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Carbon of humic substances in soil aggregates cultivated with onion under no-till and conventional tillage systems

Efecto del contenido de carbono en sustancias húmicas en suelo en un cultivo de cebolla

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ABSTRACT

Carbon (C) contents of humic substances (HS) in soil aggregates are a sensitive indicator of changes in the edaphic quality caused by soil management systems and/or cover plant species. The aim of this study was to assess total contents of C and HS of organic matter in soil aggregates cultivated with onion under a no-till vegetable production system (NTVS) and conventional tillage (CT) compared to a secondary forest area. Treatments consisted of single and mixed cover crops and onion under NTVS: spontaneous vegetation, 100% black oat; 100% rye; 100% oilseed radish; consortium of oilseed radish (14%) + rye (86%); consortium of oilseed radish (14%) + black oat (86%). An area cultivated with onion under CT (± 37 years) and a forest area (secondary forest; ± 30 years) were also evaluated. Five years after implementation of NTVS, undisturbed soil specimens were collected from the 0.0-0.05, 0.05-0.10 and 0.10-0.20 m layers and aggregates were obtained (8.00 mm > Ø ≥ 2.0 mm). In these aggregates, total organic carbon (TOC) and C of HS were determined, subdividided into a fulvic acid fraction (C-FAF), humic acid fraction (C-HAF) and humin (C-HUM). With the C values of the HS the following ratios were calculated: C-HAF/C-FAF and (C-HAF+C-FAF)/C-HUM. The highest content of TOC, C-FAF, C-HAF and C-HUM were found in the forest. In NTVS, the contents of TOC (at the 0.0-0.05 m depth) and C-HUM (0.0-0.05 and 0.05-0.10 m) were higher than those found under CT. The highest C-FAF contents were found under CT. NTVS compared to CT favors the humification process (higher C-HUM content and lower ratios of C-HAF+C-FAF/C-HUM (at the 0.0-0.05 and 0.05-0.10 m depths) and the protection of organic matter (higher TOC contents at the 0.0-0.05 m depth)). A linear relationship was observed between C-HUM and N of HS. Among the cover plant species, rye alone and rye combined with oilseed radish had the best results regarding humification of organic matter with predominance of C-HUM and C-HAF. This pattern was also observed in the control for C-HUM, due to the abundance of plant families and species, indicating that plant biodiversification is an effective way to improve the quality of soil.

Keywords: macro-aggregates, consortium of cover crops, humic acids, fulvic acids, humin, *Alliun cepa* L.

RESUMEN

Los contenidos de carbono (C) de las sustancias húmicas (SH) en los agregados del suelo son un indicador sensible de los cambios en la calidad edáfica causados por los sistemas de manejo del suelo y / o las especies vegetales de cobertura. El objetivo de este estudio fue evaluar el contenido total de C y SH de materia orgánica en agregados de suelo cultivado con cebolla bajo sistema de siembra directa de hortalizas (SSDH) y sistema de preparación convencional (SPC), comparando con una área de bosque. Los tratamientos consistieron en cultivos de cobertura solas y asociadas con cebolla bajo SSDH: vegetación espontánea, 100% avena negra; 100% centeno; 100% nabo forrajero; consorcio de nabo (14%) + centeno (86%); consorcio de nabo (14%) + avena negra (86%). También se evaluó un área cultivada con cebolla bajo SPC (± 37 años) y un área de bosque (bosque secundario, ± 30 años). Cinco años después de la implementación de SSDH, se recogieron muestras de suelo de las capas 0,0-0,05, 0,05-0,10 y 0,10-0,20 m y obtenidos los agregados (8.00 mm>; Ø ≥ 2,0 mm). En estos agregados, se determinaron el carbono orgánico total (COT) y C de SH, subdivididos en fracción ácido fúlvico (C-FAF), fracción ácido húmico (C-FAH) y humin (C-HUM). Con los valores de C de las SH, se calcularon las siguientes proporciones: C-FAH/C- FAF y (C-FAH + C-FAF)/C- HUM. El mayor contenido de COT, C-FAF, C-FAH y C-HUM se obtuvo en el bosque. En SSDH, los contenidos de COT (0,0-0,05 m) y C-HUM (0,0-0,05 y 0,05-0,10 m) fueron más altos que aquellos encontrados bajo SPC. Los contenidos más altos de C-FAF se encontraron bajo SPC. En SSDH en comparación con SPC favorece el proceso de humificación (mayor contenido de C-HUM y menores índices de C-FAH + C-FAF/C- HUM (en las profundidades de 0,0-0,05 y 0,05-0,10 m) y la protección de la materia orgánica (mayor contenido

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de COT a 0.0-0.05 m de profundidad)). Entre las especies de plantas de cobertura, el centeno solo y el centeno consorciado con nabo tuvieron los mejores resultados en cuanto a la humificación de materia orgánica con predominio de C-HUM y C-FAH. Este patrón también se observó en el control para o C-HUM, debido a la abundancia de familias de plantas y especies, lo que indica que la biodiversidad de las plantas es una forma efectiva de mejorar la calidad del suelo.

***Palabras clave**: macroagregados, consorcio de cultivos de cobertura, ácidos húmicos, ácidos fúlvicos, humin,* Alliun cepa *L.*

Introduction

Onion (*Allium cepa*) is a vegetable of major importance to Brazilian agriculture. Estimates indicate that 60 thousand hectares of land are cultivated with onion crops in Brazil yearly, with a mean yield of 28 Mg ha^{-1} and production around 1.6 million bulbs. The southern region of the country accounts for 49% of the national production; Santa Catarina (SC) has been the largest domestic producer of onion since 1990. Onion production, besides being profitable, has become increasingly sustainable as a result of studies with organic cultivation (ACATE, 2014).

Among the soil preparation methods for onion cropping, conventional tillage (CT) is still the system most commonly used in SC. This system has as main characteristics the intensive use of mechanization and constant soil disturbance (plowing, harrowing, subsoiling and scarification) (Kurtz *et al*., 2013), which favors the soil erosion process and causes alterations in soil attributes such as reduced content of nutrients and organic matter (Loss *et al*., 2015). Because of the impacts caused by CT on the soil and on several environmental conditions, conservation systems have been proposed aiming to the sustainable production of foods, fibers and energy with minimal alterations in the environment. In this context, an alternative to CT is the no-till (NT) or direct seed system; in the case of onion, the no-till vegetable production system (NTVS) (Kurtz *et al*., 2013). In these systems soil disturbance is limited to the planting lines, cover crops are used to produce mulch and these crop residues are left on the soil surface. As a consequence, as there is no soil disturbance (digging, overturning, etc) under NTVS; the volume of crop residues tend to maintain or even increase the organic matter content (OMC) in the soil; there is an improved edaphic quality especially in soils cultivated for a long time under CT (Loss et al., 2015). The NTVS is a developing technology and is different from the traditional NT systems in technological and social aspects, especially for not prescribing the use of herbicides and having the social proposal of being a potential practice for sustained agricultural development (Kurtz *et al*., 2013).

The OMC, as expressed by the content of total organic carbon (TOC) and C of the humic substances (HS), which is subdivided into C of the fulvic acid fraction (C-FAF), humic acid fraction (C-HAF) and humin (C-HUM), are considered useful indicators for the evaluation of the soil quality and detection of environmental impacts (Loss *et al*., 2010). The HS have the capacity to interact with the clay fraction and play a major role in soil fertility and structure, as well as in the immobilization of potentially hazardous elements and pesticides (Stevenson, 1994; Fontana *et al*., 2006).

Variations in the distribution of HS may indicate alterations of edaphic attributes and impacts caused by the management system on the soil quality (Fontana *et al*., 2006; Passos *et al*., 2007); the calculated ratios of the HS fractions have been suggested as indicators of environmental conditions or anthropic alterations (Fontana *et al*., 2006; Loss *et al*., 2010). According to Benites *et al.* (2010), HS reflect the changes occurred as a result of anthropic alterations and at the same time are stable in relation to short-term spatial and temporal variations compared to some biological and biochemical indicators usually employed, suggesting that HS characterization has great potential for evaluation of alterations in soil quality.

However, a significant part of the studies relating to HS evaluation are with disturbed soil samples, and few studies quantifying the C contents of the HS in undisturbed soil samples were found. Studies related to the C content of HS should also evaluate soil aggregates, because according to Loss *et al.* (2015), significant differences are found in soil aggregation between no-till and no-till vegetable cropping systems, especially in macro-aggregates (8.00 mm > Ø ≥ 2.0 mm). According to Christensen

(1996), during the aggregation process part of the OMC is physically protected in the interior of these aggregates, causing a decrease in mineralization due to lesser action of microorganisms and less diffusion of O_2 and water (Christensen, 1996). Thus keeping the OMC fractions protected in the aggregates depends on the aggregate formation and stability, while aggregate fragmentation will cause the exposure of OMC to decomposition (Adu e Oades, 1978).

Quantification of the C content of HS present in the aggregates is therefore one of the possible ways to study their dynamics, since the hierarchical levels represented by the aggregate sizes indicate the time and stability of the organic matter. Due to the higher organic matter lability present in the macroaggregates, their stability depends on the presence of plants and the constant distribution of plant residues on the soil surface. Conventionally tilled soils exhibit loss of stability, and consequently a breakdown of macro-aggregates (Loss *et al.*, 2015) and the exposure of the OMC to decay by microorganisms (Six *et al.* 2000).

Taking into account that OMC and soil quality are associated with the soil cropping system used, this study aimed to evaluate the total C content in soil and in HS (FAF, HAF and HUM) of aggregates from a Humic Distrudept cultivated with onion under CT and NTVS, compared to a forest area (secondary forest).

Materials and methods

The study was conducted at the Experimental Station of the *Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI)*, in the municipality of Ituporanga, SC, located at 27º 24’ 52”S, 49º 36’ 9” W, altitude 475 m. The climate in the region is humid subtropical (Cfa) and mesothermal (hot summers and infrequent frosts), without a defined dry season, with an annual mean temperature of 17.6 ºC and mean annual rainfall of 1400 mm.

The experiment was installed in a Humic Distrudept (Soil Survey Staff, 2006), with loamy soil texture in the 0.00-0.10 m layer with 380, 200 and 420 g kg^{-1}, of clay, silt and sand, respectively, in an area with a history of onion cultivation using CT (plowing, harrowing and scarification) for approximately 20 years until 1996. After 1996, limestone was applied to the soil surface with subsequent incorporation to raise the pH in water to 6.0. Next, a minimal onion cultivation system in rotation with cover plants (black oat - *Avena strigosa*, mucuna - *Mucuna aterrima*, millet - *Pennisetum glaucum*, brown hemp - *Crotalaria juncea*, common vetch - *Vicia sativa*), was implemented, a system that remained from 1996 to 2007. Later, a sweet potato (*Ipomoea batatas* (L.) Lam) crop was implemented and harvested until 2009. Afterwards, the experiment with onion using the NTVS was installed, one treatment being with the CT method for comparison with the NTVS.

At the time of the experiment implementation (2009), the soil at the 0.0-0.10-m depth had the following features: 23.2 g kg^{-1} of TOC, pH in water = 6.0, SMP index = 6.2; P= 26.6 mg dm^{-3} and K=145.2 mg dm^{-3} (both using Mehlich-1 extractant); Al^{3+} 0.0 $cmol_c$ kg^{-1}, Ca^{2+} 7.2 $cmol_c$ kg^{-1} and Mg^{2+} 3,4 $cmol_c$ kg^{-1} (all extracted with KCl 1 mol L^{-1}) (Tedesco *et al.*, 1995). In the same year, at the time of the experiment installation all spontaneous vegetation was desiccated using glyphosate herbicide. No further additional herbicide applications were used.

The treatments consisted of planting single and intercropping cover plants, as follows: (1) control with spontaneous vegetation (Control), comprised of 20 plant families, with predominance of the following families (85%): Amaranthaceae (10%), Asteraceae, Caryophyllaceae (10%), Convolvulaceae, Cruciferae, Cyperaceae (25%), Euphorbiaceae, Fabaceae, Lamiaceae (10%), Leguminosae, Liliaceae, Malvaceae, Oxalidaceae (10%), Plantaginaceae, Poaceae, Polygonaceae (20%); (2) 100% black oat (*Avena strigosa*) with a sowing density (SD) of 120 kg ha^{-1}; (3) 100% rye (*Secale cereale*) with SD of 120 kg ha^{-1}; (4) 100% oilseed radish (*Raphanus sativus*) with SD of 20 kg ha^{-1}; (5) mixture of oilseed radish (14%) and rye (86%) with SD of 10 and 60 kg ha^{-1}, respectively, and (6) mix of oilseed radish (14%) and black oat (86%) with SD of 10 and 60 kg ha^{-1}, respectively. In April, 2010, black oat was replaced by barley (*Hordeum vulgare*); after April 2011, barley was replaced again by black oat due to difficulty in obtaining seeds of this crop. Winter species were seeded by throwing the seeds over the soil surface in April of each year, and then a grain seeding machine passed over the field twice. The treatments consisted of randomized blocks with five replications. Each experimental unit measured 25 m^2 (5 × 5 m). In July 2009, 2010, 2011, 2012 and 2013; the seedbed for all winter species was prepared using a roller-knife, model RF240 (MBO Ltda).

Two additional treatments were evaluated, one being the original area where onion was cultivated under CT for 20 years until 1996. Adding the subsequent years from 1996 to 2013, when the soil samples were collected, a total of 37 years were cultivated under CT. The other additional treatment, a secondary forest aged ±30 years, represented the natural soil condition; it was ±500 meters distant from the experiment, with the same soil. In the CT treatment, onion was grown in rotation with millet in summer since 2007. Millet bedding was done when flowering using a roller knife, and after 30-60 days the plots were plowed and harrowed to implement the onion crop. Fertilization was done following recommendations by CQFSRS/SC (2004), with application of 165 kg ha^{-1} of P_2O_5 (triple superphosphate), 105 kg ha^{-1} of K_2O (potassium chloride) and 192 kg ha^{-1} of N (ammonium nitrate). In the CT treatment in 2010, liming was carried out to raise the pH value to 6.0, according to the SMP method (CQFSRS/SC, 2004).

In the NTVS treatment, after bedding of the winter cover plants in July of each year, 96 kg ha^{-1} of P_2O_5, in the form of natural ground Gafsa phosphate, 175 kg ha^{-1} of P_2O_5, 125 kg ha^{-1} of K_2O and 160 kg ha^{-1} of N in the form of poultry excreta were applied, half of it when the onion seedlings were planted and the remainder 30 days after planting. After the 2011 harvest no natural phosphate was used, because the contents were construed as too high (CQFSRS/SC, 2004). Afterwards, the furrows were opened using an adapted no-till machine, and seedlings of onion cv. *'Empasc 352' - Bola Precoce* were transplanted. Spacing was 0.50 m between the rows and 0.10 m between the plants, with 10 rows of onion plants per plot. Weeding was carried out 50 and 90 days after planting onion seedlings. After harvesting the onion bulbs in December of each year, black *mucuna* (*Mucuna aterrima*) was always planted in summer in the whole cultivated area, with a SD of 120 kg ha^{-1}. The seedbed for *Mucuna* was prepared in March each year to seed the cover plants in the next month (April).

The mean values of dry matter and onion bulbs produced in the areas evaluated in 2013, year when the soil samples were collected, are shown in Table 1.

In September 2013, five years after the implementation of the treatments, undisturbed soil specimens were collected from the 0.0-0.05, 0.05-0.10 and 0.10-0.20 m deep layers by opening small trenches measuring 0.40 x 0.40 x 0.40 m in each plot, using a cutting shovel. The samples were packaged in plastic bags and sent to the Laboratory of Soil at the Federal University of Santa Catarina (UFSC), where they were air dried and then manually loosened, breaking up the lumps following the crack lines or weak points, and then sieved through mesh openings measuring 8.00 mm and 4.00 mm to obtain soil aggregates, according to Claessen (1997). The undisturbed samples weighed around 900 to 1000 grams. About 60% of the aggregate particles measured between 8.00 mm > Ø ≥ 4.0 mm in the NTVS and in the forest; these were used to determine the total contents of C and N and HS.. In the CT system these particles did not exceed ±30 to 35% of the total soil mass. The values of the distribution of aggregate classes (macro-, meso- and micro-aggregates) and aggregation indices (weighed and geometric mean diameter of the aggregates) are described in Loss *et al.* (2015).

To carry out the chemical analysis, the aggregates retained in the 4.00-mm sieve mesh were broken up manually and passed through

Table 1. Production of Dry Matter of Winter Cover Crops and *Mucuna* in Summer and Onion Bulb Yield in the 2012/2013 Harvest Season.

Treatments	Dry matter		Onion bulb yield(1)
	Winter	Summer	
	— kg ha^{-1} —		Mg ha^{-1}
Spontaneous vegetation (control)	4480	2275	16.74
Black oat	5220	1951	19.94
Rye	5635	2286	18.93
Oilseed radish	4934	2155	18.68
Black oat + oilseed radish	5203	2306	18.58
Rye + oilseed radish	4656	2283	18.08
CT	–	12000	26.10

(1) The Onion Bulb Yield Is Much Higher Under The Ct System, Which Is Mainly Due To The Chemical Control Of Mildew, A Fungal Disease That Can Cause More Than 70% Losses In Onion Yields. Regarding The Ntvs, There Are Not Yet Technologies To Reduce The Effect Of This Disease.

a 2.00-mm mesh, to obtain air-dried fine earth (ADFE) particles of the aggregates. The COT and the C of the HSs were determined: humin (C-HUM), humic acid (C-HAF) and fluvic acid (C-FAF).

COT was determined by the method of humid oxidation with external heating, according to Yeomans and Bremner (1988). For the extraction and quantification of the C contents in the HS the differential solubility test in alkaline and acid medium was used, according to the methodology described by the International Humic Substances Society - IHSS, with adaptation by Benites *et al.* (2003). One gram of ADFE was added to 50-ml falcon tubes, together with 20 mL NaOH 0.1 mol L^{-1} followed by rapid manual agitation. After 24 hours of rest, the tubes were centrifuged at 5000g (g-force) for 20 min under refrigeration (*10* ºC). Afterwards, the supernatant (FAF+HAF) was transferred to another 50-ml falcon tube and 20 mL of NaOH 0.1 mol L^{-1} were added to the tube contained the material that was precipitated, which after homogenizing rested for one hour. After this time, the material was centrifuged again at 5000*g* for 20 min at *10* ºC. Finally, the supernatant was combined with the first. The precipitate obtained from the second centrifugation, which included HUM, remained at the bottom of the tube and was dried in forced-air oven under a temperature of 50 ºC for 24 hours. The pH of the supernatant (± 40 mL) was adjusted to between 0.9 and 1.1 with 20% H_2SO_4 to precipitate the HAF. The suspension rested for 18 hours, and then the HAF was separated of the soluble fraction (FAF) by centrifugation (2500*g* for 5 min.). The soluble fraction was transferred to another 50-ml falcon tube, and this volume was completed with distilled water. The precipitate (FAH) was dissolved manually with 5 mL of NaOH 0.1 mol L^{-1}, and the volume was completed to 50 mL with distilled water. To determine the C content in the HS, the dichromatometry method with external heating was used, according to Yeomans & Bremner (1988).

With the results of C-HUM, C-HAF and C-FAF the ratios to the HS (Benites *et al.*, 2003) were calculated: ratio of C of HAF to C of FAF (C-HAF/C-FAF), and the ratio of C of alkaline extract – C-EA (C-HAF + C-FAF) to C of HUM (C-EA/C-HUM).

The results were analyzed for data normality and homogeneity using tests of Lilliefors and Bartlet, respectively, and then analyzed according to the randomized blocks design with eight treatments (oat, rye, oilseed radish, oilseed radish+rye, oilseed radish+oat, spontaneous vegetation, CT and forest) and five replications. The results were subjected to analysis of variance with F-test, and the mean values, when significant, by the Skott-Knott test at 5%.

Results and Discussion

The highest TOC values in the soil aggregates were found in the forest area, at the three depths evaluated. The NTVS compared to CT indicated the highest TOC levels at the 0.0-0.05 m depth (Figure 1). The TOC contents are results of the rates of production, alteration and decomposition of organic residues, which are dependent on various factors such as temperature, humidity, aeration, pH and availability of water and nutrients. These factors are naturally associated with the pedogenetic processes, but may be also influenced by anthropic action such as soil use and management (Nascimento *et al.*, 2010). The highest content of TOC in the forest area is the result of deposition of organic matter, especially plant litter, which promotes the accumulation of C on the top of the soil as the residues are humified (Loss *et al.*, 2015).

With the introduction of agricultural systems in areas of native vegetation, there is an imbalance in OMS dynamics and consequently a rapid decrease of TOC content (Scholes & Breemen, 1997). The changes in the edaphic characteristics, on the other hand, result from the effect of the soil management system used (Machado *et al.*, 2014). In the region of the Itajaí (SC), which is considered a major onion-producing area in the SC, the use of CT in the soil preparation for onion cultivation has effectively contributed to environmental degradation, mainly due to the intensive use of plowing and harrowing, which in addition to losses in the most fertile layer, up to 0.20 m deep (Gonçalves *et al.*, 2008), also favors greater OMC mineralization due to a higher rate of oxidation caused by this system. These results were found at the 0.0-0.05 m depths for TOC and 0.0-0.05 and 0.05-0.10 m for C-HUM (Table 2), which exhibited lower values for the CT system compared to the NTVS.

In spite of the conservation practices and the amount of dry matter found in the NTVS soils (Table 1), the TOC content in the aggregates

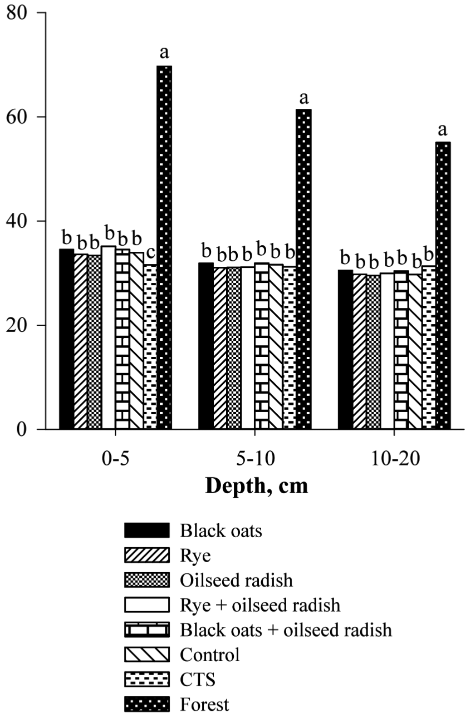

Figure 1. Total Organic Carbon (Toc) In Soil Aggregates Under No-Till And Conventional Systems With Onion Crops And Forest. Different Letters For The Same Evaluated Attribute, At Each Depth, Indicate Statistical Differences According To The Scott-Knott Test At 5%.

under this system did not reach the level of the forest area, but in the soil surface (0.0-0.05 m) it exhibited a significant increase compared to the TOC level under CT (Figure 1). Also, taking into account that under CT there is a greater yearly amount of dry mater (Table 1), the adverse impact of mechanical disturbances and pulverization on the C accumulation and storage in the soil becomes clear.

However, as the TOC content under NTVS was quite different from the content found in the forest area, this can be due to the period of use of the NTVS, which in this study was five years. According to Pereira Neto *et al.* (2007), who conducted a study about the time of consolidation of NT in maize crops between 2 and 14 years of age, the NT system is consolidated between the 9th and 10th year after implementation. These authors measured the area of the structures present in the soil cultural profile using the geographical information system and assessed them by the statistical method of main components, and found that the structures where NT was implemented less than eight years before were similar to those of CT. When NT was implemented more than nine years before, the structures were similar to the forest profile.

In the NTVS and CT cropping systems, TOC content depends, among other factors, on the quantity of dry matter (aboveground plant and root) produced by the cover crops and the management system adopted. Therefore, systems that produce and preserve dry matter on the soil surface will exhibit higher content and accumulation of TOC in the soil. This pattern was found in the NTVS

Table 2. Mean Values Of Organic Carbon Of The Humic Substances (G Kg^{-1}) Contained In Humic Distrupept Soil Under Onion Cropping Systems And Forest, Ituporanga, Sc.

Treatments	C-HUM			C-HAF			C-FAF		
	– Depths (m) –								
	0.0-0.05	0.5-0.10	0.10-0.20	0.0-0.05	0.05-0.10	0.10-0.20	0.0-0.05	0.05-0.10	0.10-0.20
Black oat	20.75b	21.71b	13.25c	4.93b	4.53c	4.04c	3.44c	3.86c	4.09c
Rye	23.99a	19.10b	12.30c	4.73b	4.97 b	4.72b	3.71c	3.53c	4.23c
Oilseed radish	21.83b	15.03c	12.88c	4.36b	5.26b	4.38c	4.28c	3.61c	3.40c
Rye+Oilseed	24.41a	14.55c	18.55b	4.99b	4.34c	4.19c	3.78c	4.13c	3.78c
Oat+Oilseed	20.41b	17.52c	14.83c	4.44b	4.23c	4.08c	3.92c	3.47c	3.70c
Control	24.87a	22.71b	11.33c	4.59b	3.94c	4.25c	3.86c	3.32c	3.59c
CT	14.28c	11.15d	12.34c	5.33b	5.24b	4.92b	5.29b	5.05b	4.94b
Forest	25.18a	26.13a	26.88a	11.23a	7.93a	5.83a	9.11a	7.31a	6.02a
CV(%)	13.37	14.42	14.29	14.64	14.73	10.69	9.27	12.53	13.54

Means Followed By The Same Letter In A Column Do Not Differ According To The Scott-Knott Test At 5%. Cv= Coefficient Of Variation. C-Hum: Carbon Of Humic Fraction, C-Haf: Carbon Of Humic Acid Fractions, C-Faf: Carbon Of Fluvic Acids Fraction.

compared to CT (Figure 1) at the 0.0-0.05 m depth of soil. In this layer, the lower TOC values found for CT are due to the increased mineralization of TOC caused by turning over the soil, causing the breakdown of plant residues and consequently contributing to the action of microorganisms. These results corroborate the studies of Six *et al.* (2000)), who reported that intense soil disruption causes TOC losses, especially because of the increased microbial activity and higher exposure of plant residues to the microorganisms and their enzymes. Under CT, even with a larger accumulation of dry matter (Table 1), soil tilling (plowing and harrowing) causes the rupture of aggregates, with consequent exposure of the TOC that were physically protected in the interior of the aggregates, and consequently leads to lower TOC content in the soil surface.

There is a tendency to increase the OMC content in management systems that use conservation practices with low soil disturbance such as the NTVS (Loss *et al.*, 2015). Thus it can be seen that five years after the implementation of NTVS there was an increase at the 0.0-0.05 m depth of up to 11.3; 9.4; 9.3; 7.4; 6.4 and 5.9%, respectively, of TOC in the aggregates of the treatments with rye+oilseed radish, black oat, black oat+oilseed radish, control, rye and oilsheed radish, in relation to CT. These results show the potential of the NTVS compared to CT to increase TOC levels and the sustainability of farming systems. The higher content of TOC under the NTVS is directly related to the higher soil aggregation indexes (mean weighted diameter and macro- and meso-aggregates) found in all treatments with this system at the 0.0-0.05 m soil depth (Loss *et al.*, 2015).

The inexistent differences at the 0.05-0.10 and 0.10-0.20 m depths between the treatments using the NTVS and CT indicates a similarity of TOC content in the cover crops. However, under CT the same TOC values (0.10-0.20 m) may be due to the incorporation of millet residues into deeper layers, homogenizing the TOC content (0.05-0.10 and 0.10-0.20 m). The same pattern of TOC accumulation was also described by Assis *et al.* (2006) for Red Latosol aggregates under NT (for 4 years) and CT (for 30 years). The authors found that the TOC content was lower in cultivated soil than in native forest (sub-deciduous forest). However, they also found that NT aggregates did not exhibit increased TOC content compared to CT aggregates.

The highest C-FAF, C-HAF and C-HUM content in the soil aggregates were found in the forest area, at the three depths of soil examined. The NTVS exhibited higher content of C-HUM at 0.0-0.05 and 0.05-0.10 m compared to CT. This system in turn yielded higher C-FAF levels at the three depths compared to the NTVS. Higher C-FAH content under CT compared to the treatments under NTVS except for the 0.0-0.05 depth was also found, except for rye (0.05-0.10 and 0.10-0.20 m) and oilseed radish (0.05-0.10 m), which did not differ under CT (Table 2).

In the HS studied there was predominance of C-HUM in all treatments and depths examined. This pattern is explained by the fact that this fraction is considered the one with the greatest carbon stock in soils and higher interaction with the mineral fraction (Stevenson, 1994; Fontana *et al.*, 2006), corroborating other studies that also found higher C-HUM content in soil aggregates under different cultivation and management systems (Passos *et al.*, 2007).

Of the treatments with NTVS cover crops, the highest C-HUM contents were found for rye, rye+oilseed radish and the area with spontaneous vegetation (control); all of them were the same as in the forest area at the 0.0-0.05 m depth. At the 0.05-0.10 m depth, the C-HUM content under the NTVS was higher in the treatments with black oat, rye and control, and the other treatments (oilseed radish, rye+oilseed radish and black oat+oilseed radish) were higher than in CT. At the 0.10-0.20 m depth, only the treatment with rye+oilseed radish exhibited higher content of C-HUM than the other NTVS and CT treatments.

The restricted soil disturbance in the NTVS favors the natural consolidation of the soil and the accumulation of crop residues on the surface, which raise the levels of OMC and biological activity. In this environment, single cover crops such as rye, and together with oilseed radish, favor the production of organic cementing substances and consequently aggregate formation and stabilization, protecting the organic matter in their interior. Rye stands out from the other crops in the chemical aspects because it has a fasciculate and dense root system, which distributes the root exudates more evenly; oilseed radish in turn causes a more physical effect on the soil when compressing it as its primary root system develops (Loss *et al.*, 2015). In the control, the diversity of families and species that composed the spontaneous vegetation also favored the physical and chemical processes related to soil aggregation, and when associated with the conservation management techniques adopted in the NTVS, allowed better protection of C contained in the aggregates and higher OMC humification rates.

Lower values of C-HUM (0.0-0.05 m) were found in the treatments with single crops of black oat and oilseed radish, and in the black oat+oilseed radish consortium compared to the treatments with rye, rye+oilseed radish and control, but all with higher values than in CT. The lower C-HUM values in the soil aggregates found in CT result from the management practices adopted in this system, since humin is the most stable fraction of OMC, composed of recalcitrant organic matter and strongly stabilized with the mineral portion (Stevenson, 1994). CT has adverse effects on the formation and stability of soil aggregates, especially the topsoil, due to excessive soil disruption and exposure of organic matter. Despite the high yield of dry matter from millet under CT (Table 1), long-term plowing, harrowing and scarification for onion cropping contributed to TOC losses (Figure 1), causing lower OMC humification rates, and consequently lower C content linked to HUM compared to the other systems evaluated at the 0.0-0.05 and 0.05-0.10 m depths (Table 2).

The highest C-HAF content was found in the forest area at the three depths assessed. At 0.0-0.05 m, there were no differences between the NTVS and CT treatments, but at 0.05-0.10 m deep, the CT, oilseed radish and rye under the NTVS exhibited the highest C-HAF content compared to the other treatments in NTVS. At 0.10-0.20 m deep, CT and the rye treatment exhibited the highest C-HAF content compared to the other treatments in NTVS. The highest C-FAF content was found in the forest area, followed by CT, and the lowest values were for the NTVS in all depths assessed (Table 2).

In the forest land, the higher C-HAF and C-FAF levels are due to the greater amount of crop residue and absence of anthropic influence, indicating the same pattern found for TOC and C-HUM. In natural environments HS formation is linked to microbial activity (Machado and Gerzabeck, 1993) and humification is the final result of the microbiological process over time. These results are corroborated by Assis *et al.* (2006), who also quantified C of HS in the soil and aggregates.

The highest content of C-HAF and C-FAF under CT, compared to some treatments under NTVS, even with breakdown of soil aggregates and subsequent exposure of OMC to the action of microorganisms, are due to the yearly addition of millet dry matter (Table 1) for a long time. These plant residues have a slow decomposition rate (high C/N ratio), which is favorable to the humification process compared to the mineralization process. Thus the HS may be chemically protected by interacting with the soil mineral fraction. These results are similar to those found by Passos *et al.* (2007), who quantified C of HS in aggregates of maize crop soils under CT for more than 30 years and one native vegetation area (*cerrado*, or savannah). The authors found the highest C-FAF and C-HAF values in the CT maize crop in detritus of *cerrado*, and this behavior was due to the yearly addition of maize residues resulting in a high C/N ratio.

Under the NTVS, the treatment with rye+oilseed radish (0.05-0.10 m) and rye (0.10-0.20 m) showed the highest C-HAF values compared to the other treatments and were not different under CT (Table 2). The results found in these NTVS treatments indicate that they contributed to the humification process and formation of molecules with greater molar mass, and therefore to the natural condensation and prevalence of C-HAF in the soil at these depths (Slepetiene and Slepetys, 2005), which contributed to the formation of macro-aggregates (Loss *et al.*, 2015) and therefore the high C-HAF content in their interior.

The values for the C-HAF/C-FAF ratio were equal to and/or higher than 1.0 for all systems under evaluation, and differences were found in the treatments at the 0.0-0.05 and 0.05-0.10 m depths. The lowest value was found for CT, but it was not different from the treatments with oilseed radish and black oat+oilseed radish at the 0.0-0.05 m depth. The other treatments had values ranging from 1.19 (control) to 1.43 (black oat). At the 0.05-0.10 m depth, the highest ratios were found in the treatments with rye and oilseed radish, and in the other treatments the values ranged from 1.04 (CT) to 1.22 (black oat+oilseed radish). At the 0.10-0.20 m depth no differences were found in the treatments; the values ranged from 0.97 (forest) to 1.29 (oilseed radish) (Table 3).

The C-HAF/C-FAF ratio indicates the humus quality because it expresses the degree of evolution of the OMC humification process (Benites *et al.*, 2003). In weathered soils, this ratio is usually less than 1.0 due to the lower degree of humification, condensation and synthesis process caused by intensive mineralization of plant residues, edaphic restrictions and the lower content of exchangeable bases, which are unfavorable to biological activity in these soils (Canellas *et al.*, 2002). It can be seen that the treatments with oat, rye, rye+oilseed radish, control and forest land at the 0.0-0.05 m depth, as well as rye and oilseed radish at 0.05-0.10 m, contribute to the OMC humification because in these systems the C-HAF/C-FAF ratios were above 1.0 and higher than the other treatments. These results indicate that the evaluated systems produce high quality matter, favoring the establishment of physical and chemical properties that are beneficial to plant development (Fontana *et al.*, 2006).

The C-HAF/C-FAF ratios above 1.0, as found in the evaluated systems (Table 3), indicate that in these areas there is predominance of C in the C-HAF relative to the C-FAF, presenting more stable organic matter. Higher C-HAF content to the detriment of C-FAF content usually indicates more preserved soils, under conservation tillage methods (Canellas *et al.*, 2002), particularly in treatments with oat (0.0-0.05 m) and rye+oilseed radish (0.05-0.10 m) compared to CT. In this system, the lower C-HAF/C-FAF ratios indicate higher C mobility in the soil, with predominance of C-FAF (more soluble), which can be associated with the soil management method (plowing, harrowing and scarification), which are unfavorable to the formation of more stable HS such as C-HAF and C-HUM.

With regard to the C-EA/C-HUM, it was found that the areas under NTVS had the lowest mean values at the 0.0-0.05 m depth compared to the CT and forest areas. At the 0.05-0.10 m depth, all systems evaluated presented values for this ratio lower than CT. The (C-FAF+C-HAF)/C-HUM ratio has been proposed as indicator of OMC stability (Benites *et al.*, 2003; Fontana *et al.*, 2006), where lower values indicate predominance of C-HUM and better OMC chemical stability in these areas. The higher values found under CT compared to the treatments under NTVS indicate that plowing, harrowing and scarification practices are unfavorable to the formation of more stable HS (C-HUM and C-HAF), and consequently higher content of the most soluble fraction (C-FAF) and less humification in CT are obtained, compared to NTVS.

In the forest area, the higher value of the C-EA/C-HUM ratio indicates better biological activity, which contributes to more polymerization of humic compounds and the formation of C-HAF relative to C-FAF, as shown in Table 2 and also mentioned by Slepetiene and Slepetys (2005).

Table 3. C-Haf/C-Faf And C-Ea/C-Hum Ratios In Humic Distrupept Aggregates
Under Different Treatments With Onion Cultivation, Ituporanga, Sc.

Treatments	C-HAF/C-FAF			C-EA/C-HUM		
	– Depth (m) –					
	0.0-0.05	0.05-0.10	0.10-0.20	0.0-0.05	0.05-0.10	0.10-0.20
Black oat	1.43a	1.17b	0.99^{ns}	0.41b	0.39c	0.62b
Rye	1.27a	1.41a	1.12	0.36b	0.45c	0.73a
Oilseed radish	1.02b	1.46a	1.29	0.40b	0.60b	0.61b
Rye+Oilseed radish	1.32a	1.05b	1.11	0.37b	0.59b	0.43c
Oat+Oilseed radish	1.13b	1.22b	1.10	0.43b	0.45c	0.53c
Control	1.19a	1.19b	1.18	0.34b	0.32c	0.70a
CT	1.00b	1.04b	1.00	0.74a	0.93a	0.81a
Forest	1.23a	1.08b	0.97	0.81a	0.60b	0.46c
CV(%)	14.40	18.90	16.42	16.37	18.54	14.03

Means Followed By The Same Letter In Column Are Not Significantly Different According To The Scott-Knott Test At 5%. Cv= Coefficient Of Variation. C-Haf: Carbon In Humic Acids Fraction, C-Faf: Carbon In Fluvic Acid, C-Ea/C-Hum: Sum Of Humic Acids Fractions And Fulvic Acids Fraction, C-Hum: Carbon Of Humin Fractions. Ns = Not Significant By F-Test At 5%.

Conclusions

The soil management methods and kind of cover crop used have an influence on the C content in the aggregates; NTVS favors the humification process (at the 0.0-0.10 m depth) and protection of the organic matter (at the 0.0-0.05 m depth) compared to CT. The conversion of CT to NTVS contributes to an increase of the TOC content, as well as their humic fractions (HUM and HAF). However, this content is still lower than those found in the forest area.

Of the species of cover crops used in the NTVS, rye alone and combined with oilseed radish contributed more effectively to the humification process of organic matter, with predominance of C in the more stable fractions. This pattern was also found in the control, due to the richness of plant families and species, indicating that plant biodiversification is a way to improve the quality of soil.

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