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SUPPLEMENTARY INFORMATION

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Degradation of terrestrially derived macromolecules in the Amazon River

Authors: Nicholas D. Ward*[1], Richard G. Keil[1], Patricia M. Medeiros[2], Daimio C. Brito[3], Alan C. Cunha[3], Thorsten Dittmar[4], Patricia L. Yager[2], Alex V. Krusche[5], Jeffrey E. Richey[1]

Supplemental Information

I. Methods Summary

Chemical measurements and dark incubations were performed in the north channel of the Amazon River mainstem near Macapá (S 00°05.400', W 51° 03.200') during four expeditions from September 2010 to September 2011. Whole water was collected with a Shurflo submersible pump from 50% river depth (annual average river depth = 28m) at the center and margins of the river channel. Bulk respiration rates were determined by measuring the consumption of oxygen in 60mL BOD bottles via Winkler titration; triplicate samples were incubated for 26-39 hours in the dark at ambient river temperature (annual average river temperature = 30 °C). Compound specific incubations were performed in triplicate for 5-7 days in 4L polycarbonate bottles. Dissolved nutrients were consumed, but remained at measureable concentrations throughout the incubations. Killed control incubations showed no statistical change in phenol concentrations. Particulate OM samples were collected on pre-combusted 142mm diameter Whatman GF/F glass fiber filters (0.7μm) and frozen. Dissolved OM samples were pre-filtered through 142mm diameter Pall 0.2um Supor filters, acidified to pH=2, and extracted at 3 mL/min on Waters Oasis HLB solid phase extraction cartridges for lignin analysis[23] and PPL cartridges for ESI-FTICR-MS analysis. ESI-FTICR-MS analysis of DOM (in 1:1 water : methanol solution) was performed in negative mode with a Bruker Daltonics 15 Tesla FT-ICR mass spectrometer at the Max Planck Research Group for Marine Geochemistry, Oldenburg, Germany. 500 broadband scans were accumulated for each sample. Particulate and dissolved lignin samples underwent CuO oxidation performed in a CEM Microwave Accelerated Reaction System[24], and were analyzed using a GC-ToF-MS after derivatizing with BSTFA (Agilent 7890A GC, Leco TruToF HT). Free lignin monomers were measured by direct

injection of the DOM extract onto the GC-ToF-MS after the same BSTFA derivatization. Bacterial abundance samples were fixed with 4% buffered formaldehyde and kept cold and dark until they could be stored frozen (within one month). Upon return to the lab, they were thawed, sonicated, stained with DAPI, and counted by epifluorescence microscopy.

Triplicate POC and PO^{13}C samples were filtered on combusted Whatman GFF filters, packed in tin capsules after drying and acid-fumigation, and analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. The filtrate was analyzed for DOC in triplicate on a Shimadzu TOC-VCPH analyzer. River discharge was measured using a Teledyne Workhorse Rio Grande 600 khz Acoustic Doppler Current Profiler (ADCP). ADCP profiles were made across the north and south mainstem channels and Belem (e.g. Tocantins) at approximately 1 hr intervals for 13 hr in order to assess river velocity over the span of a tidal cycle and calculate the total Amazon River discharge to the ocean. Total daily discharge from the Amazon River was calculated by ADCP measurements at the North and South channels of Macapá and Agência Nacional de Águas (ANA) stage/discharge records at Óbidos and the Tapajós, Xingu, and Tocantins tributaries.

Basin-wide estimates of the fate of lignin in soils and the river were made by first calculating the total amount of standing lignin biomass in the Amazon basin. Here we use conservative values of 70 Pg C for total carbon biomass[29], and multiply by 16.5%, or the fraction of total C biomass that is lignin[21]. We then estimate basin-wide annual lignin degradation rates in soils using the above value for total lignin and the empirically determined half-life of lignin phenols in the soil setting [29]. Annual river degradation rates were determined by scaling the average phenolic degradation rates measured here to total average annual Amazon River discharge. These two rates were then compared to the annual sequestration of lignin by the terrestrial biosphere to determine the relative proportion of sequestered lignin that is degraded in soils versus in the river. A value of 0.5 Pg C y^{-1} was used for annual C sequestration[12, 13] and multiplied by

16.5% to normalize to lignin[21]. Respiration in soils and the river accounted for 95% of the annually sequestered lignin; the remaining 5% was assumed to be delivered to the ocean.

Supplemental Table 1. List of the 95 phenolic BSTFA derivatives identified via GC-ToF-MS.

Compound Name	CAS #	Molecular Weight	Quant Mass
(2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone	78417-24-0	316	147
(4-Methoxy-phenyl)-(2-nitrocyclohexyl)-methanol	103077-68-5	265	137
[1,1'-Biphenyl]-4,4'-diamine, 3,3',5,5'-tetramethyl-	54827-17-7	240	240
1-(2,2-Dimethylpropyl)-1,2,3,4-tetrahydroisoquinoline	87443-62-7	203	132
1-(4-Anisidino)-2-phenyl-2-(1,2,4-thiadiazol-5-yl)ethane	153333-55-2	309	309
1,2,4-Benzenetricarboxylic acid, trimethyl ester	204-44-6	252	221
1,2,4-Trioxolane, 3,3,5-triphenyl-	23246-12-0	304	105
1,2-Benzisothiazole, 3-butoxy-	40991-40-0	207	151
1,2-Benzisothiazole-3-propanoic acid	50565-45-2	207	135
1,3-Benzenedicarboxylic acid	106450-31-1	310	295
1,3-Benzenediol, 5-iodo-	64339-43-1	236	236
1,3-Benzodioxole-5-carboxylic acid	613-95-6	280	223
1,3-Bis(trimethylsiloxy)benzene	4520-29-0	254	239
1,3-Dithiolane, 2-benzyl-2-methyl-	20137-72-8	210	119
1,4-Benzenedicarboxylic acid	4147-84-6	310	295
1,4-Butanedione, 1,4-diphenyl-	495-71-6	238	105
1-[(Pyrazinylcarbonyl)oxy]-2,5-pyrrolidinedione	342612-63-9	221	52
1-Allyldimethylsilyloxy-3-methylbenzene	532-66-2	206	165
1-Butyl(dimethyl)silyloxy-2-phenylethane	259862-61-8	236	179
1-Heptene, 1,3-diphenyl	5378-12-0	338	281
1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	3910-35-8	236	221
1H-Indole-2-carboxylic acid, 5-ethyl	74367-45-6	333	320
1-Naphthalenesulfonic acid, 5-(dimethylamino)-, phenyl ester	55837-12-2	327	327
2,2,4-Trimethyl-4-(4'-trimethylsilyloxyphenyl)chromane	155726-87-7	340	325
2,2'-Bibenzothiazole	866-23-8	268	268
2,2-Bis[4'-cyanooxyphenyl]propane	80-05-7	278	263
2,5-Dihydroxyacetophenone	490-78-8	296	281
2,6-Dihydroxyacetophenone	699-83-2	296	281

2-Butenoic acid, 4-oxo-4-[(1-phenylethyl)amino]-	108088-06-8	219	54
2-Ethyl-acridone	65753-77-7	223	208
2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl	55299-24-6	342	342
2-Iodo-3-pyridinol	40263-57-8	221	221
2-Phenyl-1,2-bispropane	294847-15-7	296	193
3,4 dihydroxybenzoic acid TMS	99-50-3	154	281
3,4-Diethoxybenzaldehyde	2029-94-9	194	166
3,4-Dimethoxyphenol	2033-89-8	226	226
3,5-Bis(4-methoxyphenyl)-1,2,4-selenadiazole	68723-61-5	346	221
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	234	219
3-Methoxyphenylpyruvic acid	27750-66-9	338	485
3-Pyridinecarboxylic acid	25436-37-7	195	180
4-(Benzyloxy)-N,N-dimethyl-1-butanamine	71126-70-0	207	58
4-Benzaldehyde	1012-12-0	194	179
4H-Pyran-4-one, 5-2-methyl	55557-21-6	286	271
4-hydroxy Benzoic acid	2078-13-9	282	224
4-hydroxyacetophenone	18803-29-7	208	151
5á-Podocarpa-8,11,13-trien-16-oic acid, methyl ester	3745-36-6	272	197
7-Oxodehydroabietic acid, methyl ester	110936-78-2	328	253
Acethydrazide, N2-[4-(thiitan-3-yloxy)benzylideno]-	9601-81-2	250	250
Acetic acid, (2-benzothiazolylthio)-	6295-57-4	225	181
Acetic acid, (3,4-dimethoxyphenyl)- methyl ester	2911-70-8	298	239
Acetic acid, phenyl(trimethylsiloxy)-, methyl ester	29233-93-0	238	179
Acetosyringone	55045-03-9	253	238
Acetovanillone	498-02-2	238	208
Anthra[1,9-cd]pyrazol-6(2H)-one	129-56-6	220	220
Anthraquinone, 7-methoxy-2-methyl-1,4,5-trihydroxy-	476-57-3	300	300
As-Indacene, 1,2,3,6,7,8-hexahydro-1,1,6,6-tetramethyl-4-(1-methylethyl)-	17465-47-3	256	241
Benzaldehyde, 2,4-bis	33617-38-8	282	267
Benzaldehyde, 3-ethoxy-4-propoxy-	350988-41-9	208	137
Benzene	18544-92-8	268	253
Benzene, 1-acetyl-3-ethyl-2-(2-ethenyl-6-ethylphenylazo)-	183-99-5	306	291
Benzeneacetic acid, 4	27750-57-8	296	73
Benzeneacetic acid, à-oxo-	55517-36-7	222	105
Benzeneacetic acid	2078-18-4	208	164
Benzenesulfonamide, N-butyl-	3622-84-2	213	141
Benzo[1,2-b:5,4-b']difuran-4,8-dione, 2-[1-(hydroxymethyl)vinyl]-5-methyl-	26962-41-4	258	258
Benzoic acid	65-35-7	194	179

Benzoic acid, 2-oxy	3789-85-3	282	439
Benzoic acid, 2-methyl	78324-00-2	250	119
Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	1421-49-4	250	235
Benzoic acid, 4-chloro-	25436-27-5	228	213
Benzoic acid, 4-methoxy-	2078-14-0	224	135
Benzoic acid, 4-methyl-, tert-butyl	345647-19-0	250	119
Benzoic acid, 5-methyl	624-53-7	296	237
Butylphosphonic acid, 3-chlorophenyl heptyl ester	6425-38-6	346	249
Carbocaine	96-88-8	246	98
Cinnamic acid, à-phenyl	63938-16-9	296	178
Cinnamic acid, m-methoxy	27750-64-7	250	235
Cinnamic acid, p-(trimethylsiloxy)-, trimethylsilyl ester	10517-30-3	308	293
Dibenzofuran-3ol, 2-methoxy-9a-(morpholin-4-yl)-5a,6,7,8,9,9a-hexahydro-	94420-49-2	305	226
Ethene, 1,2-diphenyl-1,2-bis	78375-55-0	324	309
Ferulic acid	10517-09-6	338	308
Hydrocinnamic acid, p-	27750-62-5	310	179
N(N'-Methyl-N'-nitroso(aminomethyl))benzamide	59665-02-0	193	105
p-Coumaric acid	10517-30-3	308	249
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	166273-38-7	306	191
Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4	206	206
Propiophenone, 2,2',4',6'-tetramethyl-	2040-22-4	190	147
Pyrocatechol, bis	67-946-8	338	115
Pyrrolidine, 1-[2-(4-bromophenoxy)ethyl]-	1081-73-8	269	84
Syringaldehyde	6651-62-3	254	239
Syringic acid	10517-29-0	342	297
trans-Cinnamic acid	140-10-3	220	131
Valerophenone	33342-91-5	250	193
Vanillic acid	2078-15-1	312	282
Vanillin	6689-43-6	224	209

I. Supplemental Results and Discussion

Near the mouth of the Amazon River DOC concentrations were nearly an order of magnitude greater than POC concentrations (SI Table 2). DOC and POC concentrations both appear to follow the hydrograph seasonally. The concentration of both dissolved L_8 lignin phenols and total dissolved phenolic compounds peaked in May 2011 and were lowest in September 2010 and 2011 (SI Table 2). Contrary to bulk DOC, dissolved lignin concentrations are elevated at low rising water (December 2010) relative to

low water in September (SI Table 2). The proportion of free monomers to dissolved L_8 lignin phenols is anomalously high in December, suggesting that during the early stages of rising water dissolved phenolic compounds, rich in monomeric subunits from partial degradation, are mobilized from subsurface soils into the river through stream networks (e.g. Ward et al., 2012). Free monomers were completely degraded (e.g. undetectable post-incubation) in every incubation experiment. In both dissolved and particulate phases, the ratio of vanillyl acids to aldehydes (Ad/Al(v)) increased with river discharge (SI Table 2), indicating that both the dissolved and particulate components mobilized into the river by rising and high discharge are more highly degraded than at low or decreasing flow. This trend is persistent not only in the tropics on a seasonal timescale, but has been observed on hourly timescales in temperate streams and rivers (Ward et al., 2012). Both particulate and dissolved L_8 lignin showed an elevated ratio of cinnamyl to vanillyl (C/V) phenols in September, indicating an elevated proportion of grass/leaf material (as opposed to woody material), and the lowest values in December at low-rising water. The ratio of syringyl to vanillyl (S/V) phenols was highest in May at high water and lowest in December, although the seasonal S/V variability does not appear to be statistically significant.

The absolute rates of degradation of both L_8 lignin and total phenolic compounds vary seasonally, following the hydrograph. However, when these rates are normalized to the initial L_8 and T_{phen} concentrations there is no statistically significant seasonal variability. This implies that lignin and aromatic respiration rates are likely primarily controlled by substrate availability in the river (e.g. Michaelis Menton). Bulk respiration rates, on the other hand, appear to be controlled by substrate quality since rates are inversely proportional to the hydrograph and OC concentrations.

Supplemental Table 2. Initial concentrations of dissolved (top, white) and particulate (bottom, grey) total organic carbon, Λ_8 lignin phenols, free Λ_8 monomers, and total phenolic compounds prior to incubation and the initial composition determined by the stable isotopic signature of organic carbon (δ^{13}C-POC), the ratio of syringyl to vanillyl phenols (S/V), the ratio of cinnamyl to vanillyl phenols (C/V), the ratio of vanillic acids to aldehydes (Ad/Al(v)), and the ratio of syringyl acids to aldehydes (Ad/Al (s)). Free Λ_8 monomers were completely consumed (i.e. undetectable) in all incubated samples.

Date	**DOC** (mg C L^{-1})	Λ_8 Lignin (μg C L^{-1})	Fraction of free Λ_8 lignin monomers (%)	Total aromatic DOC (mg C L^{-1})	S/V	C/V	Ad/Al (v)	Ad/Al (s)
16-Sep-10	4.8 ± 0.5	3.6 ± 0.4	0.98 ± 0.14	0.33 ± 0.05	0.37 ± 0.05	0.48 ± 0.07	0.52 ± 0.07	0.34 ± 0.05
1-Dec-10	3.5 ± 0.3	5.2 ± 0.5	6.40 ± 0.91	0.31 ± 0.04	0.21 ± 0.03	0.09 ± 0.01	0.58 ± 0.08	0.49 ± 0.07
7-May-11	5.7 ± 0.2	9.9 ± 1.0	0.04 ± 0.01	0.38 ± 0.05	0.33 ± 0.05	0.19 ± 0.03	1.25 ± 0.18	0.45 ± 0.45
13-Sep-11	3.8 ± 0.01	3.4 ± 0.3	0.73 ± 0.10	0.27 ± 0.04	0.27 ± 0.03	0.49 ± 0.06	0.42 ± 0.15	0.26 ± 0.04
Date	**POC** (mg C L^{-1})	Λ_8 Lignin (μg C L^{-1})		Total aromatic POC (mg C L^{-1})	S/V	C/V	Ad/Al (v)	Ad/Al (s)
16-Sep-10	0.45 ± 0.002	5.5 ± 0.6		0.076 ± 0.011	0.79 ± 0.11	0.15 ± 0.02	0.44 ± 0.06	0.44 ± 0.06
1-Dec-10	0.44 ± 0.002	3.4 ± 0.3		0.038 ± 0.005	0.71 ± 0.10	0.02 ± 0.00	0.46 ± 0.06	0.83 ± 0.12
7-May-11	0.56 ± 0.003	13.8 ± 1.4		0.061 ± 0.009	0.89 ± 0.13	0.09 ± 0.01	0.59 ± 0.08	0.42 ± 0.06
13-Sep-11	0.63 ± 0.003	6.8 ± 0.7		0.063 ± 0.009	0.74 ± 0.10	0.13 ± 0.02	0.40 ± 0.06	0.68 ± 0.10

Supplemental Table 3. Measured respiration rates of bulk organic carbon, dissolved (top, white) and particulate (bottom, grey) Λ_8 lignin phenols, and dissolved and particulate total phenolic compounds (T_{phen}). The normalized L_8 respiration rate is the ratio of the measured respiration rate to the initial L_8 concentration. The normalized T_{phen} respiration rate is the ratio of the rate of total phenolic respiration, based on relative GC-MS-TOF peak areas ($pA^2\ L^{-1}\ d^{-1}$), to the initial sum of peak area for total lignin-like compounds (95 identified compounds). The absolute rate of T_{phen} respiration ($\mu g\ C\ L^{-1}\ d^{-1}$) is determined by multiplying the normalized respiration rate by the ratio of total phenolic compounds to standardized L_8 lignin phenols and their initial concentration.

Date	Bulk OC respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Λ_8 Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Λ_8 respiration ($\mu g\ C\ \mu g\ C^{-1}\ L^{-1}\ d^{-1}$)	Total Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Total Lignin respiration ($pA^2\ pA^{-2}\ L^{-1}\ d^{-1}$)
16-Sep-10	55.4 ± 5.5	0.40 ± 0.05	0.11 ± 0.02	13.7 ± 2.9	0.042 ± 0.006
1-Dec-10	74.7 ± 7.5	0.40 ± 0.05	0.08 ± 0.01	21.0 ± 4.4	0.067 ± 0.010
7-May-11	39.4 ± 3.9	1.16 ± 0.14	0.12 ± 0.02	22.8 ± 4.8	0.060 ± 0.009
13-Sep-11	62.7 ± 6.3	0.72 ± 0.09	0.21 ± 0.03	14.2 ± 3.0	0.053 ± 0.008
Date	Bacterial abundance (10^9 cells L^{-1})	Λ_8 Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Λ_8 respiration ($\mu g\ C\ \mu g\ C^{-1}\ L^{-1}\ d^{-1}$)	Total Lignin respiration ($\mu g\ C\ L^{-1}\ d^{-1}$)	Normalized Total Lignin respiration ($pA^2\ pA^{-2}\ L^{-1}\ d^{-1}$)
16-Sep-10		0.53 ± 0.07	0.096 ± 0.015	5.9 ± 1.2	0.077 ± 0.012
1-Dec-10	2.37 ± 0.41	0.29 ± 0.04	0.085 ± 0.014	2.4 ± 0.5	0.064 ± 0.010
7-May-11	4.00 ± 0.7	0.83 ± 0.10	0.061 ± 0.010	3.3 ± 0.7	0.055 ± 0.008
13-Sep-11	3.90 ± 0.6	0.61 ± 0.08	0.090 ± 0.014	5.5 ± 1.2	0.087 ± 0.013