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RESEARCH ARTICLE
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Cropping systems and cover crops and their influence on the stocks and fractionation of soil carbon[1]

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ABSTRACT - No-till systems with the use of leguminous cover crops promote a greater accumulation of soil C in both the light and occluded fractions, whereas the soil disturbance from conventional tillage tends to reduce the stock. To test this hypothesis, an experiment was conducted over 11 years to evaluate the influence of cropping systems and cover crops on total organic carbon and its fractions. The main plots comprised three cropping systems (conventional tillage (CT), minimum tillage (MT) and no-till (NT)), while the subplots consisted of three cover crops (black oats (BO), forage radish (FR) and hairy vetch (HV)), in a randomised block design with three replications. The following were quantified in the soil from September to November 2022: total organic carbon (TOC), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) at a depth of 0 – 100 cm (divided into eight layers). The cropping systems had no effect on the TOC content; however, stocks were higher under CT in the 10 – 20 cm layer. HV produced greater levels and stocks of TOC in the 20 – 30 cm layer and in the cumulative total down to 100 cm. Stocks of soil C showed no increase under NT. CT reduced POC stock in the 0 – 5 cm layer only, but did not reduce the stocks of TOC or MAOC. Under HV, TOC and MAOC, stocks increased in the cumulative 0–30 cm (72.59 Mg ha^{-1} and 66.58 Mg ha^{-1}) and 0 – 100 cm (145.23 Mg ha^{-1} and 137.12 Mg ha^{-1}) profiles, with MAOC stocks increasing in the 20 – 30 cm layer (19.92 Mg ha^{-1}).

Keywords: No-till. Vetch. Particle-size fractionation. Total organic carbon.

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INTRODUCTION

The area under no-till farming in Brazil is approximately 33 million hectares (Fuentes-Llanillo *et al*., 2021). Of this total, it is estimated that at most 20% follow the recommended practices for no-till farming (Possamai *et al*., 2022), with the main neglected aspects including a failure to use cover crops and green manure combined with periods of fallow, which compromises the levels of soil organic matter (SOM). Minimal soil disturbance and the adoption of cover crops afford complementary benefits for the system, restoring the carbon (C) and nitrogen (N) originally present in the soil (Tiecher *et al*., 2020). The ability of leguminous plants to supply the system with N, favours the accumulation of soil C, suggesting that the residue produced by these plants is of higher quality (Veloso *et al*., 2018).

SOM is composed of a wide range of compounds derived from plants, microorganisms, microbial biomass and crop residue, and can be divided into particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) (Cambardella; Elliott, 1992). This is a widely used method for assessing SOM dynamics (Lavallee; Soong; Cotrufo, 2020) due to the practicality and simplicity of obtaining the SOM fractions, as well as the sensitivity of the different fractions to soil management practices (Locatelli *et al*., 2022). The main difference between the two SOM compartments relates to protection against decomposition, where MAOC is protected through association with minerals, which includes bonds between SOM and the mineral surface, as well as its occlusion in soil microaggregates (<50 – 63 µm), while POC is unprotected (Lavallee; Soong; Cotrufo, 2020). These differences allow POC and MAOC to reflect the influence of the management practices and systems in different ways. The clay content and the iron and aluminium oxides and hydroxides can affect the protective ability of the SOM, affecting its decomposition (Roscoe; Buurman, 2003), which also depends on the period of cultivation.

Piva *et al*. (2020) reported no differences in carbon stocks between no-till (NT) and conventional tillage (CT) systems after 3.5 years on a brown Latosol in the south of Brazil. Conversely, Bayer *et al*. (2004) found that NT increased the stock of total organic carbon (TOC), particularly the POC fraction, which is more sensitive than TOC over short periods (6 years), finding no effects on the MAOC fraction in an oxidic clayey Latosol, suggesting a longer evaluation period might be required.

The present study tested the hypothesis that NT results in greater stocks of soil C in both the light and occluded fractions due to the long duration of the experiment. A reduction in C stocks in the light fraction was also expected as a result of the soil disturbance under CT, with fewer alterations in the occluded fraction as this is physically protected. There is also a tendency for more C to accumulate in the soil when leguminous cover crops are grown, owing to the increased nitrogen input. The aim of this study was to assess the influence of soil management systems together with winter cover crops on the concentration and stocks of total soil organic carbon and its fractions in a long-term experiment.

MATERIAL AND METHODS

The experiment was set up in 2011 in the Experimental area of the Federal Technological University of Paraná (UTFPR) (26º10’35” S; 52º41’27” W) in Pato Branco in the state of Paraná. According to the Köppen classification, the climate in the region is subtropical (type Cfa), with an average annual temperature of 18.8 ºC and average annual rainfall of 2098 mm (IDR-Paraná, 2022). The soil is classified as a Red Latosol with a very clayey texture (770 g kg^{-1} clay), comprising kaolinite and chlorite (unpublished data), at an altitude of 730 m. Chemical analysis of the soil showed the following composition: organic matter – 49.1 g dm^{-3}; P – 15.2 mg dm^{-3} (Mehlich-1); K^+ – 0.5 $cmol_c$ dm^{-3} (Mehlich-1); Ca^{2+} – 5.73 $cmol_c$ dm^{-3}; Mg^{2+} – 3.8 $cmol_c$ dm^{-3}; H + Al^{3+} – 2.95 $cmol_c$ dm^{-3}; pH ($CaCl_2$) – 5.5; and base saturation by Ca^{2+}, Mg^{2+} and K^+ of 44%, 29% and 3.8%, respectively. The area had been used for cultivating soya beans and maize during the summer and wheat during the winter since the early 1980s. In 2004, no-till farming was introduced, and continued until the experiment was set up. The evaluations presented here cover the 11th and 12 th years after the start of the experiment (2022 and 2023).

A randomised complete block design with a split-plot arrangement and three replications was used. The cultivation systems were established in the main plots (6 m wide × 30 m long): (a) Conventional tillage (CT), prepared by one ploughing and two passes with a levelling harrow, followed by sowing using a no-till seeder-fertiliser for both the summer and winter crops; (b) No-till (NT), where sowing was carried out using a no-till seeder-fertiliser for both the summer and winter crops; (c) Minimum tillage (MT), where the winter crops were broadcast, followed by one pass of the harrow to incorporate the seeds, while the summer crop was planted using the no-till method. Ploughing was carried out using a reversible three-disc plough, with a disc diameter of 64 cm, and harrowing using a levelling harrow equipped with 26 disks, each 46 cm in diameter, arranged in two V-shaped rows. The following ground cover crops were established in the subplots (6 m wide × 10 m long): forage radish (*Raphanus*

sativus) (FR), black oats (*Avena strigosa*) (BO) and hairy vetch (*Vicia villosa*) (HV).

The cover crops were sown in May each year using a Semeato SHM 11/13 winter crop seeder at a row spacing of 17 cm and without the use of fertiliser. The seeding rates for viable seeds (adjusted for 80% germination) were 25 kg ha^{-1} for FR, 70 kg ha^{-1} for BO, and 55 kg ha^{-1} for HV; the exception was the MT system described above, where the seeds for the cover crops were broadcast and then incorporated by light harrowing. During the summer growing season, from the start of the experiment in 2011 to the end of the 2021/2022 season, the area was planted with maize, while soya beans were planted in the 2022/2023 season. When planting the summer crops, fertilisation was based on the soil analysis.

To determine the dry matter (DM) production of the winter cover crops, the plants were cut close to the ground on 22 September 2022 and 24 August 2023 using a square frame with an area of 0.25 m². Once cut, the plants were placed in paper bags and dried in a forced air circulation oven at 60 °C to constant weight, at which point they were weighed and the values extrapolated to kg DM ha^{-1}.

To evaluate the concentration and stocks of carbon (C), soil samples were collected from September to November 2022 to a depth of 100 cm, stratified into the following layers: 0 – 5, 5 – 10, 10 – 15, 15 – 20, 20 – 30, 30 – 45, 45 – 60, 60 – 80 and 80 – 100 cm. Bulk density was determined when collecting the samples using the excavation method adapted from Blake and Hartge (2018).

All the sampled layers were weighed to determine bulk density. A subsample of approximately 800 g was collected, air-dried, passed through a 2-mm mesh sieve, and placed in sealed plastic containers for later laboratory analysis. The wet combustion method (Walkley; Black, 1934), adapted from Tedesco *et al*. (1995), was used to determine the concentration of total organic carbon (TOC). Soil moisture was determined by oven-drying a sample of approximately 20 g of soil for 48 hours at 105 °C. The method described by Cambardella and Elliott (1992) was used for the particle-size fractionation of the SOM. The organic carbon stock was calculated employing the method described by Sisti *et al*. (2004) and using the black oats treatment under conventional tillage as reference, as shown in the following formula:

$$C = \sum_{i=1}^{n-1} C_i + \left[M_n - \left(\sum_{i-1}^{n} M_i - \sum_{i-1}^{n} M_{Ri}\right)\right] \cdot \left[\frac{C_n}{M_n}\right] \quad (1)$$

In Equation 1, all the variables are expressed in Mg ha^{-1}:

$\sum_{i=1}^{n-1} C_i$ is the sum of C stock fro;

M_n m the first to the penultimate layer;

$\sum_{i=1}^{n} M_i$ is the mass of the soil in the deepest layer;

$\sum_{i=1}^{n} M_{Ri}$ is the total mass of the soil from the first to the deepest layer in the reference treatment;

C_n is the C stock in the deepest layer.

The stocks of total organic carbon (TOC), mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) were calculated for each soil layer, as well as the accumulated C stock at depths of 0 – 30 cm and 0 – 100 cm.

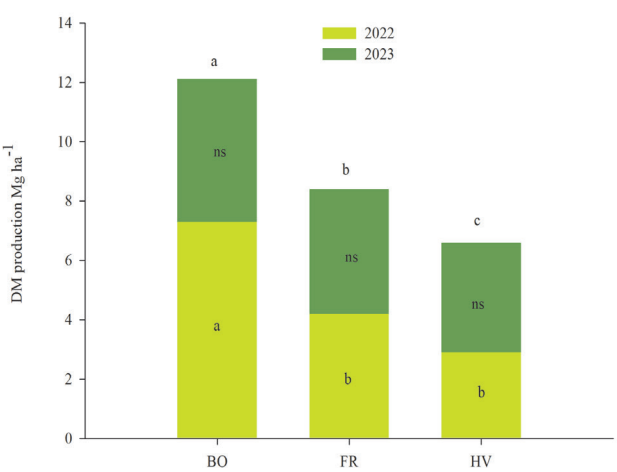
The results were submitted to an analysis of normality (Lilliefors test) and homogeneity (Bartlett's test). Whenever the assumptions were not met, the data were transformed, and an analysis of variance (ANOVA) and the F-test were carried out at a level of 5% using the Genes software. When the results were significant, Tukey's test was applied at a level of 5%. The graphs were prepared using the Excel and SigmaPlot 10.0 software.

RESULTS AND DISCUSSION

Cover crop dry matter

There was no interaction between the cover crops and cropping systems, nor was there an isolated effect of the cropping systems on the production of dry matter from the cover crops over the two years of the evaluation. In 2022, black oats outperformed the other species, producing 7.3 Mg ha^{-1} dry matter, followed by the forage radish (4.2 Mg ha^{-1}) and hairy vetch (2.9 Mg ha^{-1}). Over the two-year period, the black oats reached 12 Mg ha^{-1} and outperformed the other crops despite the cover crops having no significant effect in 2023, with a mean dry-matter production that year of 4.2 Mg ha^{-1} (Figure 1).

Figure 1 - Dry matter (DM) production as a function of the cover crops (BO = black oat; FR = forage radish; HV = hairy vetch) in 2022 and 2023. Mean values followed by the same letters in the same year or in the two-year cumulative total, do not differ significantly at p < 0.05 by Tukey's test. ns = not significant



Wolschick *et al*. (2016), in an experiment conducted on the southern plateau of the state of Santa Catarina, report similar DM production for the forage radish, albeit with greater DM production for black oats and vetch (9.91 and 9.14 Mg ha^{-1}, respectively). On the other hand, Piva *et al*. (2021) found that DM production for the vetch (2.5 Mg ha^{-1}) was similar, while for the black oats and forage radish, production was respectively lower at 2.9 and 2.1 Mg ha^{-1}, based on the five-year average of an experiment conducted in the district of Curitibanos in the central region of Santa Catarina. In the present study, cover crop DM production in 2022 was hampered by a pest attack that occurred shortly after plant emergence and which delayed development and consequently compromised DM production.

Even though there was no significant difference, DM production in the oats in 2023 (4.7 Mg ha^{-1}) was higher than that of the other crops, but 35% lower than obtained in 2022. The lower DM production in 2023 was due to greater disease pressure, particularly leaf and stem rust (*Puccinia coronata* var. *avenae*). The higher average temperature during the winter of 2023 (IDR-Paraná, 2022) exacerbated the damage caused by the disease and impaired crop development, resulting in a reduction in DM productivity.

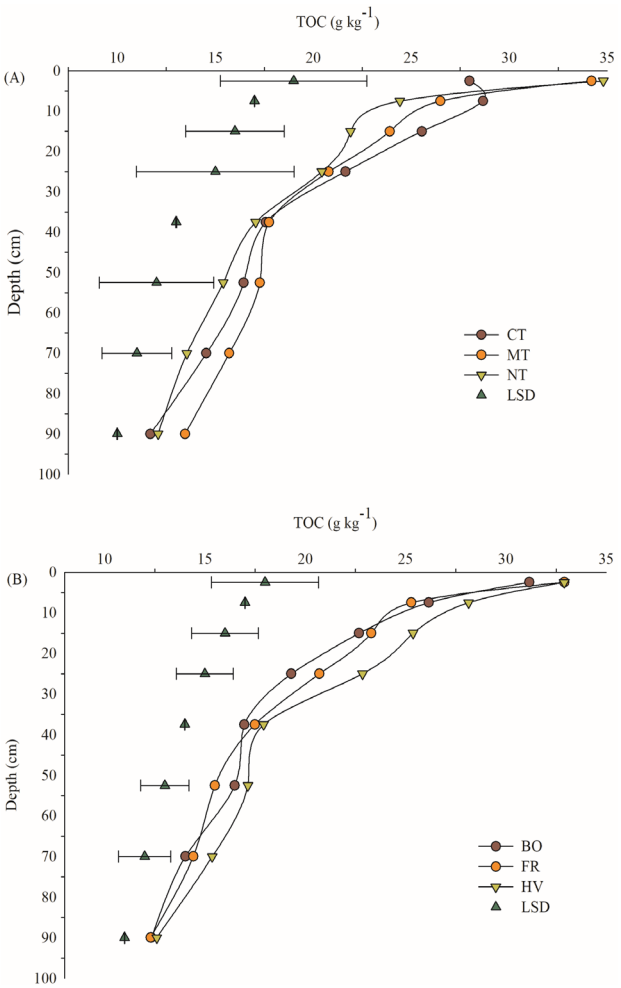
In the case of the forage radish, dry matter (DM) production was similar between 2022 and 2023, at around 4.0 Mg ha^{-1}. The greatest increase in DM production was seen in the hairy vetch, which yielded 3.7 Mg ha^{-1} in 2023, 30% more than in 2022.

TOC Concentration

The cropping systems had no effect on the TOC concentration in any of the soil layers under analysis (Figure 2A). This differs from the findings of other authors, who report that the adoption of no-till farming leads to changes in TOC concentration throughout the soil profile, with an increase in concentration in the surface layers compared to conventional tillage, decreasing as the depth increases (Tiecher *et al*., 2020; Xiao *et al*., 2020). Despite there being no significant difference, the TOC concentration in the 0 – 5 cm layer under the no-till system (34.80 g kg^{-1}) (Figure 2A) was 24.4% higher than under conventional tillage (27.97 g kg^{-1}); this is similar to the findings of Piva *et al*. (2020), who reported a TOC concentration of 34.9 g kg^{-1} in the 0 – 5 cm layer under a no-till system, 12.6% higher than under conventional tillage, in a brown Latosol in the region of Castro in the state of Paraná.

Although there was again no significant difference, higher TOC concentrations were seen in the 5 – 10 cm and 10 – 20 cm layers under the conventional tillage system compared to the no-till system, of 17.4% and 16.6%, respectively. From a depth of 20 cm down to 100 cm, the TOC concentration decreases continuously across each of the cropping systems, with no major differences between them. In the 80 – 100 cm layer, TOC levels represented 41.8% (11.69 g kg^{-1}) and 34.7% (12.08 g kg^{-1}) of the values found in the 0 – 5 cm layer under the conventional tillage and no-till systems, respectively. This shows that the influence of the cropping system on the TOC concentration occurs mainly in the surface layers of the soil. In a thirty-year experiment conducted in the subtropical climate of the state of Rio Grande do Sul, Veloso *et al*. (2018) also reported TOC concentrations that were 56% higher under the no-till system in the 0 – 5 cm layer, equal in value in the 5 – 10 cm layer, and 28.6% and 12.8% higher under conventional tillage compared to the no-till system in the 10 – 15 cm and 15 – 20 cm layers, respectively, possibly a result of soil disturbance under that system.

Figure 2 - TOC concentration (g kg^{-1}) at depths of 0 – 5, 5 – 10, 10 – 20, 20 – 30, 30 – 45, 45 – 60, 60 – 80 and 80 – 100 cm, as a function of the cropping systems (A) and cover crops (B). Horizontal bars represent the LSD by Tukey's test at $p < 0.05$



The cover crops affected the TOC concentration in the 20 – 30 cm layer only, with the highest levels for HV (22.85 g kg^{-1}) and not differing from the FR treatment (Figure 2B). Although differences were not statistically significant in the other layers, the highest TOC levels continued to be seen in the hairy vetch treatment (Figure 2B), while the black oats resulted in lower TOC levels at five of the eight depths sampled.

Locatelli *et al*. (2020), working on a clayey-textured Cambisol in the region of Curitibanos in Santa Catarina, where the mean annual temperature ranges from 11.6 °C to 20.8 °C, also reported higher TOC levels in the 30 – 45 cm and 45 – 60 cm layers with the use of hairy vetch during a four-year experiment. They also found TOC concentrations ranging from 17.29 g kg^{-1} in the 0 – 5 cm layer to 11.59 g kg^{-1} in the 45 – 60 cm layer. These values are lower than those found in the present study, which ranged from 32.90 to 12.60 g kg^{-1} for the 0 – 5 cm and 80 – 100 cm layers, respectively. This result highlights the importance of time in TOC accumulation (Tiecher *et al*., 2020), which, together with the higher clay content (770 g kg^{-1}) found in this Red Latosol, affords greater protection against degradation (Roscoe; Buurman, 2003).

Despite the lower DM production found in this study, the use of hairy vetch in the system tends to have a beneficial effect on the TOC concentration, as shown by Veloso *et al*. (2018), where replacing black oats with hairy vetch in the production system increased the TOC concentration by 29% and 18% in the 0 – 5 cm and 5 – 10 cm layers, respectively. Legumes are associated with an increase in the quantity and lability of the added residues, which promote organo-mineral associations in the fine particles of the soil (Veloso; Cecagno; Bayer, 2019).

POC Concentration

POC is highly sensitive to both environmental and management conditions. Since it is an SOM fraction composed primarily of undecomposed residue, it is natural for higher concentrations to occur in the surface layers of the soil where the crop residue is deposited. An interaction with respect to POC was found between the cover crops and cropping systems in the 0 – 5 cm layer (Table 1).

The highest POC concentration in the 0 – 5 cm layer was seen with the combination of forage radish (FR) and hairy vetch (HV). Under the NT system, hairy vetch showed the highest POC levels, while the lowest levels were found under CT, unaffected by cover crops, since ploughing and harrowing tend to increase the exposure of organic matter to degradation by soil microorganisms, which can reduce the POC concentration as this SOM fraction is the most susceptible to degradation. In the 0 – 5 cm layer, CT reduced the POC concentration by 58.6% compared to MT, confirming that this SOM fraction is highly sensitive to changes in management.

Except for the surface layer (0–5 cm), conventional tillage (CT) showed higher, albeit not significant, POC levels down to a depth of 30 cm, in line with the findings of Tivet *et al*. (2013), who reported higher POC concentrations under this system in the 10 – 20 and 20 – 40 cm layers, the result of redistribution of the TOC by successive operations with the harrow. From 30 cm down to 100 cm, the no-till system once again showed a trend towards higher POC levels compared to both conventional and minimum tillage (Figure 3A).

The cover crops had an effect on the POC in the 5 – 10 cm layer only, with higher levels seen for BO (2.61 g kg^{-1}), which did not differ from FR (Figure 3B). In the subsurface layers, there was a reduction in POC concentration for both the cropping systems and cover crops (Figure 3A, 3B).

Accumulated stocks of TOC, POC and MAOC in the 0 – 30 cm and 0 – 100 cm layers

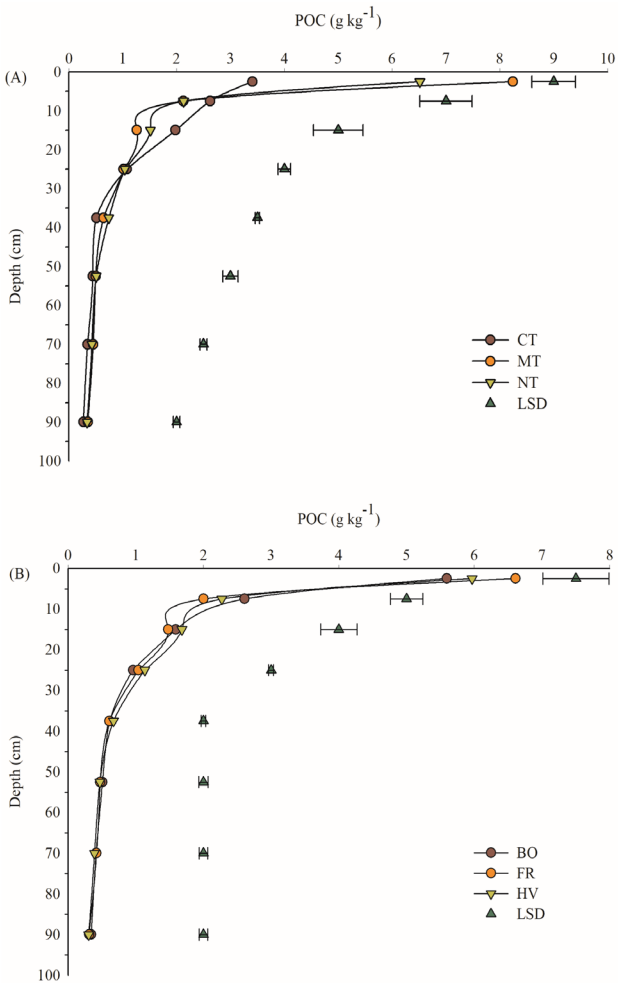
Eleven years of the no-till system were insufficient to cause any significant changes in the stocks of TOC, POC, or MAOC (Figure 4A, 4B), in contrast to the results of other studies in the south of Brazil (Sá *et al*., 2014; Tiecher *et al*., 2020); nor was there any interaction between the cover crops and cropping systems in terms of accumulated stocks in the 0 – 30 cm and 0 – 100 cm layers (Figure 4).

Table 1 - Effect of the cropping systems (conventional tillage – CT, minimum tillage – MT and no-till – NT) and cover crops (black oats – BO, forage radish – FR and hairy vetch – HV) on the Particulate Organic Carbon (POC) concentration (g kg^{-1}) at a depth of 0 – 5 cm

Cropping System	Cover Crops		
	BO	FR	HV
	---------- g kg^{-1} ----------		
CT	3.13 Ab	3.56 Ac	3.54 Ab
MT	7.38 Ba	10.51 Aa	6.83 Ba
NT	6.26 ABa	5.75 Bb	7.53 Aa

Mean values followed by the same letter, uppercase for the rows and lowercase for the columns, do not differ significantly at $p < 0.05$ by Tukey's test

Figure 3 - POC concentration (g kg^{-1}) at depths of 0 – 5, 5 – 10, 10 – 20, 20 – 30, 30 – 45, 45 – 60, 60 – 80 and 80 – 100 cm, as a function of the cropping systems (conventional tillage – CT, minimum tillage – MT and no-till – NT) (A) and cover crops (black oats – BO, forage radish – FR and hairy vetch – HV) (B). Horizontal bars represent the LSD by Tukey's test at $p < 0.05$



In terms of the TOC stock accumulated in the 0 – 100 cm layer, the value of 141.14 Mg ha^{-1} under conventional tillage is similar to that found by Veloso *et al*. (2018), who obtained values between 119.6 and 142.9 Mg ha^{-1} under CT with no nitrogen fertilisation in an Argisol in Eldorado do Sul in the state of Rio Grande do Sul, also under a subtropical climate.

The TOC stocks of 63.70 Mg ha^{-1} and 128.91 Mg ha^{-1} seen under NT for the 0 – 30 cm and 0 – 100 cm layers, respectively, were 12.2% and 8.7% lower than under CT, although the difference was not statistically significant. This result differs from other studies evaluating soil TOC (Calegari *et al*., 2008; Sá *et al*., 2014; Tivet *et al*., 2013), where no-till consistently showed greater stocks of TOC regardless of the sampling depth.

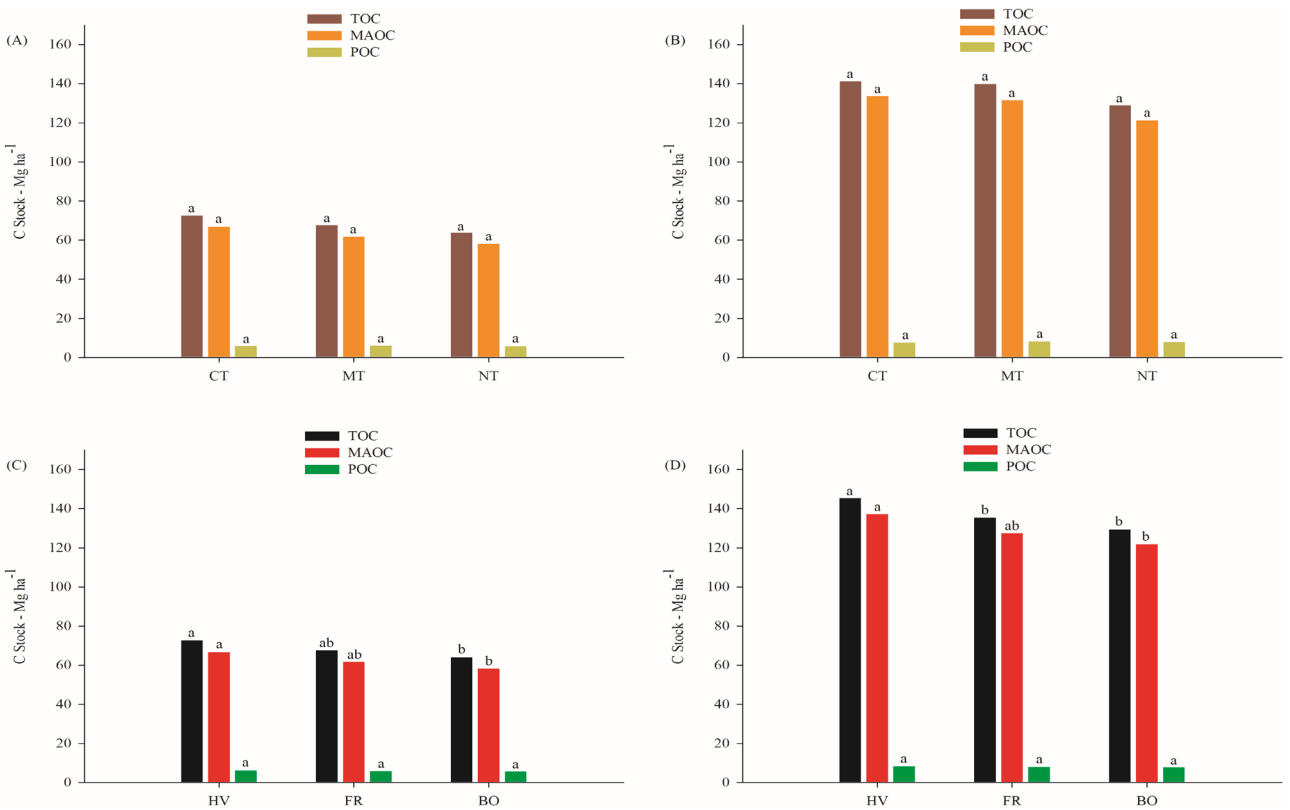
The gentle slope of the experimental site and the continuous addition of maize crop residue and winter cover crops over 11 years resulted in a significant input of carbon to the soil, which was decisive in mitigating the loss of TOC caused by the mechanical tillage operations under the CT system, leading to similar results under both NT and CT.

In this respect, considering the lower DM production obtained in this study with HV in 2022 (2.9 Mg ha^{-1}) (Figure 1), together with the 7.47 Mg ha^{-1} DM input by the maize crop that was grown each year – a value reported by Ferreira *et al*. (2018) in a study evaluating eight growing seasons on a clayey-loam Red Latosol in the region of Ponta Grossa, Paraná – the estimated average DM input is 10.37 Mg ha^{-1} yr^{-1}. This value is 30% higher than the minimum requirement for maintaining soil carbon, which is 8.0 Mg ha^{-1} yr^{-1} (Sá *et al*., 2015). The high rate of dry matter (DM) input may therefore be masking the effects of the CT system on the soil TOC, given that there was a linear increase in TOC stock in the 0 – 30 cm layer as the amount of C input into the system increased by around 2.11 Mg ha^{-1} TOC for every tonne of C input into the soil under the no-till system over the 30 years of the experiment (Veloso *et al*., 2018).

Furthermore, the mineral composition of the soil where the experiment was conducted – a Latosol with a very high clay content (770 g kg^{-1} clay), consisting of kaolinite and Fe and Al oxides – may explain the lack of difference between the cropping systems, since the ability to protect the SOM is positively correlated with the clay content of the soil (Roscoe; Buurman, 2003). The ability of clay minerals to interact with the SOM on their surface, together with the formation of aggregates with the SOM, are the primary mechanisms determining the preservation of SOM in clayey soils. According to Roscoe and Buurman (2003), the composition of the soil was a determining factor in the lack of any differences in TOC stock in the 0 – 45 cm layer of a Latosol in the Brazilian Cerrado after 30 years of cultivation.

An important point to consider when determining TOC stock is the sampling depth, as the deeper layers of the soil profile, even when not directly influenced by management practices, account for a significant portion of the TOC stock. Considering the stock of 128.91 Mg ha^{-1} under NT at a depth of 0 – 100 cm, 50.6% was found below 30 cm (Figures 4A, 4B). This is in line with the results of Tiecher *et al*. (2020), who reported that, in an experiment conducted in the same district as the present study under the same conditions of soil and climate, 61% of the TOC found at a depth of 0 – 100 cm was stored in the 20 – 100 cm layer.

Figure 4 - Accumulated stocks of TOC, MAOC and POC as a function of the cropping systems (conventional tillage – CT, minimum tillage – MT and no-till – NT) in the 0 – 30 cm (A) and 0 – 100 cm (B) layers, and of the cover crops (black oats – BO, forage radish – FR and hairy vetch – HV) in the 0 – 30 cm (C) and 0 – 100 cm (D) layers. Mean values followed by the same letters within each variable (TOC, MAOC, POC) do not differ at $p < 0.05$ by Tukey's test



The cropping systems also had no effect on POC and MAOC stocks after 11 years of cultivation, with the latter accounting for 91% and 94% of the TOC in the 0 – 30 cm and 0 – 100 cm layers, respectively (Figures 4A and 4B). Successive tillage, carried out twice a year for eleven years under the CT system, would be expected to result in a loss of soil C, reflecting in smaller stocks. However, although not statistically significant, stocks of MAOC were 10.4% greater under CT than under NT.

In line with this, Bayer *et al*. (2004) also found no difference in mineral-associated C between NT and CT in the 0 – 20 cm layer, which they attributed to the short duration of the experiment (six years) being insufficient to cause degradation of this TOC fraction, given that this fraction is chemically highly stable due to its interaction with the mineral fraction of the soil and is protected physically by its location inside microaggregates.

Despite the lack of significant results in terms of the cropping systems, Tiecher *et al*. (2020) report that the presence of cover crops promotes greater changes in C stocks than do the cropping systems. This was seen in the present study, where the cover crops had an effect on the TOC and MAOC in both the 0 – 30 cm and 0 – 100 cm layers (Figures 4C and 4D).

Greater stocks of total organic carbon (TOC) were seen in the hairy vetch (HV) treatment in both the 0 – 30 cm and 0 – 100 cm layers (Figures 4C and 4D). In the 0 – 30 cm layer, the TOC stock under HV was 13.6% greater than under black oats (BO), a similar result to that reported by Veloso *et al*. (2019), who found a 12.7% increase in TOC stock in a sandy-textured Argisol cultivated with hairy vetch compared to black oats.

Although not reported in this study, values for soil bulk density were low (close to 1.0 kg dm^{-3}) and did not differ between treatments. This behaviour is possibly related to the excavation method used to determine soil bulk density (Blake; Hartge, 2018), whose precision may be limited under certain experimental conditions. It can therefore be inferred that the greater carbon stock seen in the HV treatment is not associated with the differences in soil bulk

density, but rather with the intrinsic effects of the legume, particularly due to the nitrogen input into the system. The supply of N by legumes promotes nutrient cycling and stimulates TOC accumulation in the soil, as pointed out by Calegari *et al*. (2008).

According to Locatelli *et al*. (2020), the abundant root system of the vetch promotes C accumulation in the subsurface layers, the roots playing a more important role in adding C to the soil than do the shoots, as they grow and accumulate directly within the soil matrix, whereas shoot residue is deposited on the surface of the soil (Alburquerque *et al*., 2015) and is therefore susceptible to loss. High concentrations of TOC and its fractions are the result of the N input from crop residue, showing that under a no-till system, SOM is also affected by N availability and not solely by the C deposited by the cover crops (Raphael *et al*., 2016).

In terms of cover crops, POC stock under HV was 6.4% and 5.7% greater than under BO in the 0 – 30 cm and 0 – 100 cm layers, respectively. For Campos *et al*. (2011), the addition of legumes and cruciferous plants resulted in a 17.9% increase in POC stock in the 0 – 30 cm layer.

Among the cover crops, the greatest stocks of MAOC in the 0 – 30 cm and 0 – 100 cm layers were obtained with HV, 14.2% and 12.7% greater, respectively, than with BO. Leguminous cover crops are linked to an increase in the quantity and lability of the added residue, promoting organo-mineral associations in the fine particles of the soil, particularly when combined with no-till management (Veloso; Cecagno; Bayer, 2019).

TOC, POC and MAOC stocks by individual soil layer

There were no significant changes in TOC stock, nor was there any interaction between the cover crops and cropping systems (Table 2), except at a depth of 10–20 cm, where conventional tillage resulted in greater stocks than under the no-till system; this may be related to the incorporation of residues into the deeper layers of the soil.

Calegari *et al*. (2008) also report a reduction of 9.7% in TOC stocks in the 10 – 20 cm layer under the no-till system compared to conventional tillage. The incorporation of crop residue under conventional tillage increases contact between the carbon input by the residue and the clays present in the soil, accelerating the interaction process between them and promoting greater stocks of TOC under conventional tillage due to the C associating with the minerals in those soil layers that correspond to the working depth of the implements.

As POC is less protected from microbial activity, environmental changes that might reduce microbial inhibition or cause aggregate breakdown (soil disturbance) may immediately increase the rate of POC decomposition (Lavallee, Soong; Cotrufo, 2020). There was an interaction between the cover crops and cropping systems in terms of the POC stocks in the 0 – 5 cm layer (Table 3), albeit in none of the other layers.

The soil disturbance under CT reduced the stock of POC in the surface layer, since the lowest POC stocks were obtained under CT: a reduction of 48% to 34% compared to MT and NT, respectively. Campos *et al*. (2011) also report a reduction in POC stock of 47% in the 0 - 5 cm layer when comparing the no-till system with conventional tillage. This shows that the main factor determining POC accumulation is soil disturbance, where a reduction in soil disturbance is important for C accumulation in the particulate fraction of the SOM, which represents the main substrate that guarantees the mineralisation of nutrients by the soil biota (Souza; Figueiredo; Sousa, 2016).

There was no effect from the cropping systems or the interaction between the cropping systems and the cover crops on the stock of MAOC. There was, however, a positive effect from the cover crops in the 20 - 30 cm layer (Table 4).

The greatest stock of MAOC was found in the 20 – 30 cm layer with hairy vetch as the cover crop (19.92 Mg ha^{-1}), showing no statistical difference to the forage radish. The smallest stock of MAOC was seen with black oats (16.89 Mg ha^{-1}), which again did not differ statistically from the forage radish. There were no differences in MAOC stock between the cover crops in the other layers under analysis (Table 4).

Table 2 - Stocks of total organic carbon (TOC) (Mg ha^{-1}) at depths of 0 – 5, 5 – 10, 10 – 20, 20 – 30, 30 – 45, 45 – 60, 60 – 80 and 80 – 100 cm under the conventional tillage (CT), minimum tillage (MT) and no-till (NT) systems

Cropping System	Soil depth (cm)							
	0 - 5	5 - 10	10 - 20	20 - 30	30 - 45	45 - 60	60 - 80	80 - 100
	Stock of total organic carbon (TOC) (Mg ha^{-1})							
CT	11.46 a	14.11 a	26.27 a	20.70 a	18.29 a	18.13 a	7.50 a	14.68 a
MT	11.42 a	13.46 a	3.21 ab	19.61 a	18.39 a	17.85 a	19.47 a	16.28 a
NT	11.59 a	11.68 a	22.19 b	18.25 a	17.17 a	16.22 a	16.67 a	15.15 a

Mean values followed by the same letter in a column do not differ significantly at $p < 0.05$ by Tukey's test

Table 3 - Stocks of particulate organic carbon (POC) (Mg ha^{-1}) at a depth of 0 – 5 cm, as a function of different cropping systems (conventional tillage – CT, minimum tillage – MT and no-till – NT) and cover crops (black oats – BO, forage radish – FR, and hairy vetch – HV)

Cropping System	Cover Crops		
	BO	FR	HV
	---------- Stocks of particulate organic carbon (POC) (Mg ha^{-1}) ----------		
CT	1.21 Ab	1.74 Ab	1.34 Ab
MT	2.46 Ba	3.32 Aa	2.51 Ba
NT	2.03 Aa	1.94 Ab	2.53 Aa

Mean values followed by the same letter, uppercase for the rows and lowercase for the columns, do not differ significantly at $p < 0.05$ by Tukey's test

Table 4 - Stocks of mineral associated organic carbon (MAOC) (Mg ha^{-1}) at depths of 0 - 5, 5 - 10, 10 - 20, 20 - 30, 30 - 45, 45 - 60, 60 - 80 and 80 - 100 cm as a function of different cover crops (black oats – BO, forage radish – FR and hairy vetch – HV)

Cover Crops	Depth (cm)							
	0 - 5	5 - 10	10 - 20	20 - 30	30 - 45	45 - 60	60 - 80	80 - 100
	--------- Stock of mineral associated organic carbon (MAOC) (Mg ha^{-1}) ---------							
BO	8.73a	11.37 a	21.27 a	16.89 b	17.18 a	15.48 a	16.98 a	13.75 a
FR	9.78 a	11.86 a	21.21 a	18.81 ab	16.98 a	16.45 a	16.62 a	15.63 a
HV	9.60 a	12.64a	24.42 a	19.92 a	17.74 a	18.74 a	18.53 a	15.54 a

Mean values followed by the same letter in a column do not differ significantly at $p < 0.05$ level by Tukey's test

Including leguminous cover crops in crop rotations tends to reflect in greater stocks of MAOC, as reported by Santos *et al*. (2011) in a 17-year experiment in the district of Ponta Grossa, Paraná. Among the various combinations of pasture and/or cover crops that make up the various crop-rotation schemes, replacing black oats with vetch in the black oat/maize/wheat/soya bean rotation resulted in a 12.1% increase in MAOC stock in the 10–20 cm layer.

In the Latosol under study in this experiment, MAOC accounted for 81.4% of the TOC in the 0–5 cm layer, increasing linearly with the sampling depth to 97.2% in the 80 – 100 cm layer (average across the cover crops under the no-till system). A similar result is reported by Souza *et al*. (2016), who noted that MAOC accounted for 86% of the TOC in the 0 – 20 cm layer. A high proportion of MAOC may indicate a long soil C residence time and greater soil C storage capacity (Gulde *et al*., 2008). According to Santos *et al*. (2014), the presence of a high proportion of MAOC in the TOC demonstrates the strong interaction of organic matter with iron and aluminium oxides and with kaolinite, affording the TOC significant protection against the processes of degradation.

The high proportion of MAOC found in the present experiment suggests that changes in TOC are determined by changes in the MAOC. Given the strong interaction between clay and soil C as well as the longer time required to detect differences in TOC stocks, further studies are necessary to evaluate different conditions for inputting residues to highly clayey and oxidic soils under a no-till system in a subtropical climate.

CONCLUSIONS

1. There was no increase in the stocks of TOC, MAOC or POC under the NT system, either in the individual layers or accumulated in the 0 – 30 cm and 0 – 100 cm layers;
2. Under the TC system, the stocks of POC were reduced in the 0 – 5 cm layer only for each of the cover crops under test;
3. There was no reduction in TOC or MAOC stocks under TC, either in the individual layers or accumulated in the 0 – 30 cm and 0 – 100 cm layers;
4. The use of HV increased the stocks of MAOC in the 20 – 30 cm layer and of TOC and MAOC accumulated in the 0 – 30 cm and 0 – 100 cm layers.

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DATA AVAILABILITY

The data are available upon request to the corresponding author.

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