Char and Humin Fractions in Amazonian Dark Earths

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1. INTRODUCTION

The high and specific SOM contents of the Amazonian Dark Earths (ADE, Terra Preta de Índios), and their high contents of P and Ca provide high natural fertility to the soils. Char or black carbon (BC) plays a major role for the SOM stability in ADE soils (1) and it also provides a significant potential for carbon sequestration and sustainable ecosystems (2). There are several studies of the humic acids (HAs) of ADE soils (e.g., 3 and 4). However, because of the difficulties in isolation, there is less awareness of humin, the most abundant SOM component, and of the interaction mechanisms of recalcitrant BC with SOM (especially humin) and clays. In this study, humic materials were extracted using the IHSS procedure and by exhaustive sequential extraction procedures. Isotopic ^{13}C analyses, and solid-state ^{13}C NMR spectroscopy were used for characterizations.

2. MATERIALS AND METHODS

ADE sample 1 (500 g) was extracted by the exhaustive sequential extraction procedure using NaOH at pH 7, 10.6 and 12.6, and then with 0.1M NaOH + 6M urea (base/urea) following the procedure outlined in Song *et al* (5). Soil extracts were fractionated by the XAD-8 and XAD-4 resin-in-tandem procedure. ADE sample 2 (140 g) was treated and extracted following the procedure recommended by IHSS. Then the soil residue was extracted using 0.1M NaOH + 6M urea. HAs from 1 and from 2 were treated with 0.3M HF/0.1M HCl. Then the coarse char was carefully recovered by sieving (passing through 63 µm sieve) and sedimentation. The dry light fractions (<63 µm) from 1 and 2 were extracted with DMSO + 6% (v/v) H_2SO_4 (DMSO/H_2SO_4). The DMSO extract was adjusted pH to 2 with distilled water and further fractionated into DMSO humin (precipitate) and soluble DMSO FA (recovered by the XAD-8 resin). The humin associated with the light fraction (silt + clay) before and after DMSO/H_2SO_4 extraction was de-ashed with 10%

HF. The humic fractions were characterized by VACP-TOSS ^{13}C NMR spectroscopy (refer to Novonty *et al*. in this Conference Proceedings, and to 5).

3. RESULTS AND DISCUSSION

In the case of sample 1, the yields of HAs and FAs decreased significantly with increasing extractant pH (Table 1). The humic material isolated in base/urea was part of the humin fraction according to the classical definition. However, the isolates displayed characteristics of the alkaline humic fractions once released by base/urea. These are referred to as base/urea HAs or FAs. The HAs isolated with base and base/urea were the most abundant of the fractions (*ca*. 87%), with the FAs isolated with base and base/urea accounting for the remainder. The HAs and FAs isolated at pH 7 were the major fractions (*ca*. 57%) in the total HS isolated from base and base/urea solvents. That indicates that the ADE SOM is highly humified and oxidized. Using the urea enhanced base solvent from 8% to 19% additional humic materials were isolated. The data could suggest the humin is a mixture of HAs and FAs components that probably were trapped in, or associated with, through non-covalent bonds (e.g. H-bonds), the humic/humin core. These results support the supramolecular theory proposed by Piccolo (6), in which the HS may be regarded as associations of self-assembling heterogeneous and relatively small molecules derived from the degradation and decomposition of biomass debris. The aggregates will likely be 'held together' through a complex combination of hydrophobic associations, charge interactions, hydrogen bonds, and metal bridging (7). However, the base/urea humic fractions isolated and the DMSO humin contained relatively larger sized molecules than the conventional alkaline HAs or FAs. These are in 'stable aggregates' or 'macromolecules' (8).

Table 1. Yields of HAs, FAs, and humin isolated using base/urea and DMSO/H_2SO_4

	ADE Sample 1				ADE Sample 2	
	pH 7	pH 10.6	pH 12.6	Base/Urea	0.1M NaOH	Base/Urea
HA	3.38	1.13	0.07	1.04	17.55	1.61
FA	0.32	0.27	0.1	0.17	2.32	0.17
DMSO Humin		3.85			5.69	
DMSO FA		0.55				

The most recalcitrant SOM components associated intimately with silt and clay was isolated from DMSO/H_2SO_4. About 19 to 32% (calculated in terms of the total organic C contents of soil samples) of humin material can be extracted. With this sequence of extraction procedure, up to 74% of SOM was isolated.

The δ^{13}C values of the humic fractions indicated the humic material was mainly derived from C3 plants, with an average δ^{13}C value of *ca* -28.1‰. The δ^{13}C values of the HA fractions were slightly enriched with [13]C. The δ^{13}C decreased from -28.1‰ to -30.0‰ for DMSO humin and from -30.8 to -31.7‰ for the DMSO extracted residue, respectively. This might be explained selective utilization of organic compounds by microbes.
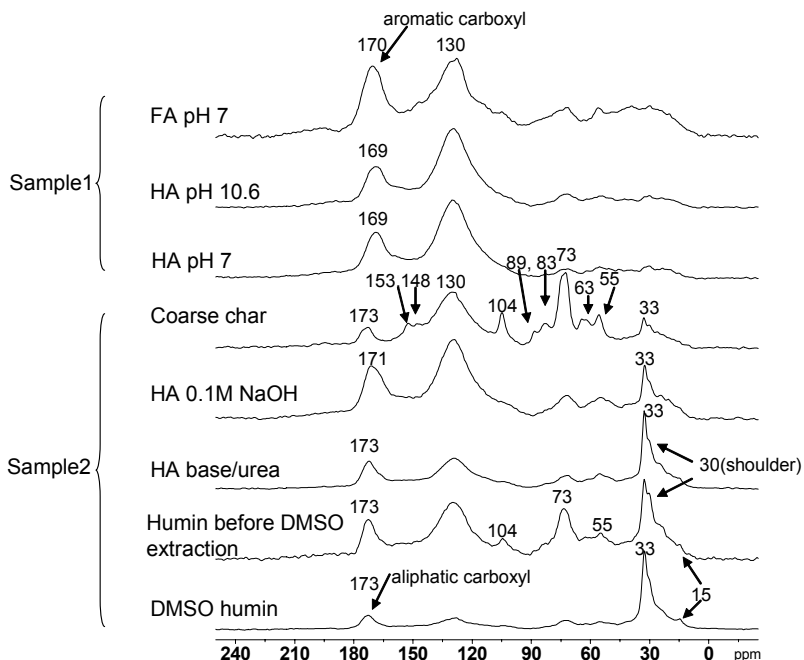

Figure 1. VACP-TOSS ^{13}C NMR spectra of HAs and FAs isolated with base, base/urea HA, humin before DMSO/H_2SO_4 extraction from light fraction, and DMSO humin.

The predominant resonances for the HAs, FAs and coarse char in 110 to 156 ppm region (Figure 1) showed hydrogen-deficient condensed aromatic C, mainly derived from char. The dipolar dephasing spectra (not shown) of the HAs and FAs isolated from 0.1M NaOH and the coarse char presented significant unprotonated C in 110 to 156 ppm region, *ca* 74%, 80% and 62%, respectively, compared to the corresponding total C area. The alkaline HAs and FAs were enriched with carboxylic groups attached to the condensed aromatic 'core', as evidenced by the relatively lower resonances of COO at *ca.* 169 to 171 ppm. The coarse char was mixed with components from lignin units (55, 148 and 153 ppm resonances), cellulose (resonances at 63, 73, 83, 89, 104 ppm) and BC (prominent aromatic C at 130 ppm). In another word, the coarse char material presents signals of

unaltered plant debris, BC and aliphatic components. The base/urea HA and DMSO humin had prominent aliphatic groups (crystalline polymethylene C at 33 ppm and amorphous polymethylene C at 30 ppm), and relatively low contents of aromatic C and carboxyl groups, suggesting significant contributions from long-chain aliphatic moieties (e.g., lipids, waxes, cutin, etc.). Compared to DMSO humin, humins before and after DMSO/H_2SO_4 extraction were very similar. It is likely that condensed BC, peptides and carbohydrate (e.g. cellulose) components were trapped in the humic matrix or physically protected by intimate association with clay, whereby both base/urea and DMSO/H_2SO_4 solvent could only partially cleave the weak bonds between BC, humin and clay minerals.

4. CONCLUSIONS

The well humified and oxidised char derived from incomplete combustion of biomass contribute the main components in ADE SOM components. The most recalcitrant BC in the humin matrix is probably embedded within the silt and clay minerals forming organic-mineral complexes, or protected by steric constraints which are difficult to cleave by base/urea and DMSO solvents. The primary recalcitrant SOM (DMSO humin) is from non-BC sources, such as long-chain fatty acids/ester, lipids, cutins, etc. containing derivatives of microbial origins.

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