GlcN/Mur ratios also decreased during the first 189 days when attached microorganisms were very active (Fig. 6). The lowest values (~4–10) were in the range of values measured in bacteria (Benner and Kaiser, 2003; Glaser et al., 2004). Intact bacterial peptidoglycan, which exhibits a 1:1 GlcN/Mur ratio, does not appear to be a major contributor of GlcN, total AS, and N (Pedersen et al., 2001). After 189 days of decomposition, the GlcN/Mur ratios increased (up to ~42) and exceeded the initial value in senescent tissues. This trend during decomposition was also observed for D-Ala/Mur ratios, which increased from 2 at 189 days to 7 after 3–4 years (Fig. 6). D-Ala and Mur are only prevalent in bacteria (Asano and Lübbehüsen, 2000; Brock et al., 1994) at ratios lower than 3 (Kaiser and Benner, unpublished data), even in Gram-positive bacteria having D-Ala-rich peptidoglycan (Schleifer and Kandler, 1972) and teichoic acids. These findings suggest that Mur is more labile than GlcN, GalN, and D-AA. Therefore, GlcN/Mur ratios, previously used to distinguish fungi from bacteria (Solomon et al., 2001; Zhang and Amelung, 1996), and D-Ala/Mur ratios appeared diagenetically sensitive. D-AA/L-AA, Mur/lignin, and GalN/lignin ratios all increased during decomposition (Fig. 6), confirming the increasing contributions of microbial compounds to the plant detritus.

3.3. Proportions of immobilized N and microbial C and N

Three independent approaches were used to quantify the percentage of detrital N derived from N-immobilization and from microorganisms. In the first approach, the percentage of immobilized N (% N_{immob}) was calculated using the following equation:

$$\%N_{immob} = 100(N_{sample} - N_{plant})/N_{sample}, \quad (1)$$

where N_{sample} is the N content of the detritus sample and N_{plant} is the N from the original plant tissue. The N_{plant} from the senescent tissues (time 0) remaining at time t was determined assuming that N_{plant} had the same reactivity as bulk C or plant lignin during decomposition:

$$N_{plant,t} = N_{plant,0}(C_t/C_0) \text{ or } = N_{plant,0}(\text{lignin}_t/\text{lignin}_0). \quad (2)$$

This "plant biomarker" approach indicated that the percentage immobilized N after 4 years of decomposition ranged from 50 to 79% (Fig. 7). The calculated proportions of immobilized N in the detritus based on lignin (L) were lower than those based on bulk C because lignin is more resistant to biodegradation. In cordgrass, this approach predicted no immobilized N at 189 days because the N content decreased more than those of C and lignin during leaching. The opposite trend (i.e., lower net N-loss than net C- and lignin-losses) in all the other samples suggests that N-immobilization occurred despite net N-losses.

The assumption that the detrital N is not more refractory than lignin appears to be valid based on studies of AA, the dominant N-containing molecules in plant tissues (Cowie and Hedges, 1992; Keil et al., 2001). Using ^{15}N as a tracer of plant N, White and Howes (1994) reported that detritus N-losses exceeded C-losses during cordgrass decomposition. In addition, bulk C includes immobilized C and thus overestimated N_{plant}. For all those reasons, this approach probably led to conservative estimates of the percentage immobilized N in the detritus.

A second approach to calculate percentage immobilized N was based on the stable N isotopic composition of the detritus (Table 1) and the following equation:

$$\delta^{15}N_{sample} = (\%N_{plant} \times \delta^{15}N_{plant}) + (\%N_{immob} \times \delta^{15}N_{immob}), \quad (3)$$

where $\delta^{15}N_{plant}$ is the isotopic composition of plant N and $\delta^{15}N_{immob}$ is the isotopic composition of the immobilized N. We assumed that the changes in $\delta^{15}N_{sample}$ were caused by immobilization of N having a different isotopic signature. The $\delta^{15}N_{sample}$ of mangrove leaf detritus decreased during leaching, so we selected the lowest $\delta^{15}N$ measured

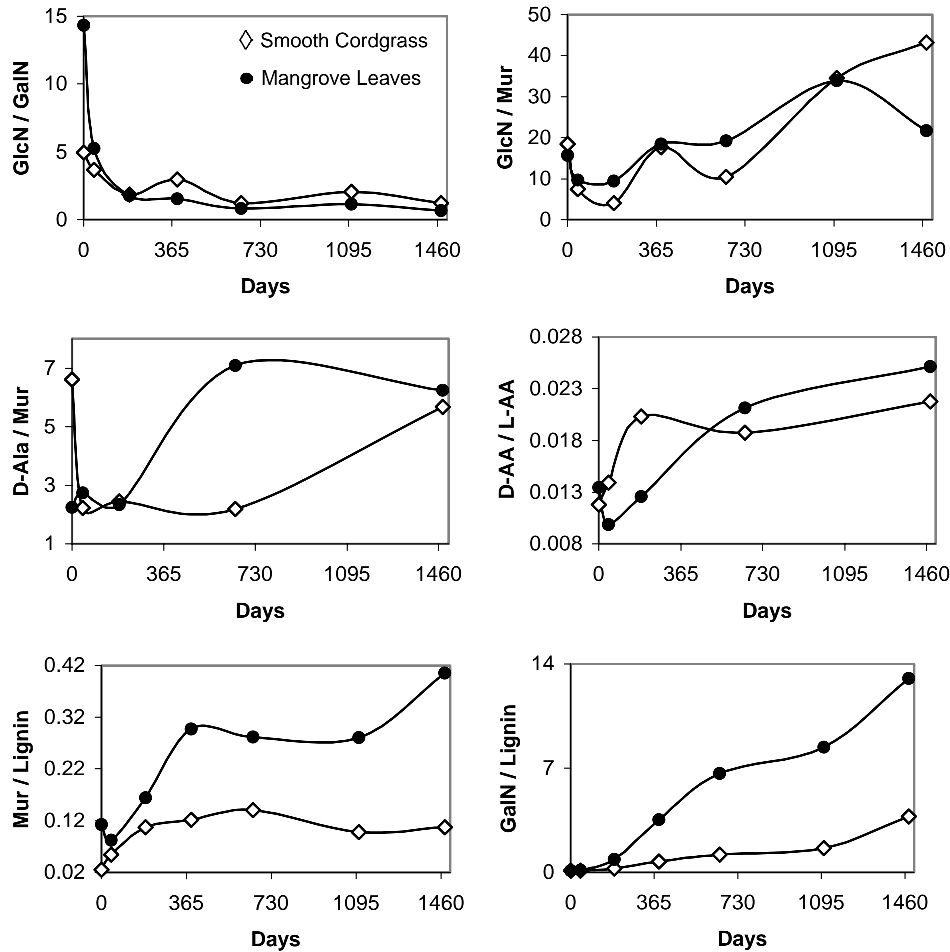

Fig. 6. Ratios of different biomolecules during the decomposition of plant detritus. Molar concentrations were used except for lignin phenols, which are expressed in milligram per 100 milligram organic carbon. See Table 2 for abbreviations.

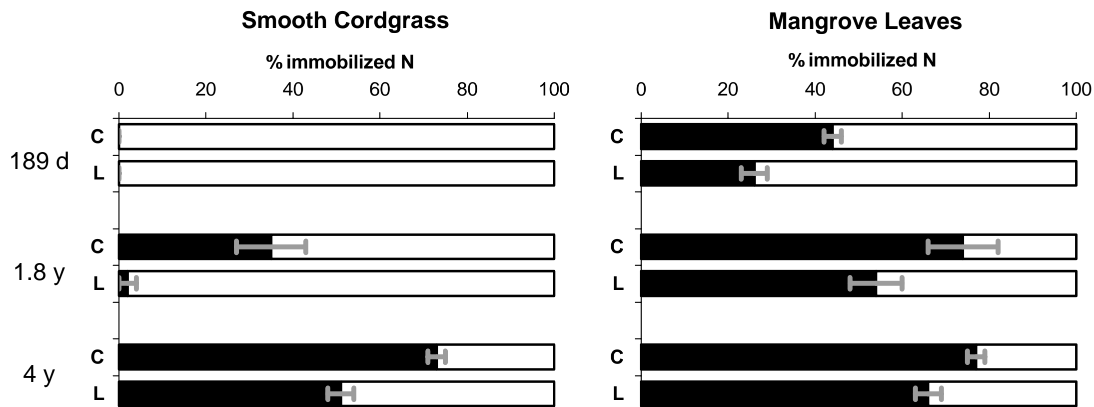

Fig. 7. Percentages of immobilized nitrogen (N) in plant detritus at three stages of decomposition. Values were calculated using Eqs. (1) and (2) (see text), and assuming that the N contribution from senescent plant tissues followed bulk C (C) or lignin phenols (L). Error bars represent percent mean deviations from duplicate samples.

(i.e., 5.7‰ at 189 days) as $\delta^{15}N_{plant}$ for this detritus instead of the senescent tissue value. Two values were estimated for $\delta^{15}N_{immob}$: 8.8‰ was chosen as the lowest limit because it equals the maximum $\delta^{15}N_{sample}$ measured in the detritus (i.e., $\%N_{immob} = 100\%$) and 11‰ was chosen as the highest limit. Values of $\delta^{15}N > 11$‰ are rarely found in particulate organic matter or in organisms of lower trophic levels (Cifuentes et al., 1989; Peterson and Howarth, 1987), because such intense fractionation is uncommon (see below). Based on these assumptions, the calculated percentages of N immobilized after 4 years of decomposition averaged 58% in cordgrass and 77% in mangrove leaves (Fig. 8). The val-

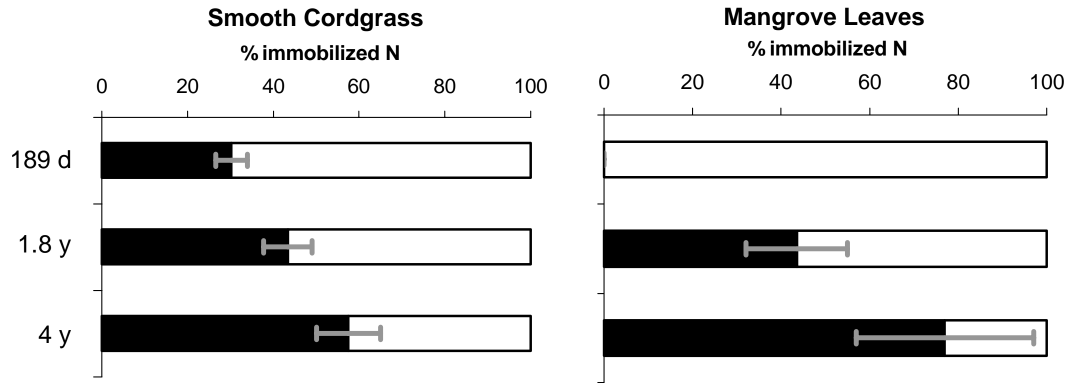

Fig. 8. Percentages of immobilized nitrogen (N) in plant detritus at three stages of decomposition. Average values from duplicate samples were calculated based on stable N isotopic compositions and Eq. (3) (see text) assuming a δ^{15}N for the immobilized N of 8.8 and 11‰. Error bars represent ranges derived from the assumed δ^{15}N of the immobilized N.

ues obtained at 189 days in mangrove leaves were zero because $\delta^{15}N_{sample}$ equals the assumed $\delta^{15}N_{plant}$.

Isotopic fractionation during decomposition could also increase $\delta^{15}N_{sample}$. However, the selective mineralization of specific and isotopically light classes of biomolecules is not supported by the present chemical characterization. A fairly constant AA composition and %N as AA were measured throughout the 4-year study in both detritus. Fractionation during microbial mineralization, after deamination and excretion of light ammonium (Macko and Estep, 1984; Freudenthal et al., 2001), was also probably of minor importance because regeneration of inorganic N is low when microbes are attached to substrates having C/N ratios greater than 14 (Pedersen et al., 1999; Tezuka, 1990). In N-depleted detritus, most of the N used by microorganisms is exogenous, more specifically nitrate and ammonium when available (Benner et al., 1988; Melillo et al., 1984). The δ^{15}N of these nutrients in coastal waters can vary from 4 to 40‰, with the more enriched values observed in nutrient poor regions under extensive biological isotopic fractionations (Cifuentes et al., 1989; Liu and Kaplan, 1989). The high nitrate and ammonium concentrations in the present waters (>20 μM) reduced the likelihood of such fractionation.

The third approach was based on the yields of Mur, GalN, and D-Ala in the detritus. Mur and D-Ala are bacterial biomarkers while GalN is also found in other microorganisms (Brock et al., 1994; Glaser et al., 2004; Sharon, 1965). Yields of these biomarkers were assumed to be proportional to bulk microbial C and N in the detritus (C,N_{microb}) by:

$$\%C,N_{microb} = 100[\text{biomarker}]_{sample}/[\text{biomarker}]_{microb}, \quad (4)$$

where $[\text{biomarker}]_{sample}$ and $[\text{biomarker}]_{microb}$ are the C- or N-normalized yields of the individual biomarker in the detritus and in heterotrophic bacteria (see Section 2.6), respectively.

All three biomarkers indicated a minor contribution of microbial C in senescent tissues (Fig. 9). The percentage of microbial C increased to 4–17% after 189 days of decomposition. Microbial C estimates in the highly decomposed detritus (4 years) were variable, with values ranging from 8 to >100% depending on the biomarker used. GalN provided the greatest estimates of microbial contribution (G in Fig. 9). Mur (M in Fig. 9) provided estimates lower than those of D-Ala (A in Fig. 9), which indicated that 23–35% of the C in 4-year detritus was of bacterial origin.

Estimates of the microbial contribution to N followed the same patterns as for C but were much greater (Fig. 9). The three biomarkers indicated that 10–36% of the N in senescent plant tissues was derived from microorganisms. When this contribution was subtracted from the senescent plant, the C/N ratios of the plant tissues increased to 68–94 for cordgrass and to ~76 for mangrove leaves. After 189 days of decomposition, all three biomarkers indicated that more than 60% of the N in cordgrass detritus was of microbial origin. The estimated percentages of microbial N were more variable (21–95%) in mangrove leaves at this time point. After 4 years, bacterial or microbial contributions to bulk N ranged from ~22% (based on Mur) to >100% (based on GalN) in both plant detritus. D-Ala provided intermediate estimates of 60–80% bacterial N in the two highly decomposed plant detritus.

Calculations based on GalN overestimated the microbial contributions (>100%) after a few years of decomposition. Lignin phenols indicated that plant C was always present in the detritus. Therefore, GalN seemed to be selectively preserved compared to bulk microbial C and N. In contrast, calculations based on Mur yields may have underestimated bacterial contributions to C and N in highly decomposed detritus. After an increase in N-normalized Mur yields during the first year of decomposition, we observed a significant decrease. In contrast, yields of the other bacterial and microbial biomarkers generally increased in the detritus. Considering that an important fraction of N came from bacteria, these findings indicate once again the relatively reactive nature of Mur. Changes in bacterial community structure during decomposition could influence the observed trends in biomarker yields. Mur and D-Ala yields vary among bacteria, particularly between Gram-

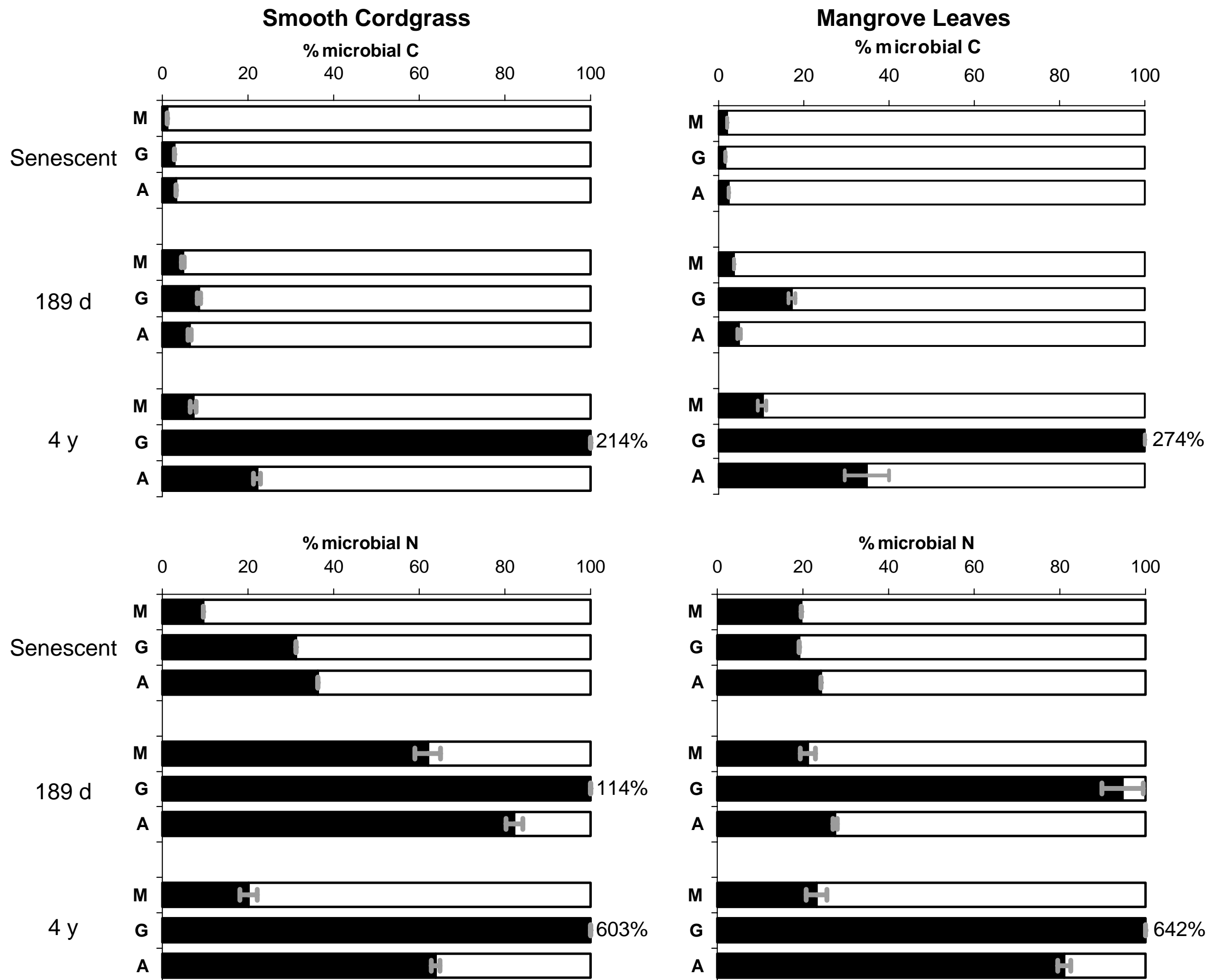

Fig. 9. Percentages of microbial or bacterial carbon (C) and nitrogen (N) in plant detritus at three stages of decomposition. Values were calculated using Eq. (4) (see text) and the C- or N-normalized yields of muramic acid (M), galactosamine (G), and D-alanine (A) measured in a mixture of heterotrophic bacteria (see Section 2 for details). Error bars represent mean percent deviations from duplicate samples.

negative and Gram-positive bacteria (Brock et al., 1994; Schleifer and Kandler, 1972). However, most bacteria in seawater and marine coastal sediments are Gram-negative (Giovannoni and Rappé, 2000; Jørgensen et al., 2003) as was assumed here (see Section 2.6).

4. Discussion

4.1. Microbial contributions to N-immobilization in plant detritus

The occurrence of exogenous N in decaying plant material was first revealed by elemental N measurements. The low initial N content of plant tissues allowed the observation of a net increase in N beyond what was possible with conservative behavior (Benner et al., 1990; Melillo et al., 1984; Rice, 1982; Rice and Tenore, 1981; White and Howes, 1994). However, the origin of this N and the mechanism of its immobilization remained speculative (Benner et al., 1991; Hernes et al., 2001; Hicks et al., 1991; White and Howes, 1994). Among the mechanisms proposed was the adsorption of inorganic N. Ammonium was not detected in significant amounts in the present study or in a previous study of aerobic decomposition (Hicks et al., 1991), indicating that N was immobilized mostly in organic form. Microorganisms have long been suspected as major sources of immobilized N (see references above). In the present study, the increase in N content after the leaching phase was positively related to the rate of detritus weight loss ($R^2 = 0.76$ and 0.95, data not shown), supporting the view that the microorganisms responsible for decomposition were also responsible for N-immobilization. However, several studies have shown that recognizable microbial cells or living bacteria and fungi only account for a small percentage of the immobilized N (Lee et al., 1980; White and Howes, 1994). Because the present experiments were conducted in subaqueous marine conditions, fungi were considered minor contributors to decomposition (Benner et al., 1986). Consequently, the

microbial contribution to N should be mostly from the remains of bacteria, including exudates like mucopolysaccharides and exoenzymes (Lee et al., 1980; Rice and Hanson, 1984).

The presence of bacterial organic matter in the detritus was confirmed here by the occurrence of biomarkers unique to bacteria (Mur), only prevalent in bacteria (D-AA), or virtually absent from higher plants (GalN) (Asano and Lübbehüsen, 2000; Brock et al., 1994; Sharon, 1965). Biomarker ratios revealed that while attached bacterial cells might have been important contributors of AS and D-AA during early stages of decomposition (42–189 days), bacterial contributions to organic matter and N in highly decomposed detritus appeared predominantly from cellular debris. This is consistent with the work done by Pedersen et al. (2001). Estimates of the percentage microbial N in highly decomposed detritus are summarized in Fig. 10. The average values obtained with the plant biomarker and the isotopic approaches were in very good agreement, with the average values estimated using bacterial biomarkers being somewhat lower. These three approaches indicated a microbial contribution to N of ~60% in cordgrass detritus and ~75% in mangrove leaf detritus. As suspected, it appears most of this N is of bacterial origin. Most of the AA and almost all of the AS found in decaying detritus were derived from bacteria. The independent nature of the three approaches greatly increases the degree of confidence in these average estimates.

The bacterial contribution to C was estimated using bacterial biomarker yields and by converting the percentage microbial N from the two other approaches using an atomic C/N ratio of 5 for bacteria. Results indicated that 20–40% of bulk C was of bacterial origin in highly decomposed detritus. These estimates are in agreement with the study of Harvey and Macko (1997) on bacterial biomass contributions to C in decaying phytoplankton.

One important assumption in the biomarker approach was that C- and N-normalized yields of the biomarkers were insensitive to diagenetic alterations. However, it appeared that the three microbial biomarkers had varying reactivities during diagenesis. The relative reactivity of Mur observed here is in agreement with previous studies (Hicks et al., 1991; Nagata et al., 2003; Ogawa et al., 2001). Mur seems to be a marker of living bacteria (Moriarty, 1977) and of relatively fresh bacterial necromass (Benner and Kaiser, 2003). On the other hand, GalN overestimated microbial contributions in highly decomposed detritus. The resistance of GalN and GlcN to decomposition relative to AA in sediments has been reported (Gupta and Kawahata, 2003). D-Ala had an intermediate reactivity, between Mur and GalN. The agreement between the bacterial biomarker approach based on D-Ala (high-limit error bars in Fig. 10) and the other independent approaches indicated D-Ala was representative of immobilized N in this study. This is consistent with the fact that AA represented a major and fairly constant percentage of bulk N. Likewise, D-Ala was also probably more representative of bacterial C dynamic than other biomarkers. Although AA are considered relatively reactive compared to bulk C (Cowie and Hedges, 1992; Keil et al., 2001), peptides containing D-enantiomers, mostly in peptidoglycan, appear to be more resistant to decomposition than those containing L-enantiomers (Jørgensen et al., 2003; Nagata et al., 2003; O'Dowd et al., 1999).

4.2. Uncharacterized N and the preservation of organic matter

About half of the bulk N in cordgrass and mangrove leaf detritus was identified as AA and the remaining half was molecularly uncharacterized throughout the 4-year study. AS accounted for a minor fraction of the bulk N in decaying plant detritus. Even though the molecular identity of the uncharacterized N remains unknown, we now know that most of this N was of bacterial origin in the highly decomposed detritus. As in the detritus, about half of the bulk N in bacterial cells is in AA (Brock et al., 1994; Cowie and Hedges, 1992). Most of the remaining N in bacteria resides in nucleic acids (Brock et al., 1994). It is intriguing to consider that nucleic acids and other

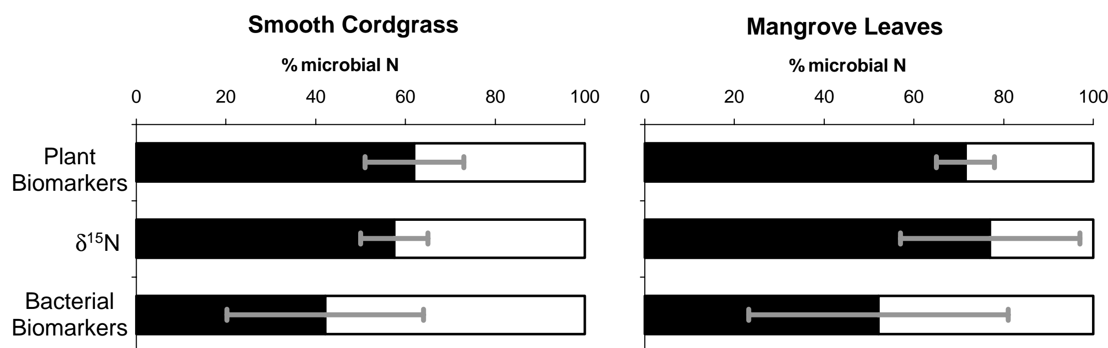

Fig. 10. Summary of the percentages of microbial nitrogen (N) in highly decomposed (4 year) plant detritus. Three independent approaches for estimating microbial N were used: C and lignin plant biomarkers, $\delta^{15}N$ values, and muramic acid and D-alanine bacterial biomarkers. Error bars represent ranges derived from different biomarkers or assumptions.

biomolecules of bacterial origin comprise a major fraction of the uncharacterized N in cordgrass and mangrove leaf detritus.

Regardless of its molecular composition, uncharacterized N had a net reactivity very similar to that of AA. The similar dynamics and tight coupling between the characterized and uncharacterized fractions of N provide additional evidence indicating the two fractions were immobilized primarily by bacterially mediated processes. This finding is supported by the relationship between bacterial activity and N-immobilization rate. These observations are inconsistent with classical models of humification processes. In those models, the organic matter composition slowly changes due to condensation reactions among low-molecular-weight decomposition products (Hedges, 1988). In the present study, there was no indication of abiotic production of uncharacterized N from AA. Instead, it appeared the detrital N acquired a biochemical composition similar to that of bacteria, the primary decomposers, and the rate of acquisition of this biochemical signature was directly related to the rate of microbial decomposition.

The observation that bacterial organic matter became prevalent during decomposition could give the impression that some components of bacterial cells are highly resistant to decomposition. The bioreactivities of bacterial C and N were calculated by estimating the amount of bacterial C and N produced during the decomposition study and the amount of bacterial C and N remaining at the end of the study. Bacterial growth efficiencies of 30% are common on plant tissues like cordgrass and mangrove leaves in estuarine waters (Benner et al., 1988). Assuming 30% C conversion efficiencies, we estimated bacterial C production based on the C loss from plant tissues during decomposition. Bacterial N production was estimated from the C production value using an atomic C/N ratio of 5. About 94–97% of the C and N produced by bacteria on both plant detritus was decomposed during the study. These are likely conservative estimates because we did not consider the production of attached bacteria utilizing dissolved organic matter. Although some individual components have varying dynamics (e.g., GalN and lignin vs. Mur), these calculations indicate that on average plant tissues and bacteria have similar bioreactivities.

Even though most of the plant and bacterial tissues were remineralized during early diagenesis, a small fraction of the organic matter from these sources was preserved during the 4-year study. The mechanisms responsible for rapidly converting reactive biomolecules into relatively unreactive organic matter remain unclear. Mineral sorption (e.g., Hedges and Keil (1995) was unlikely to be important because mineral surfaces, such as in sediments, were largely absent from the experiments. Condensation reactions between quinones, derived from phenolics, and amines have been proposed as an important mechanism in N-immobilization and preservation (Buchsbaum et al., 1991; Hernes et al., 2001; Lee et al., 1980; Melillo et al., 1984; Rice, 1982; Rice and Hanson, 1984; Suberkropp et al., 1976). The N and lignin contents in cordgrass and mangrove leaf detritus were significantly correlated ($R^2 = 0.63$ and 0.81) in the present study. However, if condensation and humification reactions between phenolics and amines occurred they were relatively rapid and did not alter the fraction of total N recognized as AA + AS.

The relationship observed herein between bacterial activity and N-immobilization is consistent with an enzymatically mediated preservation mechanism. Ogawa et al. (2001) observed the rapid production of refractory organic matter during bacterial growth on simple organic substrates. In their work only a small fraction of the organic matter produced by bacteria remained after 1 year, and this preserved organic matter had AA and AS compositions similar to those of the preserved organic matter in the present study. Ogawa et al. (2001) speculated that promiscuous or alternative enzymatic activities (see O'Brien and Herschlag, 1999) altered the structure and reactivity of biomolecules in a few days. These altered biomolecules could escape recognition during bacterial decomposition reducing their overall turnover rate. The slow remineralization of the altered biomolecules could depend on further promiscuous enzyme activities. This mechanism is appealing because it directly links the processes of microbial decomposition and organic matter preservation. Such biologically mediated processes could be important in regulating organic matter preservation and the global C cycle. Compositional similarities observed in aged organic matter from contrasting sources, such as the surprising convergence of C/N ratios (~12) (Hedges and Oades, 1997; Hedges et al., 2001), are consistent with this view. This study suggests that microbial contributions to non-living organic matter and its preservation are greater than previously suspected.

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