http://dx.doi.org/10.5935/0100-4042.20160001 *Quim. Nova,* Vol. 39, No. 1, 14-18, 2016

ORGANIC MATTER AND HUMIC FRACTIONS OF A HAPLIC ACRISOL AS AFFECTED BY COMPOSTED PIG SLURRY

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Recebido em 29/04/2015; aceito em 21/09/2015; publicado na web em 14/01/2016

The goal of this study was to investigate the effect of composted pig slurry (PS) on the organic matter concentration and distribution of humic acid (HA), fulvic acid (FA) and humin (HU) fractions. The fractions were quantified following the addition of composted PS to the soil, which was produced with no acidification (T2) or with acidification with H_3PO_4 (T3); and in soil without compost addition (T1). The HA chemical composition was analyzed by FTIR spectroscopy. The addition of the two composts did not change the soil carbon concentration but affected the distribution of the humic fractions. For the three treatments, the carbon concentration of humic substances increased until 52 days following compost addition, with more pronounced increases with the addition of non-acidified PS compost (14.5 g kg^{-1}) and acidified PS compost (15.1 g kg^{-1}). This increase was reflected in both the FA and HA concentrations. The addition of compost with PS acidification resulted in the formation of larger humic micelles (HA) with higher aromatic content and fewer functional groups than the non-acidified PS compost. These findings, together with a lower proportion of carbohydrate-type structures, indicated the presence of more stable humic micelles in the soil treated with acidified PS compost.

Keywords: compost; pig slurry acidification; humic micelles; FTIR spectroscopy.

INTRODUCTION

Pig farming contributes relevantly to the Brazilian agribusiness, and nowadays Brazil occupies the 4th position in the world rank of swine production[1]. The national pig herd comprises around 41.3 million animals[1] and approximately fifty percent of it is located in Southern Brazil[2]. Pig farms are mostly composed of small rural farms or large-scale intensive production systems, which generate large quantities of pig slurry (PS) that is applied to the soil as an organic nutrient source for plants.

In addition, adding PS to the soil to support nutrient cycling can also increase soil carbon concentrations (C_T) over time [3,4] and may change the composition of carbon and distribution of the humic fractions of the soil organic matter (SOM).[5] However, excessive and successive PS additions can result in environmental problems that may compromise the quality of soil and adjacent water sources. The composting of PS, during which it is converted into a solid matrix[6] has been used to mitigate the problems generated by PS application "in natura". The resulting compost presents a fertilizing effect and high SOM concentration, influencing the chemical, physical and biological characteristics of soil.[7] For instance, increases in the SOM, phosphorus and exchangeable potassium concentrations were observed in a Red Nitosol from Southern Brazil following the application of poultry litter and pig manure organic compost (40 t ha^{-1}).[8] Increase of the soil total organic carbon (C_T) was also observed following successive PS applications to a Red Argisol (over eight years) and after PS and compost shaving applications to a Red Latosol (over six years).[9]

A problem intrinsic to the PS composting process is the loss of nitrogen due to ammonia volatilization (NH_3), which results in reduced fertilizing capacity of the organic compost.[10,6] This problem may be avoided by PS acidification prior to its incorporation into the substrate.[11]

Humic substances (HS) constitute the most stable fraction of the SOM, with humic acids (HA) and fulvic acids (FA) playing an important role in the SOM reactions due to their functionality and reactivity.[12] The humification degree of the organic compost is directly related to the decomposition of its components and thus it affects directly the compost capacity to act as a nutrient source and /or as a soil conditioner.[13] The soil HA:FA ratio has been used as an indicator of the degree of SOM humification.[14] The addition of a compost made up by urban solid wastes decreased the HA/FA ratio of the SOM, indicating the lowering of its humification degree and therefore of its quality as a soil conditioner[15] The change of the soil humic fractions distribution after repeatedly additions of composted PS has been verified in southern Brazilian soils.[9] In an Acrisol an increase of the FA and HA fractions occurred after eight years of PS compost application while in a Red Ferralsol an increase of humin (HU) occurred after six years.[9]

The composting of PS with an agronomic purpose is an ancient technique and studies about the changes of the chemical composition of the compost during the process have been widely conducted.[16,17] Nevertheless, there are few studies about the effects of the addition of PS compost to the soil on the endogenous SOM composition. In the case of acidified PS compost, which is a recent composting strategy to mitigate nitrogen gases emissions, the information in the literature is even scarcer.[18] Therefore, the main goal of this study was to investigate the effects of the application of two PS organic compost (acidified and non-acidified) on the distribution of the SOM humic fractions and HA composition. The information obtained in this study will help to get insights about the SOM dynamics within a short time after PS compost application.

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EXPERIMENTAL PART

This experiment was performed between May and August 2012 at the Pig Farming division of the Department of Zootechnics of the Federal University of Santa Maria (UFSM), in the state of Rio Grande do Sul (RS) (29° 41' 29" South, 53° 48' 3" West). The climate is classified as Cfb based on the Köppen climate classification. The soil is classified as an Haplic Acrisol soil.[19] and its texture is composed by 350 g kg^{-1} sand, 530 g kg^{-1} silt and 120 g kg^{-1} clay. The main chemical soil characteristics of the 0-10 cm layer are given in Table 1. The exchangeable cations were determined after extraction with 0.1 mol L^{-1} KCl solution, the available P and K according to Mehlich-1 solution and soil pH in distilled water, as described in Tedesco *et al*.[20]

Both of the tested organic composts were obtained using a prototype for the simulation of PS through automated composting over 154 days using a mix of 50% sawdust and 50% shavings as a substrate.[21] For one of the composts, the PS was acidified with phosphoric acid (85% H_3PO_4, density 1.6 g m^{-3}) immediately before each addition of PS to the compost pile until a pH of approximately 5.0 was reached. The other compost was obtained without PS acidification (original pH 7.2) during composting. The chemical characteristics of the tested composts are given in Table 1. The employed methods were the same as those used for the soil. Total C and N were determined by dry combustion (Perkin Elmer 2400). The composts were incorporated (70.4 Mg ha^{-1} of non-acidified PS and 94.3 Mg ha^{-1} of acidified PS) into the superficial soil layer (0 to 0.15 m) of the plant beds during lettuce transplanting. The applied amount of the composts was defined considering the doses required for the lettuce crop (500 kg N ha^{-1})[22,23] and the N content of the respective composts (Table 1). Therefore, 333,30 mg of N kg^{-1} soil were applied via compost.[24] For further details see Cantú.[24]

A randomized blocks experimental design was used for the field experiment, with three treatments and four replicates. Before establishing the experiment, four composite samples were collected from the 0-10 cm soil layer, one from each block, to establish the original soil characteristics. This sample was used as a reference for all the treatments. The following treatments were tested: T1 (soil without compost application); T2 (soil with application of non-acidified PS); and T3 (soil with acidified PS compost applied). The lettuce (*Lactuca sativa L*) seedlings were transplanted on the day of compost application. Soil samples were collected from the 0-10 cm soil layer during the lettuce lifecycle at 40, 52 and 64 days following compost addition. The samples were subjected to manual lump breaking and sieved using a 2 mm mesh. Because the compost contained shavings larger than 2 mm, the sieving retained some of the composted material in the mesh. The C_T and total soil nitrogen (N_T) concentrations of the soil samples along the experiment and of the HA samples at 64 days were determined using dry combustion (Perkin Elmer 2400). The elemental analysis of the soil fraction > 2 mm could not be performed because it was too coarse.

Extraction, quantification and purification of humic substances

The HS were fractioned according to Swift,[25] and quantified based on Dick *et al*.[26] Thirty milliliters of 0.1 mol L^{-1} HCl was added to 1 g of soil, and the suspension was stirred for 2 h and centrifuged (1529 g, 10 min), and the supernatant was separated. The procedure was repeated 2 more times, and the total volume of extract was measured.

Thirty milliliters of 0.5 mol L^{-1} NaOH was added to the pellets and the suspension was stirred for 3 h. Following centrifugation (1529 g, 10 min), the alkaline extract containing the soluble HS was separated. This procedure was repeated until the extract became colorless (total of 5 extractions). The final volume of alkaline extract was measured, and an aliquot (10 mL) was separated for carbon analysis (C_{HS}). The alkaline solution was acidified to pH 2.0 using 4.0 mol L^{-1} HCl and was left to stand for 24 h and centrifuged (1529 g, 10 min) for separation of the FA (supernatant) and HA (pellet). The final volume of the FA fraction extract was measured. The C concentrations in the acid (C_{HCl}), soluble humic substances (C_{HS}) and fulvic acid (C_{FA}) extracts were quantified following C oxidation with K dichromate in an acid medium at 60 ºC for 4 h, and absorbance was read at 580 nm (Shimadzu UV-160 A). The C concentration occurring as soil HA (C_{HA}) was calculated as follows: $C_{HA} = C_{HS} - C_{FA}$. The C concentration occurring as humin fraction (C_{HU}) was calculated as follows: $C_{HU} = C_T - (C_{HS} + C_{HCl})$. The results were used to calculate the C_{HA}/C_{FA} ratio. The HA samples were purified using 30 mL of 5%/5% HF/HCl (v/v) solution under stirring (2 h) and were then centrifuged (1529 g, 5 min).[26] This procedure was performed 6 times. The purified HA was washed with distilled water for 30 min, with stirring, and was then centrifuged (1529 g, 5 min). This procedure was performed 5 times. The remaining mass was dried in an oven at 60 °C.

Fourier transform infrared spectroscopy (FTIR)

The purified HA was analyzed using Fourier transform infrared spectroscopy (FTIR) (Shimadzu FTIR 8300) in KBr pellets (1 mg sample: 100 mg KBr, 32 scans and 4 cm^{-1} resolution), at a spectra range between 4000 and 400 cm^{-1}. The attribution of absorption bands was performed according to Tan,[27] and the aromaticity index (I_{1630}/I_{2920}) was calculated.[28] The relative intensities of the main absorption bands were calculated according to Gerzabek *et al*.,[29] by dividing the corrected intensity of a given peak (e.g., approximately 2920, 1720, 1630, 1475 and 1070 - 1030 cm^{-1}) by the sum of the intensities of all the peaks and multiplying by 100%. The limits (cm^{-1}) for determining the base of each peak were established as follows: (base1/peak/base2) = 1800/1720/1700, 1560/1540/1490 and 1190/1070 - 1030/ 900.

Statistical analysis

The C_T, N_T and humic substance data for the tested treatments were subjected to an analysis of variance followed by a post-hoc Tukey test at p<0.05, using the Computational System for Analysis

Table 1. Main chemical characteristics of the soil and compost used in the experiment

Sample	pH H_2O	Total-N	C_T	* $P_{available}$	*K^+_{exch}	**Ca^{+2}_{exch}	**Mg^{+2}_{exch}	**Al^{+3}_{exch}	DM %	C/N
		----------------------g kg $^{-1}$---------------------				-------------- cmolc dm^{-3} --------------				
Reference (soil)	5.7	1.1	16.4	7.9	88	8.1	3.0	0	-	14
Non-acidified PS compost	7.2	27.9	410.9	13.7	36.8	-	-	-	24.7	14.6
Acidified PS compost	5.0	21.3	423.3	12.7	30.4	-	-	-	25.4	20.7

*Exchangeable cations extracted by Mehlich-1; ** exchangeable cations extracted by 0.1 mol L^{-1} solution; C_T: Total carbon; N_T: Total nitrogen; DM: dry matter.

of variance Sisvar program.[30] The data analysis were performed from the Origin 6.1 software.

RESULTS AND DISCUSSION

Soil organic carbon and nitrogen concentrations and the C/N ratio

The C_T concentrations ranged from 16 to 21 g kg^{-1} (Table 1) and did not differ between treatments or sampling times. This finding was expected because the quantity of C added via PS compost was low: 4.8 g kg^{-1} for non-acidified PS compost and 6.5 g kg^{-1} for acidified PS compost. However, in treatments with compost additions, with and without PS acidification, the C_T tended to increase from 17.8 to 20.5 g kg^{-1} between 40 and 52 days, stabilizing at approximately 21.3 g kg^{-1} after this time. This pattern may have resulted from the decomposition of some shaving residues larger than 2 mm up to 52 days, which then became part of the C_T of the fraction < 2 mm. The N_T concentrations ranged from 1.2 to 1.5 g kg^{-1} and were not affected by the addition of the two tested composts or the various sampling times. This finding was expected because the N concentration added via PS compost was very low: 0.21 g kg^{-1} for the non-acidified PS compost and 0.23 g kg^{-1} for the acidified PS compost. The C/N ratio ranged from 12.3 to 15.2 and did not differ between the treatments at the tested times (Table 2).

A possible contribution of the lettuce crop to the soil C and N contents did not occur as indicated by similar values observed for the reference and for T1 along the experiment. Furthermore, the particles of sawdust and shavings were greater than 2mm and were retained in the 2mm sieve. Thus these materials were not included in the analyses of the humified soil organic matter.

Distribution of humic fractions

In T1, where no compost added was added, the C_{HCl} concentration increased from 0.05 g kg^{-1} at 40 days, to 0.8 g kg^{-1} at 54 days and to 0.4 g kg^{-1} at 64 days (Table 2), corresponding to 0.3 to 4% of C_T (Figure 1). This extract contains substances smaller than the HS micelles.[30] Soluble compounds in this extract are considered biochemically more labile than the HS and may be weakly associated with the mineral fraction and with each other through outer sphere cation bridges.[26]

The C_{HS} concentration of T1 increased from 3.9 g kg^{-1} at 40 days to 8.1 g kg^{-1} at 52 days and remained near this value until the end of the experiment, corresponding to 24 to 50% of C_T. Contributions of C_{HA} and C_{FA} to the C_{HS} increase were, however, different. The C_{FA} concentration increased gradually from 1.9 to 5.1 g kg^{-1} (11 to 31% C_{FA}/C_T) until 64 days, whereas C_{HA} varied from 2.0 to 4.3 kg^{-1} (12 to 19% C_{HA}/C_T) (Table 2, Figure 1). Between 52 and 64 days, there was a redistribution of these two humic fractions, and the C_{HA}/C_T ratio for T1 decreased from 25% to 20%. Consequently, the C_{HA}/C_{FA} ratio decreased from 1.0 to 0.6 throughout the experiment (Table 2). Considering that the C_T concentration did not vary for this treatment, C_{HU} varied inversely with C_{HS}, decreasing from 12.5 g kg^{-1} at 40 days (C_{HU}/C_T = 75%) to 7.9 g kg^{-1} at 64 days (C_{HU}/C_T = 47%), which differed from the reference value. The humic fractions behavior observed for T1 may have resulted from a system restabilisation after setting up the experiment when the soil was plowed and, therefore, suffered disaggregation. Also, local environmental variations (e.g. temperature variation and precipitation) throughout the experiment may be responsible for the observed results in T1.

The treatment with the addition of non-acidified PS compost (T2) featured a similar variation of C_{HCl} over time as the one observed for T1 (Table 2); however, it reached 1.4 g kg^{-1} at 52 days (C_{HCl}/C = 7%), surpassing the value observed without added compost (T1). T3 (acidified PS compost added) featured higher C_{HCl} than T1 at both 40 and 52 days (C_{HCl}/C_T = 7%) (Table 2, Figure 1). Because the compounds extracted with 0.1 mol L^{-1} HCl were thought to originate mostly from microbial activity and root exudation[31,32] the higher C_{HCl} observed for treatments T2 and T3 relative to T1 may indicate a more intense microbial activity resulting from compost addition.

The C_{HS} concentrations for T2 and T3 were always higher than the reference soil and T1 (Table 2) and reached the highest levels at 52 days. This pattern may have resulted from the characteristics of the added composts, which might have featured higher proportions of HA and FA than the endogenous soil organic matter. However, the C_{HS}/C_T ratios for T2 and T3, which were always higher than in the soil without compost added, reached the highest levels at 52 days, indicating that HS formation was stimulated during this period. C_T was higher at 52 days than at 40 days, although this difference was not significant (Table 2). One hypothesis for the observed C_{HS} behavior was that the decomposition of the > 2 mm compost fraction (especially the shavings) resulted in HS production at 52 days. At 64 days, these substances became a part of the humin fraction, which increased from 4.1 at 52 days to 10.9 g kg^{-1} (C_{HU}/C_T = 53%) at 64 days. This hypothesis was supported by the C_{HCl} data for T2 and T3, which was highest at 52 days (Table 2).

Differences in C_{FA} and C_{HA} were observed between various sampling times for the treatments with compost added. T2 featured higher HA (6.3 g kg^{-1}) than FA (3.2 g kg^{-1}) at 40 days, with C_{HA}/C_{FA} = 2.0. The opposite was observed for T3 (acidified PS compost): C_{HA} = 2.3 g kg^{-1}, C_{FA} = 6.5 g kg^{-1} and C_{HA}/C_{FA} = 0.5. Intermediate C_{HA}/C_{FA} values were observed for T1 (Table 2). These results suggest that acidification of the composting medium favors FA formation and that this characteristic was transferred to SOM. During the experiment, C_{HA} tended to decrease in T2 and increase in T3. Consequently, C_{HA}/C_{FA} tended to decrease in T2, as in T1, and increase in T3, exhibiting the opposite behavior to T1 (Table 2). This result may also indicate higher SOM stabilization in T3 because it featured a higher proportion of larger humic micelles (i.e. humic acids)

These results, therefore, indicate that the two types of compost affected SOM dynamics differently because T2 and T3 exhibited different proportions of the humic fractions. For T2, the decreases were observed in the FA fraction between 52 and 64 days (C_{FA}/C_T from 40% to 27%) and in the HA fraction throughout the experiment (C_{HA}/C_T from 37 to 17%) (Figure 1). For T3, the FA fraction also decreased (C_{FA}/C_T from 37% to 20%), but C_{HA}/C_T increased from 12% to 25% between 40 and 64 days (Figure 1). A higher proportion of larger humic micelles (HA) may indicate higher SOM stability with the addition of acidified PS compost. This hypothesis is based on the proposed SOM supramolecular structure, with the SOM hydrophobic fraction located inside and the hydrophilic groups on the outside of the micelle.[33] It can be inferred that the larger the hydrophobic segment is, the larger the micelle size, and therefore, the higher its stability. The humin fraction behaved similarly to T2 and T3, varying from 7.9 to 11.6 g kg^{-1} on average over time, and the C_{HU}/C_T ratio ranged between 20% and 50% (Figure 1).

HA elemental composition and spectral behavior

For the treatments with added compost, the C concentration of HA ranged from 49 to 52% and that of N between 3 to 4% over the 64 days of the experiment and did not differ within the treatments (data not shown). The C/N ratio ranged from 15 to 16 over time.

The HA from the tested treatments featured the same pattern of FTIR spectra at 64 days (Figure 2). The following absorption bands and respective attributions were identified: a band at 3283 cm^{-1},

Table 2. C_T and N_T concentrations, C/N ratio, and the distribution of C in humic fractions for treatments without compost added (T1), with non-acidified PS compost added (T2) or with acidified PS compost added (T3) to a Haplic Acrisol

Treatments	days*	C_T	N_T	C/N	C_{HCl}	C_{HS}	C_{FA}	C_{HA}	C_{HU}	C_{HA}/C_{FA}
		g kg^{-1} soil								
Reference	0	16.4^{ns}	1.1Bb	14^{ns}	0.04BCBd	3.6BCBc	1.7CCBf	1.9BCBc	12.6ABAa	1.1AAAb
T1	40	16.5^{ns}	1.2Aa	13^{ns}	0.05Bd	3.9Bc	1.9Cf	2.0Bc	12.5Aa	1.0Ab
T2		17.8^{ns}	1.3Aa	13^{ns}	0.07Bd	9.8Ab	3.2Bde	6.3Aa	7.9Bc	2.0Aa
T3		17.8^{ns}	1.3Aa	13^{ns}	0.7Ab	8.9Ab	6.5Abc	2.3Bc	8.3Bc	0.5Bc
T1	52	16.1^{ns}	1.3Aa	12^{ns}	0.8Bb	8.1Bc	3.9Bde	4.3Bbc	7.2Ac	1.1Ab
T2		20.1^{ns}	1.4Aa	14^{ns}	1.4Aa	14.5Aa	8.0Aa	6.4Aa	4.1Bd	0.7Bc
T3		20.5^{ns}	1.5Aa	13^{ns}	1.5Aa	15.1Aa	7.4Aa	7.7Aa	4.5Bd	1.0Ab
T1	64	16.6^{ns}	1.2Aa	13^{ns}	0.4Ac	8.2Ab	5.1Acd	3.1Abc	7.9Ac	0.6Bc
T2		21.3^{ns}	1.4Aa	15^{ns}	0.5Ac	9.0Ab	5.4Abcd	3.5Abc	11.0Aab	0.8Bc
T3		21.2^{ns}	1.5Aa	14^{ns}	0.5Ac	9.7Ab	4.4Ade	5.3Aab	10.9Ab	1.2Ab

Means followed by the same capital letter do not differ between treatments in a given time, means followed by the same lower case do not differ between time for a given treatment. Tukey test at 5% probability.

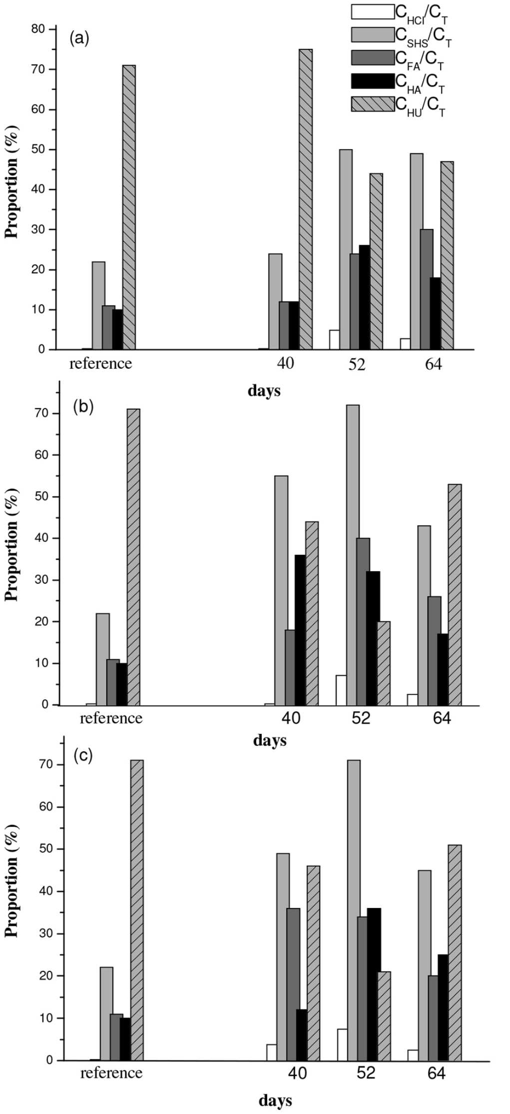

Figure 1. The proportion of C chemical compartments of soil (a) without compost added, (b) with non-acidified PS compost added, and (c) with acidified PS compost added to a Haplic Acrisol. CHCl/C_T. 0.1 mol L^{-1} HCl extractable fraction to C_T ratio; CsHS/C_T: soluble humic substances to C_T ratio; CFA/C_T: fulvic acids relative to C_T ratio; CHA/C_T: humic acids relative to C_T ratio; CHU/C_T: humin to C_T ratio

attributed to OH groups; bands at 2916-2844 cm^{-1}, attributed to aliphatic C-H stretching; a band at approximately 1728-1713 cm^{-1}, attributed to C=O carbonyl stretching of carboxylic acids; a band at 1630-1615 cm^{-1}, attributed to aromatic C=C stretching; a band at approximately 1522 cm^{-1}, attributed to N-H deformation and C-N stretching; bands at approximately 1443-1415 cm^{-1}, attributed to aliphatic C-H deformation; a band at 1386-1371 cm^{-1}, attributed to aliphatic C-H; a band at 1268-1209 cm^{-1}, attributed to C-O stretching and OH deformation of carboxylic groups; and bands at 1113-1150 cm^{-1} and 1084 - 1041 cm^{-1}, attributed to polysaccharide C-O stretching.

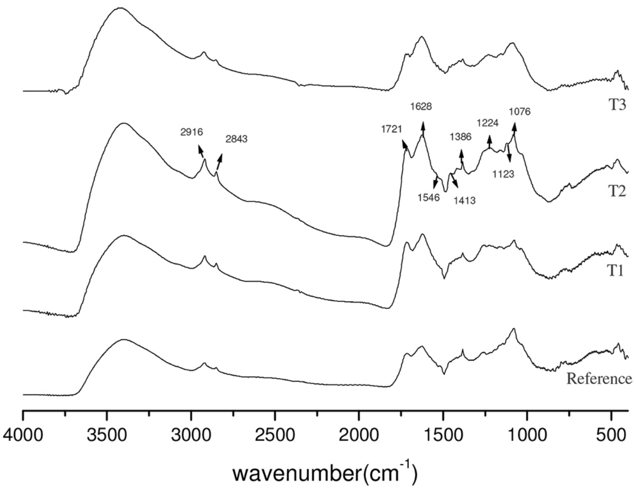

Figure 2. The FTIR spectra of the purified humic acid fraction (HA) from the reference soil, from the soil without compost added (T1), from the soil with non-acidified PS compost added (T2), and from the soil with acidified PS compost added (T3) on a Haplic Acrisol

The relative intensities calculated from the FTIR spectra are given in Table 3. HA from T1 and T2 showed similar values for the relative intensities of the most important functional groups (IR_{2921}, IR_{1726}, IR_{1630}, IR_{1067}), indicating no major alterations of the HA chemical composition due to non-acidified PS compost addition. However, HA from T3 featured lower IR_{1720} and IR_{1067}, higher IR_{1640}, and a higher aromaticity index than HA from T2 (Table 3). These findings indicate that HA from the treatment with added acidified PS compost tended to be less functionalized and more aromatic than that from the treatment with non-acidified PS compost.

These results agree with the data presented in Table 2. The

Table 3. Humic acid (HA) relative intensities and aromaticity index, calculated form the FTIR spectra, for the reference soil and treatments without compost added (T1), with non-acidified PS compost added (T2) and with acidified PS compost added (T3) to a Haplic Acrisol

Treatments	days*	IR_{2921}	IR_{1726}	IR_{1630}	IR_{1475}	IR_{1390}	IR_{1227}	IR_{1164}	IR_{1067}	I_{1630}/I_{2920}
		0-10 cm								
Reference	0	4.6	14.5	16.6	6.4	11.8	10.1	14.7	21.6	3.6
T1	64	6.4	23.3	23.3	6.4	10.6	13.9	n.i**	15.8	3.6
T2		6.9	23.8	23.4	4.0	4.7	9.5	12.08	15.3	3.3
T3		6.1	15.7	25.3	6.1	10.2	10.3	13.6	12.3	4.1

*n.i. not identified.

increase in the hydrophobicity of the humified acid fraction (extractable in 0.5 mol L^{-1} NaOH) may have led to greater formation of HA at the expense of FA. Taking into account that the HA/FA ratio is related to the humification process, our results suggest that the acidified PS compost promotes a higher stabilization degree of the soil humic fractions, in comparison to the non-acidified PS compost. Nevertheless these characteristics did not affect the lettuce productivity as verified by Cantú[24] in a parallel study conducted in the same experiment.

CONCLUSIONS

After 64 days, the single application of PS compost did not affect the soil carbon and nitrogen concentrations but did affect the SOM dynamics, favoring the formation of soluble humic substances (HA and FA) at the expense of the humin fraction.

Adding acidified PS compost promoted the formation of larger humic micelles (humic acids) that had fewer functional groups and were more aromatic than those HA from the treatment with non-acidified PS compost added or the control. These results, combined with the lower proportion of carbohydrate-type structures, indicate the presence of more stable humic micelles following the addition of acidified PS compost within a short time.

ACKNOWLEDGMENTS

The authors wish to thank CNPq and FAPERGS for the scholarships for research productivity, Master of Science and undergraduate courses.

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