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Organic matter diagenesis and bacterial contributions to detrital carbon and nitrogen in the Amazon River system

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Abstract

Amino sugars and amino acids, including the bacterial biomarkers muramic acid and D-amino acids, were measured to investigate the diagenetic alterations of and microbial contributions to detrital organic matter in the Amazon River system. Three size fractions of detrital material were analyzed: coarse and fine particulate organic matter (CPOM and FPOM), and ultrafiltered dissolved organic matter (UDOM). CPOM was depleted in amino sugars and had high percentages of total N as amino acids (32–37%), consistent with relatively fresh plant debris being a major source of CPOM. FPOM had the highest percentages of total C as amino acids (5–8%), and its composition appeared to be influenced by the preferential sorption of N-containing molecules. UDOM had the highest percentages of total N as amino sugars (up to 2.3%) indicating the relatively important contributions from microorganisms. A consistent trend of increasing proportions of D-amino acids among detrital size fractions, CPOM < FPOM < UDOM, suggests this parameter is a useful diagenetic indicator. About 4–17% of the C and 17–37% of the N in FPOM and CPOM were of bacterial origin based on D-alanine and D-glutamic acid yields. Similar bacterial contributions to UDOM were evident from the biomarker data, but they were not quantified due to insufficient information about representative yields of source biomarkers. Relationships between biomarker N and total N suggest that microbial activity influences the N content of detrital material. Bacterial contributions to detrital material were primarily as cellular remnants rather than living cells.

Biomolecules undergo major transformations in natural systems following the death of the source organisms. Heterotrophic microorganisms are the primary agents of decomposition, and they exert a strong influence on both the amount and composition of preserved organic matter. Over half of the nonliving, or detrital, organic matter in terrestrial and aquatic environments remains unidentified at the molecular level (Hedges et al. 2000*a*), confounding efforts to determine its origins and diagenetic alterations. Bacterial activity appears to play a central role in the production of uncharacterized molecules and organic matter preservation (Harvey and Macko 1997; Ogawa et al. 2001; Tremblay and Benner 2006).

Several studies have confirmed the occurrence of bacterial biomarkers in detrital organic matter from different environments (McCarthy et al. 1998; Glaser et al. 2004; Niggeman and Schubert 2006). Among these biomarkers are specific amino sugars and amino acids. The amino sugar muramic acid (Mur) is only found in the bacterial cell wall polymer, peptidoglycan (Sharon 1965; Schleifer and Kandler 1972). The D-enantiomers of amino acids (D-AA) occur in peptidoglycan as well as in other bacterial macromolecules but are not common to plants or higher organisms (Asano and Lübbehüsen 2000; Kaiser and Benner 2008). These biomarkers can be used to estimate the contribution of bacteria to detrital reservoirs of organic matter.

Variable dynamics of bacterial biomarkers have been observed (Jørgensen et al. 2003; Nagata et al. 2003) and could complicate the quantification of bacterial contributions to nonliving organic matter. For instance, the bacterial components that are selectively incorporated in particulate and dissolved organic matter (POM and DOM) can be different (Kawasaki and Benner 2006; Kaiser and Benner 2008). The biomarkers and assumptions used must be carefully selected based on knowledge of relative reactivities (Hedges and Prahl 1993). This knowledge can also be useful for assessing the degree of alteration, or diagenetic state, of organic matter.

The main objective of the present study was to use amino sugar and amino acid biomarkers to estimate bacterial contributions to and transformations of organic matter in the Amazon River system. The Amazon is the largest river in the world, accounting for nearly 20% of the global freshwater discharge and an annual discharge of 36.1×10^{12} g of organic carbon to the Atlantic Ocean (Richey et al. 1990). POM and DOM in the Amazon River system is largely derived from soils and much of it has undergone substantial diagenetic alterations (Hedges et al. 1994, 2000*b*; Aufdenkampe et al. 2007). The chemical composition and diagenetic alterations of POM and DOM in the Amazon River system have been extensively studied (*see* reviews by Devol and Hedges 2001; Mayorga and Aufdenkampe 2002). In particular, major differences in the diagenetic state were observed among three size fractions of organic matter: coarse POM (CPOM, >63 μm), fine POM (FPOM, 0.1–63 μm), and ultrafiltered DOM (UDOM, ~0.001–0.1 μm). CPOM appeared as a heterogeneous mixture of sand and relatively fresh (mean age <3 yr; Hedges et al. 1986*b*) angiosperm leaf debris. FPOM was the oldest fraction (most of FPOM is >30 yr old; Hedges et al. 1986*b*) and was relatively enriched in nitrogen relative to carbon. The preferential sorption of basic amino acids on fine particles has been seen as the

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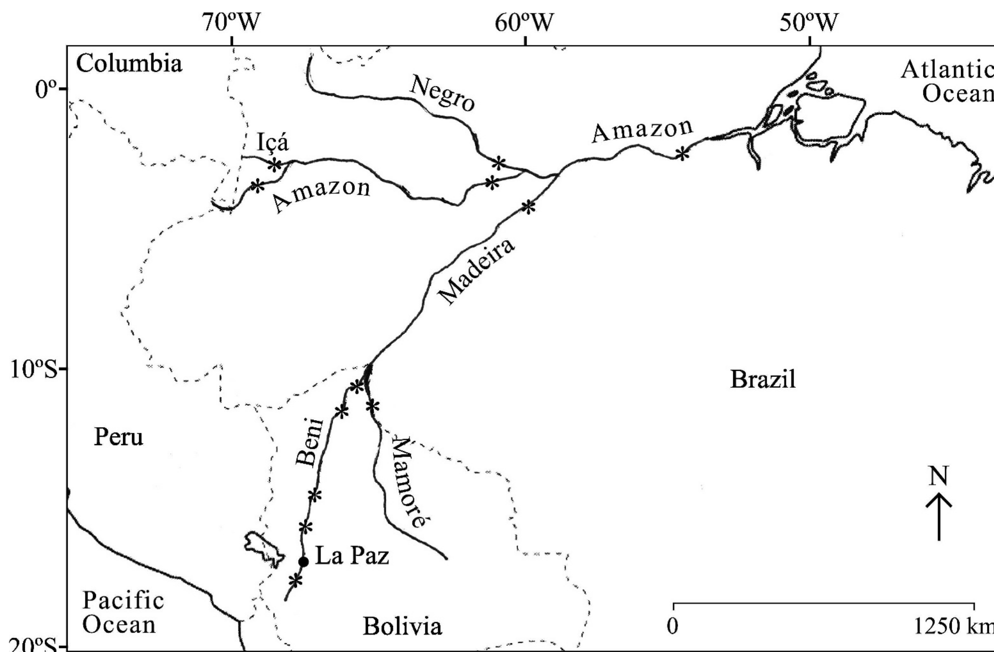

Fig. 1. Map with sampling sites marked by asterisks.

most plausible explanation for this enrichment (Hedges et al. 1994, 2000*b*; Aufdenkampe et al. 2001). UDOM was poor in nitrogen, amino acids, and aldoses, and appeared the most altered organic fraction. Surprisingly, only minor compositional differences were observed within each size fraction along a ~3000-km reach from Bolivian tributaries to the Amazon main-stem (Hedges et al. 1994, 2000*b*). Downstream trends in organic matter composition appeared more pronounced in the Peruvian headwaters (Aufdenkampe et al. 2007). Nevertheless, most of the diagenetic alterations seem to occur in soils before the organic matter enters the river system. Evidence of bacterial contributions to Amazon River organic matter have been observed, including relatively low C : N ratios compared with those in local plants and an abundance of deoxy sugars (Hedges et al. 1994, 2000*b*). However, the present study is the first to directly measure bacterial biomarkers and to estimate bacterial contributions to detrital organic matter in the Amazon River system.

Methods

Study area—A map showing the sampling sites is presented in Fig. 1. Brazilian samples come from three main-stem locations on the Amazon River and from three of its major tributaries: the Madeira, Negro, and Içá rivers. These samples were collected between April and May 1990 near the seasonal peak discharge. The other samples were isolated from waters collected in Bolivian tributaries in November 1994 in the early rising stage of the hydrograph. The Bolivian samples were collected from a first-order stream (Achumani) in the Andes and from the Beni and Mamoré rivers, which form the Madeira River. More site characteristics can be found in two previous studies (Hedges et al. 1994, 2000*b*).

Sampling—The materials studied in the present work were isolated from water samples and separated in three size fractions as detailed by Hedges et al. (1994, 2000*b*). Briefly, coarse particles were separated by sieving onto a 63-μm Nitex screen (Tetko). Fine particles (>0.1 μm) and UDOM from the Bolivian rivers were isolated by ultrafiltration on an Amicon DC-10 unit after removal of coarse particles. The water that permeated the 0.1-μm filter cartridge was then passed through tandem Amicon S10-N1 tangential flow ultrafilters with a nominal cutoff size of 1000 Daltons (~1-nm pore) to isolate the UDOM fraction. UDOM from the Brazilian rivers was isolated by ultrafiltration after passage through 3.0-μm and 0.2-μm pore-size Nuclepore cartridge filters. UDOM comprised 40% of the total dissolved organic carbon in the Achumani sample but this percentage gradually increased downstream to reach ~80% in the lower Madeira and the Amazon rivers (Hedges et al. 1994, 2000*b*).

Elemental analyses—Weight percentages of organic carbon (%OC) and total nitrogen (%N) were measured with Carlo Erba CHN analyzers models 1106 and 1108 (Hedges et al. 1994, 2000*b*; Table 1). Dissolved organic carbon (DOC) concentrations were measured on acidified and purged samples with Shimadzu high-temperature catalytic oxidation analyzers models TOC-500 and TOC-5000. All DOC concentrations were corrected for the system blank (Benner and Strom 1993). These results can be found in two previous studies (Hedges et al. 1994, 2000*b*).

Amino sugar analyses—Details of sample preparation and chromatography for amino sugars are described in Kaiser and Benner (2000). Dried particles (50–200 mg) and UDOM powders (3–5 mg) were hydrolyzed with 3 mol L^{-1} HCl at 100°C for 5 h in sealed ampoules. Tests

Table 1. Organic carbon and nitrogen contents, yields and molar compositions of amino sugars and amino acid enantiomers in samples of different size fractions. Abbreviations: OC = organic carbon; N = total nitrogen; THAS = total hydrolyzable amino sugars; GlcN = glucosamine; GalN = galactosamine; ManN = mannosamine; Mur = muramic acid; THAA = total hydrolyzable amino acids; UDOM = ultrafiltered dissolved organic matter; FPOM = fine particulate organic matter; CPOM = coarse particulate organic matter; Ó = Óbidos; M = Manacapurú; VG = Vargem Grande; RB = Riberalta-Bajo; RA = Riberalta-Arriba; R = Rurrenabaque.

River sampled	Size fraction	OC (wt %)	N (wt %)	%C as THAS	%N as THAS	GlcN (mol %)*	GalN (mol %)*	ManN (mol %)*	Mur (mol %)*	%C as THAA	%N as THAA	D-AA (mol %)†
Amazon												
Ó	UDOM	36.7	1.26	0.35	1.96	63.0	34.5	2.0	0.5	1.17	11.9	4.77
M	UDOM	26.4	0.86	0.32	1.92	60.6	35.0	3.6	0.8	1.31	13.9	4.67
VG	UDOM	24.1	0.87	0.39	2.12	62.0	35.4	2.0	0.6	1.92	18.6	3.89
Negro	UDOM	42.9	0.96	0.25	2.20	65.1	32.5	1.8	0.6	0.91	14.4	3.63
Içá	UDOM	36.2	1.26	0.42	2.34	64.2	32.6	2.5	0.7	1.57	15.5	3.52
Madeira	UDOM	28.6	1.20	0.46	2.12	63.3	32.8	3.3	0.6	1.67	14.1	4.28
Mamoré	FPOM	0.78	0.13	0.45	0.52	54.1	39.9	3.9	2.1	8.15	17.3	2.04
	UDOM	16.2	0.72	0.40	1.73	57.4	36.3	4.7	1.6	2.07	16.3	4.82
Beni												
RB	CPOM	0.25	0.05	0.34	0.33	63.4	35.1	0.2	1.3	5.52	9.5	1.61
	FPOM	0.74	0.13	0.43	0.47	53.4	43.0	1.5	2.1	8.30	17.0	2.18
	UDOM	21.9	0.96	0.36	1.59	59.7	32.8	5.2	2.3	2.65	21.1	3.25
RA	CPOM	0.29	0.05	0.26	0.29	62.0	35.5	1.0	1.5	5.91	11.8	2.22
	FPOM	0.55	0.10	0.41	0.44	52.0	44.9	1.3	1.8	7.59	14.8	2.45
	UDOM	24.8	0.97	0.34	1.66	58.8	35.7	3.8	1.7	1.71	15.4	4.52
R	CPOM	1.84	0.09	0.17	0.66	67.4	29.0	1.1	2.5	5.07	36.6	1.70
	FPOM	1.26	0.16	0.39	0.59	53.3	41.6	2.3	2.8	6.96	19.5	1.81
	UDOM	16.2	0.62	0.31	1.58	59.8	35.9	2.7	1.6	1.65	15.1	4.09
Alto Beni	CPOM	3.70	0.20	0.23	0.81	66.0	29.8	1.0	3.2	5.02	32.4	1.88
	FPOM	1.11	0.16	0.38	0.50	53.6	42.0	1.6	2.8	5.48	13.6	2.33
	UDOM	10.3	0.47	0.35	1.50	52.3	41.9	3.9	1.9	2.26	17.5	4.17
Achumani	CPOM	0.35	0.09	0.08	0.06	69.1	29.0	0.1	1.8	2.27	2.9	1.53
	UDOM	4.80	0.30	0.27	0.82	58.1	32.1	5.6	4.2	2.10	12.2	4.35

* Individual amino sugar × 100 / THAS.

† (D-serine + D-glutamic acid + D-alanine + D-aspartic acid) × 100 / THAA.

have shown that hydrolysis with 6 mol L^{-1} HCl usually gives slightly lower yields of glucosamine (GlcN) and mannosamine (ManN) and slightly higher yields for galactosamine (GalN) compared with the 3-mol L^{-1} HCl hydrolysis. During strong acid hydrolysis, acetyl groups are removed from amino sugars. Calculations of %C as total hydrolyzable amino sugars (THAS) in samples were based on the deacetylated form. Therefore, the values presented herein could underestimate %C as THAS 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 ManN, 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 ManN were separated and measured using high-performance anion exchange chromatography with pulsed amperometric detection. A Dionex 500-ion chromatography system with a Dionex CarboPac PA20 column (3 × 150 mm) with guard column (3 × 30 mm) was used for all analyses. Separation was performed under isocratic conditions with a 2-mmol L^{-1} NaOH mobile phase.

Mur was measured using reversed-phase high-performance liquid chromatography coupled with fluorescence detection. The protocol used is similar to the protocol employed for amino acid analyses (*see* below) but with the following modifications. Neutralized hydrolysates containing Mur were derivatized with *o*-phthaldialdehyde (OPA) and N-isobutyryl-L-cysteine in a borate buffer (potential of Hydrogen [pH] 9.5). Separation and detection were performed on an Agilent 1100 system with a Merck C-18 Superspher 100 RP-18 column (4 × 125 mm, 4-μm beads) and a guard column (Merck LiCrospher 100 RP-18; 4 × 4 mm, 5 μm). A linear gradient was applied, from 100% 29 mmol L^{-1} sodium acetate (pH 6.2) to 18% methanol after 37 min and 60% methanol after 42 min. Quantification was done using external and internal (2-amino-2-deoxy-gluconic acid) standards. Replicate analyses (hydrolysis and chromatography, n = 6) of muramic acid in a plant detritus sample revealed a relative standard deviation of 9.5%.

Amino acid analyses—Details of the D- and L-amino acid analysis are described in Kaiser and Benner (2005). Dried particles (50–200 mg) and UDOM extracts (3–5 mg) were hydrolyzed with 6 mol L^{-1} HCl at 110°C for 20 h in a sealed ampoule containing 0.12 μmol mL^{-1} ascorbic acid. After neutralization, precolumn derivatization with OPA and N-isobutyryl-L-cysteine (L-reagent run) or OPA and N-isobutyryl-D-cysteine (D-reagent run) was performed in a borate buffer (pH 9.5). Separation and detection were

Table 2. Carbon- and nitrogen-normalized yields of muramic acid and individual D-amino acids in the river samples, an average of six Gram-negative bacteria, and a Gram-positive bacterium typically found in soil and freshwater. Abbreviations: Mur = muramic acid; D-Ala = D-alanine; D-Glu = D-glutamic acid; D-Asp = D-aspartic acid; D-Ser = D-serine; UDOM = ultrafiltered dissolved organic matter; FPOM = fine particulate organic matter; CPOM = coarse particulate organic matter; Ó = Óbidos; M = Manacapurú; VG = Vargem Grande; RB = Riberalta-Bajo; RA = Riberalta-Arriba; R = Rurrenabaque.

River sampled	Sample types	Mur	D-Ala	D-Glu	D-Asp	D-Ser	Mur	D-Ala	D-Glu	D-Asp	D-Ser
		(nmol mg C^{-1})					(nmol mg N^{-1})				
Amazon											
Ó	UDOM	0.23	4.81	2.13	4.07	1.11	6.8	140.1	62.0	118.5	32.3
M	UDOM	0.35	5.48	2.37	4.23	1.42	10.7	167.6	72.5	129.4	43.4
VG	UDOM	0.32	6.05	2.95	5.38	1.61	9.0	167.6	81.7	149.0	44.6
Negro	UDOM	0.21	2.73	1.31	2.45	0.65	9.2	122.0	58.5	109.5	29.1
Içá	UDOM	0.41	4.78	2.23	3.53	1.25	11.9	137.3	64.1	101.4	35.9
Madeira	UDOM	0.40	6.55	2.65	4.83	1.50	9.5	156.1	63.2	115.1	35.7
Mamoré	FPOM	1.30	16.87	6.48	5.41	2.40	7.80	101.2	38.9	32.5	14.4
	UDOM	0.90	10.19	3.86	5.55	1.72	20.3	229.3	86.9	124.9	38.7
Beni											
RB	CPOM	0.61	6.84	2.94	3.51	1.07	—†	—	—	—	—
	FPOM	1.25	15.07	8.92	7.73	3.49	7.13	85.8	50.8	44.0	19.9
	UDOM	1.16	7.37	3.41	5.11	1.54	26.4	168.1	77.8	116.6	35.1
RA	CPOM	0.53	7.16	3.24	6.93	1.88	—†	—	—	—	—
	FPOM	1.00	14.95	9.09	7.92	2.91	5.47	82.2	50.0	43.6	16.0
	UDOM	0.78	7.31	2.76	4.96	1.60	19.9	186.9	70.6	126.8	40.9
R	CPOM	0.59	5.28	2.67	1.90	0.65	12.0	107.9	54.6	38.8	13.3
	FPOM	1.52	10.32	7.00	4.40	2.10	12.0	81.3	55.1	34.7	16.6
	UDOM	0.68	5.74	2.79	4.27	1.37	17.7	150.0	72.9	111.6	35.8
Alto Beni	CPOM	0.99	7.05	3.37	2.26	0.72	18.3	130.4	62.3	41.8	13.3
	FPOM	1.48	9.97	7.52	4.56	1.08	10.3	69.2	52.2	31.6	7.5
	UDOM	0.95	7.77	4.10	6.01	1.84	20.8	170.3	89.9	131.5	40.3
Achumani	CPOM	0.19	3.47	0.72	0.82	0.23	—†	—	—	—	—
	UDOM	1.52	8.98	3.98	4.44	1.54	24.3	143.7	63.7	71.0	24.6
Gram −	Bacteria*	28.4	63.4	45.3	8.1	1.1	118.6	264.3	189.0	33.7	4.6
Gram +	Bacterium*	166.7	381.9	206.7	18.9	0.0	642.9	1473.0	797.2	72.9	0.0

* Data from Kaiser and Benner (2008).

† N-normalized yields of inorganic nitrogen-rich samples were not included.

performed on an Agilent 1100 system with a fluorescence detector and 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^{-1} and the column temperature was 20°C. A mobile-phase linear gradient was applied, from 100% 40 mmol L^{-1} KH_2PO_4 (pH 6.2) to 39%, 54%, and 60% methanol : acetonitrile (13 : 1 v : v) after 50 min, 72 min, and 80 min, respectively. Yields of hydrolyzable amino acids were corrected for chemical racemization occurring during hydrolysis. Racemization rates for individual free and protein amino acids were measured (Kaiser and Benner 2005), and the average of these rates was used for corrections. Racemization correction had a negligible effect on individual L-amino acids (L-AA) yields (i.e., <2%), but the effects on D-AA yields were substantial. Peaks of D-AA leading to zero or negative values after average racemization correction were considered hydrolysis artifacts and thus rejected. D-AA peaks showing >15% variability between the L- and D-reagent runs were also rejected. Such variability indicated coelution problems. Hydrolysis converted asparagine (Asn) and glutamine (Gln) to aspartic acid (Asp) and glutamic acid (Glu), respectively. Yields of D- and L-isomers of Asp and Glu reported in this study thus included those of Asn and Gln. Yields obtained with the two runs in all the samples were different by 0.1–7.2% (mean = 3.0%) for THAA and 1.6–11% (mean = 6.8%) for total D-AA. For individual AA, the relative standard deviation was evaluated at 5–12% (n = 3; Kaiser and Benner 2005).

Biomarker yields in source bacteria—C- and N-normalized yields of bacterial biomarkers (Mur, D-alanine [D-Ala], D-Glu, and D-Asp) measured in the Amazon POM were compared to yields measured in heterotrophic bacteria. Yields of biomarkers were measured in six cultured Gram-negative bacteria from soil and freshwater as well as in a cultured Gram-positive bacterium found in soil (Benner and Kaiser 2003; Kaiser and Benner 2008, *see* Table 2). Biomarker yields were higher in the Gram-positive bacterium because these cells have thicker cell walls. Gram-positive bacteria generally represent <15% of the bacterial community in water (Giovannoni and Rappé 2000). Jørgensen et al. (2003) presented data indicating Gram-negative bacteria are the major source of cell wall biomarkers in river waters. In this study, we assumed Gram-positive bacteria comprised 5% to 15% of the bacterial community.

Quantification of bacterial contribution—Yields of bacterial biomarkers in the POM of the Amazon River system

were used to estimate bacterial contributions to bulk C and N. This approach assumes that the biomarkers are of similar reactivity as bulk bacterial C and N. The proportion of bacterial C or N was calculated by

$$\% \text{ Bacterial C or N} = 100[\text{biomarker}]_{\text{sample}}/[\text{biomarker}]_{\text{bacteria}} \quad (1)$$

where $[\text{biomarker}]_{\text{sample}}$ represents the C-normalized yields of an individual biomarker (e.g., D-Ala or Mur) in POM samples if % Bacterial C is estimated. N-normalized yields are used for estimates of % Bacterial N. $[\text{biomarker}]_{\text{bacteria}}$ represents the C- or N-normalized yield of the same biomarker in heterotrophic bacteria presented in the previous section.

Results

THAS accounted for 0.08% to 0.46% of the C and 0.44% to 2.34% of the N in the studied fractions (Table 1). The lowest values were found in CPOM. It should be noted that estimated contributions to N exclude two CPOM samples from the Beni (Riberalta-Bajo and Riberalta-Arriba) and from Achumani that are rich in inorganic N (Hedges et al. 1994, 2000*b*). The %N as THAS was relatively high in UDOM, with the maximal values (>1.9%) measured in the downstream samples.

Glucosamine accounted for 52–69% of THAS and galactosamine for 29–45% (Table 1). Calculated GlcN : GalN ratios were between 1.2 and 2.4. The two other amino sugars measured, ManN and Mur, were detected in every sample but individually accounted for <6% of THAS (Table 1). The proportion of ManN was generally low in CPOM and high in UDOM. In contrast, the proportion of Mur was very low (<1%) in the downstream UDOM samples. Measured yields of Mur were between 0.19 nmol mg C^{-1} and 1.52 nmol mg C^{-1}, corresponding to 5.47–26.4 nmol mg N^{-1} (Table 2).

Total hydrolyzable amino acids (THAA) represented 0.9–8.3% of the C and 11.9–36.6% of the N (excluding CPOM rich in inorganic N) in the studied fractions (Table 1). Major differences were observed among the three size fractions. The highest %C as THAA was found in FPOM and is attributed to the preferential sorption of amino acids onto fine particles (Hedges et al. 1994, 2000*b*; Aufdenkampe et al. 2007). In contrast, UDOM gave the lowest values. The %N as THAA was higher in CPOM (32.4–36.6%) than UDOM (11.9–21.0%) and FPOM (13.6–19.5%; Table 1). Thus, either FPOM contains an important fraction of non-hydrolyzable amino acids or it is enriched in N-molecules other than amino acids.

The THAA composition of the samples (not shown) was generally similar to those reported in other rivers as discussed in two previous studies (Hedges et al. 1994, 2000*b*). THAA composition within each size fraction varied little with location. The most noticeable differences were among the three size fractions and appeared mostly related to diagenesis. To illustrate this, degradation indices developed by Dauwe et al. (1999) for sedimentary diagenesis have been calculated. This approach clearly

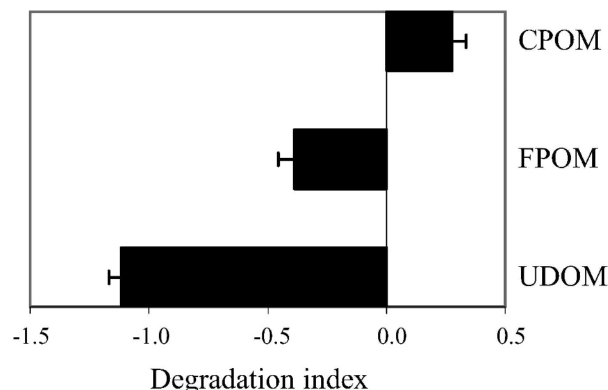

Fig. 2. Dauwe et al. (1999) degradation index for the coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. Bars represent standard deviations.

separates the three size fractions, with UDOM having the lowest value (most degraded) and CPOM the highest (least degraded; Fig. 2). Aufdenkampe et al. (2001) have shown that sorptive fractionation of amino acids, such as nonprotein amino acids (GABA, B-Ala) and basic amino acids, can complicate the use of amino acid–based diagenetic indicators. However, the removal of these amino acids had very little effect on the degradation indices calculated here (not shown). Selective incorporation of specific amino acids in DOM can also influence the degradation index of UDOM.

The D-enantiomers of Ala, Glu, Asp, and serine (Ser) were measured in all samples (Table 2). D-Ala was the most abundant D-AA (2.73–16.9 nmol mg C^{-1} or 69.2–229.3 nmol mg N^{-1}) and D-Ser was the least abundant (0.23–3.49 nmol mg C^{-1} or 7.5–44.6 nmol mg N^{-1}). As for THAA, FPOM exhibited greater C-normalized yields of D-AA but lower N-normalized yields. The greatest N-normalized yields of D-AA were measured in UDOM.

Each D-AA accounted for a larger percentage of total amino acids (D vs. D + L) in UDOM than in FPOM or CPOM (Fig. 3). In UDOM, D-Ala accounted for ~15% of total Ala. This proportion decreases to ~10% for D-Glu and D-Asp and to ~6% for D-Ser. In FPOM, %D-Ala and %D-Glu were similar (~8%).

The total mol% D-AA were 3.25–4.82% for UDOM, 1.81–2.45% for FPOM, and 1.53–2.22% for CPOM (Table 1). The mol% D-AA was plotted with known diagenetic indicators (Fig. 4; Cowie and Hedges 1994). These figures separate the three size fractions and generally reveal the expected pattern of increasing alteration along the sequence from CPOM to FPOM to UDOM.

Average yields of the biomarkers Mur, D-Ala, D-Glu, and D-Asp measured in the Amazon samples were compared with yields calculated in cultured heterotrophic bacteria from soil and freshwater (Figs. 5 and 6). D-Ser was excluded because it is not found in four of the seven cultured bacteria considered here (Kaiser and Benner 2008). Yields of Mur, D-Ala, and D-Glu were much lower in samples compared to bacteria. C-normalized yields of

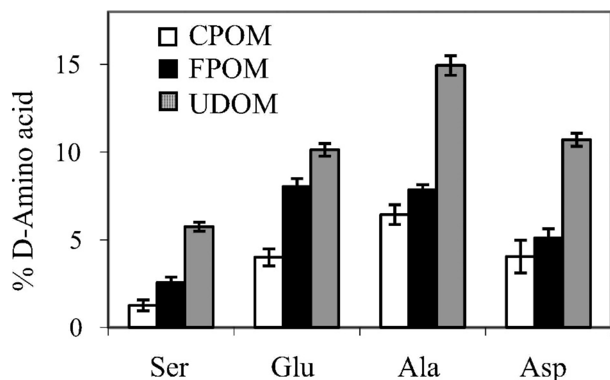

Fig. 3. Average molar percentages of D-enantiomers [D × 100 / (D + L)] for specific amino acids in the coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. Bars represent standard deviations. Ser = serine; Glu = glutamic acid; Ala = alanine; Asp = aspartic acid.

Mur were especially low. C-normalized yields of D-Asp in samples and in bacteria were more comparable (Fig. 5). N-normalized yields of D-Asp in samples were similar (CPOM and FPOM) or even greater (UDOM) than in bacteria (Fig. 6). This suggests that D-Asp is not quantitatively representative of the bacterial contribution, at least for the assemblages of bacteria considered.

The N yields (mg N per 100 mg C) of D-AA and Mur were plotted against total N yields measured in CPOM, FPOM, and UDOM (Fig. 7). Linear relationships between D-AA N and total N were found for the three size fractions (Fig. 7a). Similar correlations were observed between D-Ala N and total N (R^2 = 0.69 and 0.71, not shown) and between THAA N and total N (R^2 = 0.86, not shown). These findings indicate that changes in bacterial D-AA N and THAA N contents are reflecting changes in bulk N. Moreover, x-intercepts near zero, obtained by extrapolation, support the idea that a substantial fraction of N is associated with bacterial N. Changes in Mur N content also reflected changes in bulk N in UDOM (Fig. 7b). However, the x-intercept in this case is more important, indicating that a large fraction of total N would still be present in UDOM when no Mur is detected. This suggests differences in the proportion of dissolved D-AA and Mur produced and/or different dynamics of dissolved D-AA and Mur. In FPOM, an inverse relationship between Mur N and total N was observed suggesting bacterial processes do not control N content or that Mur is much more reactive than D-AA in FPOM.

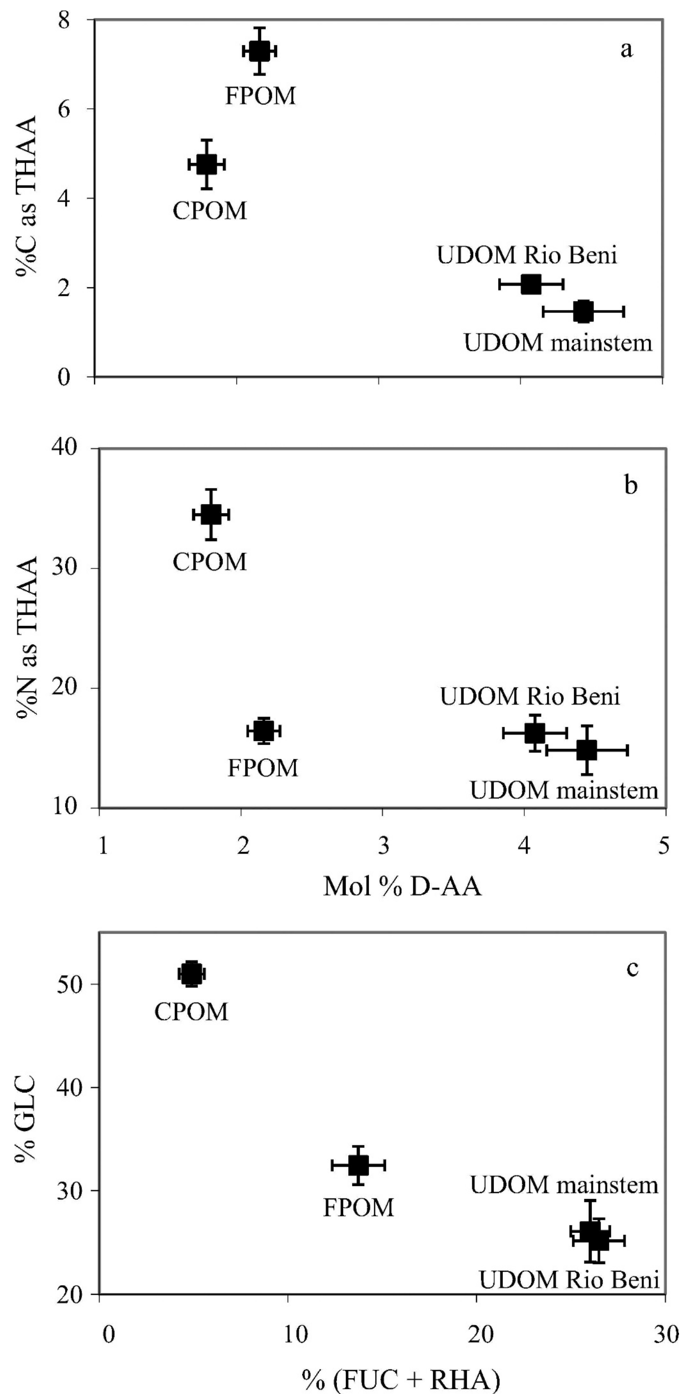

Fig. 4. (a) Percentages of total C as total hydrolyzable amino acids (THAA), and (b) percentages of total N as THAA vs. mole percentages of D-enantiomers of amino acids (total D-AA × 100 / THAA); (c) percentages of glucose (% GLC) vs. percentages of fucose plus rhamnose (% [FUC + RHA], on a glucose-free basis) from Hedges et al. (1994, 2000*b*). Values are averages among coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. CPOM samples rich in inorganic nitrogen were not included in (b). Bars represent standard deviations.

Discussion

Heterotrophic bacteria are the primary agents of the decomposition and diagenetic alterations of plant detritus in aquatic environments (Benner et al. 1986). It is therefore not surprising to measure biomarkers of bacteria associated with the remains of decaying plant material in the Amazon River system. The detection of Mur confirmed the occurrence of bacterial peptidoglycan, or its remnants, in

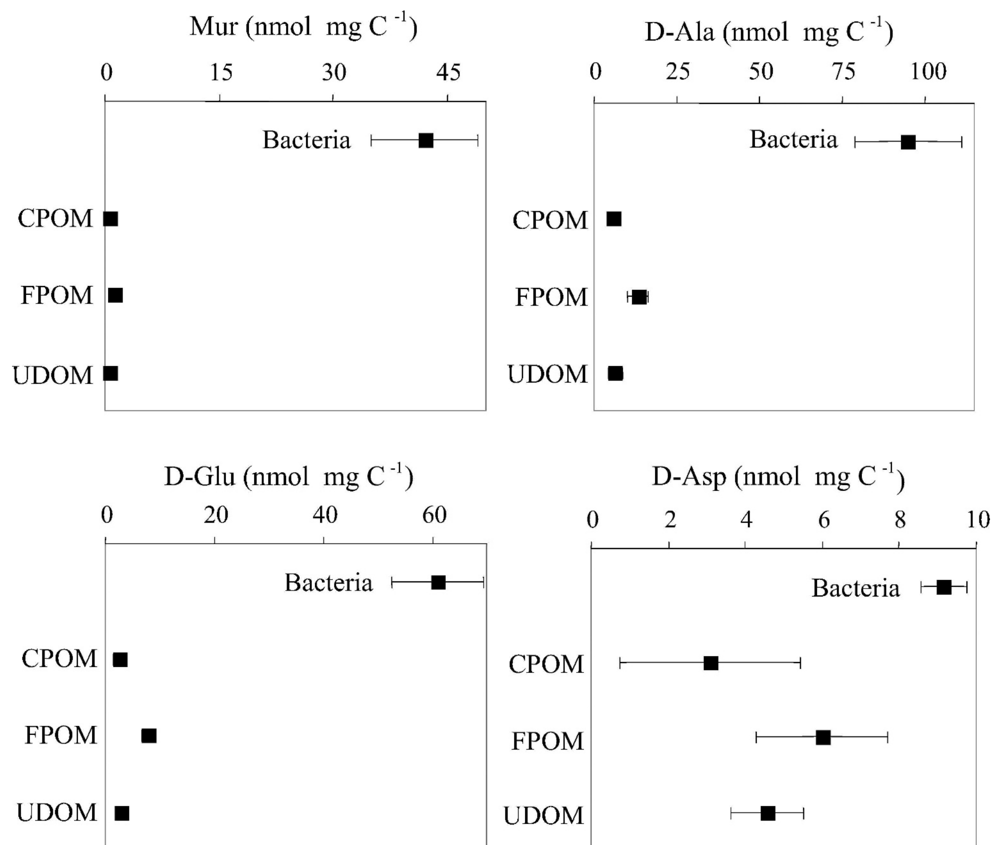

Fig. 5. Average carbon-normalized yields of muramic acid (Mur), D-alanine (D-Ala), D-glutamic acid (D-Glu), and D-aspartic acid (D-Asp) in bacteria, coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. Bars for yields in bacteria correspond to assemblages comprising 5–15% Gram-positive bacteria (*see* Methods). Bars for yields in samples represent standard deviation when greater than symbol size.

all the studied samples. The D-enantiomers of Ser, Glu, Ala, and Asp were also measured in all samples. These D-AA have been reported in peptidoglycan and in other bacterial macromolecules such as teichoic acid, lipopeptides, and lipopolysaccharides (Kaiser and Benner 2008). However, following their incorporation into POM and DOM, these N-molecules can exhibit different dynamics.

Relative reactivities of amino sugars and amino acids—Distribution patterns observed with amino sugars and amino acids can be used for the assessment of their relative reactivity. Knowledge about the diagenetic states of the samples can highlight new compositional trends or features that can be used as diagenetic indicators. Compositional features that appeared sensitive to diagenesis were mol% D-AA, THAS, and THAA contents.

D-AA contents in the Amazon samples can be compared with the few available data from other environments. The mol% of individual amino acids in UDOM from the Amazon are very similar, 1–2% greater, to values found for the same D-AA in DOM from Lake Murray (South Carolina; Kawasaki and Benner 2006). They are also similar to mol% D-AA measured in DOM from Arctic rivers (Dittmar et al. 2001), except for D-Asp (~11% vs. 21%), and from 50 Baltic rivers (Jørgensen et al. 2003; D-AA = 5.5% of THAA vs. 3.3–4.8% in Amazon UDOM). However, these last two studies did not correct for acid-induced racemization which leads to overestimation of the mol% D-AA, especially for D-Asp (Kaiser and Benner 2005). The mol% D-AA in DOM from these contrasting freshwaters are lower than those measured in seawater from the Arctic (Dittmar et al. 2001), the North Pacific and Atlantic (Kaiser and Benner 2008), and the South Pacific and the Gulf of Mexico (McCarthy et al. 1998), which have values between 27% and 45% for D-Ala and >15% for the sum of the four D-AA. Dittmar et al. (2001) have shown in Arctic DOM a consistent increasing trend of mol% D-AA with increasing marine conditions. Their reported mol% D-AA in DOM are higher than those measured in POM, in agreement with the present study.

The observed mol% D-AA pattern, CPOM < FPOM < UDOM, is consistent with the observed pattern of diagenetic alteration in these samples, with CPOM being the least altered and UDOM the most. This pattern is in agreement with the size-reactivity continuum concept (Amon and Benner 1996) and with numerous independent diagenetic indicators, including lignin phenols, carbohydrates, and amino acids (Cowie and Hedges 1994; Hedges et al. 1994, 2000*b*). Moreover, the mol% D-AA has been

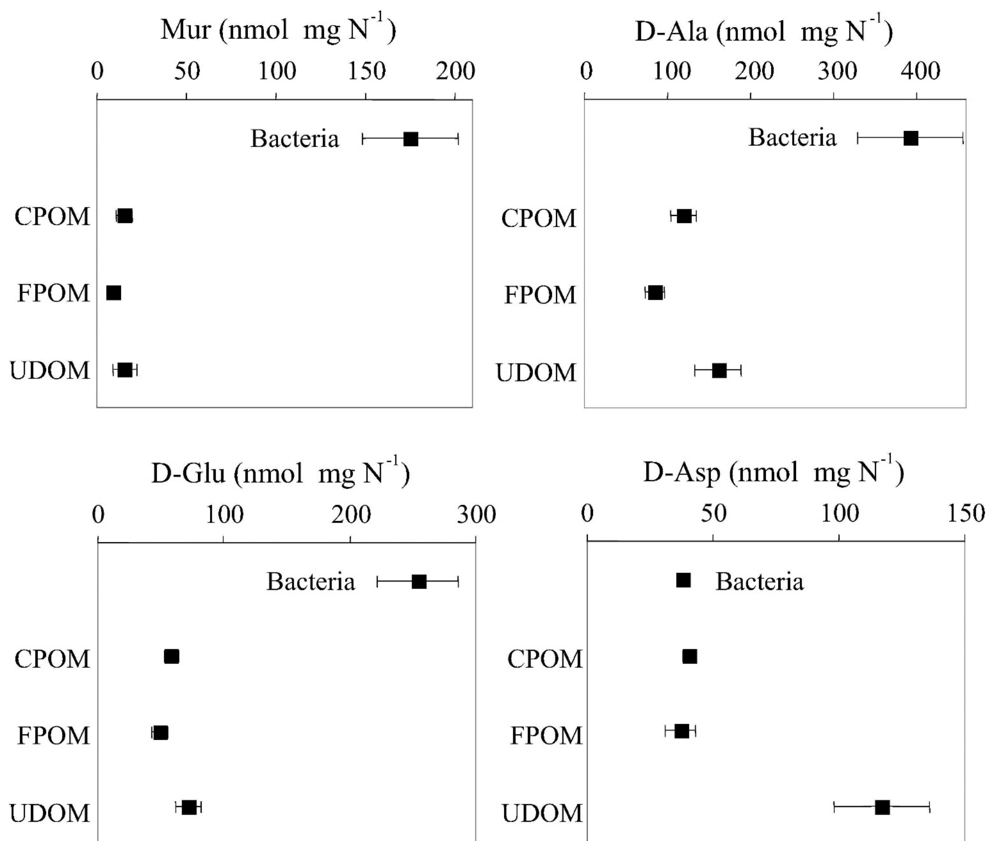

Fig. 6. Average nitrogen-normalized yields of muramic acid (Mur), D-alanine (D-Ala), D-glutamic acid (D-Glu), and D-aspartic acid (D-Asp) in bacteria, coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. Bars for yields in bacteria correspond to assemblages comprising 5–15% Gram-positive bacteria (*see* Methods). Bars for yields in samples represent standard deviation when greater than symbol size.

shown to increase with depth in sediments (not related to racemization; Pedersen et al. 2001; Lomstein et al. 2006) and with plant detritus degradation (Tremblay and Benner 2006). These results indicate the mol% D-AA can be a useful indicator of the diagenetic state of organic matter. Increases in the mol% D-AA can be attributed to greater bacterial contributions and to the lower degradation rates of the bacterial biomolecules rich in D-AA compared with proteins of various origins (Jørgensen et al. 2003; Nagata et al. 2003). In DOM, where the highest mol% D-AA are found, another factor may promote such an increase. Recent incubation experiments have shown very high mol% D-AA in bacterial DOM during cell growth attributed to the selective release of D-AA (Kawasaki and Benner 2006). Preferential release of D-AA in DOM may also occur during protozoan bacterivory (Asano and Lübbehüsen 2000) and viral lysis (Danovaro et al. 2008).

CPOM had the lowest THAS content and the highest %N as THAA. This is consistent with relatively unaltered vascular plant detritus. Plants are poor in THAS (Sharon 1965; Tremblay and Benner 2006) but THAA comprise a large fraction of the N in living organisms or fresh detritus (Cowie and Hedges 1992). The opposite trends (i.e., high THAS, low %N as THAA) were observed in UDOM. The amino sugars GlcN and GalN tend to accumulate during degradation (Kawasaki and Benner 2006; Tremblay and Benner 2006). The present results suggest that ManN is also selectively preserved, in contrast to Mur (*see* below). For FPOM, the preferential sorption of amino acids with other N-rich molecules (e.g., NH_4^+) or the transformation of amino acids into uncharacterized N, could explain high %C as THAA but relatively low %N as THAA.

Estimating bacterial contributions to detrital organic matter—Estimating bacterial contributions to organic matter in natural environments is challenging considering the diversity of bacteria and the varying reactivities of biomarkers. The approach used here is based on Eq. 1 (*see* Methods) and assumes that biomarker yields in cultured bacteria are representative of natural bacterial assemblages, and that biomarker reactivities are representative of bulk bacterial C and N reactivities. Average biomarker yields in cultured bacteria were calculated based on published values and current knowledge about bacterial assemblages typically found in soils and freshwaters (Giovannoni and Rappé 2000; Jørgensen et al. 2003; Kaiser and Benner 2008). Yields measured in bacterial cells are appropriate for estimating bacterial contributions to POM, but they are

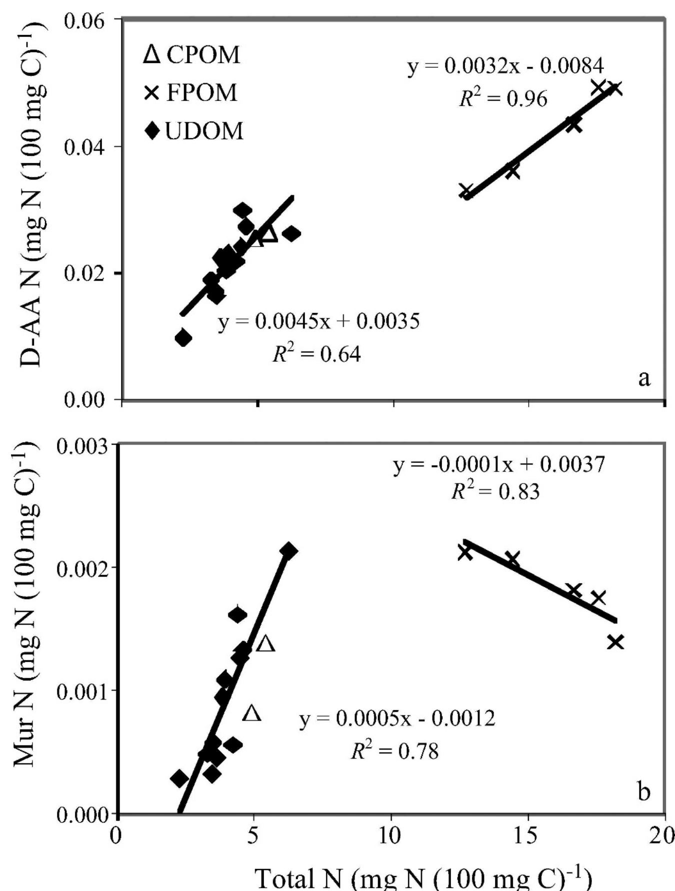

Fig. 7. Nitrogen yields in (a) D-enantiomers of amino acids (D-AA); and (b) muramic acid (Mur) vs. total nitrogen yields in the coarse particulate (CPOM), fine particulate (FPOM), and ultrafiltered dissolved organic matter (UDOM) size fractions. CPOM samples rich in inorganic nitrogen were not included.

most likely inappropriate for estimating contributions to DOM. Recent studies revealed that the molecular composition of bacteria can be quite different from that of the DOM they produce (Ogawa et al. 2001; Kawasaki and Benner 2006; Kaiser and Benner 2008). For instance, D-Ala can be selectively released from the cell during bacterial growth whereas Mur is recycled within the cell. Therefore, we use the yields of biomarkers from cultured bacteria to estimate bacterial contributions to CPOM and FPOM, but not UDOM. No experiments were conducted to determine biomarker yields in bacterial DOM, so we do not provide quantitative estimates of bacterial contributions to UDOM in this study. In addition, D-Asp and D-Ser were not used because they did not appear to be representative of bacterial contributions. They are not detected in some freshwater and soil bacterial cultures (Kaiser and Benner 2008), and they exhibited highly variable yields that were sometime greater (N-normalized) than in bacteria.

Bacterial contributions to bulk C and N in CPOM and FPOM were estimated based on Mur, D-Ala, and D-Glu yields and Eq. 1. Ranges of values depend on the proportion of Gram-positive bacteria assumed in natural assemblages (5–15%). When Mur is used, this approach indicated that only 1–2% of the C in CPOM and 3–4% of the C in FPOM are of bacterial origin. Estimates of bacterial C based on D-Ala and D-Glu were greater: 4–8% in CPOM and 11–17% in FPOM. These percentages are consistent with C primarily originating from vascular plants (Hedges et al. 1994, 2000*b*). As expected, the estimated bacterial contribution to N was greater: 20–37% for CPOM and 17–26% for FPOM according to D-Ala and D-Glu yields. Estimates of bacterial N based on Mur were 8–10% for CPOM and 4–6% for FPOM. Very little spatial variability was observed despite the vast area and contrasting river basins in the study area. This observation suggests that bacterial contributions were largely imprinted in soils before the material enters the river system.

The lower estimates of bacterial contributions obtained with Mur compared to D-Ala and D-Glu are probably caused by the relatively labile nature of Mur. Mur is known for its high reactivity relative to bulk bacterial molecules or bulk POM (Nagata et al. 2003, Tremblay and Benner 2006) and has been seen as a marker of living bacteria (Moriarty 1977) or recent bacterial necromass (Benner and Kaiser 2003; Niggeman and Schubert 2006). Therefore, Mur is probably not representative of bulk bacterial contributions in aged terrestrial detritus in rivers. This discrepancy was not observed in marine POM where a relatively recent bacterial contribution is expected (Kaiser and Benner 2008). In contrast, D-Ala and D-Glu had similar dynamics and appear to be of average reactivity relative to other bacterial molecules. Two independent approaches, based on stable N isotopic compositions and plant biomarkers, indicated that D-Ala yields were representative of the N immobilized during decomposition (Tremblay and Benner 2006). In addition, Kaiser and Benner (2008) observed no consistent pattern of selective preservation or degradation of D-Ala and D-Glu in marine POM and DOM. Unlike the other D-AA, D-Ala and D-Glu appear to be abundant in all analyzed bacteria (Schleifer and Kandler 1972; Kaiser and Benner 2008). Overall, these observations support the use of D-Ala and D-Glu as quantitative biomarkers for estimation of bacterial contributions to C and N.

This study supports the idea that CPOM is comprised largely of plant detritus (Hedges et al. 1994, 2000*b*). However, CPOM already possesses a significant proportion of bacterial N. Considering that vascular plants, the dominant source of detritus, are poor in N (Hedges et al. 1986*a*; Cowie and Hedges 1992), high proportions of bacterial N are expected. The bacterial contribution to CPOM-N is consistent with lower C : N ratios (<24) than found in local plant tissues (28–200; Hedges et al. 1986*a*, 1994) and with a mol% D-AA similar to bacteria. Estimated bacterial N contributions in slightly decomposed cordgrass and mangrove leaves (Tremblay and Benner 2006) were similar to those observed in Amazon CPOM (20–37%). The relatively low estimated bacterial contribution to FPOM-N (<26%) is somewhat surprising considering the highly degraded state of this material.

Other compositional features of detrital organic matter can be used as qualitative or semi-quantitative indicators of bacterial contributions. The observed relationships between

the N from bacterial biomarkers, N from THAA, and total N indicate that changes in bacterial N content generally reflected changes in bulk N in CPOM, FPOM, and UDOM. This finding indicates bacteria play an important role as a source of N in decomposing plant material transported by the Amazon River system. In addition to bacterial processes, the sorption of N-containing molecules onto fine particles may exert an important influence on the total N content in FPOM (Hedges et al. 2000*b*, Aufdenkampe et al. 2001).

GlcN : GalN ratios can be used as an indicator of amino sugar source considering that soil or aquatic bacteria exhibit ratios ($<\sim3$) that are lower than those found in most fungi, plants, and animals (Benner and Kaiser 2003; Glaser et al. 2004). The bacterial signature of amino sugars is rapidly imprinted in the first months of plant decomposition (Tremblay and Benner 2006). The GlcN : GalN ratios measured here, 1.2–2.4, indicated amino sugars mostly of prokaryotic origin. The bacterial cell wall polymer, peptidoglycan, has GlcN : Mur and D-Ala : Mur ratios of $\sim$1, and therefore intact peptidoglycan was not a major contributor of GlcN or N in any size fraction of Amazon River organic matter. These findings suggest other bacterial biopolymers and fragments of peptidoglycan as the main sources of amino sugars. Bacterial biomass in the waters sampled here was mostly extracted with the FPOM size fraction (0.1–63 μm), although CPOM certainly contained attached bacteria. It was possible to estimate the contributions of intact cells in FPOM based on the bacterial abundance and mass previously measured at different sites in the Amazon River system (Benner et al. 1995). Calculations indicated that bacterial cells accounted for only 0.2–2.0% of the C and 0.3–3.2% of the N in FPOM. Based on yields measured in typical assemblages of bacteria, we estimated that intact bacteria can account for 0.6–4% of GlcN, 2–8% of D-Ala, and 7–45% of Mur in FPOM. Bacterial biomass could represent an important part of particulate Mur, but not of bulk amino sugars or amino acids.

Kaiser and Benner (2008) recently estimated that $\sim$25% of the C and $\sim$50% of the N in marine POM and DOM is of bacterial origin. These estimates are higher than those calculated here for CPOM and FPOM in the Amazon River system. The same approach was used in both studies, although the estimated yields of biomarkers in marine bacteria were lower than those in freshwater and soil bacteria used here. An important ecological difference between the Amazon River system and the open ocean is that cyanobacteria are major primary producers in the ocean but not in the Amazon. It is therefore reasonable to assume that the greater contributions of bacterial C and N to detrital organic matter in the ocean are due, at least in part, to autotrophic as well as heterotrophic bacterial contributions to marine POM and DOM (McCarthy et al. 2004; Kaiser and Benner 2008). Another factor that can lower estimates of bacterial contribution is the partial alteration of biomarkers during diagenesis. Like most of the organic matter in natural systems, bacterial biomarkers can become altered by biotic and abiotic processes such as interactions with the mineral matrix (Hedges et al. 2000*a*). As a result, the approach used here could lead to underestimations of bacterial contributions. This factor is expected to be more important in highly altered organic matter such as FPOM in the Amazon.

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