

ORGANIC MATTER QUALITY IN A SOIL CULTIVATED WITH PERENNIAL HERBACEOUS LEGUMES

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ABSTRACT: Using herbaceous legumes in agricultural systems yields great quantities of plant residues, allowing changes in soil organic matter quality and content over the years. This study was conducted on an Ultisol, at Seropédica, RJ, Brazil, to evaluate the effects of different perennial herbaceous legumes on soil organic matter quality. A factorial scheme with three replications was used to evaluate the species: forage groundnut cv. BR-14951 (*Arachis pintoi*), tropical kudzu (*Pueraria phaseoloides*), and siratro (*Macroptilium atropurpureum*). After the first cut, each plot was divided into two subplots; plants were cut and left on the soil surface or cut and removed. Soil samples of a closed area covered by spontaneous vegetation (mainly C₃ plants) or by *Panicum maximum* were also analysed. Samples were collected from two layers (0-5 and 5-10 cm), processed for the fractionation of organic matter and the evaluation of structural characteristics of humic acids (HA). Evaluated legumes did not change total organic carbon contents, but promoted HA accumulation in the superficial soil layer. Humic acids may be used as indicators of the management effects on soil organic fractions, because there was significant incorporation of carbon and nitrogen derived from the legume residues, even for the short experimentation time (28 months). Residue management did not modify quantitative aspects of the distribution of the humified organic matter, but promoted, however, a higher condensation degree of humic acids evaluated by the elementary composition, IR and fluorescence spectroscopy.

Key words: FTIR, fluorescence, humic acids, humic substances, ¹³C

QUALIDADE DA MATÉRIA ORGÂNICA DE UM SOLO CULTIVADO COM LEGUMINOSAS HERBÁCEAS PERENES

RESUMO: O uso de leguminosas herbáceas em sistemas agrícolas permite o aporte de quantidades expressivas de fitomassa, possibilitando alterações no teor e na qualidade da matéria orgânica do solo ao longo dos anos. Este trabalho avalia a qualidade da matéria orgânica de um Argissolo Vermelho Amarelo localizado em Seropédica, RJ, e cultivado com diferentes espécies de leguminosas herbáceas perenes: amendoim forrageiro cv. BR-14951 (*Arachis pintoi*), cudzu tropical (*Pueraria phaseoloides*) e siratro (*Macroptilium atropurpureum*), num delineamento fatorial com três repetições, considerando a manutenção da parte aérea depois do corte na parcela ou a retirada da parte aérea da área experimental. Foram também analisadas amostras de uma área adjacente com vegetação espontânea (mata de capoeira) e capim colônia (*Panicum maximum*). As amostras de solo foram coletadas em duas profundidades (0-5 e 5-10 cm), realizando-se o fracionamento da matéria orgânica e a avaliação das características estruturais dos ácidos húmicos. As leguminosas avaliadas não alteraram o conteúdo de carbono orgânico total, mas promoveram acúmulo de ácidos húmicos (AH) na camada superficial. Os AH podem ser utilizados como compostos indicadores dos efeitos do manejo sobre a fração orgânica do solo, pois apesar do pouco tempo de implementação do experimento (28 meses) foi verificada incorporação significativa de carbono e nitrogênio provenientes dos resíduos orgânicos das leguminosas. O manejo dos resíduos vegetais não alterou aspectos quantitativos da distribuição de matéria orgânica humificada, mas conferiu maior grau de condensação aos ácidos húmicos avaliados pela análise da composição elementar, espectroscopia de IV e de fluorescência.

Palavras-chave: IVTF, fluorescência, ácidos húmicos, substâncias húmicas, ¹³C

INTRODUCTION

Benefic effects on soil properties resulting from the use of legumes in crop rotation or intercropped with other plant species has been observed by several authors (Girma & Endale, 1995; Szott & Palm, 1996; Seganfredo et al., 1997; Espindola, 2001). Despite of great potential as cover crops, scanty information exist on the use of perennial herbaceous legumes in tropical soils and their effects in the quality of soils organic matter (SOM).

Organic matter composition and structural features of the humic substances have provided quite useful information to describe and to establish parameters on the capacity of agrosystems to support agricultural practices (Mielniczuck, 1999). The use of SOM as indicator of quality is based on the premise that it has an important role in soil fertility (affects chemical, physical and biological properties), and their humified fraction is a stable chemical marker of soil history (suffers influences of soil formation factors like time, origin material, climate, relief and anthropomorphic action).

Humic acids (HA) assume a key role as indicators of SOM quality since during the humification process (organic residues stabilization), the humified fraction is the one that undergoes the largest structural changes (Kögel-Knaber et al., 1988). Zech et al. (1997) confirm these observations, basing their conclusions on the fact that the spectroscopic behavior of fulvic acids is essentially similar to polysaccharides, and the humins are little modified because of their association with the soil mineral fraction and their apolar characteristics.

Changes in the functionality and molecular composition of HA, like chemical characteristics and spectroscopic behavior, can be used in the evaluation of the management impact on agricultural environment (Volkoff et al., 1988; Arshad & Schnitzer 1989; Martin et al., 1998; Canellas et al., 2001). The use of Fourier-Transformed Infrared (FT-IR) spectroscopy is an useful tool for the analysis of SOM evolution in tropical soils (Haberhauer et al., 2000), especially for the observation of oxygen-

ated functional groups (MacCarthy & Rice, 1985). Additionally, the region of absorption between 900 and 1400 cm^{-1} is known as a fingerprint region of an organic substance (Nakanish, 1965). It is, therefore, possible to establish relative comparisons between the different types of HA and the effects of cover crops through the analysis of this absorption region. In the same way, the fluorescence spectroscopy can also be used to evaluate the presence of fluorophors and to correlate them to SOM quality (Bayer et al., 2002). The effects of soil cover with different perennial herbaceous legumes on the content of humic substances of an Ultisol were herein investigated, as well as the chemical characteristics of humic acids, in order to access SOM quality.

MATERIAL AND METHODS

Characterization of the site - The trial was carried out on an Ultisol of Seropédica, RJ, Brazil ($43^{\circ} 41' \text{ W}$, $22^{\circ} 45' \text{ S}$; altitude 33 m). Soil chemical properties of the 0-20 cm layer were analysed according to Embrapa (1997): pH (H_2O) = 5.6; Al = 0.0 $\text{mmol}_c \text{ dm}^{-3}$ (titration against NaOH); Ca = 30.0 $\text{mmol}_c \text{ dm}^{-3}$ and Mg = 14.0 $\text{mmol}_c \text{ dm}^{-3}$ (titration against EDTA); P = 2.0 mg dm^{-3} (Mehlich 1 extraction and colorimetric determination); K = 72.0 mg dm^{-3} (Mehlich 1 extraction and flame photometric determination).

Trial was set up in a completely randomized block design, 3 x 4 factorial scheme, with three replications. After previous evaluation for species adaptation to soil and climate conditions (Espindola, 2001), three legumes species were chosen: forage groundnut cv. BR-14951 (*Arachis pintoii* Krap. & Greg.), tropical kudzu (*Pueraria phaseoloides* (Roxb) Benth.), and siratro (*Macroptilium atropurpureum* (OC.) Urb), major vegetal composition characteristics shown in Table 1. After the first cut, each plot was divided into two subplots: plants were cut and left on the soil surface or were cut and removed. Six cuts of *A. pintoii* and seven of *M. atropurpureum* and *P. phaseoloides* were made during the

Table 1 - Quality of different plant residues obtained from the cover crops.

Cover crops	Dry mass Mg ha^{-1}	C ----- g kg^{-1} -----	N ----- g kg^{-1} -----	C/N -	Lignin ----- g kg^{-1} -----	Cellulose ----- g kg^{-1} -----
<i>A. pintoii</i> - A	13.1	459	28.6	16.1	120	152
<i>A. pintoii</i> - B	12.6					
<i>M. atropurpureum</i> -A	12.3	472	21.3	22.7	127	269
<i>M. atropurpureum</i> -B	15.3					
<i>P. phaseoloides</i> - A	15.2	478	25.3	19.2	88	207
<i>P. phaseoloides</i> - B	12.7					
Spontaneous vegetation	11.1	459	10.6	43.9	88	297

Adapted from Espindola (2001). Carbon contents were determined by muffle heating at 550°C , and N by Micro-Kjeldahl method. Lignin and cellulose were obtained by Van Soest & Wine method (1968). (A): plants were cut and left on the soil surface; (B): plants were cut and removed from the plots.

experimental period. Plot size was 3.0 m x 7.0 m, and subplot size was 3.0 m x 3.5 m. Thirty months after trial installation, soil samples were collected at the depths of 0-0.05 and 0.05-0.10 m with (A) or without (B) plant residues on the soil surface. Soil samples were also collected at the same depths in a closed area covered by secondary vegetation (mainly *C₃* plants) or by *Panicum maximum* cv. KK-16.

Fractionation of soil organic matter - The humified organic matter fractionation was carried out according to the Belckkova-Kononova methods, described in details by Guerra & Santos (1999). Previous pre-treatment of samples were carried out with H_3PO_4 2 mol L⁻¹ to extract the fraction known as free fulvic acids (FFA). Humic material was extracted by adding 100 mL of NaOH 0.1 mol L⁻¹ + $\text{Na}_4\text{P}_2\text{O}_7$ 0.1 mol L⁻¹ to subsamples with 10 g of soil. The air was displaced by N_2 from flasks, which were then stoppered and shaken for 16 hours at room temperature. The supernatant solution was separated from the residual soil by centrifugation at 3000 g for 30 min and decanted. This procedure was repeated until obtaining a colorless extract evaluated by absorbance at 465 nm. The alkaline extraction solution was acidified to pH 1.0 with HCl 6 mol L⁻¹. After 12 hours at 8°C, the extract was separated into soluble and insoluble parts. The carbon contents in all humified fractions were determined by dicromatometry through colorimetric procedures on a Shimadzu spectrophotometer at 600 nm (Anderson & Ingram, 1993). Because of the low content, the FFA fraction was associated to the FA fraction.

Extraction of humic acids for spectroscopic analysis - The extraction of the humic substances was carried out according to the IHSS procedure (Ceretta, 1995), with pre-treatment of soil samples with HCl 0.1 mol L⁻¹ and extraction with NaOH 0.5 mol L⁻¹ under a N_2 atmosphere (1:10 soil sample: solvent ratio; v/v). The isolation of HA was made by the addition of HCl 6 mol L⁻¹ to pH 1.0. The redissolution and reprecipitation of HA was repeated three times. HA purification was performed using a diluted mixture of HF 0.3 mol L⁻¹ + HCl 0.1 mol L⁻¹, centrifuged at 3000 g for 20 min and dialysed against deionized water (14 kDa cut-off membranes Thomas Sci., Inc.). Finally, HA was freeze-dried.

Characterization of humic acids

Isotopic and elemental composition of humic acids - The C and N contents were determined in 1-3 mg of humic acids samples by dry combustion, and the isotopic composition (d^{13}C) by an elemental analyser coupled to mass spectrometer (Finigrann Mat Model Delta-E). The Dumas method was used for calculation procedures. Ash contents were obtained by incineration of samples at 700°C using three replications.

Carboxylic, phenolic and total acidity - The total acidity of humic acids was determined by the $\text{Ba}(\text{OH})_2$ method with titration of HCl excess, and the carboxylic acidity by adding $\text{Ca}(\text{OAc})_2$ and determining the OAc liberation with NaOH titration according to Schnitzer & Gupta (1965). The phenolic acidity was obtained by difference between total and carboxylic acidity values.

E_4/E_6 ratio - E_4/E_6 ratios were determined by dissolving 4 mg of each HA in 10 mL of NaHCO_3 0.05 mol L⁻¹ and the pH was adjusted to 8.3 with diluted NaOH. The absorbances at 465 nm and 665 nm were measured on a Shimadzu spectrophotometer. The ratio of the absorbance values gave the E_4/E_6 ratio.

Infrared spectroscopy - FT-IR spectra of HAs were recorded on KBr pellets in the 400-4000 cm⁻¹ wave number using a Shimadzu 83000 spectrophotometer. A mixture of 1 mg HA and 300 mg KBr was pressed, under reduced pressure, to obtain the KBr pellets, with three replications per treatment. The subtraction spectra procedure was used to infer the H_2O and CO_2 contaminations (absorption region at 3300-3400 cm⁻¹ and 2340 ± 10 cm⁻¹, respectively). To compare one spectrum to another, data were corrected using baselines at 4000 cm⁻¹, 2000 cm⁻¹ and 860 cm⁻¹, considered as zero absorbance (A). The absorbance ratios were obtained dividing A values of a certain wave number by another, according to recommendations of Inbar et al. (1989), and Freixo et al. (2002).

Fluorescence spectroscopy - Fluorescence spectra of humic acids were obtained in aqueous solution with 100 mg L⁻¹ concentration. The pH of the solution was adjusted to 8.0 with NaOH 0.05 mol L⁻¹, using a Hitachi F-4500 spectrometer. The excitation spectra was obtained by scanning the range of 270-500 nm with emission at 465 nm, 5 nm slit, scan velocity 120 nm min⁻¹ and automatic spectra. Total fluorophors intensity in the spectra was obtained by automatic electronic integration of the spectrometer software.

RESULTS AND DISCUSSION

The climate of the site is classified as type Aw, characterized by high annual means of temperature and precipitation during the rainy season (from October to March), followed by a warm and dry season (from April to September). These conditions favoured a rapid plant residue evolution in the soil, up to CO_2 and H_2O (primary mineralization).

Values of the constant of decomposition (k , days⁻¹) for different plant residues varied, as measured by Espindola (2001), between 0.006 for spontaneous vegetation and 0.024; 0.016 and 0.011 for *A. pintoi*, *M. atropurpureum* e *P. phaseoloides*, respectively. These re-

sults corresponded, in theory, to half-lives of 28, 43, 63 and 116 days for *A. pintoi*, *M. atropurpureum*, *P. phaseoloides* and the spontaneous vegetation.

A consequence of this markedly oxidant natural environment is the low content of carbon on soil. Soil covering with different perennial herbaceous legumes did not modify the total content of organic carbon in experimental plots. A summary of analysis of variance for this experiment is presented in Table 2. The character of the alkaline-soluble, humified organic matter in this study is essentially fulvatic, with predominance of fulvic acid fractions, since the HA/FA ratio for the plots covered with legumes was always smaller than 1 (Table 3). The fulvatic character was more accentuated for *A. pintoi* at 0-0.05 m, because of the high content of the FA fraction, and for *P. phaseoloides* at 0.05-0.10 m, because of the small content of HA. The FAs are organic compounds with higher oxidation degree than HA, and their high content in soils may be result from partial oxidation of organic matter, with relative enrichment of this fraction (Ortega, 1983). The FA formation from the HA fraction was experimentally obtained by Aleksandrova & Naidenova "apud" Bambalov & Belen'kaya (1998).

The major fraction of humified SOM was represented by the humins, between 21.6-43.3 % of the soil organic carbon. These values are within the range ordinarily observed in other tropical soils (Dabin, 1981). The prolonged dry season in the region (April to September) favours preservation of the the low transformed biopolymer through the interaction with the mineral soil fraction, and the humic substances formation is favoured during the rainy season by intense microbial activity (Santos & Camargo, 1999). According to the analysis of variance (Table 2), the management of perennial herbaceous legumes have not effects on SOM humified distribution.

The HA/FA ratios of soil samples from the areas with grass (*Panicum maximum*) and spontaneous vegetation, both adjacent to experimental plots, were used only as reference values, not participating in the statistical design. The HA/FA ratio were lower for the legumes, in relation to the spontaneous vegetation. It is possible that soil mobilization due to tillage modified the content of humified SOM fractions (Table 3). This behaviour was observed for both soil depths. The composition of the humus can be used as an appropriated indicator of the effects of management on soil quality (MacCallister & Chien, 2000).

Table 2 - Summary of the analysis of variance (F test) for soil total organic carbon (TOC), carbon in fulvic acids (C_{FA}), humic acids (C_{HA}) and humins (C_H), humic acid/fulvic acid ratio (HA/FA) and degree of humification (DH).

Variation Sources	TOC	C_{FA}	C_{HA}	C_H	HA/FA	DH
Legume	NS	*	*	*	*	**
Residues management	NS	*	NS	NS	NS	NS
Depth	NS	**	NS	NS	NS	NS
Management x depth	NS	NS	NS	NS	NS	NS

*Significance level at 0.05; **Significance level at 0.01; NS = not significant ($P > 0.05$); DH = degree of humification, calculated by the equation $(HS/TOC) \times 100$ (Kononova, 1982).

Table 3 - Total soil organic carbon (TOC), carbon in fulvic acids (C_{FA}), humic acids (C_{HA}) and humins (C_H), humic acid/fulvic acid ratio (HA/FA) and degree of humification (DH).

Cover crops	TOC	C_{FA}	C_{HA}	C_H	HA/FA	DH
----- g kg ⁻¹ -----						
0-0.05 m						
<i>A. pintoi</i>	18.3 A	2.24 A	0.53 AB	6.51 A	0.24 B	52.14 A
<i>M. atropurpureum</i>	14.5 B	1.83 AB	0.63 A	4.76 A	0.34 A	53.16 A
<i>P. phaseoloides</i>	18.2 A	1.45 B	0.48 B	4.42 A	0.33 A	38.98 B
<i>P. maximum</i>	14.9	1.43	0.78	4.21	0.55	40.5
Spontaneous vegetation	17.8	0.86	0.24	3.84	0.28	27.8
0.05-0.10 m						
<i>A. pintoi</i>	14.3 A	2.19 A	0.81 A	5.87 A	0.37 A	65.01 A
<i>M. atropurpureum</i>	13.0 A	2.04 A	0.55 AB	4.68 A	0.27 B	60.14 AB
<i>P. phaseoloides</i>	14.1 A	1.49 B	0.35 B	5.68 A	0.23 B	52.23 B
<i>P. maximum</i>	12.7	1.25	0.86	5.24	0.69	57.5
Spontaneous vegetation	13.6	0.74	0.66	5.89	0.89	53.4
CV (%)	27.73	33.26	39.45	35.39	63.34	34.30

Means with the same letter within a depth and column do not differ by Tukey test ($P = 0.05$).

The highest values of HA/FA were observed for the plots with *Panicum maximum*, what suggests that a more ramified root system may favor the formation of more condensed, alkaline-soluble humic substances. Besides changes in the relative composition of humified organic matter, qualitative changes were observed for the humic acids by the modification of their structural properties.

The C contents of the HA (Table 4) were comparable to the contents ordinarily found for FA fractions (Stevenson, 1994), suggesting the low degree of evolution of this humified fraction. In the humification process, there is an increase of condensation reactions (molecules joined by water loss and increased C atoms and decreased O and H contents). On the other hand, the N contents of the HA were typical of highly humified substances, even for plots without legumes. Flaig (1971) postulated that phenol insolubilization and condensation of quinones, the major precursors of humic substances, are resultant of reactions mediated by N compounds. As a result, N accumulation occurs during the humification process.

Kuwatsuka et al. (1978) verified low correlation between C contents of 39 different HA samples and the humification degree evaluated by spectroscopic methods. However, there was high and significant correlation with N content. The values of the C/N ratio for the HA were low, between 10.9 and 13.2, indicating that it is possible

to use HA as the energy source for soil biota (enhance of secondary mineralization). Ceretta (1995) observed that the C/N ratio of the HA fraction decreased with the intercropping of corn and legumes, as compared to crop rotation with grasses and bare soil.

The ^{13}C isotopic dilution analysis of HA samples showed that, even for the reduced period of experimentation, C incorporation from legumes to humified material occurred (Table 4). The mean values of $\delta^{13}\text{C}$ ‰ from HA isolated in the plots with *Panicum maximum* was -17.02 ± 0.28 , and in the HA isolated from legume -19.13 ± 0.38 . Balesdent et al. (1987) states that the isotopic composition of C_4 plants (eg grasses) range from -9 to -19 ‰, while for C_3 plants (eg legumes), from -23 to -40 ‰. The incorporation of organic materials derived from legumes in the HA was higher in the superficial layer, and justifies the use of structural changes of HA to monitor effects of soil management on organic matter.

Total acidity was in the typical range for HA (Stevenson, 1994) and the carboxylic groups presented values similar to phenolic groups (Table 5). The smallest values for total and carboxylic acidity were observed for the HA samples isolated from plots with *P. phaseoloides*. The lowest content of lignins of this legume may be an indication that the quality of organic residues can modify reaction properties of humic substances.

Table 4 - Elemental composition*, molecular atomic relations and isotopic relations of humic acids isolated from an Ultisol covered with different plant species, the residues of which were cut and left on the soil surface (A) or they were cut and removed from the plots (B).

Cover crops	C	N	C/N	^{13}C
	----- g kg ⁻¹ -----			‰
0-0.05 m				
<i>A. pinto</i> - A	443.5 ± 1.30	45.0 ± 1.13	11.0	-19.16 ± 0.01
<i>A. pinto</i> - B	462.5 ± 0.99	39.8 ± 0.53	11.6	-18.55 ± 0.02
<i>M. atropurpureum</i> - A	453.8 ± 4.12	32.4 ± 0.57	10.9	-21.77 ± 0.02
<i>M. atropurpureum</i> - B	467.1 ± 1.41	41.2 ± 0.42	11.3	-20.15 ± 0.04
<i>P. phaseoloides</i> - A	448.6 ± 1.02	40.7 ± 0.45	11.0	-20.97 ± 0.03
<i>P. phaseoloides</i> - B	466.6 ± 0.14	41.2 ± 0.42	11.3	-18.16 ± 0.04
<i>P. maximum</i>	429.4 ± 2.29	32.7 ± 0.30	13.2	-17.30 ± 0.01
Spontaneous vegetation	489.0 ± 0.10	39.7 ± 0.45	12.3	-18.62 ± 0.01
0.05 - 0.10 m				
<i>A. pinto</i> - A	449.1 ± 2.53	40.8 ± 0.28	11.0	-17.38 ± 0.01
<i>A. pinto</i> - B	451.0 ± 1.13	39.7 ± 0.42	11.4	-17.38 ± 0.40
<i>M. atropurpureum</i> -A	449.5 ± 1.84	38.5 ± 0.71	11.7	-18.92 ± 0.54
<i>M. atropurpureum</i> -B	454.0 ± 1.43	37.6 ± 0.74	12.1	-19.27 ± 0.29
<i>P. phaseoloides</i> - A	450.5 ± 0.93	39.9 ± 0.29	11.3	-18.56 ± 0.09
<i>P. phaseoloides</i> - B	489.0 ± 1.41	41.4 ± 0.28	11.8	-19.97 ± 0.03
<i>P. maximum</i>	445.5 ± 0.68	35.1 ± 1.27	12.7	-16.74 ± 0.28
Spontaneous vegetation	481.0 ± 1.44	42.3 ± 0.71	11.4	-17.85 ± 0.44

*C and N contents were recalculated for a basis free of ash, which varied from 0.98 % to 1.15 %.

The use of UV-VIS spectroscopy for HA evaluation provided E_4/E_6 ratios that, according to Chen et al. (1977), are inversely related to the contents of free radicals, C, O, COOH and total acidity, and are mainly influenced by the molecular weight/size. This ratio can be used as a humification degree (Anderson, 1979). The values found for E_4/E_6 were high (6.5 to 9.0) when compared to the range ordinarily found for FA fractions (Stevenson, 1994), indicating a low humification degree of these HA. Among each plant species, values of E_4/E_6 decreased with soil depth, suggesting a greater stability of the HA. Bravard & Righi (1991) found high values for E_4/E_6 of HA extracted from superficial horizons in Oxisols, diminishing in depth, an indication that the interaction with clay minerals like caulinite, Fe and Al oxides and hydroxides, can stabilize the alkaline-soluble humic substances, protecting them against humification reactions.

Based on Nakanish (1965), a summary of the major absorption bands of FT-IR spectroscopy is presented. The FT-IR spectra presented similar features, with major absorption at 3740 cm^{-1} ($n_s\text{O-H}$ and N-H free); $3200\text{-}3500\text{ cm}^{-1}$ with the center at 3300 cm^{-1} (nO-H intramolecular binding); 2900 cm^{-1} ($n_s\text{C-HC}$ in alkyl groups), shoulder between $2600\text{-}2400\text{ cm}^{-1}$ (CO_2H), 1716 cm^{-1} (nC=O carboxylic groups and/or carbonyl); 1500 cm^{-1} (nC=C aromatic, dN-H), absorption band between $1635\text{-}1645\text{ cm}^{-1}$ (C-C aromatic on C=O , COO and/or quinones

in conjugation); 1450 cm^{-1} (dC-H alkyl), 1250 (dO-H from CO_2H groups and/or nC-O from phenolic groups); 1100 cm^{-1} (nC-O-H from polysaccharides).

In general, an enlargement of absorption was observed in FT-IR spectra of HA isolated from legumes plots, for bands between $3300\text{-}3400\text{ cm}^{-1}$ and lower intensity in the absorption band at 3700 cm^{-1} , with increase in soil depth. The presence of intramolecular H bonding and the decrease of free H-O or H-N are possible indications of the presence of more complex structures in soil depth (verified by the decrease of acidity and E_4/E_6 ratio). At the superficial layer, absorption bands with high intensity were observed at 1110 cm^{-1} , and attributed to the presence of polysaccharides. A smaller intensity of this band, for the 5-10 cm layer, is another indication of the presence of more HA complex, structural substances with soil depth. In the plots with *P. phaseoloides*, independent of plant residue management, absorption bands related to nO-H in intramolecular bonding were larger, which implies in more vibrational energy, a characteristic of more rigid systems.

Relative indexes of hydrophobicity and condensation of HA were obtained with the ratio of absorbance between apolar and polar groups, and between alkyl and aromatic groups, respectively. Inbar et al. (1989) and Freixo et al. (2002) successfully used indexes obtained from FT-IR to evaluate the organic matter evolution dur-

Table 5 - Total (Ba(OH)_2 method), carboxilic (CaAcO method) and phenolic (obtained by subtraction) acidities and E_4/E_6 ratios of humic acids isolated from an Ultisol covered with different plant species, the residues of which were either cut and left on the soil surface (A) or were cut and removed from the plots (B).

Cover crops	Total acidity	Carboxilic	Phenolic	E_4/E_6 ratio
----- mmol _(c) Kg ⁻¹ de AH -----				
0 - 0.05 m				
<i>A. pintoi</i> - A	10521 A	6418 A	4103	6.8
<i>A. pintoi</i> - B	7984 A	4231 A	3752	7.5
<i>M. atropurpureum</i> - A	9139 A	4661 A	4478	6.5
<i>M. atropurpureum</i> - B	7393 A	4362 A	3031	6.2
<i>P. phaseoloides</i> - A	6282 B	3769 A	2513	7.5
<i>P. phaseoloides</i> - B	5239 B	2043 B	3196	7.6
<i>P. maximum</i>	10138	5170	4968	7.6
Spontaneous vegetation	7318	3878	3439	8.3
0.05 - 0.10 m				
<i>A. pintoi</i> - A	9497 A	5983 A	3514	6.6
<i>A. pintoi</i> - B	7304 A	4456 A	2849	6.5
<i>M. atropurpureum</i> - A	7408 A	4593 A	2815	5.5
<i>M. atropurpureum</i> - B	6449 AB	3740 AB	2708	4.9
<i>P. phaseoloides</i> - A	5480 AB	3343 AB	2137	7
<i>P. phaseoloides</i> - B	4843 B	2712 B	2131	7.3
<i>P. maximum</i>	7260	3775	3485	7.3
Spontaneous vegetation	7302	4016	3286	7.8

Means with the same letter within a depth and column do not differ by Tukey test ($P = 0.05$).

ing the composting process and the organic matter quality in aggregates of soils under different tillage. The ratios I2900/3300 and I2900/1600 are regarded in relation to the relative contents between C-H (low polarity) and O-H e COO- (high polarity), respectively. On the other hand, the ratio I3300/1700 indicates the proportion between O-H and C=O systems, which is an indicator of condensation. This is an important index because humification generally increases the number of aromatic systems and decreases the number of OH.

The HA isolated from plots with *M. atropurpureum* presented the highest values of the ratios 2900/3300 cm^{-1} and 2900/1600 cm^{-1} , presenting greater participation of hydrophobic structures at their molecular composition (Table 6). These differences were also observed in the area with *Panicum maximum*, but they were not evident in the area with spontaneous vegetation. Plots with *M. atropurpureum* presented the highest condensation degree obtained through derived indexes from IR spectra. Values of E_4/E_6 comparatively smaller for these HA (Table 5), support the hypothesis derived from IR indexes. However, systematic studies should be performed to corroborate the use of these indexes for soil organic matter studies. The effects of residue management were particularly detected for the plots of *P. phaseoloides*. There was an enlargement of the absorption bands of HA isolated from plots where residues of this species were

removed, what may be attributed to the higher intensity of superposition of absorption bands from polymerized structures. Such effect was also observed, with lower intensity, for HA isolated from plots with *M. atropurpureum*, but not for those isolated from plots with *A. pintoii*.

Fluorescence spectra of HA (not shown) agree with the observed data obtained through the chemical methods UV-VIS and FT-IR spectroscopy. The higher content of fluorophors at deeper layers (Figure 1) did not depend on soil cover and its management, indicating greater participation of conjugated systems with increasing depth as a consequence of the higher humification degree.

Soil cover with perennial legumes alters the quantitative distribution of humified fraction of soil organic matter, promoting differences in HA contents in relation to legume species, specially for the depth of 5-10 cm. Humic acids may be used as marker compounds to evaluate the effects of soil management on organic fraction, because there was an incorporation of C and N derived from legume residues despite of the short conduction time of the trial (28 months). Residue removal did not change quantitative aspects of the distribution of humified organic matter, but promoted a higher degree of condensation to humic acids evaluated by total acidity and fluorescence spectroscopy.

Table 6 - Structural parameters obtained from semi-quantitative analysis of FT-IR. Values represent the relation between absorbance at different wave numbers (cm^{-1}) of humic acids isolated from an Ultisol covered with different plant species, the residues of which were cut and left on the soil surface (A) or they were cut and removed from the plots (B).

Cover crops	Hydrophobicity		Condensation
	2900/3300 cm^{-1}	2900/1600 cm^{-1}	3300/1700 cm^{-1}
0 - 0.05 m			
<i>A. pintoii</i> - A	1.1	1.1	1.0
<i>A. pintoii</i> - B	1.0	0.9	1.0
<i>M. atropurpureum</i> - A	1.9	1.5	0.6
<i>M. atropurpureum</i> - B	1.7	1.4	0.6
<i>P. phaseoloides</i> - A	1.1	1.0	0.9
<i>P. phaseoloides</i> - B	1.2	1.1	0.9
<i>P. maximum</i>	1.1	1.1	1.0
Spontaneous vegetation	1.2	1.1	0.9
0.05 - 0.10 m			
<i>A. pintoii</i> - A	1.0	1.1	1.0
<i>A. pintoii</i> - B	1.2	1.0	0.8
<i>M. atropurpureum</i> -A	1.7	1.5	0.6
<i>M. atropurpureum</i> -B	1.5	1.5	1.0
<i>P. phaseoloides</i> -A	1.2	1.0	0.9
<i>P. phaseoloides</i> B	1.6	1.2	0.6
<i>P. maximum</i>	1.1	1.0	0.9
Spontaneous vegetation	1.0	1.1	1.1

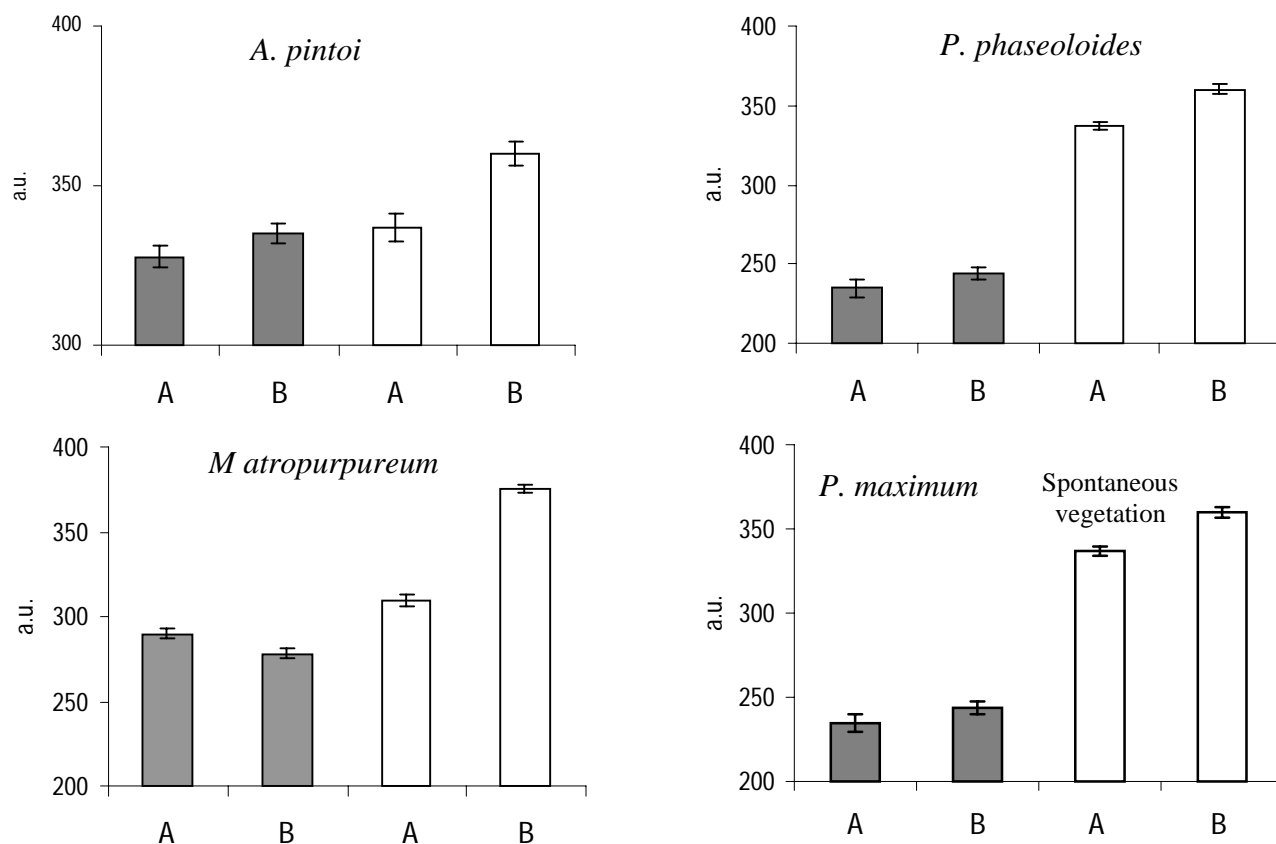


Figure 1 - Intensity of fluorophors (arbitrary unity - a.u.) contained at humic acids isolated from an Ultisol covered with different plant species, the residues of which were cut and left on the soil surface (A) or they were cut and removed from the plots (B). Soil samples were collected at the depths of 0-0.05 m (gray bars) and 0.05-0.10 m (white bars).

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