REDOX INDEX OF SOIL CARBON STABILITY[1]

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SUMMARY

As an alternative to the relatively complex and expensive spectroscopic methods, the redox properties of humic acids, determined by potentiometric titrations, have been used to evaluate the stability of soil organic C. The objective of the present study was to establish a Redox Index of C Stability (RICS) and to correlate it with some properties of the humic acids extracted from different modal soils in Brazil (distinct weathering stages or management) to facilitate system comparison. The RICS was efficient for soil comparison and variations were comparable to those of the chemical and spectroscopic methods used for humic acid characterization. The values of soil pH, point of zero salt effect, sum of bases, exchangeable Ca content, weathering index, as well as the humic acid O/C ratio, quinone and semiquinone free radical contents, aromatic C and fluorescence intensity were closely related with the RICS. The RICS was higher in less weathered soils, with more active clays and higher fertility. The RICS values of soils under long-term sugarcane management were ranked in decreasing order: unburned, burned with vinasse, burned without vinasse.

Index terms: soil chemistry, soil organic matter, humic substances, oxidation capacity.

RESUMO: *ÍNDICE REDOX DE ESTABILIDADE DO CARBONO DO SOLO*

Como alternativa aos relativamente complexos e dispendiosos métodos espectroscópicos, as propriedades redox dos ácidos húmicos, determinadas por titulação potenciométrica, vêm sendo usadas na avaliação da estabilidade do C do solo. O objetivo deste estudo foi estabelecer um índice redox de estabilidade do C (RICS) e correlacioná-lo com alguns atributos de diferentes solos modais brasileiros (distintos estádios de intemperismo ou manejo), a fim de mais facilmente comparar sistemas. O RICS foi eficiente para comparação de solos e variou similarmente com os métodos químicos e espectroscópicos usados na caracterização dos ácidos húmicos. Os valores de pH, ponto de efeito salino nulo zero, soma de bases, teor de cálcio trocável, índice de intemperismo, bem como de razão entre oxigênio e carbono, teores de quinonas e de radicais livres semiquinonas, carbono aromático e intensidade de fluorescência dos ácidos húmicos, mostraram estreitas relações com o RICS. Os solos menos intemperizados, com argilas mais ativas e mais alta fertilidade apresentaram maior RICS. Os solos sob longo tempo de manejo com cana-de-açúcar mostraram RICS seguindo a ordem decrescente: cana crua, cana queimada com vinhaça, cana queimada sem vinhaça.

Termos de indexação: química do solo, matéria orgânica do solo, substâncias húmicas, capacidade de oxidação.

INTRODUCTION

Organic matter preservation is a first step towards a recovery of the soil biological, chemical and physical properties, with a view to the reduction of erosion and environmental pollution and increased soil fertility (Zech et al., 1997). The humus reactivity is determined by the functional groups found in humic substances (HS) (Schnitzer & Gupta, 1965; Schnitzer & Riffaldi, 1972; Baldotto et al., 2007, 2008; Sposito, 2008).

Humic acids (HA) participate in redox reactions with chemical species existing in soils, e.g., Fe (Lovley, 1997; Lovley et al., 1996, 1998, 2000; Scott et al., 1998), Mn (Sunda & Kieber, 1994; Lovley, 1995), Hg (Alberts et al., 1974; Mathiessen, 1996), and V (Wilson & Weber, 1979), catalyzed by microorganisms. Other studies indicated that the quinone moieties in the HA may participate in the redox reactions as electron transfers (Mathiessen, 1995; Scott et al., 1998; and Struyk & Sposito, 2001). These groups, regardless of the low concentrations, contribute to the HA activity in most redox reactions occurring in natural systems.

The Semiquinone-type Free Radical Concentration (SFRC) in humus has been used to evaluate soil C stability. The SFRC was quantified by electron spin resonance (ESR) spectroscopy and correlated with indexes estimated by ultraviolet-visible (E_4/E_6), fluorescence intensity (FI), nuclear magnetic resonance (NMR ^{13}C) and infra-red spectroscopies (Milori et al., 2002; Perez et al., 2004; Canellas et al., 2007, 2008) of HA.

Kononova (1961) introduced an index of humification degree based on Russian soils, the E_4/E_6 ratio, i.e., the relation of absorbances at 465 and 665 nm, and attributed low E_4/E_6 values to a high humification degree. Milori et al. (2002) and Senesi (1990) studied organic matter quality by fluorescence spectroscopy. The fluorescence intensity at 465 nm is believed to promote energy absorption by electrons in condensed aromatic rings, and/or highly unsaturated alkyl chains (Milori et al., 2002) in HA. The order of the fluorescence intensity values was inverse to that observed for the E_4/E_6 ratio.

The ^{13}C NMR spectra of HA indicated the increase of hydrophobicity (sum of aromatic and alkyl C) with increasing soil C stability (Piccolo, 2001; Ikeya et al., 2004; Spaccini et al., 2006; Xiaoli et al., 2007; Conte et al., 2007; Canellas et al., 2008). This hydrophobicity in HA as a result of increased stability had previously been shown in association with the quinone moieties (Milori et al., 2002; Perez et al., 2004; Canellas et al., 2007, 2008) of HA.

The reduced quinone moieties in HA can be oxidized by I_2, using redox titrations (Struyk & Sposito, 2001). Thus, as an alternative to the relatively complex and expensive spectroscopic methods, redox titrations have been used, as in this study, to evaluate soil C stability (Baldotto et al., 2007, 2008). Positive correlations between redox capacity and this aromaticity were reported by Scott et al. (1998) and Baldotto et al. (2007, 2008) and support the hypothesis that the quinone moieties are significant redox groups in HA.

Baldotto et al. (2007, 2008) used redox titrations (I_2) and the techniques of methylation or acetylation to evaluate the functional groups involved in redox properties of HA and showed that a greater and similar decrease of oxidation capacity (~ 91 %) was verified in both the methylated and acetylated derivatives in comparison with the original HA, suggesting that the free quinones and phenolic groups, that can be blocked by derivatization, were the

principal participants in the redox reactions. Baldotto et al. (2007) also verified by infrared spectroscopy of HA samples that the absorption attributed to C-H stretching (2.927–2.942 cm^{-1}) increased with HA methylation or acetylation. This confirmed that the oxidation capacity of HA is associated with phenolic and quinonic aromaticity.

An index incorporating the redox properties of HA would be useful for a more precise evaluation of the soil C stability or persistence. This index of stability, in a first approach, would be defined combining the oxidation capacity and the stock of soil HA. The oxidation capacity (mol_c kg^{-1}) of humic acids is defined as the amount of charge that compost can transfer to an oxidant (e.g., I_2) and can be determined by redox titrations (Struyk & Sposito, 2001; Baldotto et al., 2007, 2008). The humic acid pool (Mg ha^{-1}) is obtained based on the HA concentration in a sampled soil layer with determined density (Canellas et al., 2007).

The objective of this study was to establish a Redox Index of C Stability - RICS - and to correlate it with some chemical properties of the HA extracted from 15 different Brazilian soils.

MATERIAL AND METHODS

Brazilian soils

Modal soil samples were collected from the surface horizons of 11 sites located in a soil weathering sequence. These sites were described and characterized (Table 1) by Baldotto et al. (2007, 2008), Canellas et al. (2008) and Dobbss et al. (2008, 2009). The study also included an Entisol under continuous

sugarcane cultivation over the course of 55 years (Canellas et al., 2003, 2008; Busato et al., 2005a,b; Baldotto et al., 2008). In this case the soil plots differ only in the practice of burning or not burning sugarcane at harvest or in vinasse applications (with crop burning). The soil plots were adjacent to each other and part of the same morphologically uniform flat area (data corroborated in fieldwork interviews with landowners). Since no spatial variability was found at soil sampling, differences between the plots were attributed to changes induced by the different long-term management practices of sugarcane cultivations (burned vs. unburned; burned with vinasse vs. burned without vinasse).

Sampling and characterization of soil and humic acids

All soil samples were taken from the 0.00–0.20 m layer. The samples were air-dried, ground, sieved (2 mm) and stored for characterization in the laboratory. Details of soil organic matter fractionation, HA isolation, purification and elementary composition, functional group contents, oxidation capacity, as well as ultraviolet-visible (E_4/E_6), fluorescence (FI), nuclear magnetic resonance (NMR ^{13}C) and electron spin resonance (ESR) spectroscopy for the HA have previously been presented and discussed by Baldotto et al. (2007, 2008), Canellas et al. (2008) and Dobbss et al. (2008, 2009). Some of the soils and HA properties were selected and are shown in the tables 2 and 3, respectively.

Redox titrations

The redox titration data had been reported previously by Baldotto et al. (2007, 2008). Since these results constituted the main variable underlying the

Table 1. Classification, vegetation, coordinates and location of the soil samples

Soil sample N°	Classification[1] USDA	Brazilian	Vegetation	Coordinate Latitude	Coordinate Longitude	Location
1	Typic Calciustoll	Chernossolo Rêndzico Saprolítico típico	Grass	21° 25'	41° 41'	Rio de Janeiro
2	Vertic Argiustoll	Chernossolo Argilúvico órtico vértico	Grass	21° 13'	41° 49'	Rio de Janeiro
3	Typic Kanhaplustult	Luvissolo Cromico pálico abrúptico	Grass	21° 12'	41° 50'	Rio de Janeiro
4	Ultic Paleustalf	Argissolo Vermelho Amarelo distrófico	Grass	21° 13'	41° 50'	Rio de Janeiro
5	Typic Haplustox	Latossolo Amarelo coeso típico	Grass	21° 43'	41° 20'	Rio de Janeiro
6	Haploperox	Latossolo Bruno distroférrico rúbrico	Grass	28° 30'	50° 56'	Rio Grande do Sul
7	Rhodic Humic Hapludox	Latossolo Vermelho perférrico húmico	Savana (Cerrado)	20° 04'	43° 58'	Minas Gerais
8	Sombrihumox	Latossolo Vermelho-Amarelo distroférrico húmico	Tropical Forest	22° 16'	42° 31'	Rio de Janeiro
9	Rhodustalf	Latossolo Vermelho acriférrico típico	Tropical Forest	28° 17'	54° 15'	Rio Grande do Sul
10	Hapludox	Latossolo Vermelho Amarelo distrófico típico	Tropical Forest	22° 31'	43° 43'	Rio de Janeiro
11	Rhodic Hapludox	Latossolo Vermelho distrófico típico	Savana (Cerrado)	15° 47'	47° 55'	Brasília
	Entisol:					
12	Unburned at harvesting	Cambissolo Háplico Ta eutrófico vertissólico				
13	Burned at harvesting		Sugarcane	21° 00'	41° 30'	Rio de Janeiro
14	With vinasse					
15	Without vin					

[1] Classification: Soil Survey Staff (2006) and Brazilian System of Soil Classification (Embrapa, 2006), respectively.

Table 2. Chemical and physical properties of the soil samples

Soil sample	Soil attributes													
	C	FA	HA	H	HA/FA	C_S	Ki	pH H_2O	pH KCl	ΔpH	PZSE	Sand	Loam	Clay
Class	——— g kg^{-1} ———					Mg ha^{-1}						—— g kg^{-1} ——		
Typic Calciustoll	22.6	1.21	3.44	19.2	2.9	47.9	2.6	7.0	6.8	-0.2	3.0	400	180	420
Vertic Argiustoll	17.2	2.42	3.66	10.8	1.5	35.8	1.9	6.6	5.8	-1.2	3.5	300	230	470
Typic Kanhaplustult	16.2	3.33	3.11	6.6	1.0	48.6	1.8	6.6	5.0	-1.6	3.5	400	330	270
Ultic Paleustalf	13.6	3.32	1.90	9.3	0.6	27.5	1.8	6.4	5.4	-1.0	4.1	600	160	240
Typic Haplustox	13.1	2.14	0.83	8.9	0.4	35.6	1.6	5.5	4.3	-1.2	4.1	600	20	380
Haploperox	43.0	0.63	0.05	33.2	0.1	85.1	1.8	6.1	4.2	-1.9	5.8	50	230	720
Rhodic Humic Hapludox	12.8	0.14	0.02	8.5	0.2	25.6	0.1	5.8	4.8	-1.0	4.0	280	290	430
Sombrihumox	29.5	0.32	0.08	26.9	0.3	67.9	0.7	5.2	4.4	-0.8	4.9	350	160	490
Rhodustalf	10.7	0.31	0.06	7.2	0.2	25.9	2.0	6.8	5.9	-0.9	5.2	130	250	620
Hapludox	12.5	0.22	0.06	8.4	0.3	28.3	1.5	5.3	4.5	-0.8	5.1	400	110	490
Rhodic Hapludox	25.4	0.38	0.07	19.8	0.2	62.5	1.6	5.1	4.6	-0.5	5.0	140	110	750
Entisol:														
Unburned at harvesting	22.3	0.99	0.96	12.7	0.9	59.3	2.5	6.7	6.0	-0.7	3.1	270	380	350
Burned at harvesting	13.1	0.97	0.36	9.2	0.4	36.2	2.5	6.5	6.1	-0.4	3.7	230	400	370
With Vinasse	18.3	0.71	0.39	7.0	0.6	52.0	2.5	5.9	5.3	-0.6	3.3	150	470	380
Without Vinasse	15.7	0.52	0.28	5.1	0.5	48.4	2.5	6.0	5.5	-0.5	3.5	160	520	320
	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}	H + Al	SB	t	T	V	m		
	—— mg dm^{-3} ——		———————— cmol$_c$ kg^{-1} ————————								—— % ——			
Typic Calciustoll	10.2	46.8	43.3	0.4	0.14	0.0	0.8	44.0	44.0	44.8	98	0		
Vertic Argiustoll	7.3	81.9	18.2	0.4	0.10	0.0	2.8	18.9	18.9	21.7	87	0		
Typic Kanhaplustult	7.5	39.0	9.9	0.2	0.06	0.0	2.4	10.3	10.3	12.7	81	0		
Ultic Paleustalf	5.4	58.5	4.7	2.6	0.09	0.0	3.3	7.5	7.5	10.8	70	0		
Typic Haplustox	5.3	74.1	1.8	1.9	0.09	0.1	4.6	4.0	4.1	8.6	46	2		
Haploperox	1.5	187.2	1.0	0.2	0.06	3.9	3.0	1.7	4.7	5.6	30	64		
Rhodic Humic Hapludox	0.0	31.2	0.6	0.4	0.03	0.2	0.9	1.1	1.3	2.0	55	15		
Sombrihumox	8.0	27.3	0.4	0.3	0.06	1.2	1.0	0.8	2.0	1.8	44	60		
Rhodustalf	1.5	214.5	0.8	0.3	0.08	1.1	4.7	1.7	2.8	6.4	26	39		
Hapludox	0.0	54.6	1.2	0.7	0.04	1.3	3.1	2.1	3.4	5.2	41	38		
Rhodic Hapludox	1.0	35.1	0.6	0.4	0.02	0.8	3.3	1.1	1.9	4.4	25	42		
Entisol:														
Unburned at harvesting	546.2	585.0	8.3	2.6	0.34	0.0	2.2	12.7	12.8	15.0	85	0		
Burned at harvesting	6.3	85.8	4.8	2.9	0.18	0.0	2.3	8.1	8.1	10.4	78	0		
With Vinasse	21.7	315.9	5.3	2.4	0.11	0.0	3.7	8.6	8.4	12.2	69	0		
Without Vinasse	6.1	171.6	4.9	2.2	0.11	0.0	3.2	7.7	7.8	11.0	71	0		

Properties: C, FA, HA e H: total carbon, fulvic acids, humic acids and humins, respectively; C_S: carbon stock (0–0.20 m); Ki: SiO_2/Al_2O_3 molar ratio; pH: soil: solvent 1: 2.5; ΔpH: $pH_{KCl} - pH_{H2O}$; PZSE: point of zero salt effec; Sand, Loam and Clay: granulometric fracions; P e K^+: Mehlich-1 extractor; Ca^{2+}, Mg^{2+} e Al^{3+}: KCl 1 mol L^{-1} extractor; H + Al: CaOAc 0.5 mol L^{-1} pH 7.0 extractor; SB: sum of bases; t and T: effective and potential (pH 7.0) cation exchange capacity, respectively; m: Al^{3+} saturation; V: base saturation.

proposal of RICS in this study, the methods were revised (Struyk & Sposito, 2001) and described here again.

Titrations of the HA samples under argon gas in aqueous suspension were performed by oxidation with I_2. For each titration the oxidant solution was standardized with sodium thiosulfate. The titration vessel was a hermetic 200 mL flask, maintained at room temperature under continuous water flow, with a lid prepared to install a pH measurement equipment or (Quimis-Q400A) a platinum redox combination electrode (Analyser-6A05-GK AgCl, Ag | | Pt), an argon (Ar) gas inlet tube, and a 2 mL microburet (Gilmont®

GS-1200-A, with 2 µL subdivisions). For each titration, the pH meter was calibrated using pH 4 and pH 7 buffers and the redox-combined electrode was checked with a ferric-ferrous redox buffer (Light, 1972).

For each titration, 125 mL of a combined 0.05 mol L^{-1} potassium chloride (KCl), phosphate buffer solution (Na_2HPO_4 0.05 mol L^{-1} and $NaH_2PO_4.H_2O$ 0.05 mol L^{-1}) were added to the titrated vessel. The pH was adjusted with HCl or NaOH to pH 5 or 7, and then purged with argon for 60 min for oxigen gas depletion. HA was added to reach a suspension concentration of 50.0 mg L^{-1} (6.25 mg HA

Table 3. Chemical properties of humic acid fractions isolated from the soil samples

Soil	C/N	O/C	Carboxilic	Phenolic	Quinone	Semiquinone	E₄/E₆	F.I.	C-sp²
						Humic acids attribute[1]			
Class					mol$_c$ kg^{-1}				135-160 ppm
Typic Calciustoll	14.1	0.5	2.90	1.65	0.74	4.92 x 10^{-4}	5.8	8,798	36
Vertic Argiustoll	8.3	0.5	1.90	1.29	0.71	3.42 x 10^{-4}	5.9	9,850	40
Typic Kanhaplustult	13.9	0.6	2.10	1.42	0.73	8.47 x 10^{-5}	7.0	6,857	42
Ultic Paleustalf	14.1	0.6	3.45	1.61	0.55	4.15 x 10^{-5}	7.3	6,588	45
Typic Haplustox	11.9	0.8	1.08	0.55	0.47	1.66 x 10^{-5}	7.5	5,624	41
Haploperox	10.7	0.7	1.97	1.23	-	-	6.9	_159_	_16_
Rhodic Humic Hapludox	11.8	0.9	0.71	1.90	-	-	6.7	_81_	_18_
Sombrihumox	8.7	1.2	1.84	1.21	-	-	4.0	_149_	_12_
Rhodustalf	11.7	1.0	0.69	1.49	-	-	6.5	_82_	_15_
Hapludox	8.6	0.7	3.54	0.94	-	-	5.4	_115_	_14_
Rhodic Hapludox	11.8	0.8	2.23	1.51	-	-	8.9	_113_	_21_
Entisol:									
Unburned at harvesting	15.1	0.8	4.77	2.70	0.85	4.14 x 10^{-4}	3.5	_17,804_	_34_
Burned at harvesting	11.3	0.5	2.07	0.79	0.43	5.65 x 10^{-5}	4.3	_8,954_	_10_
With Vinasse	12.7	0.7	4.60	2.62	0.77	2.16 x 10^{-4}	3.5	_10,754_	_20_
Without Vinasse	11.5	0.6	2.15	0.84	0.51	5.98 x 10^{-5}	4.1	_9,505_	_19_

[1] C/N and O/C: ratios from elementary composition; Carboxilic, phenolic and quinone functional groups; Semiquinone: free radicals; E₄/E₆: ratio of UV-vis absorbance at 465 and 665 nm; F.I.: fluorescence emission intensity at 465 nm (u.a.: arbitrary unity); C-sp²: aromatic and olefinic groups including quinones (NMR ^{13}C). F.I. and C-sp² are semiquantitative data, from different analytical series, indicated by normal, underlined and italic letters, respectively.

suspended in 125 mL 0.05 KCl, 0.05 phosphate buffer solution), and the resulting suspension was bubbled for 30 min. After deaerating the HA suspension, the pH was checked and readjusted to 5.00 or 7.00 prior to the titration procedure. When the change in cell electromotive force (emf$_{cell}$) did not exceed 2.0 mV min^{-1}, the pH value, emf$_{cell}$, and volume of iodine (I$_2$) solution remaining in the microburet were recorded as starting values.

In titration, 2 μL increments of 0.025 mol kg^{-1} I$_2$ solution were added, and after each addition of the oxidizer, the solution was stirred and bubbled with argon for 30 s to facilitate mixing. The gas inlet tube was raised from the solution after stirring. Then, the electrodes were lowered into the supernatant solution and electromotive force of cell titration (emf$_{cell}$) was measured by a potentiometer until the drift was no more than 2.0 mV min^{-1}, usually after a period of 10–15 min. Titrations were continued until the values of emf$_{cell}$ were virtually constant after the addition of the I$_2$ solution. Each titration required approximately 2.5–5 h.

Titration data consisted of emf$_{cell}$, which is a function of the moles of charge transferred per unit mass of HA (mol$_c$ kg^{-1}). Smooth curves were drawn through plots of emf vs. mol$_c$ kg^{-1} using the interpolation function of the software package. An inflection point was located on each curve and its position was used to determine the oxidation capacity. Cell titrations were performed in blank solutions without HA (Struyk & Sposito, 2001).

Redox index of carbon stability - RICS

The RICS (mol$_c$ ha^{-1}), in a first approach was defined by equation 1:

$$RICS = OC . S_{HA} \qquad (1)$$

where OC is the oxidation capacity of the HA (mol$_c$ kg^{-1}) and S$_{HA}$ is the HA stock in the soil sample (Mg ha^{-1}) obtained by equation 2:

$$S_{HA} = HA . layer . sd . 10 \qquad (2)$$

where HA is the concentration of humic acid fraction (g kg^{-1}), the layer of the soil sampled (m), and sd is the soil density (Mg m^{-3}).

Thus, the sum of the product between the oxidation capacity (OC$_{Fraction}$) and stock (S$_{Fraction}$) for each i to n fractions (not necessarily of the chemical fractionation) could be using the following refined expression:

$$RICS = \sum_{i}^{n} (OC_{Fraction}).(S_{Fraction}) \qquad (3)$$

Therefore, for a given system (e.g., soil, sediment) this equation 3 for RICS comprises the global sum of the transferable electrons of the total C pool.

Data analysis

The properties of soil and HA samples (Tables 2 and 3) were examined to discover trends in the RICS data. Conditions for the statistical significance of the correlation coefficient values were described by Steel

& Torrie (1960). The grouping test using the minimization of variance (Euclidean distance) was obtained by multivariate analysis of data (Cruz, 2006).

RESULTS AND DISCUSSION

The RICS varied significantly with sample origin (Table 4). The RICS values of soils in early and intermediate weathering stages were higher (14.35 to 46.87 mol_c ha^{-1}) than of highly weathered soils (< 0.57 mol_c ha^{-1}). Depending on the management, the RICS of the Entisol under continuous sugarcane cultivation varied drastically (from 1.63 to 16.45 mol_c kg^{-1}), decreasing in the following order: unburned, burned, burned with vinasse and burned without vinasse.

Some chemical properties of soil and humic acids (Tables 2 and 3) were significantly linearly correlated with RICS (Table 4). RICS presented correlations with soil pH ($r = 0.64$ $p < 0.01$), point of zero salt effect (PZSE) ($r = -0.60$ $p < 0.01$), sum of bases ($r = 0.82$ $p < 0.01$), Ca^{2+} content ($r = 0.84$ $p < 0.01$) as well as with the O/C ratio ($r = 0.57$ $p < 0.05$), quinone ($r = 0.58$ $p < 0.05$) and semiquinone free radicals ($r = 0.77$ $p < 0.01$) in the HA. Higher RICS values were accompanied by higher C-aromatic (NMR) ($r = 0.69$ $p < 0.01$). The fluorescence intensity data of HA samples was discussed in a different study (Baldotto et al., 2007, 2008; Canellas et al., 2008; Dobbss et al., 2008, 2009) and the correlations were obtained separately, with an average of coefficients equal to $0.70^{p < 0.05}$. RICS (Table 4) was also associated with the weathering stage (Ki) (Table 2) and morphogenetic properties of the soils (Baldotto, 2006). The RICS values in decreasing order followed the sequence of the soil structure in the diagnostic horizon: big blocks forming prisms (vertic horizons) > Simple with big blocks (argillic horizons with high-activity clays) > Simple with small blocks (argillic horizons with low-activity clays) ~ poorly formed/granular blocks (oxic horizons).

Complex and highly condensed aromatic structures are believed to stabilize quinone moieties in HA (Schnitzer & Levesque, 1979; Senesi, 1990; Martin-Neto et al., 1998; Bayer et al., 2002). In this study, the HA values for these aromatic compositions (Table 4) were considerably different and varied with the sample origin. Highest values were found for HA from the least weathered soils, whereas the lowest values were observed in soils with the most advanced weathering stage. Concomitantly, the variations in RICS accompanied the weathering stage.

The dendrogram (Figure 1) grouped the soils differently. The oxidic soils appear separated from those with higher activity clays and less oxides. Ultic Paleustalf and Typic Haplustox were grouped with the less weathered soils. On the other hand, Typic Calciustoll was grouped together with Entisol under longstanding unburned sugarcane. Multivariate analysis also showed that soils under continuous vinasse inputs are not different from those without vinasse applications or burnt at harvest. The separation based on RICS was similar.

Two of the least weathered soils have a chernozemic horizon (Table 1). This horizon is an indicator of high base saturation (more than 65 %), with the prevalence of the Ca and Mg (Embrapa, 2006). The prismatic structure of these soils is coherent with high-activity clays and natural fertility. These factors contributed to the accumulation and predominance

Table 4. Redox Index of Carbon Stability (RICS) and other variables

Soil	Variable [1]				
	OC	S_{HA}	SD	RICS	Weathering stage
	mol_c kg^{-1}	Mg ha^{-1}	Mg m^{-3}	mol_c ha^{-1}	
Typic Calciustoll	6.43±0.06	7.29	1.06	46.87	Early/intermediate
Vertic Argiustoll	5.78±0.05	7.61	1.04	44.00	Early/intermediate
Typic Kanhaplustult	4.11±0.10	9.33	1.50	38.35	Int ermediate
Ultic Paleustalf	3.74±0.08	3.84	1.01	14.35	Intermediate
Sombrihumox	2.72±0.08	0.18	1.15	0.50	Advanced
Hapludox	3.51±0.09	0.14	1.13	0.47	Advanced
Typic Haplustox	2.69±0.07	0.24	1.36	0.36	Advanced
Rhodustalf	2.43±0.09	0.15	1.21	0.35	Ad vanced
Rhodic Hapludox	1.72±0.08	0.17	1.23	0.29	Advanced
Haploperox	1.94±0.13	0.10	0.99	0.19	Advanced
Rhodic Humic Hapludox	2.90±0.12	0.04	1.00	0.12	Advanced
Entisol:					
Unburned at harvesting	6.44±0 .06	2.55	1.33	16.45	
Burned at harvesting	1.64±0 .08	0.99	1.38	1.63	Intermediate
Burned with vinasse	4.99±0 .06	1.11	1.42	5.53	
Burned without vinasse	2.37±0 .06	0.86	1.54	2.04	

[1] Variables: OC, S_{HA} and SD: oxidation capacity, humic acid stock and soil density, respectively.
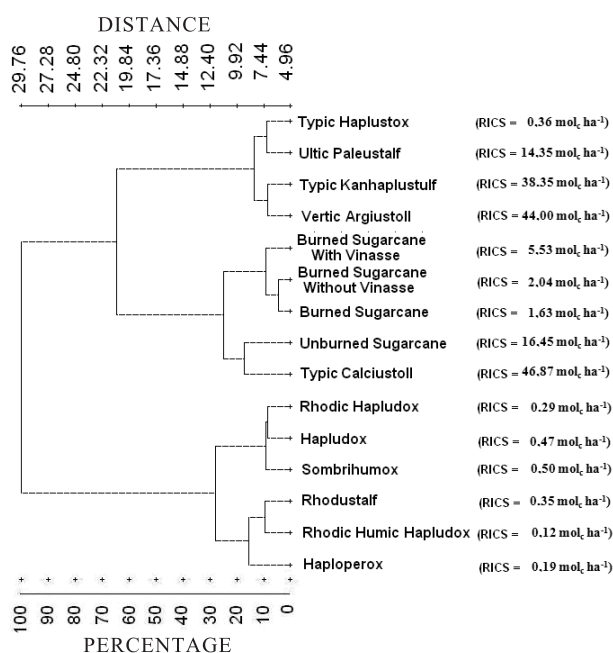
Figure 1. Dendrogram for the minimum variance based on Euclidean distances obtained by multivariate analysis of all variables for the group of studied samples and Redox Index of Carbon Stability (RICS).

of stable forms of organic matter in the soil surface with increasing RICS values. Sposito (2008) stated that the C protection increases simultaneously with the soil CEC and the available bases. Cunha et al. (2005) studied several Brazilian Oxisoils and verified that the AH/AF relationship was inversely correlated with base saturation. The high base saturation (e.g., Ca^{2+}) is another factor associated to the increase of C stability in the soil system (Mendonça & Rowell, 1996). Thus, the Ca^{2+} can tie two negative surfaces , organic or mineral. The leaching of this basic cation as weathering advances leads to the progressive acidification of the soils.

The genesis of these weathered soils occurred under extreme climatic factors and generated soils with low water and nutrient retention capacity and high acidity. These results indicate that in the soils where is the larger need of the soil organic matter (SOM), e.g., those described as advanced weathering stage (Table 4), which the amount and quality of humus were the lower, with the smallest values of RICS for all samples.

In the special case of the Typic Haplustox, the soil was grouped together with the less weathered and appears distant from the very leached soils in the dendrogram. This soil was formed by neogenic action on colluvial sediments of the tertiary period. It occupies the coastal landscape of Brazil, which in the past was covered by Atlantic Forest. Nowadays, unfortunately, few areas are preserved. These soils

characteristically have a clay-sand texture with a weak presence of loam and predominance of kaolinite in the clay fraction.

The addition of organic matter over 55 years of continuous sugarcane cultivation, through the maintenance of the cane trash on soil surface (unburned sugarcane) resulted in the presence of more reactive humic acids with higher RICS (Table 2), increased soil CEC and soil C content and decreased PZSE, compared to the sugarcane management with burning. The values of the same soil properties in plots of this Entisol under longstanding use of vinasse were intermediary. The separation based on RICS was similar (Table 4).

In the commonly used management forms of sugarcane cultivation the concern about soil protection and conservation is not a priority. The intensive use of heavy machinery during soil preparation, combined with crop burning to facilitate harvesting contributes to the degradation of soil organic matter and the decrease of soil fertility and crop yields (Bayer et al., 2002; Canellas et al., 2003; Busato et al., 2005a,b; Canellas et al., 2007; Baldotto et al., 2008). As a consequence of the high temperature on soil surface during burning, the oxygenated functional groups (González-Pérez et al., 2004), C, N, and O concentrations of SOM are diminished (Fernandez et al., 2004; Garcia-Gil et al., 2004). Similar alterations were observed in this study (Table 3). An additional disturbance of the burned sugarcane management is CO_2 emission to the atmosphere.

Finally, the relationships between variables of a group involving different samples, mainly of soils, requires caution in applicability. This study showed an estimative of correlations for all soils together. However, the contrasting samples presented data with magnitude more discrepants. This observation indicates difficulties in the adjustment of predictive models to evaluate the quality of humic substances. This is the case when the differences between sample environments are minor, e.g., among weathered soils, perhaps because these systems tend to equilibrium. This use of RICS is intended as a thermodynamic function, as an indicator of the oxidation tendency of the C stock. It would be expected that a soil C stock with high reducing power is more likely to persist in the organic matter of a given system.

CONCLUSIONS

1. The RICS was efficient for soil C stability comparison.

2. The variations in results of chemical and spectroscopic methods (UV-Vis, Fluorescence, RPE and NMR) used for humic acid characterization were similar to those of RICS.

3. The soil pH, PZSE, sum of bases, Ca^{2+} content, weathering index Ki as well as the humic acid O/C ratio, quinone and semiquinone free radicals, aromatic C, and fluorescence intensity were closely related with RICS.

4. The RICS values of less weathered soils with more active clays and higher fertility are higher.

5. Under long-term sugarcane management the RICS values could be ranked as follows: unburned > burned with vinasse > burned without vinasse.

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LITERATURE CITED

ALBERTS, J.J.; SCHINDLER, J.E. & MILLER, R.W. Elemental mercury evolution mediated by humic acid. Science, 184:895-896, 1974.

BALDOTTO, M.A. Propriedades redox e grupos funcionais de ácidos húmicos. Campos dos Goytacazes, Universidade Estadual do Norte Fluminense, 2006. 100p. (Tese de Doutorado)

BALDOTTO, M.A.; CANELLAS, L.P.; CANELA, M.C.; SIMÕES, M.L.; MARTIN-NETO, L.; FONTES, M.P.F. & VELLOSO, A.C.X. Propriedades redox e grupos funcionais de ácidos húmicos isolados de adubos orgânicos. R. Bras. Ci. Solo, 3:465-475, 2007.

BALDOTTO, M.A.; CANELLAS, L.P.; CANELA, M.C.; REZENDE, C.E. & VELLOSO, A.C.X. Propriedades redox e grupos funcionais de ácidos húmicos isolados de solo cultivado com cana-de-açúcar por longo tempo. R. Bras. Ci. Solo, 3:1043-1052, 2008.

BAYER, C.; MARTIN-NETO, L.; MIELNICZUK, J.; SAAB, S.C.; MILORI, D.M.P. & BAGNATO, V.S. Tillage and cropping system effects on soil humic acid characteristics as determined by ESR and fluorescence spectroscopy. Geoderma, 105:81-92, 2002.

BUSATO, J.G.; CANELLAS, L.P. & VELLOSO, A.C.X. Fósforo num Cambissolo cultivado com cana-de-açúcar por longo tempo. I - Fracionamento seqüencial. R. Bras. Ci. Solo, 29:935-944, 2005a.

BUSATO, J.G.; CANELLAS, L.P.; RUMJANEK, V.M. & VELLOSO, A.C.X. Fósforo num Cambissolo cultivado com cana-de-açúcar por longo tempo. II - Análise de ácidos húmicos por RMN ^{31}P. R. Bras. Ci. Solo, 29:945-953, 2005b.

CANELLAS, L.P.; VELLOSO, A.C.X.; MARCIANO, C.R.; RAMALHO, J.F.G.P.; RUMJANEK, V.M.; REZENDE, C.E. & SANTOS, G.A. Propriedades químicas de um Cambissolo cultivado com cana-de-açúcar, com preservação do palhiço e adição de vinhaça por longo tempo. R. Bras. Ci. Solo, 27:935-944, 2003.

CANELLAS, L.P.; BALDOTTO, M.A.; BUSATO, J.G.; MARCIANO, C.R.; MENEZES, S.C.; SILVA, N.M.; RUMJANEK, V.M.; VELLOSO, A.C.X.; SIMÕES, M.L. & MARTIN-NETO, L. Estoque e qualidade da matéria orgânica de um solo cultivado com cana-de-açúcar por longo tempo. R. Bras. Ci. Solo, 31:331-340, 2007.

CANELLAS, L.P.; ZANDONADI, D.B.; BUSATO, J.G.; BALDOTTO, M.A.; SIMOES, M.L.; MARTIN-NETO, L.; FAÇANHA, A.R.; SPACCINI, R. & PICCOLO, A. Bioactivity and chemical characteristics of humic acids from tropical soils sequence. Soil Sci., 173:624-637, 2008.

CONTE, P.; SPACCINI, R.; ŠMEJKALOVÁ, D.; NEBBIOSO, A. & PICCOLO, A. Spectroscopic and conformational properties of size-fractions separated from a lignite humic acids. Chemosphere, 69:1032-1039, 2007.

CRUZ, C.D. Análise multivariada e simulação. Viçosa, MG, Universidade Federal de Viçosa, 2006. 175p.

CUNHA, T.J.F. Ácidos húmicos de solos escuros da Amazônia. Seropédica, Universidade Federal Rural do Rio de Janeiro, 2005. 140p. (Tese de Doutorado)

DOBBSS, L.B.; CANELLAS, L.P.; ALLEONI, L.R.F.; REZENDE, C.E. FONTES, M.P.F. & VELLOSO, A.C.X. Eletroquímica de Latossolos brasileiros após a remoção da matéria orgânica humificada solúvel. R. Bras. Ci. Solo, 32:985-996, 2008.

DOBBSS, L.B.; RUMJANECK, V.M.; BALDOTTO, M.A.; VELLOSO, A.C.X. & CANELLAS, L.P. Caracterização química e espectroscópica de ácidos húmicos e fúlvicos isolados da camada superficial de Latossolos brasileiros. R. Bras. Ci. Solo, 33:51-63, 2009.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos. 2.ed. Rio de Janeiro, Embrapa Produção de Informação, 2006. 412p.

FERNANDEZ, I.; CABANEIRO, A. & GONZALEZ-PRIETO, S.J. Use of ^{13}C to monitor soil organic matter transformations caused by a simulated forest fire. Rapid Communications in Mass Spectrometry, 18:435-442, 2004.

GONZÁLEZ-PÉREZ, J.A.; GONZÁLEZ-VILA, F.J.; ALMENDROS, G. & KNICKER, H. The effect of fire on soil organic matter: A review. Environ. Inter., 30:855-870, 2004.

GARCÍA-GIL, J.C.; CEPPIB, S.B.; VELASCOB,M. I.; POLOA, A. & SENESI, N. Long-term effects of amendment with municipal solid waste compost on the elemental and acidic functional group composition and pH-buffer capacity of soil humic acids. Geoderma, 121:135-142, 2004.

IKEYA, K.; YAMAMOTO, S. & WATANABE, A. Semiquantitative GC/MS analysis of thermochemolysis products of soil humic acids with various degrees of humification. Org. Geochem., 35:583-594, 2004.

KONONOVA, M.M. Soil organic matter. Its nature, its role in soil formation and in soil fertility. New York, Pergamon Press, 1961.

LIGHT, T.S. Standard solution for redox potential measurements. Anal. Chem., 44:1038-1039, 1972.

LOVLEY, D.R. Microbial reduction of iron, manganese and other metals. Adv. Agron., 54:175-231, 1995.

LOVLEY, D.R.; COATES, J.D.; BLUNT-HARRIS, E.L.; PHILLIPS, E.J.P. & WOODWARD, J.C. Humic substances as electron acceptors for microbial respiration. Nature, 382: 445-448, 1996.

LOVLEY, D. Microbial Fe (III) reduction in subsurface environments. FEMS Microbiol. Rev., 20:305-313, 1997.

LOVLEY, D.R.; WOODWARD, J.C.; BLUNT-HARRIS, E.L.; HAYES, L.; PHILLIPS, E.J.P. & COATES, J.D. Humic substances as mediator for microbially- catalyzed metal reduction. Acta Hydrochim. Hydrobiol., 26:152-157, 1998.

LOVLEY, D.R.; KASHEFI, K.; VARGAS, M.; TOR, J.M. & BLUNT-HARRIS, E.L. Reduction of humic substances and Fe(III) by hyperthermophilic microorganisms. Chem. Geol., 169:289-298, 2000.

MARTIN-NETO, L.; ROSELL, R. & SPOSITO, G. Correlation of spectroscopic indicators of humification with mean annual rainfall along a temperate grassland climosequence. Geoderma, 81:305-311, 1998.

MATTHIESSEN, A. Determining the redox capacity of humic substances as a function of pH. Vom Wasser, 84: 229–235, 1995.

MATTHIESSEN, A. Kinetic aspects of the reduction of mercury ions by humic substances. J. Anal. Chem., 354:747-749, 1996.

MENDONÇA, E.S. & ROWELL, D.L. Mineral and organic fractions of two Oxisols and their influence on effective cation-exchange capacity. Soil Sci. Soc. Am. J., 60:1888-1892, 1996.

MILORI, D.M.B.P.; MARTIN NETO, L.; BAYER, C.; MIELNICZUCK, J. & BAGNATO, V.S. Humification degree of soil humic acids determined by fluorescence spectroscopy. Soil Sci., 167:739-749, 2002.

PÉREZ, M.G.; MARTIN-NETO, L.; SAAB, S.C.; NOVOTNY, E.H.; MILORI, D.M. B.P.; BAGNATO, V.S.; COLNAGO, L.A.; MELO, W.J. & KNICKER, H. Characterization of humic acids from a Brazilian Oxisol under different tillage systems by EPR, 13C nmR, FTIR and fluorescence spectroscopy. Geoderma, 118:181-190, 2004.

PICCOLO, A. The supramolecular structure of humic substances. Soil Sci., 166:810-832, 2001.

SCHNITZER, M. & GUPTA, U.C. Determination of acidity in soil organic matter. Soil Sci. Soc. Am. Proc., 29:274-277, 1965.

SCHNITZER, M. & RIFFALDI, R. The determination of quinone groups in humic substances. Soil Sci. Soc. Am. Proc., 36:772-777, 1972.

SCHNITZER, M. & LEVESQUE, M. Electron spin resonance as a guide to the degree of humification of peats. Soil Sci., 127:140-145, 1979.

SCOTT, D.T.; MCKNIGHT, D.M.; HARRIS, E.; KOLESAR, S. & LOVLEY, D. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ. Sci. Technol., 32:2984-2989, 1998.

SENESI, N. Application of electron spin resonance (ESR) spectroscopy in soil chemistry. Adv. Soil Sci., 14:77-130, 1990.

SOIL SURVEY STAFF. Keys to soil taxonomy. 10.ed. Washington, USDA National Resources Conservation Service, 2006.

SPACCINI, R.; PICCOLO, A.; CONTE, P. & MBAGWU, J.S.C. Changes of humic substances characteristics from forested to cultivated soils in Ethiopia. Geoderma, 132:9-19, 2006.

SPOSITO, G. Chemistry of soil. 2.ed. New York, Oxford University Press, 2008. 330p.

STEEL, R.G.D. & TORRIE, J.H. Principles and procedures of statistics with special reference to the biological sciences. New York, McGraw-Hill Book Company, 1960. 453p.

STRUYK, Z. & SPOSITO, G. Redox properties of standard humic acids. Geoderma, 102:329-346, 2001.

SUNDA, W.G. & KIEBER, D.J. Oxidation of humic substances by manganese oxides yields low-molecular-weight organic substrates. Nature, 367:62-65, 1994.

XIAOLI, C.; SHIMAOKA, T.; XIAOYAN, C.; QIANG, G. & YOUCAI, Z. Spectroscopic studies of the progress of humification processes in humic substances extracted from refuse in a landfill. Chemosphere, 69:1446-1453, 2007.

WILSON, S.A. & WEBER, J.H. An EPR study of the reduction of vanadium (V) to vanadium (IV) by fulvic acid. Chem. Geol., 26:1240-1246, 1979.

ZECH, W.; SENESI, N.; GUGGENBERGER, G.; KAISER, K.; LEHMANN, J.; MIANO, T. M.; MILTNER, A. & SCHROTH, G. Factors controlling humification and mineralization of soil organic matter in the tropics. Geoderma, 79:117-161, 1997.