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Carbon and nitrogen stocks in a Rhodic Nitisol under different tillage methods and mineral and organic fertilizers

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ABSTRACT: Changes in soil management, for example by more vigorous crops, adoption of conservation tillage and optimization of fertilization, can increase soil organic carbon (SOC) and total nitrogen (TN) stocks. We hypothesized that corn - black oat rotation under no-tillage (NT) and adequate soil fertilization can increase these stocks, compared to conventional tillage (CT). This study compared these two tillage methods and organic with mineral fertilizers, regarding their effects on C and N cycling and SOC and TN stocks in a Rhodic Nitisol in southern Brazil. The study started in 2012, in a pasture area, which was converted into corn (*Zea mays* L.) - black oat (*Avena strigosa* Scherb.) rotation. The treatments were applied in a 2 × 5 factorial arrangement, consisting of two soil tillage methods (NT and CT) and five fertilizers (pig slurry (PS); biodigested PS (PS-B); composted PS (PS-C); mineral fertilizer; and a control). From 2019 onwards, treatment PS-B was replaced by injected PS (PS-I) and PS-C by poultry litter (PL). A randomized block design was used in a split-plot arrangement, where the plots corresponded to soil tillage and subplots to fertilization. In every year of the study, corn was fertilized with 140 kg N ha^{-1} and at least 115 kg P_2O_5 ha^{-1} and 77 kg K_2O ha^{-1}. Total SOC and TN stocks were determined in six soil layers (0.00-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40 and 0.40-0.60 m) whereas the soil particulate (POC and PN) and mineral-associated (MAOC and MAN) fractions were evaluated in the four upper layers (0.00-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30m) at the beginning of the study (2012) and after nine years (2021). The cumulative values under NT showed that SOC stocks nearly doubled, compared to those under CT. These increases occurred in the most labile POC and PN fractions. However, no difference in response to the different fertilizers was observed in these stocks. The studied factors indicated a marked effect of soil tillage on alterations in C and N stocks. No-tillage increases SOC and TN stocks, mainly in the most labile fractions (POC and PN) of Rhodic Nitisols in southern Brazil, under corn - black oat rotation.

Keywords: soil management, organic fertilizers, particulate organic matter, mineral-associated organic matter.

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INTRODUCTION

Soil is one of the largest active terrestrial carbon (C) reservoirs, as it contains approximately 2,400 Pg of soil organic carbon (SOC), a constituent of soil organic matter (SOM), whereas the atmosphere contains 760 Pg and vegetation 550 Pg of carbon (IPCC, 2007). Therefore, changes in soil management, such as the implementation of vigorous annual crops and conservation tillage systems, can positively affect C stocks and distribution in the soil profile (Vezzani and Mielniczuk, 2011; Man et al., 2021; Tiefenbacher et al., 2021).

With regard to C and N stocks, conservation and regenerative soil and agricultural management systems can increase inputs and reduce losses from SOM and enlarge these stocks. These systems can contribute to mitigate greenhouse gas emissions (GGE) and favor the adaptation of agricultural systems to climate changes, due to the numerous co-benefits (Tiefenbacher et al., 2021).

Soil conservation tillage systems, such as no-tillage (NT), contribute to SOC accumulation by enhancing the SOM content. This is the result of physical protection mechanisms within soil aggregates, since these structures are preserved due to the absence of soil disturbance. This absence prevents aggregate breakage, and the maintenance of crop residues on the soil surface reduces soil exposure to rainfall impact (Six et al., 1999; Bayer et al., 2000; Lovato et al., 2004). A study of Pinheiro et al. (2015) on tropical soils showed that SOM accumulation under NT is higher than under CT, even when crop residue are scarce, which reinforces the importance of conservation practices.

Apart from tilling or not tilling, a sound soil fertility management, along with high C input from the crop biomass (Ferreira et al., 2018), can also increase or recover SOC. Organic fertilization (Nicoloso., 2009; Mafra et al., 2014), soil acidity correction (Inagaki et al., 2017) and soil erosion reduction (Bertol et al., 2003) are examples of practices that favor SOC storage.

Soil fertilization, mainly with nitrogen (N), directly influences soil C and N input and accumulation, promotes the vegetative (root and shoot) plant development and improves biomass production (Diekow et al., 2005; Zanatta et al., 2007). Soil fertilization with organic fertilizer, alone or combined with mineral fertilizer, has a higher capacity to increase SOC stocks than the exclusive use of mineral fertilizer (Mafra et al., 2014; Rodrigues et al., 2021). In addition, the combination of organic fertilization with NT and the use of fertilizers with high organic matter stability can recover SOC quickly (Nicoloso et al., 2016). Thus, organic fertilizers with high dry matter content and C : N ratio, as those based on pig slurry (PS) and poultry litter (PL), add high quantities of C, which contributes to soil C stocks (Romanyà et al., 2012; Domingo-Olivé et al., 2016). Applications of PS by injection can reduce C and N losses in form of atmospheric emissions and volatilization, respectively, contributing to SOC storage (Federolf et al., 2017; Francisco et al., 2022).

In view of the importance of a more in-depth understanding of the separate and combined effects of soil tillage and fertilization on the temporal dynamics of soil C and N stocks, this study analyzed the effect of different soil tillage and fertilizers on C and N cycling and stocks in six layers (0.00-0.60 m) of a Rhodic Nitisol (*Nitossolo Vermelho distroférrico*) from 2012 to 2021.

MATERIALS AND METHODS

Characterization and history of the experimental area

The study was conducted in an experimental area in Concórdia, Santa Catarina State, Brazil (27° 18’ 53” S; 51° 59’ 25” W). The regional climate is humid subtropical (Cfa), according to Köppen’s classification system, with annual means of 18 °C and 1,800 mm rainfall. The soil was classified as Rhodic Nitisol, according to the World Reference

Base for Soil Resources (WRB) (IUSS Working Group WRB, 2022), which corresponds to a *Nitossolo Vermelho distroférrico*, by the Brazilian Soil Classification System (SiBCS) (Santos et al., 2018).

This study is part of a long-term experiment initiated in April 2012, in an area previously covered with natural grassland. The pasture consisted mainly of the subtropical grass *Paspalum notatum,* which was glyphosate-desiccated in April 2012. Before that, at the beginning of the study in March 2012, soil samples were collected from the surface layer (0.00-0.10 m) using a Dutch auger, to determine particle-size and chemical properties (Claessen, 1997). Clay, silt and sand contents were found to be 250, 460 and 290 g kg^{-1}, respectively, and the chemical properties: pH(H_2O) 5.3; pH_{SMP} 5.8; Al^{3+} 0.3 $cmol_c$ dm^{-3}; organic matter 39.0 g kg^{-1}; $P_{Mehlich-1}$ 6.6 mg dm^{-3}; $K_{Mehlich-1}$ 250 mg dm^{-3}; Ca^{2+} 7.5 $cmol_c$ dm^{-3}; Mg^{2+} 3.3 $cmol_c$ dm^{-3}; cation exchange capacity (CEC) 11.9 $cmol_c$ dm^{-3}; and base saturation 68 % (Grave et al., 2015).

Two weeks after pasture desiccation, the soil was plowed with a disc plow to incorporate 2.0 Mg ha^{-1} of limestone into the 0.00-0.20 m layer, to raise the soil pH to 6.0. Then, black oat (*Avena strigosa* L. (Scherb) was planted for mulch production. Treatments were first applied in October 2012, when corn (*Zea mays* L.) was planted for the first time. Corn-winter black oat was rotated in all plots in all experimental years. Corn was sown between September 15 and October 31, according to the climate conditions of each year. Black oat was sown between March 15 and April 15 and was glyphosate-desiccated at full flowering, approximately 20 days before sowing corn.

Treatments and experimental design

Treatments consisted of combinations of two soil tillage practices and five fertilizers. Tillage methods were conventional tillage (CT) and no-tillage (NT); and the fertilizers tested from 2012 to 2018 were pig slurry (PS), biodigested PS (PS-B); composted PS (PS-C); combined mineral fertilizer (NPK); and a control treatment (CTR). From 2019 onwards, treatment PS-B was replaced by injected PS (PS-I) and PS-C by poultry litter (PL). Soil under CT was prepared by disc plowing once and harrowing once before planting corn and harrowing twice before sowing black oat. The sources of N, P and K in the mineral fertilizer treatment were urea, triple superphosphate and potassium chloride, respectively.

Organic fertilizers PS and PS-I were excretes from finishing pigs raised on a pig farm of the Brazilian Agricultural Research Corporation (Embrapa Swine and Poultry), collected from open anaerobic storage tanks. All fertilizers were broadcast on the soil surface, except for PS-I, which was injected into the soil. The latter procedure was carried out with a liquid fertilizer distributor with an incorporator (Mepel), which opens a furrow in the soil into which the fertilizer is injected. Poultry litter was acquired from broiler farms, prioritizing chicken beds with at least seven finished lots.

Prio to application, the organic fertilizers were analyzed for dry matter content at 65 °C, total carbon (C) and nitrogen (TN) by dry combustion, TN and ammoniacal nitrogen ($N-NH_4$) by the Kjeldahl method and nitrate ($N-NO_3$) and nitrite contents ($N-NO_2$) by flow injection. Nutrient contents were also determined: phosphorus (P) by spectrophotometry; potassium (K) by plasma spectrometry; calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn) by atomic absorption and pH by potentiometry (Table 1). All extraction methods and analyses followed the standard protocol (Brasil, 2014).

Fertilizers were applied once a year, broadcast on the soil surface, always after black oat desiccation and soil tilling in the CT plots. Fertilizer rates were established to ensure the same amount of total N (140 kg N ha^{-1}) in the treatments, except the control treatment, which was not fertilized. It was considered that 100 % of the manure N is available for the crop to be fertilized, i.e., equivalent to mineral. From 2019 onwards, PS-C was replaced by PL and the application rate was raised to 200 kg N ha^{-1} to ensure an equivalent amount (140 kg) to the other fertilizers, based on the N release index of this fertilizer of 70 % (CQFS-RS/SC, 2016).

Table 1. Characteristics and application rates of fertilizers used in the treatments (2012-2021)

Fertilizer	Year	Characteristics											Rate
		DM	VS	TOC	TN	C/N	ON	NH_4-N	NO_3-N	Min-N	P	K	
		%	kg m^{-3}							%	kg m^{-3}		m^3 ha^{-1}
	2012	7.4	45.9	29	4.4	6.6	1.7	2.7	ND	62.0	0.7	0.9	31.7
	2013	ND	ND	15.6	4.1	3.8	0.9	3.2	ND	78.4	1.2	1.2	34.1
	2014	ND	17.2	9.3	3.0	3.1	1.0	2.0	ND	67.4	0.7	0.9	46.9
PS[(1)]	2015	ND	112	51.5	5.7	9.1	2.4	3.3	ND	58.2	2.1	0.9	24.7
	2016	ND	11.8	6.1	2.4	2.6	0.8	1.6	ND	65.6	0.3	0.7	59.1
	2017	9.9	ND	38.0	5.8	6.6	2.7	3.1	ND	53.8	1.6	0.9	24.2
	2018	ND	15.4	8.3	3.7	2.2	1.1	2.6	ND	71.1	0.8	1.1	38.1
	2019	ND	4.7	2.7	1.7	1.6	0.3	1.5	ND	85.0	0.2	0.8	81.2
	2020	ND	ND	ND	2.3	ND	0.4	1.9	ND	ND	ND	ND	71.1
	2021	ND	ND	ND	6.7	ND	ND	ND	ND	ND	ND	ND	23.9
	2012	6.5	38.4	17.7	5.2	3.4	2.5	2.6	ND	ND	3.2	0.9	27.1
	2013	ND	ND	6.3	2.6	2.5	0.5	2.1	ND	81.1	0.2	1.2	54.8
	2014	ND	8.0	4.3	1.9	2.3	0.5	1.3	ND	71.5	0.45	0.9	75.6
PS-B[(1)]	2015	ND	7.1	4.0	1.9	2.2	0.3	1.6	ND	82.7	0.23	1.0	74.5
	2016	ND	4.2	1.9	1.8	1.1	0.2	1.6	ND	88.6	0.11	0.7	78.7
	2017	0.8	ND	2.3	1.8	1.2	0.2	1.6	ND	87.4	0.1	0.9	76.0
	2018	ND	3.7	1.9	1.9	1.0	0.2	1.7	ND	91.1	0.23	1.1	73.5
	2019	ND	4.7	2.7	1.7	1.6	0.3	1.5	ND	85.1	0.22	0.8	81.2
PS-I[(1)]	2020	ND	ND	ND	2.3	ND	0.4	1.8	ND	ND	ND	ND	71.1
	2021	ND	ND	ND	6.7	ND	ND	ND	ND	ND	ND	ND	23.9
		%	g kg^{-1}							%	g kg^{-1}		Mg ha^{-1}
	2012	29.1	ND	317	16.6	19.1	15.1	1.2	0.2	8.9	3.2	5.5	29.0
	2013	47.3	ND	250	23.6	10.6	23.5	0.1	0.0	0.5	10.7	4.6	12.5
	2014	43.9	ND	378	21.6	17.5	19.8	0.8	1.0	8.5	4.6	4.8	14.8
PS-C[(2)]	2015	42.0	ND	325	18.3	17.8	18.2	0.1	0.0	0.3	6.9	3.9	18.3
	2016	30.0	ND	299	17.5	17.1	17.0	0.0	0.5	3.1	3.7	3.26	26.7
	2017	34.7	ND	268	15.7	17.0	ND	ND	ND	ND	5.5	2.92	25.7
	2018	42.2	ND	302	15.2	19.8	ND	ND	0.0	6.1	4.1	3.65	21.7
	2019	71.5	ND	308	34.0	9.1	ND	ND	0.1	19.7	9.7	14.1	8.2
PL[(2)]	2020	79.5	ND	356	42.5	8.4	ND	ND	ND	12.9	ND	ND	9.5
	2021	79.5	ND	356	42.5	8.4	ND	ND	ND	12.9	ND	ND	9.5

PS: pig slurry; PS-B: biodigested PS; PS-I: PS injected into the soil; PS-C: composted PS; PL: poultry litter; ND: not determined; DM: dry matter; VS: volatile solids; TOC: total organic carbon; TN: total nitrogen; ON: organic nitrogen; NH_4-N: ammoniacal nitrogen; NO_3-N: nitric nitrogen; C/N: total carbon to total nitrogen ratio. [(1)] expressed on a fresh basis; [(2)] expressed on a dry basis.

Nitrogen rate was calculated for an expected corn yield of 8.7 Mg ha^{-1}, corresponding to the average yield in the region (CQFS-RS/SC, 2016). As the fertilizer rate was calculated to adjust N rates, the P and K quantities varied, depending on the fertilizer source. Therefore, when the quantity of the latter two nutrients in the organic fertilizers was lower than recommended for corn, complementary mineral fertilizer (triple superphosphate and potassium chloride, respectively) was added. Thus, in all treatments except the control, 140 kg N ha^{-1} and at least 115 kg P_2O_5 ha^{-1} and 77 kg K_2O ha^{-1} were applied annually (CQFS-RS/SC, 2016).

Corn and black oat were sown using a seeder with cutting discs, subsoiler shanks and double disc openers. The plant density of corn and black oat were 60.000-65.000 ha^{-1} and 200 - 250 m^{-2}, respectively. Corn was mechanically harvested and crop residues were left on the soil surface. All other cultural practices were applied according to the usual technical recommendations for each crop.

In a split-plot arrangement, a randomized block design with four replications was used for the experiment. The 10 × 25 m plots corresponded to the two soil tillage methods and the 10 × 5 m subplots to the fertilizer sources.

Estimated carbon and nitrogen input

Carbon and N inputs via biomass production were estimated by annual sampling and determination of total dry matter produced by corn and black oat. Corn grain was sampled at physiological maturity and at harvest; four random plants per subplot were cut at the ground level. Grains of both crops were sampled and dried to constant weight at 65 °C.

Corn yield was determined by harvesting the grain of two meters of the planting row, at three points of the subplot, to blend a composite sample of each subplot. The grains were threshed and weighed; a subsample was dried at 65 °C to constant weight to determine the moisture content. The grain moisture content was adjusted to 13 % and corn yield expressed in Mg ha^{-1}

These data were used to determine the harvest index (HI), which is the grain-to-shoot weight (stalk, leaves and cob) ratio of the plant. The HI was calculated by equation 1.

$$HI = \frac{G}{(G + SW)} \quad \text{Eq. 1}$$

in which: G and SW are the grain and shoot weight, respectively (kg). Dry grain weight was used to estimate corn shoot weight per area (Mg ha^{-1}), based on the HI.

Black oat was sampled at full flowering, that is, soon before desiccation, to determine shoot dry weight. The plants were cut at the ground level in each subplot, in an area of 0.25 m^2 delimited by a metal square. Samples were dried at 65 °C to constant weight and shoot weight was expressed in Mg ha^{-1}.

In 2014, corn roots were sampled to determine the root-to-shoot weight ratio at physiological maturity. Trenches were opened to collect root samples on the edge of each subplot under CT and NT treated with mineral fertilizer. Data of these samples were extrapolated to the other treatments and years to estimate root weight in each year. Root samples were collected from soil blocks (0.80 × 0.50 m) in the layers 0.00-0.05, 0.05-0.10, 0.10-0.20 and 0.20-0.30 m. Soil blocks with 3-4 corn plants were taken from the center corn rows in the sampled area. Corn shoots were removed and then the soil blocks were crumbled by hand to preserve the roots.

Soil was removed from the roots by washing the material in tap water on a 2-mm sieve. Shoots and roots were dried at 65 °C to constant weight. The C and N contents of all shoot, grain and root samples were analyzed in the laboratory, by the same methodology as described for organic fertilizer.

Soil sampling and analyses

Soil was sampled in March 2012 and May 2021, to determine total organic carbon (TOC), total nitrogen (TN), particulate organic carbon (POC), particulate N (PN), mineral-associated organic carbon (MAOC) and mineral-associated N (MAN) contents. Samples were collected using a hydraulic auger; 5-cm diameter undisturbed soil cylinders were taken to the depth of 0.60 m. Two samples per subplot were collected, from the 0.00-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40 and 0.40-0.60 m layers. Soil particulate (POC and PN) and mineral-associated (MAOC and MAN) fractions were evaluated only in the four upper layers (0.00-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30m). Each cylinder was immediately measured and separated according to the evaluated layers, avoiding contamination between layers. The two subsamples of each layer were joined in one composite sample per subplot and weighed soon after collection.

Aliquots of approximately 10 g of each soil sample were dried at 105 °C to constant weight to determine the moisture content. This was the basis to determine the dry weight of the entire sample and, then, bulk density and soil weight of each layer (Mg m^{-3} and Mg ha^{-1}, respectively) (Wendt and Hauser, 2013).

The remaining soil of the samples was crumbled by hand, air-dried, sieved (<2 mm); roots and plant fragments were removed and the samples were stored for later analysis. Subsamples of approximately 5 g were ground in an agate mortar to determine total organic carbon (TOC) and N contents in an elemental analyzer.

Particle-size of SOM was analyzed by the method described by Cambardella and Elliott (1992). Twenty grams of soil were dispersed in 100 mL flasks containing 60 mL of a sodium hexametaphosphate solution (5 g L^{-1}). The flasks were shaken for 16 h and the dispersed soil was sieved (<53 µm) under distilled water. The retained soil fraction was transferred to an aluminum tray and dried at 65 °C to constant weight. The through-sieved fraction (>53 µm) was collected in another aluminum tray and dried at 65 °C to constant weight. Both fractions were ground in an agate mortar and then analyzed to determine soil C and N contents by dry combustion.

Carbon and N contents in the >53 µm fraction were termed particulate organic C (POC) and particulate N (PN). Carbon and N contents in the fraction <53 µm were denominated mineral-associated organic C and mineral-associated N (MAOC and MAN). Total organic carbon and TN stocks and other particle-size fractions were calculated at equivalent soil mass (Wendt and Hauser, 2013), based on the reference values of 2012 of each soil layer.

Statistical analysis

Data were subjected to analysis of variance and the means compared by Tukey's test at 5 % probability.

RESULTS

Carbon input to the soil surface

Carbon added in the crop shoots throughout the nine experimental years, together with fertilizer C, contributed to soil C stocks (Table 2). Soil tillage methods (CT and NT) did not differ in amount of C added by corn; however, in general, the fertilizers NPK, PS and PS-I increased C soil input (4.60 to 4.73 Mg ha^{-1} yr^{-1}) over the CTR. Carbon inputs were lowest in the treatments PL and CTR (4.05 and 3.67 Mg ha^{-1} yr^{-1}, respectively).

Carbon input by black oat did not differ either between the tillage methods (Table 2). However, with respect to the fertilizer effect, regardless of the soil tillage method, PS-I added 1.85 Mg C ha^{-1} yr^{-1}, which was significantly more than the control and the chemical fertilizer (NPK) treatments.

Total C input to the soil included the contribution of the fertilizers and of corn and black oat dry matter (Table 2). No difference was found between the tillage methods, but differences were observed among fertilizers. Poultry litter was the fertilizer that most added C over time (mean of 2.68 Mg ha^{-1} yr^{-1}), followed by PS and PS-I (0.92 and 0.48 Mg ha^{-1} yr^{-1}, respectively). This carbon input reflected on the total amounts, with higher means in response to PL than to the other treatments (total of 8.08 Mg C ha^{-1} yr^{-1}). The means of the other organic fertilizers, PS and PS-I, were lower than that of PL, but higher than those of NPK and CTR, resulting in total inputs of 6.95 and 6.85 Mg C ha^{-1} yr^{-1}, respectively.

Total soil C and N stocks

Evaluations in 2021 detected no significant differences in total organic carbon (TOC) and total N (TN) stocks among the fertilizer treatments. However, significant effects of soil tillage on C stocks were found in the layers 0.00-0.05, 0.05-0.10 and 0.10-0.20 m (Figure 1).

Carbon stocks in the 0.00-0.05 and 0.05-0.10 m layers were higher under NT; the C stock in the 0.10-0.20 m layer was highest under CT (Figure 1a). In the 0.00-0.05 m layer, after nine years of agricultural use, NT induced an increase of nearly 1 Mg C ha^{-1}, and CT a decrease of approximately 0.5 Mg C ha^{-1}, compared to the initial stocks of 2012. In the 0.05-0.10 m layer, the difference was also significant, with an increase of approximately 0.8 Mg C ha^{-1} under NT and preserved C stocks under CT.

Carbon stocks in the 0.10-0.20 m layer in 2021 under both soil tillage forms were higher than those in 2012. However, CT stored approximately 0.3 Mg ha^{-1} more C than NT in this layer. In the 0.20-0.30 m layer, the soil tillage treatments were not significantly different from each other, but increased the C stocks compared to 2012. No changes in the C stocks of 2012 were observed in layers below 0.30 m, with no significant difference between soil tillage methods (Figure 1a). In 2021, TN was not different among the samples of the different soil layers (Figure 1b). However, N stocks increased down to a depth of 0.40 m, compared to those in 2012, mainly in the 0.20-0.30 m layer.

Table 2. Above–ground carbon input on the surface of a Rhodic Nitisol treated with different soil tillage and fertilization methods, between 2012 and 2021

Sources	Soil tillage	N source					Mean
		CTR	NPK	PS	PS-B/PS-I	PS-C/PL	
		Mg ha^{-1} yr^{-1}					
Fertilizers	CT/NT	0.00	0.00	0.92	0.48	2.68	n/d
Corn	CT	3.87	4.67	4.69	4.60	4.14	4.39ns
	NT	3.47	4.52	4.59	4.89	3.95	4.28
	Mean	3.67 b[(1)]	4.60 a	4.64 a	4.73 a	4.05 b	4.34
Black oat	CT	1.61	1.61	1.69	1.92	1.70	1.70ns
	NT	1.53	1.56	1.69	1.79	1.75	1.67
	Mean	1.57 b	1.59 b	1.69 ab	1.85 a	1.73 ab	1.69
Total	CT	5.47	6.29	7.00	6.79	8.15	6.74ns
	NT	5.00	6.08	6.91	6.92	8.01	6.59
	Mean	5.24 d	6.18 c	6.95 b	6.85 b	8.08 a	6.66

[(1)] Means followed by the same letter in a row were not significantly different from each other by Tukey's test (<0.05). CT: conventional tillage; NT: no-tillage; CTR: control; NPK: mineral fertilizer; PS: pig slurry; PS-I: injected pig slurry; PL: poultry litter; ns: not significant by the F test.

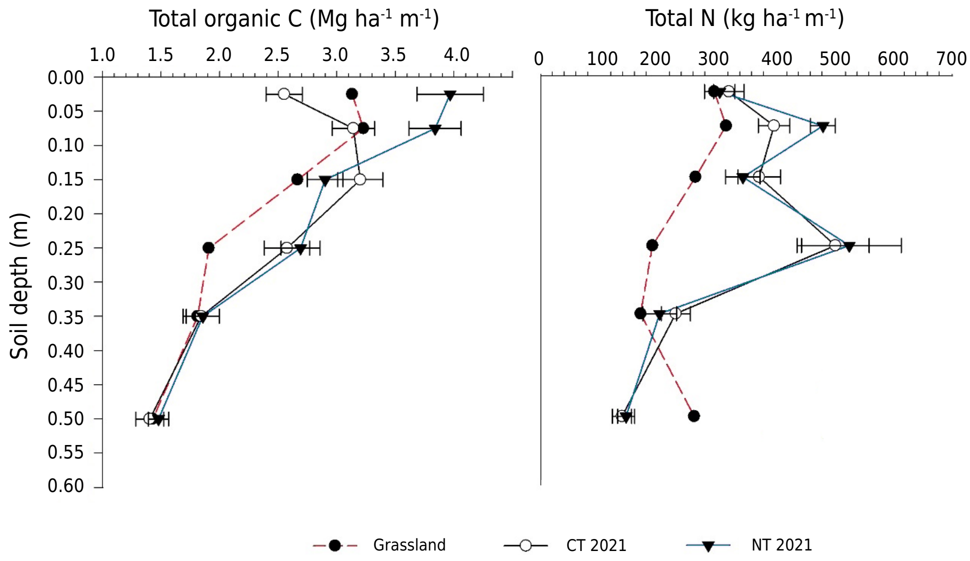

Figure 1. Total organic C stocks (a) and total N (b) in the 0.00-0.60 m soil layer of a Rhodic Nitisol in 2012, under natural pasture and in 2021, after nine years of corn - black oat rotation, under conventional tillage (CT) and no-tillage (NT) management.

For the total C and N stocks, i.e., the additional C and N accumulation in the period from March 2012 to May 2021, in the 0.00-0.30 m layer, soil tillage affected total organic C significantly (Figure 2a). In 2021, NT had accumulated approximately 17.4 Mg C ha^{-1}, twice as much as under CT (8.7 Mg ha^{-1}). Total organic C stocks accumulated in the 0.00-0.60 m layer were not significantly different between soil tillage methods. Nevertheless, the numerical values of the sum of accumulated C in all evaluated layers were different; the lack of statistical difference between soil tillage methods was attributed to the high coefficient of variation for the means.

The sum of cumulative TN did not differ significantly between soil tillage methods (Figure 2b). However, both tillage methods resulted in higher N contents in the soil profile than in 2012 (baseline value of 0 Mg ha^{-1}). Although the different sources of organic soil fertilizers had no significant effect on the soil C and N stocks, the variation in the results indicated

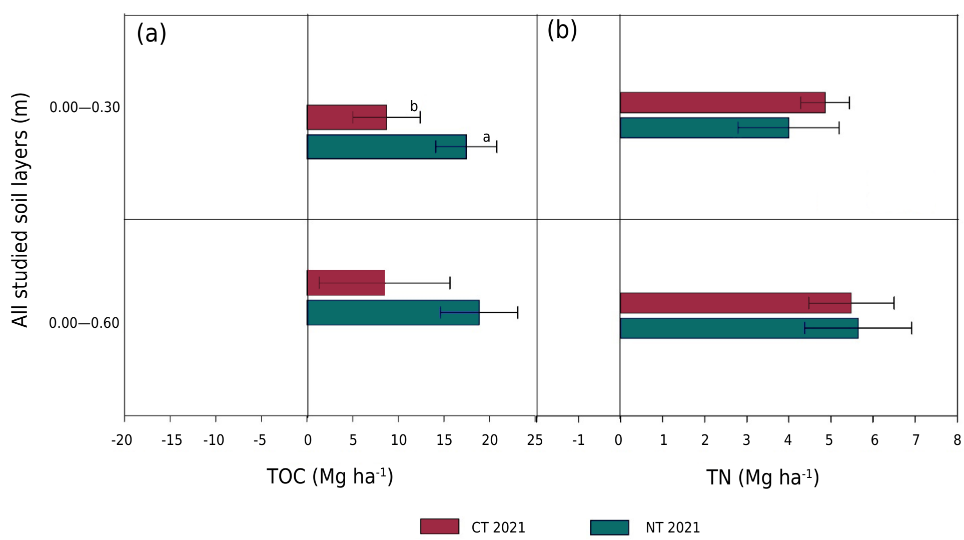

Figure 2. Variation in total organic carbon stocks (a) and total N (b) in the 0.00-0.30 and 0.00-0.60 m layers of a Rhodic Nitisol after nine years of corn - black oat rotation under conventional tillage (CT) and no-tillage (NT).

that long-term monitoring is interesting to determine the effects of organic fertilization on the C and N stocks.

Carbon and nitrogen stocks in particulate organic matter

In 2021, the C and of N stocks of the particulate fraction (>53 µm) were not significantly affected by the applied fertilizers. Under NT, POC stocks in the surface layers (0.00-0.05 and 0.05-0.10 m) were higher, exceeding the initial values (2012) and approximately 2 Mg ha^{-1} cm^{-1} higher than under CT. In the layers below 0.10 m, soil tilling maintained the POC levels, which were higher than the initial contents (Figure 3a).

The PN followed the same trend as POC, with higher accumulation in the surface layers (0.00-0.05 and 0.05-0.10 m) and higher stocks under NT than CT, accumulating approximately 170 kg N ha^{-1} cm^{-1} in 0.00-0.05 m and 50 ha^{-1} cm^{-1} in the 0.05-0.10 m layer (Figure 3b).

Accumulated POC and PN in the 0.00-0.30 m layer were higher in 2021 than at the beginning in 2012, under both tillage methods, but significantly higher under NT than CT (Figure 4). In this layer, POC and PN under NT had increased around 11.9 Mg ha^{-1} and 1.2 Mg ha^{-1}, respectively, both with higher increases than under CT. The comparison between TOC and TN stocks of each layer (Figure 3) and the accumulated stocks (Figure 4) indicated that the difference in accumulated stocks in the 0.00-0.30 m layer was due to increases in POC and TN contents in the two uppermost layers (0.00-0.05 and 0.05-0.10 m). This shows that the tillage management influences the upper layers and that SOC accumulation owing to the management system occurs mainly in more recent formations, as in the case of POC, which is physically protected within soil aggregates.

Soil mineral-associated C and N stocks

The effect of the treatments (fertilizers and soil tillage methods) was not significant for the C and N stocks associated with minerals (MAOC and MAN). However, both NT and CT decreased MAOC contents (Figure 5a) in the surface layers (0.00-0.05 and 0.05-0.10 m), compared to the baseline values (2012); for MAN, this result was found only in the 0.00-0.05 m layer. The MAOC and MAN contents increased in the 0.10-0.20 m layer under CT and in the 0.20-0.30 m layer under both tillage methods (Figure 5b).

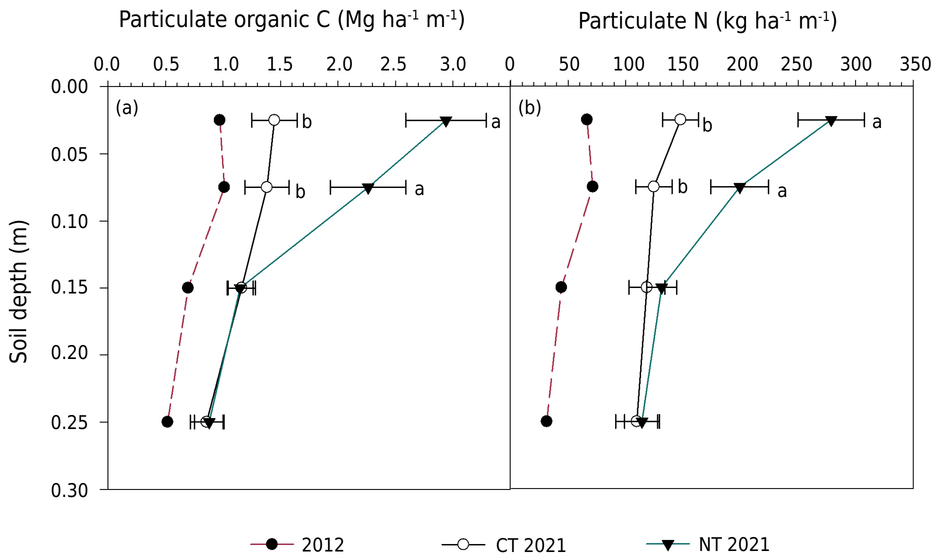

Figure 3. Organic C stocks (POC) (a) and N (PN) (b) contained in the particulate fraction (>53 µm) of the 0.00-0.30 m layer of a Rhodic Nitisol under grassland in 2012 and after nine years of corn - black oat rotation under conventional tillage (CT) and no-tillage (NT) management.

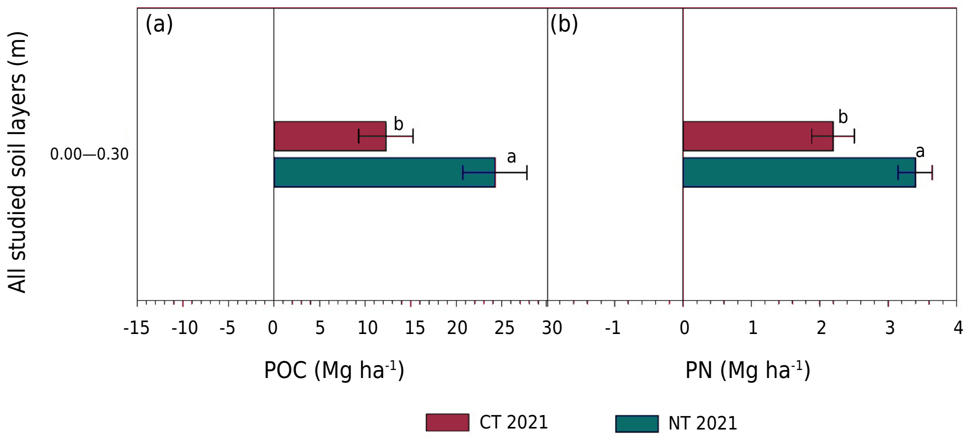

Figure 4. Variation in stocks of particulate organic carbon (POC) (a) and particulate nitrogen (PN) (b) in the 0.00-0.30 m layer of a Rhodic Nitisol after nine years of corn - black oat rotation under conventional tillage (CT) and no-tillage (NT) management.

No significant difference between soil tillage methods were found in the 0.00-0.30 m layer; however, higher MAOC losses were detected under NT, compared to the results found in 2012 (Figure 6a). The MAN fraction increased compared to the baseline values, with an approximately 1.2 Mg ha^{-1} higher MAN storage under CT than NT (Figure 6b).

Fertilizer treatments had significant effects on MAN in the 0.00-0.30 m layer (Table 3), with lower values in the CTR than the NPK, PL and PS treatments under CT. Soil under NPK, PL, PS and PS-I approximately 3.18, 2.28, 2.16 and 1.31 Mg ha^{-1} more MAN, respectively, was stored than under CTR. Fertilizers had no significant effect on MAN under NT. Considering the soil tillage methods for each fertilizer, MAN was higher in the treatments NPK and PS (2.96 and 2.27 Mg ha^{-1}, respectively), under CT than NT.

DISCUSSION

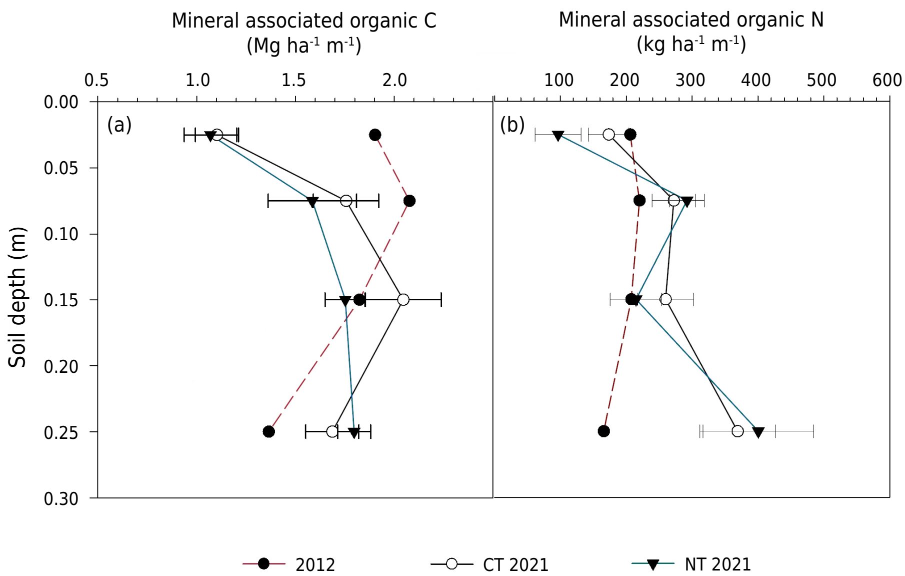

Figure 5. Stocks of mineral-associated organic carbon (MAOC) (a) and mineral-associated N (MAN) (b) in the 0.00-0.30 m layer of a Rhodic Nitisol under grassland in 2012 and after nine years of corn - black oat rotation under conventional tillage (CT) and no-tillage (NT) management.

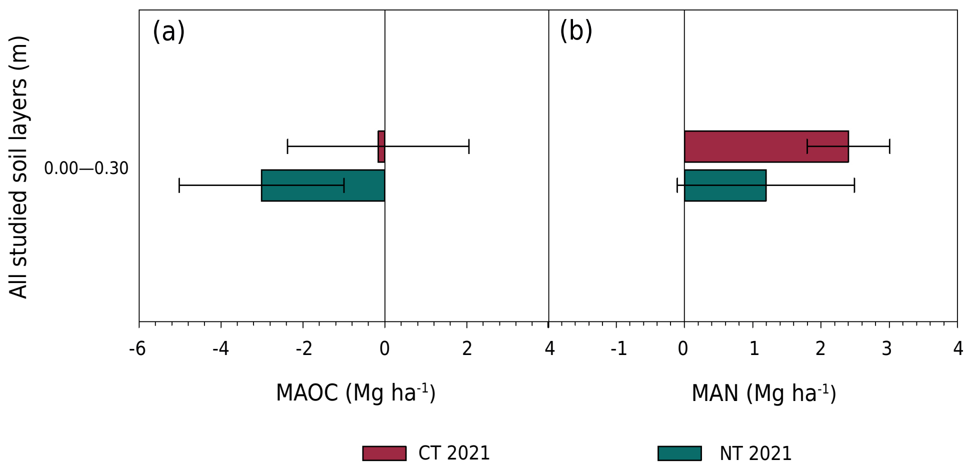

Figure 6. Variation in stocks of mineral-associated organic carbon (MAOC) (a) and nitrogen (MAN) (b) in the 0.00-0.30 m layer of a Rhodic Nitisol after nine years of corn and black oat rotation under conventional tillage (CT) and no-tillage (NT) management.

Table 3. Stocks of mineral-associated nitrogen (MAN) in response to different fertilizers in the 0.00-0.30 m layer of a Rhodic Nitisol after nine years of corn and back oat rotation under conventional tillage (CT) and no-tillage system (NT)

Soil management	CTR	NPK	PS	PS-I	PL	Mean
	Mg ha^{-1}					
CT	6.48 aB[(1)]	9.66 aA	8.64 aA	7.79 aAB	8.76 aA	8.27
NT	7.92 aA	6.70 bA	6.37 bA	6.99 aA	7.30 aA	7.06
Mean	7.21	8.18	7.51	7.39	8.03	

[(1)] Means followed by the same letter were not significantly different from each other by Tukey's test (<0.05). CT: conventional tillage; NT: no-tillage; CTR: control; NPK: mineral fertilizer; PS: pig slurry; PS-I: injected pig slurry; PL: poultry litter; ns: not significant by the F test.

Total soil C and N stocks

Increases or decreases in soil C and N contents are mainly related to the entries into and exits from (inputs and losses) the system. If inputs exceed the losses, C and N stocks increase. Different fertilizers represented inputs with different quantities of C (Table 2). However, although the initial C input was higher from PS-C and PL, this was not necessarily a key factor that resulted directly in higher C stocks. Several factors, mainly crop biomass yield can be related to this result. Thus, the high N contents in stable organic forms, with low mineralization (Giacomini and Aita, 2008; Rogeri et al., 2016) in PS-C and PL fertilizers resulted in a lower corn biomass production and, consequently, lower C input.

With regard to the sum of C inputs by fertilizers and crop biomass production (Table 2), the fertilizers did not result in different cumulative C and N stocks, although the treatments PS-C and PL added higher amounts of C throughout the nine experimental years. Probably, more time would be needed to be able to show significant effects of organic fertilization in evaluations (Rodrigues et al., 2021). However, the effects of soil tillage methods on SOM dynamics are usually higher than those of N fertilization (Man et al., 2021).

After nine experimental years, C contents in the surface layers (0.00-0.05 and 0.05-0.10 m) were higher under NT than under CT and compared to the baseline values (2012), since NT is a conservation tillage method that preserves soil macroaggregates that constitute a physical protection for C (Six et al., 2004). In addition, soil tillage methods with no plowing or minimal soil disturbance reduce aeration and increase soil temperature, factors that can stimulate the microbial activity, accelerating SOM decomposition and mineralization, which occur intensely under CT. Soil managements that contribute to the preservation of recently-formed macroaggregates lead to greater SOM stabilization in microaggregates contained within stable macroaggregates form (Six and Paustian, 2014). Under NT, high crop residue inputs and little soil disturbance favor soil aggregation and increase the physical protection of C and N in the aggregates. This is also favorable for the organo-mineral interaction, as it reduces the oxidative potential of SOM by increasing C and N stocks, compared to CT (Tiecher et al., 2020). Soil aggregate formation can also be stimulated by organic fertilization (Nicoloso et al., 2018) and green manure (Tiecher et al., 2020). In addition, N fertilizer managements associated with conservation managements that stimulate crop residue input to the soil account for increases in soil organic carbon (SOC) stocks, mainly in the surface layers (Ferreira et al., 2018).

Total organic carbon stocks were higher in the upper layers under NT, as reported elsewhere (Sá et al., 2014; Rodrigues et al., 2021). In a 24-year study on a Gleysol under different N fertilizer rates and soil tillage methods in Canada, no changes in total SOC contents were observed (Man et al., 2021). This suggests that climate and environmental factors also affect these dynamics; thus, studying these factors under different soil and climate conditions is important. Another study showed that winter cover crops influence soil C and N accumulation rates more than the soil tillage method (Tiecher et al., 2020).

In the 0.10-0.20 m layer, the C stock was higher under CT than NT. This can be attributed to soil plowing, by which organic surface residues are mechanically reincorporated into soil subsurface layers, contributing to the redistribution of TOC from the surface to deeper layers (Jagadamma and Lal, 2010).

In the other layers (0.20-0.30, 0.30-0.40 and 0.40-0.60 m), TOC was not affected by the soil tillage method. It was expected that deeper layers would be less influenced by tillage methods and fertilization, but evaluating them is important nonetheless, to assess the dynamics of C stocks or losses in the entire profile to avoid over- or underestimation (Blanco-Canqui et al., 2021). Monitoring the entire soil profile over time allows for a precise assessment of the C and N dynamics, since after many years of evaluation, SOC stocks in the surface layer (0.00-0.20 m) may continue to increase linearly under NT. An evaluation of the entire soil profile (0.00-1.00 m) on the other hand, detected no differences between soil tillage methods, as the stocks are redistributed across the soil profile (Veloso et al., 2019; Tiecher et al., 2020; Locatelli et al., 2022).

Total accumulated N (TN) was not affected by the soil tillage methods. However, both methods led to higher cumulative N in the soil profile after nine experimental years, compared to the baseline values. This may be a result of the annual soil fertilization, which contributed to maintain SOM. These findings indicate that NT contributes to raise TOC and TN stocks, mainly in the soil surface layers.

Particulate and mineral-associated soil C and N

Particulate organic C (POC) and particulate N (PN) are more sensitive fractions of SOM than the mineral-associated organic carbon (MAOC) and mineral-associated organic nitrogen (MAN) fractions. They represent >53-µm diameter particles, which corresponds to the soil sand fraction (Cambardella and Elliott, 1992). This fraction has a faster turnover rate and was formed more recently, mainly by the incomplete decomposition of plant residues and fertilizers. The percentage of this fraction depends on continuous replenishment by plant residue inputs, which is the reason why it is used as indicator of short-term effects of soil tillage methods (Bayer et al., 2001, 2002). This effect was observed in this study, where increases in TOC stocks under NT also occurred in the POC fraction, mainly in the surface layers.

Changes in soil use and management can reduce soil C and N stocks; however, conservation practices can reduce or avoid these decreases (Wuaden et al., 2020; Locatelli et al., 2022).

A previous study that evaluated the same experiment five years after implementation showed increases in POC in response to conservation practices (Wuaden et al., 2020). The POC and PN stocks increased mainly in the surface layers, both in the same proportion. Changes in N contents in different soil layers are usually reflected in alterations in C levels (Cambardella and Elliott, 1992).

Minimum tillage and high input of organic matter contribute to increase the concentrations of labile C fractions (POC) in the soil (Bongiorno et al., 2019). This increase can be ascribed to the characteristics of organic matter deposition, the biochemical composition, vegetation type, soil biodiversity, biomass input, management practices, and climate and soil conditions (Derrien et al., 2023).

Particulate organic matter accumulation is higher in regions with colder climates, e.g., in coniferous forests of northern Europe and regions with frequent floods. It is however worth mentioning that this accumulation may be a result of microbial inhibition under specific regional conditions (Lugato et al., 2021).

Mineral-associated soil fraction is considered more stable, with medium- to long-term formation and accumulation. However, MAOC and MAN decreased in the upper layers. This effect can be attributed to changes in the soil use, from native vegetation to agriculture, which may decrease C contents associated with the silt+clay fraction due to aggregate breakdown in agricultural areas, increasing the exposure of C to microbial action (Andrade et al., 2013). Another effect resulting from the land-use change from native vegetation to NT is the decrease in MAOC in the 0.30-0.60 m layer and increase in POC in surface layers. This study showed that the losses in MAOC were compensated by POC increases in the upper layers, as also stated by Locatelli et al. (2022).

Experimental duration (9 years) may be another factor that can explain these facts, as long-term experiments have shown increases in POC and mineral-associated organic matter contents under NT due to the constant deposition of crop residues on the soil surface (Ferreira et al., 2020). The higher residue input over time and little soil disturbance under NT improves the physical protection of C and N in the aggregates and favors organo-mineral interaction, which reduces the soil oxidative potential and increases C and N stocks (Tiecher et al., 2020). Moreover, there is evidence that in temperate climates, in relatively organic-matter-poor soils, C tends to be stored as mineral-associated organic matter rather than as particulate organic matter (Cotrufo et al., 2019).

Persistence of particulate organic matter is controlled mainly by microbial and enzymatic inhibition and some short-term occlusion in aggregates. Mineral-associated organic matter, on the other hand, is protected from decomposition by organo-mineral interactions with amorphous Al, Fe and Mn and may be susceptible to changes in pH caused by land-use conversion (Pulleman et al., 2004; Lavallee et al., 2020).

A study of soils in different countries showed the importance of mineral protection for the preservation of organic C (Hemingway et al., 2019). However, C storage in the MAOC fraction is limited by the maximum saturation (Cotrufo et al., 2019; Georgiou et al., 2022), whereas the size of particulate organic C (POC) seems to be unlimited. This fraction is therefore interesting for additional C storage and may be a promising option of increasing soil C storage with few mineral reactive phases. However, the C storage capacity of POC is still controversial, since the limited duration of this storage prior to POC degradation by decomposers may hamper its contribution to long-term increases in SOC stocks (Derrien et al., 2023).

CONCLUSIONS

No-tillage increased carbon and nitrogen stocks in the soil surface layers (0.00-0.30 m), mainly in the most labile fractions (particulate organic carbon and particulate nitrogen). However, the nine experimental years were probably insufficient to clearly reflect the contributions of organic fertilizations combined with no-tillage management, by increases in soil C and N stocks, which requires further evaluations.

Soil tillage was the factor that most affected the carbon and nitrogen stocks in a Rhodic Nitisol in southern Brazil, under corn-black oat rotation, throughout the nine experimental years.

No-tillage crops, combined with fertilization to increase plant biomass production, increase soil C and N stocks, mainly in the most labile fractions, whereas there was no difference between the effects of organic and mineral fertilizers in this regard.

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