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# Quantification of the Carbon Content of the Fractions of Humic Substances and Total Organic Carbon in Different Production Systems

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**Abstract**

Soil organic matter is degraded and easily altered by the type of management. The objective of this work is to determine the total organic carbon and humic substance fractions in the organic matter of the soil with different management types and depths in the western region of Paraná, Brazil. The work was carried out in the Experimental Farm "Professor Antônio Carlos dos Santos Pessoa", belonging to the State University of the West of Paraná. Five soil management systems were evaluated: one area with corn cultivation for silage (CS); other area with succession of crops, with soybean in summer and corn in winter (SC); the next area also with succession of crops, with soy in the summer and oat in the winter (SO); the following area with permanent pasture with Tifton (PP); and the last area with crop-livestock integration (ILC). For each management system, four plots were randomly selected, in each plot three simple samples were collected in a diagonal direction to form a composite sample for the depth of 0.00-0.05 m, 0.05-0.10 m and 0.10-0.15 m. Total organic carbon, fractionation of the humic substances and the AH/AF and EA/HUM ratios were calculated. For most of the analyzed variables, it was verified that there were significant differences ($P < 0.05$) between the systems evaluated in the studied depths. In the evaluated areas, the PP, SO and ILC systems presented the highest carbon content for all attributes analyzed.

**Keywords:** recalcitrant fraction, soil management organic matter

## 1. Introduction

The search for alternatives to increase crop productivity in recent years has prompted the agricultural sector to seek alternatives to produce more sustainably and thus preserve soil, water and environmental resources (Loss et al., 2009). The soil being a natural resource in constant transformation, the agricultural activity can promote processes of degradation and loss of physical, chemical and biological quality, when adequate management and conservation practices are not considered (Chaer & Tótola, 2009).

Some systems have been developed in response to this issue, with the common objective of providing a more sustainable management of the soil, as in the case of the no-tillage system (SPD), which advocates the constant maintenance of mulch on the soil, among other objectives; and integrated systems, such as crop-livestock integration (ILC), which combines annual crops and pasture in the same area (Andrioli et al., 2008).

Among the advantages of using these management systems, in relation to the soil factor, we can mention the better soil protection against weathering, especially erosion; maintenance of soil moisture, improvement of soil structural and nutritional characteristics and increase the microbiological activity of the soil (Andrade et al., 2009; Loss et al., 2011). These improvements are related to the contribution of organic matter, which is an attribute that indicates the quality of the soil, directly affecting the structural part and the chemical dynamics of the essential elements (Carneiro et al., 2009).

The organic matter of the soil has in its composition living and non-living beings, the living fraction corresponding to the roots of plants and soil organisms, constituting approximately 4% of the total. The non-living components of organic matter are represented by decomposing plant residues, humid and non-humidified substances. In general, the soil organic matter content can vary from 0.5 to 5% in the mineral horizons in most of the soils (Primo et al., 2011).

The soil organic matter is altered by the type of management, but also the crop species and environmental factors, such as temperature, light and humidity. Organic matter in turn is present in the soil in the form of unmethylated acidic compounds and humified macromolecules (humic substances). The humic substances correspond to 85 to 90% of the soil organic matter and are responsible for most of the cation-exchange capacity (CEC) of the soil of organic origin, being the main reasons that make the organic matter fraction of soil the main focus in studies and researches (Barreto, 2008).

It is clear, therefore, that the systems that recommend the maintenance of straw and the use of fodder tend to promote increase of the total soil organic carbon, as well as the proportion of humic substances that improve the quality of the soil. Based on this, the objective of this work is to determine the total organic carbon and humic substance fractions of the soil organic matter with different soil management types and in different depths in the western region of Paraná, Brazil.

## 2. Material and Methods

Soil samples were collected in commercially operated fields with different soil management systems, in place different lengths of time, and conducted on rural properties in the municipality of Marechal Candido Rondon, Western Paraná, Brazil. The climate of the region, according to the classification of Köppen, is Cfa type, subtropical humid, subtropical of dry winter, with rains well distributed throughout the year and hot summers, average annual temperature between 22 to 23 ºC and total precipitation of 1600 to 1800 millimeters (Caviglione et al., 2000). Due to proximity of all areas, the soil is classified as eutrophic Red Latosol (Santos et al., 2013) and in the granulometric analysis we obtained 52.52 g $kg^{-1}$ of sand, 266.48 g $kg^{-1}$ of silt and 681.00 g $kg^{-1}$ of clay for the layer of 0.00 to 0.10 m and 49.39 g $kg^{-1}$ of sand, 199.11 g $kg^{-1}$ of silt and 751.50 g $kg^{-1}$ of clay for the layer of 0.10 to 0.20 m.

Five areas were evaluated, using a completely randomized design, with four replications. These areas were: an area with productive management of first and second corn crop for silage (CS); an area with succession of crops with soybean in the summer and corn in the winter in no-tillage system for ten years (SC); an area with succession of crops, with soybean in the summer and oat in the winter, in a system of direct sowing (SO), where the oats are desiccated to form cover, this system implemented for five years; an area with permanent pasture with Tifton 85 for harvesting (PP) for ten years; and an area with soybean in the summer and oat in the winter, and grazing animals on oats, five years of establishment (ILC).

Regarding the management of agricultural crops in this area, recommendations were made for fertilization and cultural treatments, due to the specific need for each crop. In relation to the oat cultivation with direct grazing for the ILC system, it started when the plants' height was between 0.25 and 0.35 m and the animals were removed when their height was 0.15 m, so that there was no damage to the apical meristem, and it would grow again for the formation of straw sufficient for direct planting of soybeans in succession (Fontaneli et al., 2012).

For each management area, four sites were randomly selected; within this area, a composite sample was collected in the diagonal direction, formed by the collection of three sub-samples at depths of 0.00-0.05 m; 0.05-0.10 m and 0.10-0.20 m, subsequently homogenized. The collected samples were air dried, heat dried, macerated and passed through a 2 mm mesh sieve (TFSA), to perform the following analysis: total organic carbon (TOC), determined by hot oxidation with potassium dichromate (Yeomans & Bremner, 1988); and chemical fractionation of the humic substances, carried out following the technique of differential solubility, separating the fulvic acids (AF), the humic acids (AH) and the humins (HUM) as established by the International Society of Humic Substances and adapted by Benites et al. (2003).

After separation of the humic substances, the respective total organic carbon contents were determined by hot oxidation with potassium dichromate (Yeomans & Bremner, 1988), after determination of the contents of each fraction, the following ratios were calculated: AH/AF (indicates soil carbon mobility) and alkaline extract (EA)/HUM (indicates organic matter illuviation in the soil profile), the alkaline extract is the sum of humic acids and fulvic acids.

The degrees of freedom for treatment were decomposed into four orthogonal contrasts within each depth. We worked with average contrasts, dividing the result of each contrast by the respective coefficient (Table 1). The significance of the contrasts was tested by the F test ($P < 0.05$), using the statistical software SISVAR (Ferreira, 2014), from the mean square of the combined residue, and the effect of a given characteristic increased or decreased when signs of the estimated contrasts were positive or negative, respectively.

Table 1. Comparisons of interest, contrasts and coefficients established between treatments

| Comparisons of Interest | Contrasts | Treatments[1] | | | | |
|---|---|---|---|---|---|---|
| | | PP | CS | SO | ILC | SC |
| Grazing by Agricultural crops | C1 | 4 | -1 | -1 | -1 | -1 |
| Grain crop by Silage crop | C2 | 0 | 3 | -1 | -1 | -1 |
| Integrated System by System non-Integration | C3 | 0 | 0 | -1 | 2 | -1 |
| Succession of oats-soybean by corn-soybean succession | C4 | 0 | 0 | 1 | 0 | -1 |

*Note*. [1] PP: permanent pasture; CS: corn for silage; SO: succession of soybeans and oats; ILC: crop-livestock integration; SC: succession of soybean and corn.

The contrast C1 establishes a comparison between treatments of permanent pasture and agricultural crops; contrast C2 compares the treatments of the crop for grain and for silage; contrast C3 compares the areas that had management with integration of agriculture with grazing on the winter crop, within the same area and an area non-integration; and finally contrast C4 was established to compare the treatments that had succession of oats in winter with soybean in summer and succession of corn in winter with soybean in summer.

## 3. Results and Discussion

Table 2 presents the results of total organic carbon, carbon fractions of humic substances, relationships of interest and results of mean contrasts for each treatment and depth studied.

Table 2. Total organic carbon (TOC), fulvic acid (AF), humic acid (AH), humina (HUM), humic acid and fulvic acid ratio (AH/AF), alkaline extract and humina ratio (EA/HUM) and estimates of mean contrasts established for each management system and depth

| Treatments | TOC | AF | AH | HUM | AH/AF | EA/HUM |
|---|---|---|---|---|---|---|
| | ------------------------------ g $kg^{-1}$ -------------------------------- | | | | | |
| *Depth of 0.00-0.05 m* | | | | | | |
| PP | 24.46 | 2.96 | 3.00 | 16.62 | 1.02 | 0.37 |
| CS | 14.77 | 2.72 | 2.16 | 11.16 | 0.80 | 0.44 |
| SO | 24.37 | 2.78 | 3.10 | 14.16 | 1.13 | 0.42 |
| SC | 19.81 | 2.59 | 2.41 | 14.59 | 0.95 | 0.35 |
| ILC | 20.05 | 2.73 | 3.14 | 14.06 | 1.15 | 0.42 |
| Contrasts | | | | | | |
| C1 | 4.71* | $0.26^{ns}$ | $0.30^{ns}$ | 3.12* | $0.01^{ns}$ | $-0.04^{ns}$ |
| C2 | -6.65** | $0.03^{ns}$ | -0.72** | -3.12* | -0.27** | $0.05^{ns}$ |
| C3 | $-2.04^{ns}$ | $0.04^{ns}$ | $0.38^{ns}$ | $-0.31^{ns}$ | $0.12^{ns}$ | $0.04^{ns}$ |
| C4 | $4.56^{ns}$ | $0.18^{ns}$ | 0.68** | $-0.42^{ns}$ | $0.18^{ns}$ | $0.07^{ns}$ |
| CV (%) | 16.0 | 13.8 | 11.6 | 13.5 | 13.0 | 14.2 |
| *Depth of 0.05-0.10 m* | | | | | | |
| PP | 20.63 | 2.72 | 2.65 | 13.07 | 0.99 | 0.41 |
| CS | 17.16 | 2.50 | 1.79 | 11.23 | 0.71 | 0.38 |
| SO | 18.11 | 2.60 | 3.07 | 13.44 | 1.18 | 0.42 |
| SC | 18.15 | 2.35 | 2.31 | 13.96 | 1.05 | 0.34 |
| ILC | 18.37 | 2.80 | 2.86 | 13.10 | 1.02 | 0.44 |
| Contrasts | | | | | | |
| C1 | $2.68^{ns}$ | $0.16^{ns}$ | $0.14^{ns}$ | $0.14^{ns}$ | $0.00^{ns}$ | $0.02^{ns}$ |
| C2 | $-1.05^{ns}$ | $-0.08^{ns}$ | -0.95** | -2.28* | -0.37** | $-0.02^{ns}$ |
| C3 | $0.24^{ns}$ | $0.32^{ns}$ | $0.17^{ns}$ | $-0.60^{ns}$ | $-0.09^{ns}$ | $0.05^{ns}$ |
| C4 | $-0.03^{ns}$ | $0.24^{ns}$ | 0.76** | $-0.52^{ns}$ | $0.14^{ns}$ | $0.08^{ns}$ |
| CV (%) | 14.0 | 15.9 | 12.9 | 12.0 | 19.5 | 15.3 |
| *Depth of 0.10-0.20 m* | | | | | | |
| PP | 17.14 | 2.63 | 2.61 | 11.97 | 1.01 | 0.44 |
| CS | 12.69 | 2.32 | 1.93 | 9.730 | 0.83 | 0.44 |
| SO | 17.55 | 2.29 | 2.91 | 13.38 | 1.27 | 0.39 |
| SC | 17.59 | 2.12 | 1.81 | 12.44 | 0.87 | 0.32 |
| ILC | 15.75 | 2.26 | 2.68 | 13.38 | 1.20 | 0.37 |
| Contrasts | | | | | | |
| C1 | $1.25^{ns}$ | $0.38^{ns}$ | $0.28^{ns}$ | $-0.26^{ns}$ | $-0.03^{ns}$ | $0.06^{ns}$ |
| C2 | -4.27* | $0.10^{ns}$ | -0.54* | -3.34** | -0.28* | $0.08^{ns}$ |
| C3 | $-1.82^{ns}$ | $0.06^{ns}$ | $0.32^{ns}$ | $0.47^{ns}$ | $0.13^{ns}$ | $0.02^{ns}$ |
| C4 | $-0.04^{ns}$ | $0.17^{ns}$ | 1.10** | $0.94^{ns}$ | 0.40** | $0.08^{ns}$ |
| CV (%) | 20.0 | 14.5 | 17.5 | 12.1 | 18.4 | 16.8 |

*Note*. The row data represent the analysis for each contrast established in the analyzed variables. CV: coefficient of variation. ** significant at 1% by the F test. * significant at 5% by the F test. ns: not significant by the F test, according to the established contrasts.

Regarding the results of the comparisons made for TOC in the depth of 0.00-0.05 m, there was a difference ($P < 0.05$) in the C1 contrast, and the average pasture area was 4.71 g $kg^{-1}$ of carbon in the soil more than the other areas. In the comparison performed by the C2 contrast, there was statistical difference ($P < 0.01$), in which the grain cultivation areas averaged 6.65 g $kg^{-1}$ of carbon more than the area of corn cultivation for silage. For the comparisons made by the C3 and C4 contrasts there was no difference ($P > 0.05$) between the comparisons made (Table 2).

The comparisons made for TOC in the depth of 0.05-0.10 m, did not present statistical difference ($P > 0.05$). For the depth of 0.10-0.20 m there was a statistical difference ($P < 0.05$) only in the comparison carried out by the C2 contrast, with the average grain yield of 4.27 g $kg^{-1}$ of carbon higher than in the maize cultivation area for silage (Table 2).

The TOC content depends essentially on the input of material on the soil surface and on the process of decomposition or mineralization of the soil organic matter (Martins et al., 2015). The high TOC values present in the pasture are not only related to the amount of material produced, but also to the fact that their material components, such as lignins and polyphenols, are more resistant to degradation, which can guarantee a longer permanence of TOC in a system, when comparing with other (Dortzbach et al., 2015). On the other hand, the low TOC content in the corn area for silage is explained by the removal of all the aerial biomass produced in the crop area, so that the only input of organic matter comes from the roots and therefore in this system it was lower in all depths (Wendling et al., 2005).

For systems in which the soil is turned over, the organic matter of the soil is distributed throughout the arable layer, which means that the TOC contents in larger depths can be similar or even higher (Ussiri & Lal, 2009). Carneiro et al. (2009), found similar results so that TOC of planted pastures was higher than several other management systems such as natural pasture and ILC.

Regarding the results of the comparisons made for AF, in all comparisons and depths there was no statistical difference ($P > 0.05$). As this matter is easily degraded by the edaphic microorganism and also because the soil has been turned over and has incorporated the vegetal remains, a practice that normally increases the levels of AF in relation to AH (Loss et al., 2010), the levels were equal between the areas and in the comparisons made.

For all the depths and in the contrasts C2 and C4 the AH had a significant difference, having a mean of 0.72 g $kg^{-1}$ and 0.68 g $kg^{-1}$, for the depth of 0.00-0.05 m, 0.95 g $kg^{-1}$ and 0.76 g $kg^{-1}$, for the depth of 0.05-0.10 m, 0.54 g $kg^{-1}$ and 1.10 g $kg^{-1}$, to the depth of 0.10-0.20 m, respectively, for each comparison. The results obtained in C2 show that on average the areas of grain cultivation are higher in relation to the area of maize for silage and for C4 they show that on average the crop with succession of oats-soybean is higher than the succession corn-soybean.

According to Loss et al. (2010), the non-disturbance of the soil by tillage, and the accumulation of organic matter due to the no-tillage system and the use of forages, allows an increase of AH in relation to AF. This explains the fact that the areas of grain crops had AH values above the area of maize for silage, since the accumulation of organic matter is higher in these systems.

The fractions AF and AH are less stable because they contain lower molecular mass, so can be easily mineralized, translocated to subsurface or polymerized depths, reducing their soil content (Valladares et al., 2011). The highest values of AH in comparison to AF are a consequence of the intense humification and the rapid mineralization of the organic material deposited in the soil (Cunha et al., 2007).

In relation to the levels of HUM in the depth of 0.00-0.05 m in the C1 contrast, there was a difference between the comparison performed, with the permanent pasture area being, average, 3.12 g $kg^{-1}$ of carbon higher than the other areas; in contrast C2, there was a difference in all depths, with the grain yield being, 3.12 g $kg^{-1}$ of carbon average more than the cultivation for silage at a depth of 0.00-0.05 m; 2.28 g $kg^{-1}$ carbon, at a depth of 0.05-0.10 m, and 3.34 g $kg^{-1}$ carbon at a depth of 0.10-0.15 m. The rest of the comparisons made at different depths did not show significant differences.

There is predominance of the humina fraction in comparison with the fractions of humic and fulvic acids, independently of the management system used and the depth analyzed. Humina is the fraction of the carbon that is most closely associated with the mineral colloids of the soil, being randomly distributed in the profiles (Canellas et al., 2000). According to Grinhut et al. (2007), this predominance of the HUM fraction is related to its insolubility and resistance to biodegradation, favored by the formation of stable clay-humic complexes. The predominant mineral composition of soils in studies, composed of Fe and Al oxides and hydroxides, favors organo-mineral interactions, increases the protection of the functional groups of easy decomposition of the HUM fraction and contributes to increase their carbon stock (Dick et al., 2003; Dick et al., 2008).

The results found for the AH/AF ratio in all depths, both in the C1 and C3 contrasts, did not show differences ($P > 0.05$). In contrast C4 there was a significant difference only in the depth of 0.10-0.20 m, having the oats-soybean succession system on average a ratio 0.40 higher than the soybean-corn succession system. For the C2 contrast, there was difference in all the depths, with the area of grain cultivation averaging a ratio of 0.27, 0.37

and 0.28, at depth 0.00-0.05 m, 0.05-0.10 m and 0.10-0.15 m, respectively, higher than for silage. This difference has the same relation as explained by Loss et al. (2010) for AH and AF, previously discussed.

The AH/AF ratio is an indicator of humus quality, since it expresses the degree of evolution of the organic matter humification process, where values lower than 1.0 indicate selective loss of AF or less synthesis and accumulation of the most stable fraction (AH) (Fontana et al., 2005; Bonifácio et al., 2006), which is common in tropical soils, indicating low rates of humification and mineralization of organic matter, being a reflection of low base content, soil acidity and restriction of microbial activity (Miranda et al., 2007). The relationships higher than 1.0 are explained by soil and climate conditions, where the polymerization and condensation processes are favorable (Valladares et al., 2007).

For comparisons for the EA/HUM ratio at all depths there was no difference ($P > 0.05$). The low values of the EA/HUM ratio indicate the high stability between the organic matter and the mineral matrix of these soils, provided by the interaction between 2:1 clays and the Ca and Mg ions with the ionized COOH and OH functional groups, mainly of organic matter (Canellas et al., 2008).

## 4. Conclusion

The permanent pasture area had higher levels of TOC and HUM than the other systems evaluated, mainly in the 0.00-0.05 m depth.

Grain cultivation areas had higher TOC, AH, HUM and AH/AF levels than the maize cultivation area for silage in all depths evaluated.

The comparison between integrated system and non-integration system for all variables and depths analyzed did not show differences.

The soybean-soybean succession area had only higher AH contents than the corn-soy succession area.

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