de Moraes, Gislane M.; Alisson da Silva Xavier, Francisco; de Sá Mendonça, Eduardo; de Araújo
Filho, João Ambrósio; Senna de Oliveira, Teógenes

CHEMICAL AND STRUCTURAL CHARACTERIZATION OF SOIL HUMIC SUBSTANCES UNDER
AGROFORESTRY AND CONVENTIONAL SYSTEMS

Comissão 2.4 - Química do solo

CHEMICAL AND STRUCTURAL CHARACTERIZATION OF SOIL HUMIC SUBSTANCES UNDER AGROFORESTRY AND CONVENTIONAL SYSTEMS[1]

Gislane M. de Moraes[2], Francisco Alisson da Silva Xavier[3], Eduardo de Sá Mendonça[4], João Ambrósio de Araújo Filho[5] & Teógenes Senna de Oliveira[6]

SUMMARY

Studies have proven that the agroforestry systems in the semi-arid region of the State of Ceará, Brazil, induce an increase in soil organic C levels. Notwithstanding, there is no information if this increase also results in qualitative changes in different pools of soil organic matter. The objective of this study was to verify the possible chemical and structural alterations in fulvic and humic acids of a Luvisol in areas adopting agroforestry, traditional intensive cultivation and native forest in a long-term experiment conducted in the semi-arid region of Ceará State, Brazil. The study was conducted in an experimental area of the National Goat Research Center (Embrapa) in Sobral, CE. The following treatments were evaluated: agrosilvopasture (AGP), silvopasture (SILV), intensive cultivation under fallow (ICF), and areas with native forest (NF). Soil fulvic and humic acids fractions were extracted from the 0–6 and 6–12 cm layers and characterized by elemental composition, thermogravimetry and infrared spectroscopy analyses. The elemental composition analysis of humic acids confirmed the data found for fulvic acids, showing reduction in the C, H and N levels, followed by an increase in O contents in the AGP and ICF treatments over SILV and NF. In all treatments, except to SILV

in the 0–6 cm layer, the percentage of mass loss was highest (300–600 °C) for humic acids in the thermally most stable region. Despite the similarity between infrared spectra, soil fulvic acids in the SILV treatment extracted from 6–12 cm depth decrease the absorption bands at 1708 and 1408 cm^{-1} followed by an increase in the absorption band at 1608 cm^{-1} attributed to aromatic C=C groups. This behavior suggests an increase in the aromatic character of the structure. The AGP and ICF treatments, which increase the soil tilling, favored the maintenance of humic substances with a more aromatic character in the soil than SILV and NF. The less aromatic humic substances in the SILV treatment resulted in an increase of exchange sites of soil organic matter, indicating improved nutrient cycling and maintenance of productivity in the system.

Index terms: semi-arid, silvopasture, agrosilvopasture, spectroscopy.

RESUMO: *CARACTERIZAÇÃO QUÍMICO-ESTRUTURAL DE SUBSTÂNCIAS HÚMICAS DO SOLO SOB SISTEMAS AGROFLORESTAIS E CONVENCIONAL*

Estudos comprovam que na região semiárida cearense sistemas agroflorestais aumentam os teores de C orgânico do solo. Entretanto, não há registro se tal incremento implica também mudanças qualitativas nos diferentes compartimentos da matéria orgânica do solo. O objetivo deste estudo foi verificar possíveis alterações das características químico-estruturais das frações ácidos fúlvicos e ácidos húmicos de um Luvissolo em áreas sob sistemas agroflorestais, cultivo intensivo e vegetação nativa em experimento de longa duração, instalado na região semiárida cearense. O estudo foi conduzido no campo experimental do Centro Nacional de Pesquisa de Caprinos da EMBRAPA, município de Sobral-CE, onde foram avaliados os seguintes tratamentos: agrossilvopastoril (AGP), silvopastoril (SILV), cultivo tradicional intensivo em pousio (CIP) e áreas sob mata nativa (MN). As frações ácidos fúlvicos (AF) e ácidos húmicos (AH) foram extraídas das camadas de 0–6 e 6–12 cm e caracterizadas por meio das análises de composição elementar, termogravimetria e espectroscopia na região do infravermelho. A análise da composição elementar da fração AH confirma o comportamento dos dados da fração AF, mostrando redução dos teores de C, H e N e aumento do teor de O nos tratamentos AGP e CIP em relação aos sistemas SILV e MN. A fração AH apresentou maiores percentuais de perda de massa na região de maior estabilidade térmica (350–600 °C) em todos os tratamentos, à exceção do SILV na camada de 0–6 cm. A despeito da similaridade entre os espectros de infravermelho, no tratamento SILV a fração AF extraída da camada de 6–12 cm sofreu diminuição das bandas de absorção em 1.708 e 1.408 cm^{-1}, seguida de aumento da banda de absorção em 1.608 cm^{-1}, atribuída a grupamentos aromáticos C = C. Tal comportamento sugere o aumento do caráter aromático da estrutura. Os sistemas AGP e CIP, por promoverem maior revolvimento do solo, favorecem a permanência de substâncias húmicas de maior caráter aromático em comparação com os sistemas SILV e MN. O menor caráter aromático das substâncias húmicas do solo no sistema SILV implica aumento dos sítios de troca da matéria orgânica do solo, implicando na melhoria da ciclagem de nutrientes e na manutenção do processo produtivo do sistema.

Termos de indexação: semi-árido, silvopastoril, agrossilvopastoril, espectroscopia.

INTRODUCTION

International concerns about climate changes attributed to land use have triggered discussions on proposals of environmentally less harmful agricultural production systems. Among the possibilities of environmental-friendly agroecosystems, agroforestry (AGF) is considered a viable option for a better soil use, favoring environmental functions (Jose, 2009) and increasing C sequestration (Montagnini & Nair, 2004; Takimoto et al., 2009).

In the semi-arid Northeastern region of Brazil, AGF may be considered a promising alternative to restore the soil quality of areas degraded by intense soil cultivation (Araújo Filho & Carvalho, 2001). These agroecosystems can improve nutrient cycling and enhance soil structure (Maia at al., 2006), reducing water erosion (Aguiar et al., 2010) and increasing soil organic C stocks (Maia et al., 2007).

Among several hypotheses proposed by Young (1997) that involve the adoption of AGF for soil conservation, one specifically states that these systems maintain satisfactory levels of soil organic matter (SOM) for adequate soil fertility. In global terms, it is considered that AGF has a significant potential to increase soil C sequestration (Montagnini & Nair, 2004; Oelbermann et al., 2004); however, this potential must be assessed on local and regional scales. For the specific semi-arid conditions of Ceará State (Brazil) it has been shown that AGF systems affect different pools of SOM and increase total soil organic C levels (Maia et al., 2007). Nevertheless, in spite of this increase, little is known about qualitative changes in SOM as a result of the adoption of AGF, mainly in the systems developed in the semi-arid region of Brazil.

Studies evaluating the effects of soil management on SOM quality are focused on the investigation of the chemical and structural characterization of humic substances, which represent around 90 % of total soil organic C (SOC), constituting the major organic reserve in the soil (Stevenson, 1994). A study conducted by Maia et al. (2007) showed that AGF systems, especially agrosilvopasture and silvopasture, caused quantitative changes in the contents of soil humic substances. However, in Brazil there are no conclusive studies so far showing the occurrence of qualitative changes as well.

Chemical and structural properties of humic substances have been studied by techniques such as: elemental composition analysis, Fourier transform infrared spectroscopy (FTIR), thermogravimetry and ^{13}C nuclear magnetic resonance (^{13}C NMR) (Dell'Abate et al., 2002; Castilhos et al., 2008; Gonzaléz-Pérez et al., 2008).

Due to the constant input of organic material from arboreous and herbaceous components, it was assumed that AGF induces chemical and structural changes in humic substances compared to areas under traditional intensive cultivation or native forest, which in turn result in the formation of humic substances with a less humified character. To test this hypothesis, the objective of this study was to characterize fulvic and humic acids fractions of a Luvisol in areas under agroforestry (e.g. adopting agrosilvopasture and silvopasture), intensive cultivation, and native forest in a long-term experiment conducted in a semi-arid region of Ceará State.

MATERIALS AND METHODS

General description of the study site

The study was conducted in an experimental area of the Fazenda Crioula that belongs to the Centro Nacional de Pesquisa de Caprinos of EMBRAPA, State of Ceará, Brazil, in the semi-arid region of Ceará (3º41' S and 40º 20' W; 70 m asl), where the average annual temperature is 27 ºC and rainfall 822 mm (Figure 1). The rainy season is mainly concentrated between February and May (IPECE, 2005). The soils are typical ortic Chromic Luvisol and typical ortic Hypochromic Luvisol (Maia et al., 2007) (soil texture and chemical description in Table 1). The predominant native vegetation in the area is 'Caatinga', composed mostly by deciduous species that lose their leaves during the dry season, between June and January (Araújo & Tabarelli, 2002).

Description of the selected agroecosystems

Since 1997, AGF are being evaluated in a long-term experiment, as an alternative to the traditional and commonly used conventional agricultural systems in the region. These agroecosystems were previously characterized by Maia et al. (2008). In this study, the following systems were selected and evaluated:

Agrosilvopasture (AGP) – treatment in two adjacent areas (1.6 and 1.8 ha) under an alley cropping system, with approximately 200 native trees per hectare, representing a soil cover of 22 %. The predominant tree species belong to the families: Borragonaceae and Caesalpinaceae, although Papilionaceae, Cactaceae, Apocinaceae, Mimosoidea and others can be also found. Part of the wood of the native forest was cut in 1997 and deposited on the soil surface in rows spaced 3 m apart, perpendicular to the slope, and the rest was used on the farm or sold on the local market.
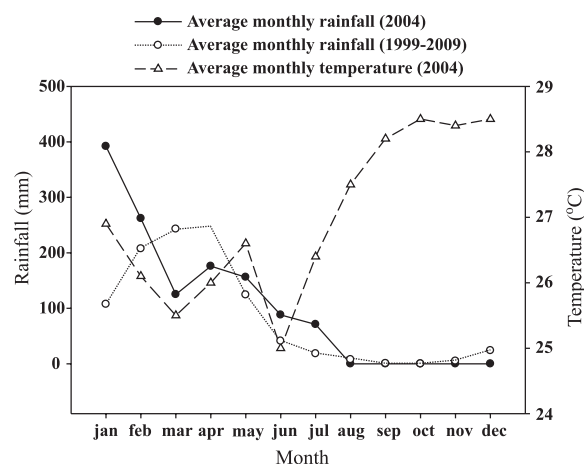

Figure 1. Average monthly rainfall and temperature in Sobral, Ceará.

Table 1. Selected physical and chemical properties of a Chromic Luvisol in the 0–6 and 6–12 cm layers under agroforestry, intensive cultivation, and native forest

Depth/treatment	Coarse sand	Fine sand	Silt	Clay	BD	pH H_2O	CEC	BS
		g kg^{-1}			g cm^{-3}		cmol$_c$ dm^{-3}	%
0-6 cm								
AGP	274	348	272	106	1.42	7.3	27.4	27.0
SILV	491	203	231	75	1.46	6.7	17.4	15.1
ICF	372	329	209	90	1.49	6.8	19.1	17.5
NF-1	414	211	245	130	1.41	7.0	32.4	30.4
NF-2	473	307	157	63	1.55	6.4	17.1	14.5
6-12 cm								
AGP	242	355	263	140	1.64	7.1	27.8	27.1
SILV	499	192	223	86	1.67	6.4	11.7	9.7
ICF	323	323	227	127	1.64	6.9	19.1	17.5
NF-1	339	233	255	173	1.64	6.8	33.0	31.1
NF-2	468	290	175	67	1.67	6.2	14.0	11.6

BD: soil bulk density; CEC: cation exchange capacity; BS: percentage of base saturation; AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.

Leucaena leucocephala (Lam.) de Wit was cultivated in 0.5 m plant spacing on either side of the rows, and maize (*Zea mays* L.) was cultivated between the rows with a spacing of 0.3–0.5 m. No chemical fertilizers were applied and the soil was prepared by hand-hoeing. In the dry season, the areas were used for grazing (20 ewes h^{-1} day^{-1} in the morning). The annual input of organic residues consisted of: (1) wood and leaves cut at the beginning of the experiment; (2) tree leaves dropped during the dry season; (3) biomass from pruning of Leucaena and from native trees during the rainy season; (4) herbaceous biomass; and (5) manure produced by the ewes. The annual removal of biomass from this area consisted of: (1) crop residues (grain, straw); (2) part of the Leucaena to produce hay; and (3) leaves and branches by grazing.

Silvopasture (SILV) – treatment consisted of two adjacent areas (4.8 ha) on which the number of native trees had been reduced to approximately 260 trees per hectare, corresponding to a soil cover of 38 %. The predominant trees families were Borragonaceae and Mimosoidea. During the year this area and the adjacent native forest were used for grazing by 20 ewes. The annual input of organic residues consisted of: (1) wood and leaves cut at the beginning of the experiment; (2) fallen tree leaves in the dry season; (3) manure produced by the ewes. The biomass removal from this area was represented by grazing.

Intensive cultivation under fallow (ICF) - This treatment consisted of a 1.0 ha area that had been deforested and burned in 1997, and cultivated intensively with maize (*Z. mays*) from 1998 to 2002, with a spacing of 0.3–0.5 m, in a traditional agricultural system commonly adopted in the region. No chemical fertilizers were applied and the soil was prepared by hand-hoeing. After maize harvest in 2002, the area was left under fallow to regenerate the soil cover which was dominated by Andropogon spp at the time of soil sampling. The organic residue inputs consisted of: (1) residues from weeding from 1998 to 2002; and (2) crop residues. The maize harvest represented the biomass removal from this area.

Native forest (NF) - This area was used as a reference in comparison with the cropping systems. The native vegetation, known as 'Caatinga', is peculiar to the Brazilian semi-arid region. It consists of short and thorny trees that shed their leaves seasonally, and is associated with arid-adapted grasses. There were, on average, 1.73 thousand trees per hectare; the most abundant species belong to the families Mimosoidea, Borragonaceae, and there are representatives of the Caesalpinaceae, Combretaceae, Euforbiaceae, and other less abundant families. This deciduous forest is adapted to unpredictable rains and arid conditions. The area was part of a rotational system, used as pasture for wood supply during the dry season. The annual input of organic matter consisted mainly of leaves from the trees and herbaceous biomass. A part of the herbaceous biomass was removed by grazing. The NF areas were selected for their proximity to the cropland under study; NF1 was used in comparisons with AGP and SILV, whereas NF2 was used as a reference for the ICF treatment.

Soil sampling, extraction and purification of humic substances

Soil samples were collected in September 2004 from the 0–6 and 6–12 cm layers, with five replications. For a more representative soil sampling procedure, each replicate consisted of a composite

sample of four randomly collected subsamples on each plot. For the humic substance extractions, the five replicates were mixed, originating a composite sample for each depth separately. The criteria to eliminate the replicates per plot was based on two factors: (1) the analyses involving spectroscopy, thermogravimetry and elemental composition used in this study are relatively costly and the extraction and purification procedures of humic substances lengthy; and (2) the variations in results between replicates in similar analyses to those carried out in this study were not significant (Mao et al., 2000).

The humic substances were extracted using the technique of differential solubility in acidic media, separating the fractions of fulvic (FA) and humic acids (HA), according to concepts of the humic substances established by the International Humic Substance Society (Swift, 1996). The purification of the FA fraction was carried out using a percolation column containing Amberlite XAD-8 resin. The percolated material was discarded and the absorbed material reverse-eluted with 0.1 mol L^{-1} NaOH and collected. The total solute was immediately adjusted to pH 2 and passed again through a column of Amberlite 120^+ resin to remove salt excess, and freeze-dried to a low-ash form. Purification of the HA fraction was carried out through successive washing with a solution of 10 % HF + 0.5 % HCl to eliminate the influence of paramagnetic ions (Gonçalves et al., 2003). After shaking, the samples were centrifuged at 10,000 g for 20 min and the supernatant was discarded. This procedure was repeated three times, and at the end of the third cycle the samples were dialyzed using cellulose membranes (Visking dialysis tubing 36/32) until free of chlorine and then freeze-dried.

Soil and humic substance analyses

Total soil organic carbon (SOC) was quantified by wet oxidation of organic matter using a mixture of potassium dichromate and sulfuric acid under external heating (Yeomans & Bremner, 1988).

The elemental composition (C, H, N and O contents) of the FA and HA fractions was determined in a Perkin-Elmer 2400 elemental analyzer with 1 g samples of the corresponding fraction. The C, H and N measurements were carried out in duplicate, and O content was determined by the difference from 100 %.

Thermogravimetry was assessed using a TGA-50 SHIMADZU thermogravimetric analyzer. The experiment was conducted in a O_2 atmosphere with a heat ramp from 5 °C min^{-1} to a final temperature of 600 °C. Ash content was determined at the end of the experiment after 3 h at 600 °C (Huffman & Stuber, 1985). Based on the thermal behavior of thermograms, and considering main weight losses in the ranges 105–350 and 350–600 °C, the thermogravimetry index (TGI) was calculated between two zones of mass loss as follows: Zone (I) = [mass loss at 105–350 °C]/[total

mass loss at 105–600 °C] and Zone (II) = [mass loss at 350–600 °C]/[total mass loss at 105–600 °C]. The TGI was accessed by the ratio Zone(II)/Zone(I), used as an index of thermal lability of humic structures (Dell'Abate et al., 2002).

Infrared spectra of the FA and HA fractions were recorded using Fourier transform infrared sprectroscopy (FTIR) in a Perkin-Elmer, Spectrum 1000 apparatus by the KBr pellet technique. KBr pellets were prepared by grinding and thoroughly mixing a portion of 2 mg of FA or HA with 100 mg KBr (both dried at 60 °C for 24 h) and vacuum pressing at 10.000 kg cm^{-2} for 10 min. Spectra were recorded within a range of 400 to 4000 cm^{-1} wavenumbers at 4 cm^{-1} resolution. In all cases, 20 scans were averaged to reduce noise and corrected against the spectrum with ambient air as background. The FTIR spectra were interpreted based on various studies available in the literature (Stevenson, 1994; Sánchez-Monedero et al., 2002; González Pérez et al., 2004).

The treatment effect was analyzed based on average means of their dispersion measurements (standard deviation).

RESULTS AND DISCUSSION

Total organic C contents in soil and humic substances

The results were analyzed based on means and dispersion measures (standard deviation) because a specific experimental design could not be established to this on-farm long-term experiment involving trees, animals and agricultural crops; this would make it impossible to establish replicates of the experimental units in the space. In conventional statistical analysis, pseudo-repetition would have been adopted (Hurlbert, 1984; Millar & Anderson, 2004), which uses statistical inferences to test treatment effects in situations in which there are no treatment replicates in space and no statistical independence among the replicates, as in the case of the present study. Total organic C contents in the soil and humic substances are shown in table 2. SOC contents varied from 8.0 to 25.7 g kg^{-1} among the evaluated treatments. The SOC level in the upper soil layer was highest in the SILV treatment, with similar C content to the reference (NF-1), suggesting a greater potential to maintain SOM levels. In the AGP treatment, SOC content was reduced by 41 % when compared to the reference NF-1. This reduction may be attributed to the oxidation of organic matter caused by increased soil tilling in this treatment, which contributes to the exposure of protected organic matter, favoring the microbial attack and accelerating the biological oxidation process (Maia et al., 2007).

Table 2. Total organic C contents in the soil and in humic substance fractions in the 0–6 and 6–12 cm layers under agroforestry, intensive cultivation, and native forest. Means ± standard deviation

Depth/treatment	SOC	C_{FA}	C_{HA}	Ratio	
				C_{FA+HA}/SOC	C_{HA}/C_{FA}
0-6 cm		g kg^{-1}			
AGP	15.7 ± 2.0	2.5 ± 0.10	4.2 ± 0.18	0.43	1.68
SILV	23.8 ± 3.4	3.0 ± 0.17	4.8 ± 0.27	0.33	1.60
ICF	14.5 ± 2.6	2.5 ± 0.17	4.4 ± 0.69	0.48	1.76
NF-1	25.7 ± 3.5	1.4 ± 0.12	2.5 ± 0.06	0.15	1.79
NF-2	18.3 ± 5.0	1.0 ± 0.08	2.1 ± 0.20	0.17	2.10
6-12 cm					
AGP	8.8 ± 1.5	1.9 ± 0.13	2.9 ± 0.12	0.55	1.53
SILV	10.3 ± 1.0	1.6 ± 0.12	3.2 ± 0.36	0.47	2.00
ICF	8.0 ± 1.6	2.0 ± 0.16	3.5 ± 0.72	0.69	1.75
NF-1	12.6 ± 0.9	0.8 ± 0.12	1.5 ± 0.05	0.18	1.88
NF-2	9.0 ± 1.7	0.4 ± 0.11	1.2 ± 0.10	0.18	3.00

SOC: total soil organic carbon; C_{FA}: carbon content in the fulvic acids; C_{HA}: carbon content in the humic acids; AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.

The C contents in the FA and HA fractions varied from 0.4 to 3.0 and 1.2 and 4.8 g kg^{-1}, respectively, being higher in cultivated areas than in native forest (Table 2). In the topsoil layer, C levels were highest in the SILV treatment in both humic fractions, corroborating with SOC data. In cultivated areas, the sum of the C content in FA and HA fractions (C_{FA+HA}) represented around 41 and 57 % of total SOC in the 0–6 and 6–12 cm layers, respectively. In the native forest areas (NF-1 and NF-2), the percentages were 16 and 18 %, respectively. Carbon content in HA was predominant over FA in all treatments, as shown by the HA/FA ratio (Table 2). The values of this ratio varied from 1.53 to 3.00, evidencing the humic character of SOM. Predominance of the HA fraction may be related to the synthesis process through formation of more complex structures from a structural rearrangement of less condensed molecules, such as FA. On the other hand, it could be the result of the degradation process of the FA fraction, which is more easily utilized as energy source by soil microorganisms (Stevenson, 1994).

Elemental composition of humic substances

The elemental composition (C, H, N and O contents) of the FA and HA fractions and the C-to-N atomic ratio is shown in table 3. In the topsoil layer, AGP and ICF treatments showed lower C, H and N and higher O contents in the FA fraction than NF-1. On the other hand, SILV treatment presented higher C, H and N and lower O contents than NF-1, AGP and ICF treatments, suggesting a less oxidized structure, which explains the lower HA/FA humification ratio (Table 2). On the other hand, in the 6–12 cm layer, C, H and N contents in the FA fraction of SILV were substantially lower than in the other treatments, which can explain the higher HA/FA ratio in this

treatment. The elemental composition of the HA fraction confirms the data obtained for the FA fraction, with lower C, H and N and higher O contents in AGP and ICF treatments than in SILV and NF. In the cultivated areas, the SILV treatment also showed HA with less oxidized structure, evidenced by higher C and H and lower O contents, compared to the other treatments. Since the majority of exchange sites of SOM are attributed to humic substances, the ability of the soil to develop negative charges correlates strongly with FA and HA fractions (Mendonça et al., 2006). Thus, the SILV treatment, to maintain these fractions, can be considered an interesting option of soil management to generate negative charges in the soil, indicating a greater potential for nutrient cycling and increasing soil fertility. The less aromatic character of the humic fractions in the SILV treatment could be related to the constant cycling of recent organic residues from the herbaceous substrate that exist in this treatment but not in AGP and ICF. The greater degree of oxidation of the humic fractions in AGP and ICF treatments may be associated with more intense soil tilling during soil cultivation , which intensifies the SOM transformation processes.

The C-to-N atomic ratio of the humic fractions Table 3) shows that chemically more stable organic forms (ratios near to 10) are predominant in the upper soil layer compared to those extracted from the deeper layer. These results show that SOM transformation processes occurred more intensely in the top soil, given the influence of climate, biological and biochemical factors, especially when intensified by anthropic activities in cultivated areas.

Figure 2 shows the relationship between atomic ratios H/C and O/C of the FA and HA fractions extracted from 0–6 and 6–12 cm depths. Humic

Table 3. Elemental composition and C/N atomic ratio of fulvic and humic acid fractions of a chromic Luvisol in the layers 0–6 and 6–12 cm under agroforestry, intensive cultivation, and native forest

Depth/ treatment	Elemental composition				Atomic ratio C/N
	C	H	N	O	
	g kg^{-1}				
0-6 cm		Fulvic acid			
AGP	281.7	26.3	18.0	674.0	18
SILV	515.4	45.2	36.3	403.2	17
IC	194.2	17.3	12.4	776.1	18
NF-1	412.6	35.1	21.5	530.9	22
NF-2	547.0	47.7	28.7	376.6	22
6-12 cm					
AGP	198.4	21.0	8.4	772.2	28
SILV	89.8	7.2	3.5	899.5	30
IC	496.0	47.7	26.2	430.1	22
NF-1	285.6	26.8	10.7	676.9	31
NF-2	313.0	28.3	8.7	650.1	42
0-6 cm		Humic acid			
AGP	594.9	51.0	45.6	308.5	15
SILV	675.0	54.9	47.8	222.2	16
ICF	584.6	49.0	41.7	324.7	16
NF-1	645.7	56.1	53.5	244.7	14
NF-2	696.8	56.9	52.6	193.7	15
6-12 cm					
AGP	618.2	48.9	36.6	296.3	20
SILV	668.4	48.4	32.0	251.2	24
IC	579.7	45.6	35.1	339.7	19
NF-1	643.1	53.9	40.1	262.8	19
NF-2	644.2	57.2	37.6	261.0	20

AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.

fractions with low H/C and high O/C atomic ratios suggest a higher oxidation (humification) degree in their structure, and vice versa. In general, it was observed that AGP and ICF treatments were situated farther right in the graph (greater O/C ratio) than the other treatments, reflecting the more aromatic character of both FA and HA. This fact indicates a lower potential of these fractions to generate SOM exchange sites, suggesting that in this aspect AGP and ICF systems are less advantageous than SILV. The FA fraction extracted from the 6–12 cm layer was an exception, with the lowest H/C ratio and highest O/C ratio in the SILV treatment (Figure 2), suggesting an increase in the oxidation degree of the organic structure. In this same treatment, the clay content in the subsurface layer was lower than in the other areas (Table 1), indicating lower physical protection of the FA fraction by clays. The reduced physical protection by clays intensifies a process of SOM transformation due to an increase of exposure of the organic material to microbial attack and oxidation processes (Balesdent et al., 2000).

Thermogravimetry

Thermograms of FA and HA fractions are shown in figure 3. In general, three distinct mass-loss regions were observed. The first occurred in the interval between 30 and 105 °C due to water loss; the second between 105 and 330 °C associated to thermal degradation of the aliphatic chains; and the third between 350 and 600 °C due to loss of thermally more stable aromatic nuclei (Dell'Abate et al., 2002; Trompowsky et al., 2005).
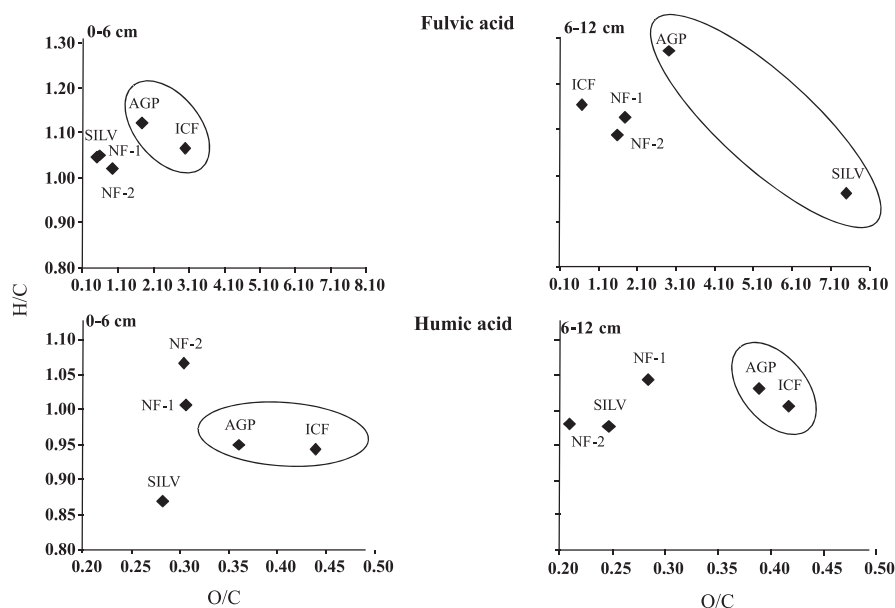

Figure 2. Relationships between H/C and O/C atomic ratios of the fulvic and humic acids extracted from the layers 0–6 and 6–12 cm of a chromic Luvisol under agroforestry, intensive cultivation, and native forest. AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.
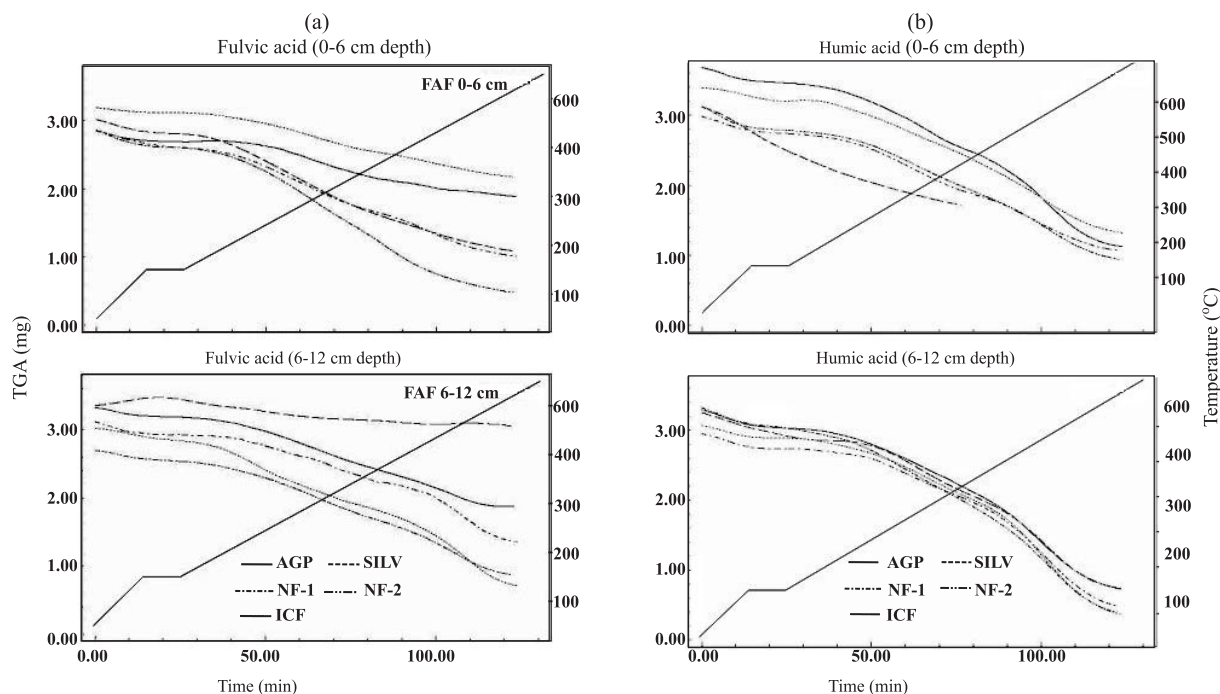
Figure 3. Thermograms of fulvic acid (a) and of humic acid (b) extracted from the 0–6 and 6–12 cm layers of a Chromic Luvisol under agroforestry, intensive cultivation, and native forest. AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.

Mass loss percentage by thermogravimetry and the thermogravimetry index (TGI) are summarized in table 4. Mass losses in the FA fraction extracted from the 0–6 cm layer were relatively similar in both temperature ranges in all treatments, with exception of SILV, where mass loss between 105 and 350 °C (55 % of total loss) was higher than in the other treatments. Degradation of lateral aliphatic chains is predominant in this region (Dell'Abate et al., 2002). This behavior reinforces our previous results, indicating the effect of the SILV treatment in generating less aromatic humic structures. A similar trend was also observed in the FA fraction extracted from 6–12 cm depth, where in SILV 75 % of mass was lost in the thermally less stable region (Table 4). Greater mass loss in the thermally less stable range (105–350 °C) explains reduction of TGI in the SILV treatment in the two evaluated soil layers. The HA fraction generally had higher percentages of mass loss in the thermally more stable range (350–600 °C) in all treatments with exception of SILV in the 0–6 cm layer, with around twice as high losses between 105 and 350 °C when compared to AGP and ICF treatments, reducing TGI to 0.54 (Table 4). This result confirmed the influence of SILV on the genesis of humic substances in the soil, favoring more aliphatic structures which could mean greater availability of exchange sites of SOM (Stevenson,

1994), improving nutrient cycling in the soil and acting as energy source for soil microorganisms.

Occurrence of humic substances with predominance of aliphatic character in the SILV treatment could be analyzed from two points of view. The first is that the constant cycling of plant material from the herbaceous stratus associated with less intense soil tilling may favor the formation of more labile C chains in FA and HA structures. The second is that the more aromatic character of the humic substances in AGP and ICF treatments seems to be related to a more intense soil tilling process in those treatments. This intensifies the oxidation of more labile organic structures given the greater exposure of the material to microbial attack. From this point of view, an increase in the lability of the humic fractions in the SILV treatment seem to be better related with the greater intensity of SOM oxidation process in the AGP and ICF treatments, where soil disturbance is more intense, than necessarily with the *in situ* formation of less aromatic C nuclei in the SILV treatment.

In spite of the soil tilling in the AGP and ICF treatments, it is important to emphasize that soil disturbance was not as intensive as in mechanized agriculture (e.g. no machinery was used), restricted only to the use of animal plow and/or hoe. In this study,

Table 4. Thermogravimetry: humidity, ash content, weight loss, and thermogravimetry index (TGI) in function of different ranges of temperature of solid-state humic and fulvic acids extracted from the 0–6 and 6–12 cm layers of a Chromic Luvisol under agroforestry, intensive cultivation, and native forest

Depth/treatment	Humidity[1]	Ash	Loss of mass		TGI[3]
			105-350 °C	350-600 °C	
	——— % ———		——— %[2] ———		
0-6 cm			Fulvic acid		
AGP	5.83	12.5	45.97	54.03	1.18
SILV	6.76	9.5	54.73	45.27	0.83
ICF	2.25	15.3	49.95	50.05	1.00
NF-1	8.75	9.1	50.41	49.59	0.98
NF-2	8.62	10.0	49.84	50.16	1.01
6-12 cm					
AGP	4.04	10.2	47.64	52.36	1.10
SILV	3.03	5.3	75.31	24.69	0.33
ICF	6.14	12.7	42.41	57.59	1.36
NF-1	5.73	10.0	35.18	64.82	1.84
NF-2	5.63	9.4	40.73	59.27	1.45
0-6 cm			Humic acid		
AGP	6.06	5.9	36.89	63.11	1.71
SILV	15.81	6.6	64.78	35.22	0.54
ICF	6.31	6.9	37.76	62.24	1.65
NF-1	7.86	11.4	44.51	55.49	1.25
NF-2	10.53	14.3	39.61	60.39	1.52
6-12 cm					
AGP	7.97	7.6	32.54	67.46	2.07
SILV	9.94	9.8	33.39	66.61	1.99
ICF	5.67	3.0	28.21	71.79	2.54
NF-1	7.17	12.5	28.46	71.54	2.51
NF-2	8.58	13.2	35.44	64.56	1.82

[1] Relative mass loss from 0 to 105 °C. [2] Ash-free basis. [3] TGI = Zone II/Zone I, where: Zone I = [mass loss at 105–350 °C]/[total mass loss at 105–600 °C]; Zone II = [mass loss at 350–600 °C]/[total mass loss at 105–600 °C]; AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.

intensive cultivation can be defined as a traditional agroecosystem with continuous soil cultivation under slash-burning, monoculture, without adoption of soil conservation practices. Nevertheless, although the level of soil disturbance in the ICP treatment was not similar to that in highly mechanized agriculture, it was higher than in AGP. These differences between the two areas were corroborated by the structural analysis of humic substances. For instance, it was observed that FA and HA fractions in the AGP treatment suggest less structural transformation in relation to the ICF (Figure 2). This analysis leads to the conclusion that the semi-arid environment is sensitive to recent changes caused by soil management, even though the parameter to evaluate these changes may be considered highly stable, e.g., humic substances. In other environments, e.g., in the Brazilian Cerrado, the interaction of organic matter with the mineral matrix of highly weathered soils is largely controlled by physical and chemical factors, so that small changes in soil management are not easily detectable in the analysis of stable SOM pools. It may therefore be concluded that the type of soil management in the semi-arid environment has a strong influence on its components, including SOM. This analysis is fundamental in decision making on the most suitable type of agroecosystem, since its implications are directly associated with the environmental quality.

Infrared spectroscopy (FTIR)

Infrared spectra of FA and HA fractions are shown in figure 4. Descriptions of the absorption bands (Table 5) were given as proposed elsewhere (Stevenson, 1994; Sánchez-Monedero et al., 2002; González Pérez et al., 2004). In general, the FTIR spectra in soil FA and HA extracted from different treatments were very similar. The complex composition of organic components that make up humic material simplify the infrared spectrum (Canellas & Santos, 2005), explaining the similarity between spectra in the present study.

Regardless of the similarity between the spectra, there were changes in absorption of some specific peaks in FA samples extracted from the 6–12 cm layer. In the SILV treatment there was a reduction in absorption bands at 1708 and 1408 cm^{-1}, corresponding to
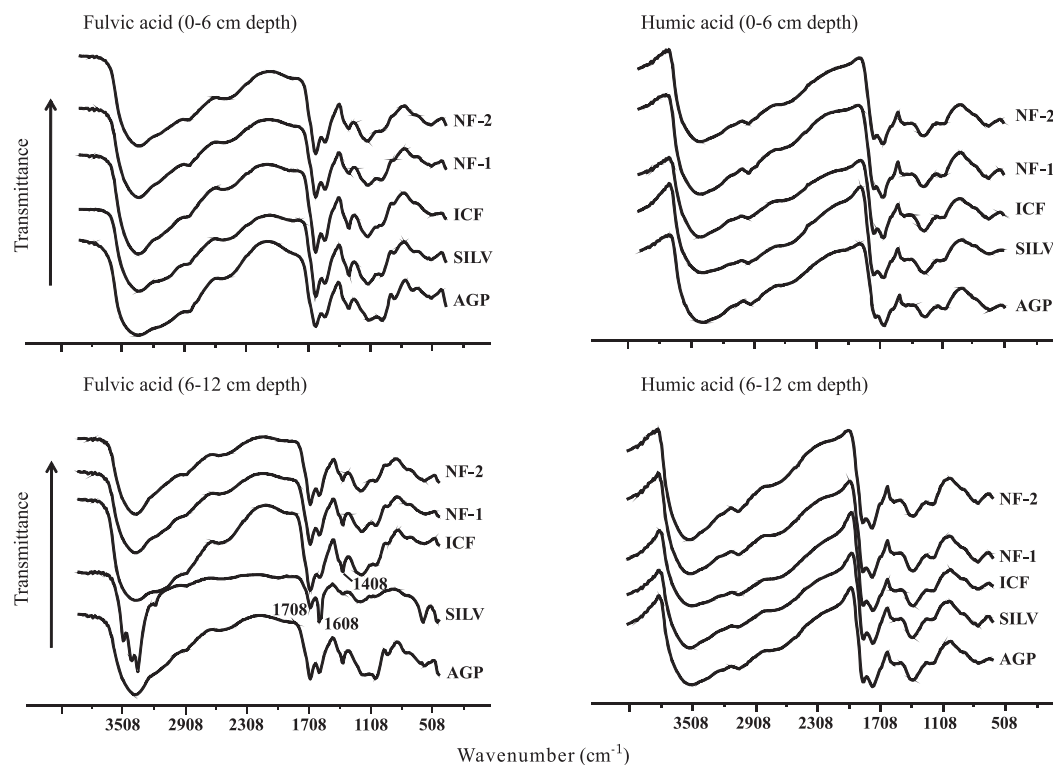
Figure 4. Infrared spectra of fulvic and humic acids extracted from the 0–6 and 6–12 cm layers of a Chromic Luvisol under agroforestry, intensive cultivation, and native forest. AGP: agrosilvopasture; SILV: silvopasture; ICF: intensive cultivation under fallow; NF-1: native forest compared to AGP and SILV treatments; NF-2: native forest compared to the ICF treatment.

Table 5. Assignment of Fourier transform infrared (FTIR) spectral peaks indicating the main functional groups of fulvic and humic acids extracted from the 0–6 and 6–12 cm layers of a Chromic Luvisol under agroforestry, intensive cultivation, and native forest

Wave number	Proposed assignment[1]
cm^{-1}	
3690 - 3619	stretching of free OH
3400 - 3300	stretching of OH, NH inter and intra-molecular, NH from amide and OH from phenol
2920 - 2840	stretching of aliphatic CH
1725 - 1720	stretching of C=O from COOH and ketone
1660 - 1630	stretching of C=O from amide, C=O from quinone and/or C=O linked to H from paired ketones, aromatic C=C
1630 - 1600	stretching of aromatic C=C group
1560 - 1540	deformation of NH and stretching of C=N
1400 - 1380	deformation of OH and stretching of CO from phenolic OH
1280 - 1200	CO stretching, deformation of OH from COOH and CO stretching
1190 - 1127	stretching of aliphatic OH and stretching of C-O-C from alcohol, ether and phenols
1000 - 530	stretching of CO from polysaccharides, aromatic CH or out of plane

[1] Attributions as proposed in the literature (Stevenson, 1994; Sánchez-Monedero et al., 2002; González Pérez et al., 2004).

vibrations of ketone C=O groups and/or COOH carboxyl groups, stretching of phenol OH, deformation of CH_2 and CH_3 groups and COO$^-$ stretching (Stevenson, 1994; Canellas et al., 2001), followed by an increase in the 1606 cm^{-1} absorption band, which is attributed to aromatic C=C groups. These results suggest an increase in the aromatic character of the structure, which corroborate with data obtained by

elemental composition analysis, that indicated an increase in the degree of oxidation of the humic structure as shown by the decrease in the H/C atomic ratio and increased O/C ratio (Figure 2). The increase of the aromatic character of the FA fraction in the subsurface in the SILV treatment was attributed to the mechanism of SOM protection. In the 6–12 cm layer, there were higher sand and lower clay contents in this treatment than in the others (Table 1). Since there was less protection of the FA by clays, it seemed to favor or intensify oxidation processes of the structure by exposure of organic material to microbial attack, which explains reductions in the structural C in this fraction (Table 3). Similar behavior did not occur in the HA fraction, given its greater chemical complexity compared to FA, which is more easily altered. Nevertheless, in the topsoil layer, the formation of more labile humic structures or with a less aromatic character in the SILV treatment may be attributed to the effect of cycling of the organic residues derived from herbaceous vegetation as well from arboreous strata. In addition, the less intense soil tilling contributed to the maintenance of humic substances in the system, with relevant consequences in the process of nutrient cycling in the soil.

CONCLUSIONS

1. The chemical and structural composition of soil fulvic and humic acids is influenced by agroforestry.

2. Soil tilling, common in agrosilvopasture systems, and intensive cultivation systems, lead to the genesis of more aromatic humic substances, whereas the constant input of organic matter in silvopasture reduces the intensity of humification.

3. The less aromatic character of soil humic substances in the silvopasture system implies in an increase in exchange sites of soil organic matter, generating negative charges and improving nutrient cycling and the maintenance of productivity.

4. Changes in the chemical and structural properties of soil humic substances (FA and HA) suggest that the semi-arid is a sensitive environment to land use changes.

ACKNOWLEDGEMENTS

LITERATURE CITED

AGUIAR, M.I.; MAIA, S.M.F.; XAVIER, F.A.S.; MENDONÇA, E.S.; ARAÚJO FILHO, J.A. & OLIVEIRA, T.S. Sediment, nutrient and water losses by water erosion under agroforestry systems in the semi-arid region in northeastern Brazil. Agrofor. Syst., 79:277-289, 2010.

ARAÚJO, E.L. & TABARELLI, M. Estudos de ecologia de populações de plantas do nordeste do Brasil. In: ARAÚJO, E.L.; MOURA, A.N.; SAMAPAIO, E.S.V.B.; GESTINARI, L.M.S. & CARNEIRO, J.M.T., eds. Biodiversidade, conservação e uso sustentável da flora do Brasil. Imprensa Universitária, Recife, PE, Brasil, 2002. p.135–142.

ARAÚJO FILHO, J.A. & CARVALHO, F.C. Sistemas de produção agrossilvipastoril para o semiárido nordestino. In: CARVALHO, M.M.; ALVIM, M.J. & CARNEIRO, J.C., ed. Sistemas agroflorestais pecuários: Opções de sustentabilidade para áreas tropicais e subtropicais. Juiz de Fora, Embrapa Gado de Leite, 2001. p.101-110.

BALESDENT, J.; CHENU, C. & BALABANE, M. Relationship of soil organic matter dynamics to physical protection and tillage. Soil Tillage Res., 53:215-230, 2000.

CANELLAS, L.P. & SANTOS, G.A. Humosfera: Tratado preliminar sobre a química das substâncias húmicas. Seropédica, Universidade Estadual do Norte Fluminense, 2005. 309p.

CANELLAS, L.P.; SANTOS, G.A.; RUMJANEK, V.M.; MORAES, A.A. & GURIDI, F. Distribuição da matéria orgânica e características de ácidos húmicos em solos com adição de resíduos de origem urbana. Pesq. Agropec. Bras., 36:1529-1538, 2001.

CASTILHOS, R.M.V.; DICK, D.P.; CASTILHOS, D.D.; MORSELLI, T.B.A.G.; COSTA, P.F.P.; CASAGRANDE, W.B. & ROSA, C.M. Distribuição e caracterização de substâncias húmicas em vermicompostos de origem animal e vegetal. R. Bra. Ci. Solo, 32:2669-2675, 2008.

DELL'ABATE, M.T.; BENEDETTI, A.; TRINCHERA, A. & DAZZI, C. Humic substances along the profile of two Typic Haploxerert. Geoderma, 107:281-296, 2002.

GONÇALVES, C.N.; DALMOLIN, R.S.D.; DICK, D.P.; KNICKER, H.; KLAMT, E. & KÖGEL-KNABNER, I. The effect of 10 % HF treatment on the resolution of CPMAS ^{13}C NMR spectra and on the quality of organic matter in Ferralsols. Geoderma, 116:373-392, 2003.

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

GONZÁLEZ-PÉREZ, M.; TORRADO, P.V.; COLNAGO, L.A.; MARTIN-NETO, L.; OTERO, X.L.; MILORI, D. M.B.P. & GOMES, F.H. ^{13}C NMR and FTIR spectroscopy characterization of humic acids in Spodosols under tropical rain forest in southeastern Brazil. Geoderma, 146:425-433, 2008.

HURLBERT, S. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr., 54:187-211, 1984.

HUFFMAN, E.W.D. & STUBER, H.A. Analytical methodology for elemental analysis of humic substances. In: AIKEN G.R.; MCKNIGHT D.M.; WERSHAW R.L. & MACCARTHY, P. Humic substances in soil, sediment and water: Geochemistry, isolation and characterization. New York, John Wiley & Sons, 1985. p.433-455.

INSTITUTO DE PESQUISA E ESTRATÉGIA ECONÔMICA DO CEARÁ - IPECE. Perfil Básico Municipal, Sobral. Fortaleza, SEPLAN – Secretaria do Planejamento e Coordenação, Governo do Estado do Ceará, 2005. 10p.

JOSE, S. Agroforestry for ecosystem services and environmental benefits: An overview. Agrofor. Syst., 76:1-10, 2009.

MAIA, S.M.F.; XAVIER, F.A.S.; OLIVEIRA, T.S.; MENDONÇA, E.S. & ARAÚJO FILHO, J.A. Organic carbon pools in a Luvisol under agroforestry and conventional farming systems in the semi-arid region of Ceará, Brazil. Agrofor. Syst., 71:127-138, 2007.

MAIA, S.M.F.; XAVIER, F.A.S.; OLIVEIRA, T.S.; MENDONÇA, E.S. & ARAÚJO FILHO, J.A. Impactos de sistemas agroflorestais e convencional sobre a qualidade do solo no semi-árido cearense. R. Árvore, 30:837-848, 2006.

MAIA, S.M.F.; XAVIER, F.A.S.; OLIVEIRA, T.S.; MENDONÇA, E.S. & ARAÚJO FILHO, J.A. Frações de nitrogênio em Luvissolo sob sistemas agroflorestais e convencional no semi-árido cearense. R. Bras.Ci. Solo, 32:381-392, 2008.

MAO, J.D.; HU, W.G.; SCHMIDT-ROHR, K.; DAVIS, G.; GHABBOUR, E.A. & XING, B. Quantitative characterization of humic substances by solid-state carbon-13 nuclear magnetic resonance. Soil Sci. Soc. Am. J., 64:873-884, 2000.

MENDONÇA, E.S.; ROWELL, D.L.; MARTINS, A.G. & SILVA, A.P. Effect of pH on the development of acidic sites in clayey and sandy loam Oxisol from the Cerrado Region, Brazil. Geoderma, 132:131-142, 2006.

MILLAR, R.B. & ANDERSON, M.J. Remedies for pseudoreplication. Fish. Res., 70:397-407, 2004.

MONTAGNINI, F. & NAIR, P.K.R. Carbon sequestration: An underexploited environmental benefit of agroforestry systems. Agrofor. Syst., 61:281-295, 2004.

OELBERMANN, M.; VORONEY, R.P. & GORDON, A.M. Carbon sequestration in tropical and temperate agroforestry systems: A review with examples from Costa Rica and southern Canada. Agric. Ecosyst. Environ., 104:359-377, 2004.

SÁNCHEZ-MONEDERO, M.A.; ROIG, A.; CEGARRA, J.; BERNAL, M.P. & PAREDES, C.; Effects of HCl – HF purification treatment on chemical composition and structure of humic acids. Europ. J. Soil Sci., 53:375-381, 2002.

STEVENSON, F.J. Humus chemistry: Genesis, composition, reactions. 2.ed. New York, John Wiley & Sons, 1994. 496p.

SWIFT, R.S. Organic matter characterization. In: SPARKS, D.L.; PAGE, A.L.; HELMKE, P.A.; LOEPPERT, R.H.; SOLTANPOUR, P.N.; TABATABAI, M.A.; JOHNSTON, C.T. & SUMMER, M.E., ed. Methods of soil analysis. Madison, Soil Science Society of America/American Society of Agronomy, 1996. Part 3. p.1011-1020. (Soil Science Society of America Book, Series, 5).

TAKIMOTO, A.; VIMALA, D. & NAIR, P.K.R. Contribution of trees to soil carbon sequestration under agroforestry systems in the West African Sahel. Agrofor. Syst., 76:11-25, 2009.

TROMPOWSKY, P.M.; BENITES, V.M.; MADARI, B.E.; PIMENTA, A.S.; HOCKADAY, W.C. & HATCHER, P.G. Characterization of humic-like substances obtained by chemical oxidation of eucalyptus charcoal. Org. Geochem., 36:1480-1489, 2005.

YEOMANS, J.C. & BREMNER, J.M. A rapid and precise method for routine determimation of organic carbon in soil. Comm. Soil Sci. Plant Anal., 19:1467-1476, 1988.

YOUNG, A. Agroforestry, soil management and sustainability. In: YOUNG, A., ed. Agroforestry for soil management. 2.ed. Nairobi, CAB international, ICRAF, 1997. p.1-22.