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Humic Substances and Chemical Properties of an Acrisol Amended with Vermicomposted Vegetal and Animal Residues

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ABSTRACT: Vermicomposted vegetal and animal residues are rich in nutrients and humic substances and thus can be applied to the soil as a source of functionalized organic matter (OM) and as an alternative to chemical fertilizers. In Southern Brazil, many animal and vegetal residues are easily accessible. Therefore, this study aimed to evaluate alterations in soil chemical properties and OM chemical composition in response to the application of local vermicomposted residues. The vermicomposts of: cattle manure (CM), sheep manure (SM), rice parboiling sludge (PS), CM+PS; SM+PS; CM+food waste (FW); SM+FW; CM+fruit waste (FRW); and SM+FRW were mixed with Acrisol (*Argissolo*) samples collected at 0.00-0.20 m layer, and the samples were incubated in plastic bags for 182 days. Soil samples without vermicomposts were also incubated (control). Overall, vermicomposts increased soil pH(H_2O), exchangeable Ca and available P and K contents. The treatments with CM or SM with FW promoted carbon accumulation in the soil compared to the control, especially as humin. The humic acids of SM and PS were more oxygenated than the others, and oxygenation was apparently associated with aromatic structures. Fulvic acids of CM+PS showed the greatest oxygenation and aliphatic character. The relationship between vermicompost origin (animal or vegetal) and soil properties or OM composition was not clear. Aromaticity degree (H/C) and C/N ratio were similar in fulvic and humic acids, and oxygenation degree (O/C) as well as polarity index [(O+N)/C] were more efficient parameters to distinguish these humic fractions. We concluded that vermicomposts derived from cheap and easily available vegetal and animal residues in Southern Brazil have great potential as alternative fertilizers as well as sources of functionalized OM when applied to the soil, especially vermicomposts of rice parboiling sludge and cattle or sheep manure mixed with food waste.

Keywords: incubation, infrared spectroscopy, elemental analysis, polarity index.

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INTRODUCTION

The improper allocation of solid residues in the environment is known to lead to the contamination of soil and underground water, especially when the residues are not pre-treated (Ceretta et al., 2010; Dahan et al., 2014).

Due to the increasing world population, from near 7.2 billion in 2013 to a projected 9.6 billion people by 2050 (UN DESA, 2013), the production of vegetables and animal-derived products has been substantially increased in order to feed the growing population. Concomitantly, greater amounts of organic residues have been generated. This scenario represents not only a human health safety issue but also an environmental concern, which requires special attention and strategies for its remediation (Santos et al., 2008).

In Southern Brazil, many vegetal and animal-derived residues are available in large amounts and have low cost. Nevertheless, they are usually discarded. The utilization of these products as soil conditioners or fertilizers represents a win-win strategy, mitigating environmental impacts from their incorrect disposal and boosting agriculture on a more sustainable basis. In fact, several studies have reported that organic fertilizers can promote higher crop yields of rice, soybean, wheat, and corn, in comparison to mineral fertilizers (Cassol et al., 2012; Sartor et al., 2012; Novakowiski et al., 2013; Grohskopf et al., 2016; Shahid et al., 2017). These crops are extremely relevant for feeding the global population as they are together responsible for producing nearly 66 % of global agricultural calories (Ray et al., 2013). Additionally, improvement of soil structure, water and nutrient retention, and chemical properties as well as attenuation of the negative effects of heavy metals on plant growth are benefits usually observed in soils amended with organic materials (Fernández-Gómes et al., 2012; Ibrahim et al., 2015; Rong et al., 2016; Erdal and Ekinci, 2017).

The treatment of organic residues before their addition to the soil is crucial to minimize possible negative effects on human, soil, and plant health, such as contamination with pathogenic organisms (fungi, bacteria, viruses, and helminths), nitrogen immobilization due to the high C/N ratio of original residues, and undesired variations in soil pH in response to the residue’s alkalinity or acidity. In this sense, vermicomposting is a low energy-demanding process that stabilizes the pH and lowers the C/N ratio of organic residues in a shorter time than the regular composting method (Castilhos et al., 2008; Cotta et al., 2015). The final product is more stable and nutrient-enriched than the original material, which are crucial properties for maximization of the fertilizing effect of vermicomposts and for optimization of machinery operations when these products are applied in the field (Hashemimajd and Jamaati-Somarin, 2011; Baldotto et al., 2012). Moreover, vermicomposts are rich in humic substances (HS) resulting from the humification of the plant and animal products. These substances are rich in C and functional groups, which play an important role in soil pH buffering and plant nutrition (Dong et al., 2009; Latifah et al., 2018).

Combining quantification and chemical characterization of HS from vermicompost-amended soils allows acquisition of the quality and functionality status of soil organic matter (OM) (Stevenson, 1994). Testing the effect of vermicomposts on soil OM composition and chemical properties permits identification of the most suitable product to be applied to the soil, depending on its edaphic deficiencies.

In this way, vegetal and animal residues easily available in the context of the South region in Brazil were previously subjected to vermicomposting and we aimed to quantify and chemically characterize HS extracted from an Acrisol (*Argissolo*) amended with these products and, moreover, to link these characteristics to changes in soil chemical agronomical properties.

MATERIALS AND METHODS

Soil, treatments, and incubation experiment

Soil sampling was performed at Palma Experimental Station (Federal University of Pelotas - UFPEL), Capão do Leão county, Rio Grande do Sul State, Brazil. The soil is classified as *Argissolo Vermelho-Amarelo* (Santos et al., 2013), Acrisol (IUSS Working Group WRB, 2006). The samples were collected at 0.00-0.20 m soil layer and analyzed according to Tedesco et al. (1995) for main soil properties: pH(H_2O) = 4.7; available phosphorous (P) = 3.7 mg dm^{-3} and potassium (K) = 30 mg dm^{-3}; exchangeable calcium (Ca) = 0.6 $cmol_c$ dm^{-3} and magnesium (Mg) = 0.4 $cmol_c$ dm^{-3}; OM = 1.2 %; and clay = 23 %.

The treatments consisted of vermicomposted residues of: cattle manure (CM); sheep manure (SM); rice parboiling sludge (PS); CM+PS; SM+PS; CM+food waste (FW); SM+FW; CM+fruit waste (FRW); and SM+FRW. Mixture of vermicomposts was performed in 1:1 proportion. The vermicomposts were mixed and incubated with the soil samples, as described below. Additionally, soil samples without vermicompost addition were incubated (control). The manures were collected at Palma Experimental Station, UFPEL, the PS at local rice processing industries, the FW at the UFPEL restaurant, and the FRW at local canning industries.

The vermicomposting was carried out under greenhouse conditions, in 90-L wood boxes (0.5 × 0.6 × 0.3 m) containing between 60 and 70 kg of organic residue/box. The boxes were arranged in a randomized block design with four replicates. Residues were vermicomposted for 131 days. When the temperature of the materials was stabilized, 42 days after the beginning of the vermicomposting process, earthworms (*Eisenia fetida*) were added to the composts at a density of 200 units/box, as recommended by Morselli (2009). The humidity of the composts was kept between 50 and 60 % during vermicomposting, by manual watering. The C/N ratio of the PS vermicompost was near to 6, whereas it was near to 10 in all other vermicomposts at the end of the vermicomposting process (Antunes et al., 2016).

The incubation experiment was performed in a complete randomized design with three replicates. The vermicomposts were manually mixed with 1 kg of 2.4 mm-sieved soil (Acrisol). The mixture was placed into plastic bags (kept closed) and incubated for 182 days. Vermicomposts were added to the soil at a dose corresponding to 45 kg ha^{-1} of N. Table 1 shows the chemical composition of the vermicomposts, and the amount of vermicompost effectively added to each experimental unit (in g kg^{-1} and Mg ha^{-1}) as well as the corresponding doses in kg ha^{-1}. During incubation, the mixtures were aerated weekly and their humidity was kept at 18 g of water per 100 g of soil, which corresponded to 80 % of the water retained in the soil at 0.3 bar pressure.

At the end of the incubation (182 days), the mixtures were dried out at room temperature (25-35 °C) and milled in an agate mortar for chemical analysis and HS extraction. The soil pH(H_2O), pH(SMP), exchangeable Al, Na, Ca, and Mg, and available Fe, Mn, P, and K contents were determined according to Tedesco et al. (1995).

Humic substances extraction, quantification, and purification

The HS were separated based on their solubility in acidic and alkaline mediums, according to Dick et al. (1998). To 20 g of soil+vermicompost mixture, 60 mL of HCl 0.5 mol L^{-1} were added, and the suspension was horizontally shaken for 2 h. The suspension was centrifuged at 2,500 rpm for 15 min, and the acid (HCl) extract (corresponding to the suspended fraction) was collected. This procedure was performed three times, and the final volume of this extract was quantified. Thereafter, 60 mL of NaOH 0.5 mol L^{-1} were added to the fraction remaining in the centrifuge tube, and this suspension was shaken for 3 h followed by centrifugation at 2,500 rpm for 15 min. This process was repeated until

Table 1. Chemical properties of the vermicompost, amount of vermicompost applied to each experimental unit, and the respective amounts of chemical elements applied to each experimental unit

Treatments[1]	Vermicompost's properties						Vermicompost applied		Chemical elements applied to each experimental unit					
	C_{ox}[2]	N	P	K	Ca	Mg			C_{ox}	N	P	K	Ca	Mg
	g kg^{-1}						g kg^{-1}	Mg ha^{-1}	kg ha^{-1}					
Control	-[3]	-	-	-	-	-	-	-	-	-	-	-	-	-
CM	143.4	10.8	4.5	8.6	82.5	1.2	12.2	24.4	3,499	264	110	210	2,013	29.3
SM	174.6	15.0	2.9	20.1	137.4	4.6	8.9	17.8	3,108	268	52	358	2,446	81.9
PS	147.8	25.1	42.0	10.2	263.9	26.0	5.3	10.6	1,567	266	445	108	2,797	275.6
CM+PS	141.9	14.5	16.6	17.7	181.4	4.6	9.1	18.2	2,583	264	302	323	3,301	83.7
SM+PS	157.7	15.4	11.5	20.1	159.4	4.7	8.6	17.2	2,712	264	197	346	2,742	80.8
CM+FW	178.7	16.0	22.0	28.7	280.4	19.6	8.2	16.4	2,931	263	361	471	4,599	321.4
SM+FW	139.3	11.2	3.1	15.6	126.4	1.9	11.9	23.8	3,315	266	75	371	3,008	45.2
CM+FRW	180.1	14.9	6.5	15.8	131.9	2.1	8.9	17.8	3,206	265	116	282	2,348	37.4
SM+FRW	162.5	15.4	3.8	18.3	88.0	1.5	8.6	17.2	2,795	264	66	314	1,514	25.8

[1] Treatments: Control = without vermicompost; CM = cattle manure; SM = sheep manure; PS = rice parboiling sludge; CM+PS = cattle manure + rice parboiling sludge; SM+PS = sheep manure + rice parboiling sludge; CM+FW = cattle manure + food waste; SM+FW = sheep manure + food waste; CM+FRW = cattle manure + fruit waste; and SM+FRW = sheep manure + fruit waste. [2] Tedesco et al. (1995): C_{ox} (oxidizable carbon) = determined by the Walkley-Black method; N (total) = digestion with 2 mL of concentrated H_2SO_4; P and K extracted with HCl (0.05 mol L^{-1}) + H_2SO_4 (0.0125 mol L^{-1}) solution; Ca^{2+} and Mg^{2+} extracted with KCl 1 mol L^{-1} solution. [3] Vermicomposts were not applied to the control treatment.

the supernatant became colorless. After each extraction, the alkaline extract (containing the HS) was deposited into a bottle and its final volume measured. The precipitated material remaining into the centrifuge tube after HS extraction corresponded to the humin (HU) fraction + soil. The HS extract was acidified with HCl solution (4 mol L^{-1}) to pH 2.0 and left to stand for 24 h. Afterwards, this solution was centrifuged in order to obtain the fulvic acid (FA, supernatant) and humic acid (HA, precipitate) fractions. Aliquots of 20 mL of the HCl extract, HS, and FA were collected for C determination. The C content in the fractions: acid extract (C_{HCl}), HS (C_{HS}), and FA (C_{FA}) was determined by spectroscopy, measuring the absorbance at 580 nm after reaction with acidic dichromate solution, with K 1.25 mol L^{-1} for 4 h at 60 °C, using a standard D-anhydroglucose curve of 0 to 250 mg L^{-1} C (Dick et al., 1998).

Soil C content was determined by the Walkley-Black method (Tedesco et al., 1995). Bearing in mind that this method underestimates total soil C since it is not able to oxidize all OM of the soil as is the case for dry combustion (Fernandes et al., 2015), the C content determined by the Walkley-Black method in this study will be considered as corresponding to more easily oxidizable OM and henceforth it will be depicted as oxidizable C (C_{ox}).

The C content in the HA (C_{HA}) and HU (C_{HU}) fractions was calculated as $C_{HA} = C_{HS} - C_{FA}$ and $C_{HU} = C_{ox} - (C_{HCl} + C_{HS})$, respectively.

Purification of HA was carried out as follows: each HA sample was shaken twice with 30 mL of HCl+HF 5 %/5 % (volume/volume) solution. The suspensions were centrifuged, and the supernatants discarded. The precipitated material was lyophilized. Thereafter, it was washed with distilled water until the test with $AgNO_3$ at 10 % for chloride ions was negative. The FA fraction was purified in a granulated adsorption resin of polymethylmethacrylate (XAD-8) in a glass column at pH 2.0. The FA retained in the column was washed with distilled water (volume of water equivalent to the volume of the column) and eluted with NaOH 0.1 mol L^{-1} solution. Elution was ceased when the FA started to be collected, and thereafter the pumping of distilled water into the column was started. The collected solution containing the FA was acidified to pH 1.5 with HCl 1 mol L^{-1} solution, and its volume reduced by drying it in an oven at 60 °C. Finally, the FA extract was placed into dialysis membranes (molecular weight cut-off 1 kDa) for the removal of sodium (Dick et al., 1998). Considering that FA molecules can be <1 kDa (Stevenson, 1994) and thus could pass through the dialysis membrane used, henceforth the FA fraction thereby separated will be depicted as FA >1 kDa. The purified HA and FA (>1 kDa) samples were dried in a vacuum oven at 60 °C for 24 h. The three replicates of HA or FA were blended to form composite samples which were used for Fourier-transform infrared spectroscopy (FTIR) and elemental analyses. The C, hydrogen (H), and N contents were determined by dry combustion (Perkin Elmer 2400). The ash content was determined gravimetrically after calcination at 750 °C for 24 h (Dick et al., 1998). After ash analysis, the oxygen (O) content was determined by difference. The C/N ratio, H/C, and O/C atomic ratios and the polarity index [(O+N)/C] were calculated based on the molar proportions (Torrents et al., 1997).

FTIR spectroscopy

The purified HA and FA (>1 kDa) composite samples were blended with potassium bromide (KBr) in the proportion 1:100 (sample:KBr) and pressed into pellets. The pellets were subjected to FTIR analysis, using Shimadzu FTIR 8300 equipment, in the range of 4,000-750 cm^{-1}, resolution of 4 cm^{-1} and 21 scans min^{-1}. The FTIR spectra interpretation and assignment of the absorption bands were performed according to Dick et al. (2003, 2006, 2008), Dias et al. (2009), Moraes et al. (2011), and Tan (2014).

Statistical analysis

Because the soil chemical properties data pH(H_2O), pH(SMP), C_{ox}, exchangeable Al, Ca, Mg, and Na, and available Fe, Mn, P and K contents could be correlated, multivariate analysis of variance (MANOVA) was performed. When treatment effect was significant, *post-hoc* Tukey test at 5 % probability with Bonferroni correction was used to compare means of treatments and to avoid an increase in Type I error due to successive univariate tests.

The C content data for OM fractions were submitted to analysis of variance (ANOVA) and analyzed in a linear mixed model where the fractions were considered repeated measures since C_{HCl}, C_{HA}, C_{FA}, and C_{HU} contents are components of C_{ox} content. Treatment means were compared for each OM fraction (C_{HCl}, C_{HA}, C_{FA}, and C_{HU}) by Tukey test at 5 % probability when the interaction treatment*fraction was significant.

All statistical analyses were performed using R software (R Development Core Team, 2018).

RESULTS AND DISCUSSION

Soil chemical properties and C_{ox} content

The four MANOVA tests (Wilk's, Roy's, Hotelling, and Pillai) indicated a significant effect of treatments on the soil chemical properties and C_{ox} data set (Table 2).

All treatments, except PS, significantly increased the soil pH(H_2O) in comparison to the control, on average by 0.5 pH units. The highest pH(H_2O) value was observed for CM+FW (5.6), which did not differ from SM+FW (5.5) and CM (5.4) (Table 3). The increase in soil pH(H_2O) after addition of the vermicomposts can be assigned to the pH(H_2O) of the original residues used in the vermicomposting process, which varied from 6.1 (FW) to 8.6 (SM), as shown in a previous work (Antunes et al., 2015). The alkaline properties of vermicomposts with SM, CM, or PS can be attributed to the $CaCO_3$ (added to the diet in the case of SM and CM) contained in these residues, which may dissociate in the soil leading to pH increase, as suggested by Eghball (2002), Lourenzi et al. (2011), and Vieira et al. (2011) who reported an increase of pH after the application of cattle manure, pig slurry, and rice parboiling sludge, respectively, to acidic soils. Plants eventually grown in the Acrisol of the present study could benefit from lower Al toxicity as a result of the liming effect of the vermicomposts and the complexation of Al by organic molecules (Wu et al., 2014). Plants could also take advantage of greater P availability due to the

Table 2. Multivariate analysis of variance of soil chemical property data from all treatments

Criterion	Test	F	Pr (> F)
Wilk's lambda	$4.7319e^{-08}$	10.394	$< 2.2e^{-16***}$
Pillai's trace	4.9245	2.2958	$1.595e^{-06***}$
Lawley–Hotelling	945.5	96.884	$< 2.2e^{-16***}$
Roy's largest root	823.42	1564.5	$< 2.2e^{-16***}$

*** Significant at 0.01 %.

Table 3. Chemical properties of an Acrisol amended with vermicomposts and incubated for 182 days

Treatments[1]	pH		C_{ox}	Al^{3+}	Ca^{2+}	Mg^{2+}	Fe	Mn	P	K	Na
	H_2O	SMP									
			mg g^{-1}	cmol$_c$ dm^{-3}			mg dm^{-3}				
Control	4.8 g	6.7 ab	5.1 b	0.2^{ns}	0.7 c	0.6^{ns}	0.1^{ns}	26.3 ab	5.70 e	40.0 g	15.7 e
CM	5.4 abc	6.8 ab	5.8 ab	0.1	1.4 ab	1.4	0.1	11.0 c	32.9 d	145.3 bc	25.0 cd
SM	5.2 def	6.7 ab	5.7 ab	0.2	1.1 bc	1.1	0.1	11.7 c	24.1 de	103.7 e	20.3 de
PS	5.0 fg	6.6 b	5.4 ab	0.2	1.6 a	1.3	0.1	37.0 a	105.4 a	73.0 f	35.3 a
CM+PS	5.3 bcd	6.8 ab	5.6 ab	0.2	1.7 a	1.3	0.1	16.7 bc	72.3 b	127.7 d	32.0 ab
SM+PS	5.3 bcde	6.7 ab	5.3 ab	0.1	1.3 ab	1.2	0.1	16.7 bc	54.5 bc	131.0 d	27.3 bc
CM+FW	5.6 a	6.8 ab	5.8 a	0.1	1.7 a	1.2	0.1	8.70 c	69.9 b	165.7 a	30.7 ab
SM+FW	5.5 ab	6.9 a	6.0 a	0.1	1.3 ab	1.2	0.1	10.7 c	34.6 cd	153.0 b	23.7 cd
CM+FRW	5.1 ef	6.7 ab	5.5 ab	0.2	1.0 bc	1.4	0.1	14.3 c	26.4 de	136.7 cd	27.3 bc
SM+FRW	5.2 cde	6.8 ab	5.8 ab	0.1	1.2 b	0.9	0.1	12.7 c	32.9 d	112.7 e	27.0 bc

[1] Treatments: Control = without vermicompost; CM = cattle manure; SM = sheep manure; PS = rice parboiling sludge; CM+PS = cattle manure + rice parboiling sludge; SM+PS = sheep manure + rice parboiling sludge; CM+FW = cattle manure + food waste; SM+FW = sheep manure + food waste; CM+FRW = cattle manure + fruit waste; and SM+FRW = sheep manure + fruit waste. Mixture of vermicomposts was performed in 1:1 proportion. Tedesco et al. (1995): pH(H_2O) = proportion of 1:1 of soil:water; pH(SMP) = 5 mL of SMP solution added to the proportion of 1:1 of soil:water; C_{ox} (oxidizable carbon) = determined by the Walkley-Black method; Al^{3+}, Ca^{2+}, Mg^{2+}, and Mn extracted with KCl 1 mol L^{-1} solution; Fe = extracted with ammonium oxalate ($C_2H_8N_2O_4$) 0.2 mol L^{-1} solution; P, K, and Na extracted with HCl (0.05 mol L^{-1}) + H_2SO_4 (0.0125 mol L^{-1}) solution. Means followed by the same letter in a column do not differ statistically according to Tukey's test at 5 % probability. ns = not significant.

increase of soil pH resulting from vermicompost amendment (Lourenzi et al., 2014). Additionally, besides the significant increase in bases content (Mg, Ca, K, and Na) in the soil observed for most of the treatments compared to the control (Table 3), the increase in soil pH(H_2O) after vermicompost addition can be also attributed to a coupled effect resulting from the vermicomposting process: i) secretion of NH_4^+ ions reducing the H^+ pool; and ii) increment of $CaCO_3$ in the vermicomposts promoted by the fixation of CO_2 as $CaCO_3$ due to the activity of calciferous glands of earthworms that catalyze this process, hindering pH decrease, as indicated by Pattnaik and Reddy (2010) who observed a gradual increase in pH from substrate to compost to vermicompost of same materials. The pH(SMP) ranged from 6.6 (PS) to 6.9 (SM+FW) (Table 3), and, for practical aspects, the significant differences observed between treatments are not relevant.

In general, the vermicomposts increased exchangeable Ca and Na and available P and K contents in comparison to the control (Table 3), indicating that these products can potentially be used as soil fertilizers. These data corroborate those of Yagi et al. (2003), who observed an increase in available P and K and exchangeable Ca when cattle manure vermicompost was applied to a Typic Hapludox. Furthermore, the authors observed a linear increase of nutrient content in the soil in response to increasing doses of vermicompost. During vermicomposting, organic materials undergo physical decomposition and biochemical changes due to enzymatic and enteric microbial processing in the worm's gut, increasing nutrient availability and absorbability, as reported by Pattnaik and Reddy (2010) for N, P and K.

According to CQFS-RS/SC (2016), the available P content in the control (5.7 mg dm^{-3}) was considered i) very low: for moderate P-demanding and high P-demanding plant species; and ii) low: for low P-demanding plant species. Interestingly, the available P content in the treatments with vermicompost amendment ranged from 24.1 to 105.4 mg dm^{-3} (Table 3), and therefore was classified as "high" or "very high", depending on the plant species P demand. Special attention is given to PS, which increased the available P content by 19-fold compared to the control. The P content in the PS vermicompost (before its application to the soil) was, on average, 666 % higher than that observed in the other vermicomposts, which may explain such results. High P content in PS residues can be attributed to residues of fertilizers and crop protection products, to the leaching of P-containing compounds to the waterlogging water, and to the hydrolysis of phytin during parboiling, which releases P as phosphate (Faria et al., 2006; Pinto, 2009). These data are in line with those of Vieira et al. (2011) who observed that applying a dose of PS residue equivalent to 25 % of the N demand of corn to the soil (Acrisol) increased the available P content by 270 % compared to the control (without fertilization). According to the authors, the high P content of the PS residue (42.4 g kg^{-1}) contributed to these results. Adjusting the P content of the control and the doses of PS application of that study to the values of our study, that increase (25-fold) would be close to the increase observed in our study, 19-fold. Together with the P content of the vermicomposts, the higher pH(H_2O) of some treatments with vermicomposts compared to the control may have increased P availability.

Similar to the available P content, the exchangeable K content in the control (40 mg dm^{-3}) (Table 3) was classified as "low". With the application of SM, PS or SM+FRW, this classification was shifted to "high" (CQFS-RS/SC, 2004). When CM, CM+PS, SM+PS, CM+FW, SM+FW or CM+FRW were applied, the K content in the soil was shifted from "low" (control) to "very high" (CQFS-RS/SC, 2004). The highest K content was observed for the CM+FW treatment, which was 4-fold higher than that of the control (Table 3). This result is explained by the higher K content observed in the CM+FW vermicompost compared to the other vermicomposts (Table 1). Based on these results, adequate supply of both P and K to plants could be expected with the utilization of the vermicomposts tested in this study, highlighting the great potential of these products as alternative fertilizers. In fact, in a previous study by Antunes et al. (2016), it was observed that CM+FW vermicompost promoted higher shoot biomass of *Acacia mearnsii* in comparison to the control and performed similarly to mineral fertilizers. Additionally, the authors observed that CM+FW and mineral fertilizer promoted similar N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu content in the shoots of *A. mearnsii*, a species of great economic importance in Southern Brazil (Farias et al., 2008).

Even though vermicomposts did not promote a significant increase in the exchangeable Mg content when compared to the control (Table 3), all the vermicomposts, except SM+FRW, shifted Mg content in the soil from "medium" (control) to "high" (CQFS-RS/SC, 2016).

The highest available Mn content in the soil was observed for the PS treatment (37.0 mg dm^{-3}), which did not differ from the control (Table 3). All other treatments presented lower available Mn content in comparison to the control, on average 51 % lower. This decrease is probably associated with the increase of soil pH(H_2O) in response to application of the vermicomposts, as previously discussed, and to the complexation of Mn by organic molecules. Nevertheless, the Mn content in the soil was considered "high" (>5 mg dm^{-3}) for all treatments according to CQFS-RS/SC (2016).

The vermicomposts increased the exchangeable Ca content in the soil by between 42 and 143 % in comparison to the control. However, the Ca content status in the soil was still classified as "low" (<2 mg dm^{-3}) for all treatments (CQFS-RS/SC, 2016).

The C_{ox} content ranged from 5.1 to 6.0 mg g^{-1} soil (Table 3). Only CM+FW and SM+FW treatments significantly increased C_{ox} content compared to the control (Table 3), on average by 16 %. The C_{ox} content for all other treatments did not differ either from CM+FW and SM+FW or from the control (Table 3). No significant correlation was observed between the C content in the vermicomposts and the C_{ox} of the soil (data not shown), suggesting that the vermicomposts may have suffered different decomposition rates during incubation, probably due to the nutrient content and OM composition of each vermicompost facilitating or hindering the activity of microorganisms.

Carbon distribution in organic matter fractions

The interaction treatment*fraction was significant at 0.1 % according to ANOVA ($p < 2.2e^{-16}$, data not shown), and therefore treatment means were compared for each OM fraction.

In general, the C_{HCl} content varied slightly, from 0.20 to 0.30 mg g^{-1} soil, and differences between treatments were not observed (Table 4). The C_{HCl} fraction is composed of labile organic compounds, mainly from microbial activity and root exudates, which have weak association with soil minerals and with each other, and therefore are easily decomposable (Stevenson, 1994; Leal et al., 2015). In fact, the C_{HCl} fraction represented only 4-5 % of the soil C_{ox} (Figure 1). Most probably, vermicomposting of the organic materials for 131 days followed by incubation of the vermicomposts with soil for 182 days led to the decomposition of these labile compounds, and thus differences between treatments were not observed.

The C_{FA} content in the soil amended with vermicomposts ranged from 0.93 to 1.12 mg g^{-1} soil and did not differ from that observed in the control (0.92 mg g^{-1} soil). The contribution of C_{FA} to C_{ox} varied from 17 to 20 % (Figure 1). In a previous work, Antunes et al. (2015) investigated the C_{FA} content in the final vermicomposts alone (not mixed) and verified that FRW showed the greatest content. Differences in C_{FA} between CM, SM, PS, and FW were not observed by the authors. In our study, the soil C_{FA} content of the treatments did not differ, apparently reflecting the similarity of the vermicomposts regarding C_{FA}, even after their incubation with soil. Unlike for C_{FA}, the vermicomposts affected the C_{HA} content in the soil. The highest C_{HA} content was observed for CM (1.34 mg g^{-1}) and SM+FW (1.32 mg g^{-1}) treatments, while the lowest C_{HA} content was observed for CM+FW (0.84 mg g^{-1}) and SM+PS (0.93 mg g^{-1}) (Table 4). The control, PS, SM, and CM+FRW treatments did not differ either from the treatments with the highest or from

Table 4. Carbon content in the acid extract (C_{HCl}), as fulvic acid (C_{FA}), humic acid (C_{HA}), and humin (C_{HU}), of an Acrisol after 182 days of incubation with different vermicomposts

Treatments[1]	C_{HCl}	C_{FA}	C_{HA}	C_{HU}
	mg g^{-1}			
Control	0.20^{ns}	0.92^{ns}	1.14 abc	2.78 e
CM	0.25	1.11	1.34 a	3.07 cde
SM	0.26	1.12	1.15 abc	3.14 cd
PS	0.29	1.10	1.05 abc	2.95 de
CM+PS	0.30	1.06	1.00 bc	3.27 bc
SM+PS	0.28	1.02	0.93 c	3.11 cd
CM+FW	0.24	0.97	0.84 c	3.76 a
SM+FW	0.23	0.93	1.32 a	3.53 ab
CM+FRW	0.23	0.99	1.07 abc	3.19 cd
SM+FRW	0.24	0.97	1.26 ab	3.33 bc

(1) Treatments: control = without vermicompost; CM = cattle manure; SM = sheep manure; PS = rice parboiling sludge; CM+PS = cattle manure + rice parboiling sludge; SM+PS = sheep manure + rice parboiling sludge; CM+FW = cattle manure + food waste; SM+FW = sheep manure + food waste; CM+FRW = cattle manure + fruit waste; and SM+FRW = sheep manure + fruit waste. Mixture of vermicomposts was performed in 1:1 proportion. ns = not significant. Means followed by the same letter in a column do not differ statistically according to Tukey's test at 5 % probability.

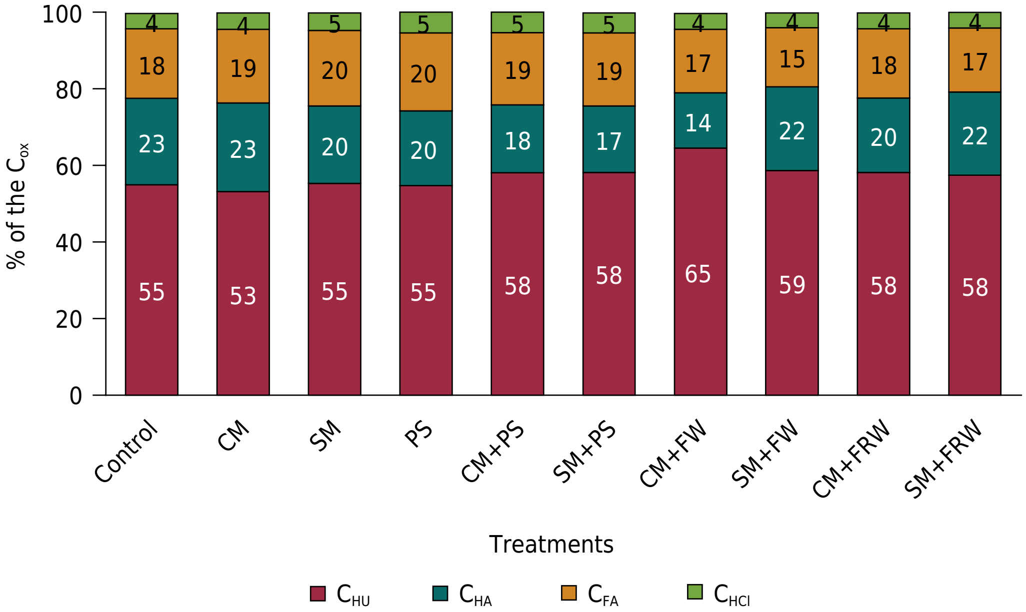

Figure 1. Contribution (%) of the carbon content as humin (C_{HU}), humic acid (C_{HA}), fulvic acid (C_{FA}), and acid extract (C_{HCl}) to the oxidizable carbon (C_{ox}) content of an Acrisol amended with vermicomposts. (1) Treatments: Control = without vermicompost; CM = cattle manure; SM = sheep manure; PS = rice parboiling sludge; CM+PS = cattle manure + rice parboiling sludge; SM+PS = sheep manure + rice parboiling sludge; CM+FW = cattle manure + food waste; SM+FW = sheep manure + food waste; CM+FRW = cattle manure + fruit waste; and SM+FRW = sheep manure + fruit waste. Mixture of vermicomposts was performed in 1:1 proportion.

the treatments with the lowest C_{HA} contents (Table 4). It seems that the origin (vegetal or animal) of the vermicompost did not influence remarkably the C_{HA} content in the soil. In general, the C_{HA} content for treatments with vermicomposts did not differ from that of the control (1.14 mg g^{-1}). The contribution of C_{HA} to C_{ox} ranged from 14 to 23 % and was comparable to that of C_{FA} (Figure 1).

The highest C_{HU} content was observed for CM+FW (3.76 mg g^{-1} soil), which was 34 % higher than that of the control (2.78 mg g^{-1}) (Table 4). In general, vermicomposts with FW (CM+FW and SM+FW) led to higher C_{HU} content in the soil compared to the control. The C_{HU} content in CM and PS did not differ from that of the control (Table 4). The HU fraction contributed to the majority (55-65 %) of C_{ox} (Figure 1). The chemical recalcitrance of this fraction and its interaction with soil minerals probably led to the accumulation of C as HU, either for the control treatment or in the soil amended with vermicomposts.

Elemental composition and FTIR analysis of humic and fulvic acids

In general, the elemental composition (Table 5) and FTIR spectra of HA and FA (Figure 2) for the control treatment were similar to those noticed for the treatments with vermicompost amendment. However, differences between the various treatments were perceptible. That said, clear distinction of treatment effects on OM composition resulting from vermicompost origin (animal or vegetal) was not evidenced. These data corroborate the lack of or slight differences between treatments with respect to C_{ox} and C content in OM fractions (Table 4).

The elemental composition, ash %, C/N ratio, H/C and O/C atomic ratios, and the polarity index [(O+N)/C] of HA and FA are reported in table 5. The C, H, N, and O % values of HA (C = 45.7-54.5 %, H = 4.7-5.9 %, N = 4.1-5.0 %, O = 34.7-45.6 %) and FA (C = 27.0-44.2 %, H = 3.7-4.6 %, N = 2.5-4.4 %, O = 46.8-66.6 %) are in agreement with values commonly observed for HS extracted from soil (Dick et al., 1998; Canellas et al., 2001; Dick et al., 2003; Tan, 2014; Lüdtke et al., 2016).

The HA of the CM+PS treatment showed the greatest C and N % values, higher H % compared to all treatments except CM+FRW, and the lowest O %, suggesting a less oxidized HA (Moraes et al., 2011). In fact, the HA of CM+FRW presented the lowest O/C atomic ratio (0.48) and polarity index (0.56), indicating the lower oxygenation degree of this HA in comparison to the HA of the other treatments. Moreover, these results indicate that the HA of CM+FRW was poorer in functional groups (carboxylic or phenolic), suggesting that the humic acids of the other treatments have greater potential to generate soil OM exchange sites (Dick et al., 2003), contributing to improve soil chemical agronomical properties, an effect particularly important in low-OM content soils, as in the case of this study. In this sense, the HA of SM showed the lowest H/C and highest O/C atomic ratios, as well as the highest polarity index (0.83) (Table 5) in comparison to the rest of the treatments, indicating the presence of aromatic domains with polar functional groups in the HA of SM (Chen et al., 2011; Moraes et al., 2011). Similar behavior was verified for PS.

The HA C/N ratios ranged between 10.2 and 12.5 (Table 5), indicating well-developed HA (Tan, 2014). According to the author, during the decomposition process, the C/N ratio tends to decline due to C loss as CO_2 and to the incorporation of N in humic molecules. In the present study, the OM decomposition process during the incubation experiment and, moreover, the previous vermicomposting of the materials may have contributed to these results.

Table 5. Elemental composition (C, H, N, O), ash content (Ash), C/N ratio, H/C, and O/C atomic ratios and polarity index [(O+N)/C] of humic acids (HA) and fulvic acids (>1 kDa) (FA) of an Acrisol amended with vermicomposts and incubated for 182 days

Treatments[1]	C	H	N	O	Ash	C/N	H/C	O/C	(O+N)/C
	%								
					HA				
Control	49.6	5.54	4.55	40.3	6.60	10.9	1.34	0.61	0.69
CM	52.0	5.14	4.17	38.7	18.5	12.5	1.19	0.56	0.63
SM	45.4	4.90	4.12	45.6	0.00	11.0	0.77	0.75	0.83
PS	45.9	4.69	4.35	45.0	4.26	10.6	1.23	0.74	0.82
CM+PS	54.5	5.79	5.01	34.7	10.5	10.9	1.27	0.48	0.56
SM+PS	48.7	5.07	4.52	41.7	0.00	10.8	1.25	0.64	0.72
CM+FW	48.7	5.38	4.71	41.2	0.00	10.3	1.33	0.63	0.72
SM+FW	45.7	5.12	4.21	44.9	2.51	10.9	1.34	0.74	0.82
CM+FRW	50.3	5.90	4.94	38.9	1.71	10.2	1.41	0.58	0.66
SM+FRW	48.3	5.30	4.62	41.7	0.00	10.5	1.32	0.65	0.73
					FA				
Control	31.5	3.75	2.64	62.1	11.6	11.9	1.43	1.48	1.55
CM	37.0	3.96	3.41	55.6	20.8	10.9	1.28	1.13	1.20
SM	44.2	4.55	4.42	46.8	18.6	10.0	0.81	0.79	0.88
PS	43.2	4.49	4.13	48.2	22.6	10.5	1.25	0.84	0.92
CM+PS	27.0	3.87	2.48	66.6	23.3	10.9	1.72	1.85	1.93
SM+PS	37.0	3.95	3.32	55.7	20.0	11.2	1.28	1.13	1.20
CM+FW	37.8	4.13	3.72	54.3	21.6	10.2	1.31	1.08	1.16
SM+FW	31.5	3.85	2.90	61.7	6.36	10.9	1.46	1.47	1.55
CM+FRW	31.6	3.68	2.98	61.7	4.76	10.6	1.40	1.46	1.55
SM+FRW	30.8	3.72	2.78	62.7	5.41	11.1	1.45	1.53	1.61

(1) Treatments: Control = without vermicompost; CM = cattle manure; SM = sheep manure; PS = rice parboiling sludge; CM+PS = cattle manure + rice parboiling sludge; SM+PS = sheep manure + rice parboiling sludge; CM+FW = cattle manure + food waste; SM+FW = sheep manure + food waste; CM+FRW = cattle manure + fruit waste; and SM+FRW = sheep manure + fruit waste. Mixture of vermicomposts was performed in 1:1 proportion.

The largest contrast in FA elemental composition was noticed between SM and CM+PS. The SM showed the greatest C, N, and H % and lowest O %, while CM+PS presented the lowest C and H % and the greatest O % of all treatments (Table 5). These data indicate greater aromaticity and lower oxygenation of FA in SM. In fact, SM showed the lowest H/C (0.81) and O/C (0.79) atomic ratios and polarity index (0.88) of all treatments (Table 5). Opposite H/C, O/C, and polarity index values, 1.72, 1.85, and 1.93, respectively, were noticed for CM+PS, suggesting greater oxygenation and aliphatic character of its FA. The weak peak at 1,710 cm^{-1} and the strong peak at 1,109 cm^{-1} in the FA FTIR spectrum of CM+PS corroborate these data, suggesting that FA oxygenation is preferentially associated with aliphatic rather than with aromatic structures in this treatment. Although the peak at 1,109 cm^{-1} could also be attributed to impurities in the samples, as discussed below, it is less relevant in this case, since the FA of SM and CM+PS had comparable ash content, 19 and 23 %, respectively (Table 5).

In the present study, the O/C atomic ratio and the polarity index differentiated more clearly HA from FA samples compared to the H/C atomic ratio and to the C/N ratio. On average, the O/C atomic ratio and polarity index values in FA were 2-fold higher than in HA. This can be attributed to the greater oxygenation of FA compared to HA due to carboxylic groups, either associated with aromatic structures as indicated by the more pronounced peak at 1,710 cm^{-1} in FA (mainly in SM+FRW, CM+FRW, SM+FW, and SM+PS spectra) or with aliphatic structures, since the H/C atomic ratio was similar in FA and HA. The greater O/C atomic ratio of FA compared to HA has previously been reported in the literature, mostly attributed to the higher contribution of functional groups (carboxylic or phenolic) generally associated with lower aromaticity (higher H/C atomic ratio) in FA compared to HA molecules (Schiavo et al., 2007; Moraes et al., 2011; Coelho et al., 2013).

The FTIR spectra of HA and FA fractions exhibited a similar shape (Figure 2) and are in accordance with the FTIR spectra of HS extracted from Brazilian soils, either under natural conditions or subjected to agricultural management (eventually receiving external organic amendments), as reported by authors applying HS extraction and purification procedures similar to that used in the present work (Dick et al., 1999; Dias et al., 2009; Moraes et al., 2011; Santana et al., 2011; Coelho et al., 2013).

The main absorption bands in the FTIR spectra of HA (Figure 2a) and their respective assignments were: a band at 3,410 cm^{-1} attributed to the stretching of O-H; bands at 2,920 and 2,850 cm^{-1} due to aliphatic C-H stretching (CH_3 and CH_2), which is confirmed by the absorption band at 1450 cm^{-1} (aliphatic C–H); a shoulder at 1,710 cm^{-1} that can be attributed to -C=O stretching of -COOH groups of humified OM conjugated with more condensed structures compared to fresh OM, when this absorption band would appear at a higher wave number (Dick et al., 2006); followed by an absorption band at 1,640 cm^{-1} due to stretching of C=O of COO^-, and stretching of aromatic C=C; a band at 1,540 cm^{-1} assigned to N-H deformation and stretching of C=N; a band at 1,380 cm^{-1} assigned to deformation of O-H and stretching of C-O of phenol OH, deformation of CH_2 and CH_3, and COO^- in asymmetric stretching; an absorption band at 1,260 cm^{-1} attributed to stretching of C-O of carboxylates, O-H deformation of COOH and stretching of N-O of nitrates; and absorption bands at 1,085 and 1,030 cm^{-1} assigned to stretching of C-O of polysaccharides, phenols or aliphatic groups. Overall, the soil HA spectra of the treatments were similar, and only slight differences in some absorption peaks were identified. In the HA spectra of PS and SM, the absorption bands at 1,710 and 1,640 cm^{-1} were more pronounced compared to the rest of the HA spectra (Figure 2a), corroborating H/C and O/C atomic ratios and polarity index data previously discussed, which indicate the presence of aromatic domains with polar functional groups in SM mainly, and in PS as well.

The absorption bands observed in the FA spectra (Figure 2b) were 3,410, 2,920, 2,850, and 1,710 cm^{-1}, and bands near to 1,640 and 1,380 cm^{-1} as reported for HA spectra

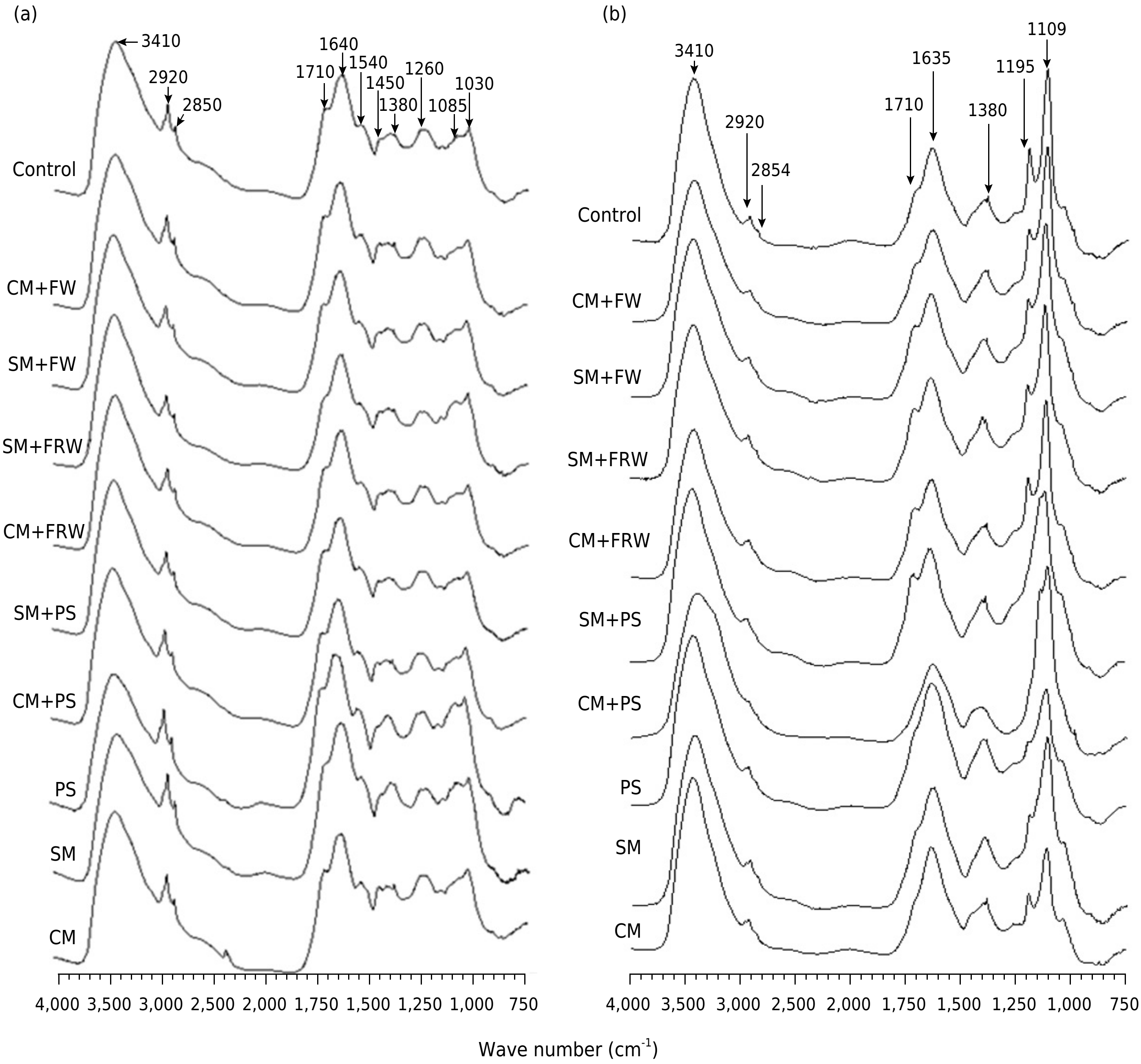

Figure 2. Fourier-transform infrared spectroscopy of humic acids (a) and fulvic acids (>1 kDa) (b) extracted from an Acrisol amended with vermicomposts. Control = without vermicompost; CM = cattle manure; SM = sheep manure; PS = rice parboiling sludge; CM+PS = cattle manure + rice parboiling sludge; SM+PS = sheep manure + rice parboiling sludge; CM+FW = cattle manure + food waste; SM+FW = sheep manure + food waste; CM+FRW = cattle manure + fruit waste; and SM+FRW = sheep manure + fruit waste. Mixture of vermicomposts was performed in 1:1 proportion.

(Figure 2a) and yet: there were absorption bands at 1,195 and 1,109 cm^{-1}, that can be assigned to C-O stretching of polysaccharides or even to impurities from silicate, as suggested by the high ash content observed in the FA samples, ranging from 4.8 to 23 % (Table 5). Such band attribution is also supported by the low intensity of aliphatic CH bands at 2,920 and 2,850 cm^{-1}, which would be more evident in the case of preferential assignment of these bands to polysaccharides. Similar to the HA spectra, the FA spectra of the treatments showed the same pattern, differing only with respect to some absorption bands, particularly at 1,710 cm^{-1} (Figure 2b). In the FA spectra of SM+FRW, CM+FRW, SM+FW, and SM+PS, this peak was more pronounced, suggesting that the greater oxygenation of FA in these treatments compared to most of the others, as corroborated by the O/C atomic ratio data (Table 5), probably occurred, at least partially, as COOH groups conjugated to more condensed structures.

The differences between the FA and HA spectra obtained in this study are in line with those obtained by Tan (2014). Compiling a range of soil HS FTIR spectra, the author reported that, in contrast to FA, HA spectra usually have stronger absorption bands in the regions of 2920, 2980, 1720, and 1,650 cm^{-1}, and lack a strong band at 1,000 cm^{-1}, which is ordinarily associated with impurities when present in HA spectra. Thus, since the HA spectra of all treatments lacked a peak at 1,000 cm^{-1}, it can be assumed that the purification of HA with HCl-HF was efficient. However, HA showed a broader absorption band at 3,410 cm^{-1} compared to FA, indicating a more acid material analyzed due to H bonded to OH groups (Dick et al., 2003; Dias et al., 2009). The precipitation and purification of HA in acid solutions may explain these observations.

CONCLUSIONS

In general, the vermicomposts improved important soil agronomical properties, mainly soil pH(H_2O), available P and K, and exchangeable Ca contents, compared to the soil without vermicompost.

Vermicomposts of cattle or sheep manure mixed with vermicompost from food waste promoted carbon accumulation in the soil, especially as humin.

The humic acids of SM and SP were more oxygenated than the other humic acids, with polar functional groups being apparently associated with aromatic structures. Contrarily, the greatest oxygenation degree of fulvic acids, observed in CM+SP, seems to be preferentially associated with aliphatic structures.

Degree of aromaticity (H/C) was similar in humic and fulvic acids, as was the C/N ratio. The O/C atomic ratio and polarity index were better indicators to distinguish humic and fulvic acids, with values being, on average, about 2-fold greater in the fulvic fraction.

Effects on soil chemical properties, soil humic acids, and humin content as well as on soil fulvic and humic acid chemical composition depending on vermicompost origin (animal or vegetal) were not clear.

Overall, we conclude that the local and easily available organic residues tested in our study, after vermicomposting, have great potential as alternative fertilizers as well as sources of functionalized organic matter when applied to the soil, especially vermicomposts of rice parboiling sludge and cattle or sheep manure mixed with vermicompost from food waste. Alternatively, these products could be used to accelerate soil quality recovery in degraded areas.

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