**CSIRO** PUBLISHING

*Soil Research*

https://doi.org/10.1071/SR19346

**Aggregation index, carbon, nitrogen, and natural abundance of 13C and 15N in soil aggregates and bulk soil cultivated with onion under crop successions and rotations**

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**Abstract.** Use of soil cover crops of different families in crop rotation or succession under no-tillage system (NTS) foronion production results in higher soil quality compared to land use systems with less plant diversity. The objective was to evaluate the effect of using different combinations of plant species from different botanical families in rotation and succession of soil cover crops in NTS for onion production on formation of macroaggregates, mesoaggregates, and microaggregates, and on total organic C (TOC) and N (TN) contents, including isotopic forms of C and N, in soil aggregates and bulk soil. The treatments (T) evaluated were maize/onion (NTS-T1); cover plants (winter)/onion (NTS-T2); maize/winter grasses/onion (NTS-T3); velvet bean/onion (NTS-T4); millet/cover plants (winter)/onion (NTS-T5); velvet bean/rye/onion (NTS-T6); maize/onion in conventional tillage system (CTS-T7); and intercrop cover plants (summer)/onion (NTS-T8). We evaluated macroaggregates (8.0–0.25 mm), microaggregates (<0.25 mm), and bulk soil (<2.0 mm) at depths of 0–5, 5–10, and 10–20 cm, in a nine-year field experiment. The greater plant diversity in T2–T6 and T8 resulted in higher geometric mean diameter (GMD) of aggregates compared to T1 and T7. The T8 was more efficient in increasing GMD in the 10–20 cm soil depth than the other treatments. The T1 was more efficient in improving the evaluated soil physical and chemical attributes than T7. The use of NTS with plants of the Poaceae and Fabaceae families in single or intercrop systems for onion production resulted in higher TOC and TN contents in the 0–5 and 5–10 cm soil depths compared to CTS. Isotope 15N measurements showed that C and N were more protected in microaggregates in all evaluated treatments and depths compared to macroaggregates and bulk soil. Macroaggregates had more TOC and TN than microaggregates.

**Additional keywords:** conventional tillage system, macroaggregates and microaggregates, natural abundance of15N,no-tillage system, onion production, soil cover plants.

Received 26 November 2019, accepted 17 June 2020, published online 23 July 2020

**Introduction**

Onion (*Allium cepa*) is a member of the Amaryllidaceae family and has wide use in human food (Souza and Lorenzi [2012](#page5)). Onion is grown all over the world; China, India, and the USA are the main producing countries. Brazil is the ninth largest onion-producing country, with an estimated production of 1.72 million tonnes in 2017, which decreased to 1.66 million tonnes in 2018 (The Daily Records [2019](#page5); IBGE [2019](#page5)).

The state of Santa Catarina (SC) in Brazil has the largest national production, with ~630 000 tonnes, in an area of more than 20 000 ha in 2017 (SEAP 2017). Onion production in SC is predominantly in the Upper Itajai River Valley, especially in

the municipality of Ituporanga, which has the largest national onion production in the country (Menezes Junior *et al*. [2013](#page5)). The conventional tillage system (CTS) of the soil is traditionally used in SC for onion production, with intensive soil turning and use of highly soluble fertilisers and agrochemicals. Therefore, the soil physical, chemical, and biological attributes show intense degradation, making it necessary to seek alternatives for soil management, such as the no-tillage system (NTS) for onion production (Souza *et al*. [2013](#page5); Loss *et al*. [2015](#page5); Santos *et al*. [2017](#page5); Ferreira *et al*. [2018](#page5)).

One of the principles of NTS is protection of the soil surface and structure, thus avoiding the effects of surface

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erosion and favouring the formation of larger aggregates. Soil aggregates can be divided according to their diameter (Ø) into three classes: Ø 2.0 mm (macroaggregates), 2.0 > Ø 0.25

1. (mesoaggregates), and Ø < 0.25 mm (microaggregates). This division by size classes favours the understanding of the distribution of chemical and physical fractions of organic matter in each of these classes. However, further information is still needed on which of these classes of aggregates is responsible for the greater accumulation of carbon (C) and nitrogen (N), and which land use and management are more significant in this process (Fabrizzi *et al*.[2009](#page5);Fernández *et al*.[2010](#page5);Costa Junior *et al*.[2012](#page5);Loss *et al*. [2014](#page5), Ferreira *et al*. [2018](#page5)).

Recent studies compared C and N distribution in soil aggregates and bulk soil. According to Zhong *et al*. ([2017](#page5)), soil aggregates result in less accessibility to organic substrates for microorganisms, which can reduce the soil microbial activity and physically protect the organic C from decomposition. These authors conducted an experiment on a Rhodic Hapludox under a planted forest with *Schima* sp. in south-west China and evaluated the capacity of the physical protection of soil aggregates to stabilise total organic C (TOC) and, consequently, reduce TOC loss rates. They evaluated soil TOC, total N (TN), C and N in the microbial biomass (CMB and NMB), dissolved organic C (DOC), and hot water extractable organic C (HWEOC) in soil aggregates and bulk soil. They found that NMB in soil aggregates was 61.79–69.86% lower than in bulk soil; the CMBs in the 1–2, 2–5, and 5–8 mm layer aggregates were 20.69%, 15.74%, and 13.36% lower than those in bulk soil respectively; and the DOC and HWEOC concentrations in soil aggregates were 41.02–66.40% and 91.30–104.45% higher than those in bulk soil respectively. These results showed a decrease in the microbial activity with the physical protection of soil aggregates, which prevented TOC decomposition and resulted in higher DOC and HWEOC concentrations compared to bulk soil.

The importance of soil aggregation in soil TOC protection from decomposition was pointed out by Li *et al*. ([2016](#page5)). They collected soil samples from representative forests along an altitude gradient in the Wuyi Mountain, south-eastern China, to quantify soil TOC in different soil aggregate fractions and evaluate the effects of temperature and soil aggregation on the TOC distribution in different soil fractions. The authors

concluded that soil TOC presents a high correlation (*r* = 0.90) with soil aggregation (weighted mean diameter), probably because soil aggregation provides physical protection and, therefore, prevents soil TOC decomposition by the microbiota.

NTS is recommended to increase soil C and N contents and, consequently, soil organic matter (SOM). This system consists in minimising soil turning, and use of practices such as crop rotation or succession, intercrop of plant species, and use of green manure and soil cover plants. The soil chemical, physical, and biological conditions are improved as SOM contents increase, including nutrient availability, cation exchange capacity, complexation of toxic and micronutrient elements, water infiltration and retention, aeration, soil microbial activity and biomass, and soil aggregation (Liu

*et al*.[2005](#page5);Hoorman,[2009](#page5);Loss *et al*.[2009*a*](#page5),[2009*b*](#page5),[2015](#page5); Coutinho *et al*. [2010](#page5); Vezzani and Mielniczuk [2011](#page5); Tivet *et al*. [2013](#page5); Ferreira *et al*. [2018](#page5)).

The use of plant species of different botanical families in rotation or succession of crops in NTS can generate better edaphic attributes, especially compared to systems that use soil turning and do not use soil cover crops (Comin *et al*. [2018](#page5); Ferreira *et al*. [2018](#page5)). Furthermore, cover crops combined with NTS can have a strong positive impact on C and N pools, increasing the stocks inside aggregates and mineralisable rates in the bulk soil (Jian *et al*. [2020](#page5); White *et al*. [2020](#page5)). However, few studies have evaluated the effect of these soil cover plants on C and N dynamics in soil aggregates and compared them with those in bulk soils.

In addition to the quantification of total soil C and N contents, the isotopic characterisation of these two elements can be used to understand the C and N dynamics in different soil use systems. These isotopes are 13C and 15N; specific values of

13C are obtained according to the photosynthetic metabolism of plants, with varied values of 13C in C3 (–24‰ to –34‰) and

C4 (6‰ to –19‰) plants (Smith and Epstein [1971](#page5)). The 15N of the air (0.3663%), which is between –10‰ and +10‰, is used as the standard for 15N concentration variation; these variations are related to plant physiological processes, such as the associations with microorganisms, N sources, and plant N demand (Högberg [1997](#page5)). Thus, evaluations of total C and N contents and their isotopic forms (13C and 15N) in soil and aggregates indicate the effect of the management system (NTS and CTS) on agricultural areas and on SOM fractions. Organic matter is also an important compartment for soil N, and the combination of the amounts of 13C with the measurements of 15N isotopes contributes to the understanding of the plant dynamics (Mendonca¸ *et al*. [2010](#page5)).

The objective of this work was to evaluate, in a long-term experiment, the effect of using different combinations of plant species from different botanical families in the rotation and succession of soil cover crops in NTS for onion production on the formation of macroaggregates, mesoaggregates, and microaggregates, and on the total C and N contents, including isotopic forms of C and N, in soil aggregates and bulk soil.

**Material and methods**

*Location and characterisation of the area of study, and planning of the experiment*

The experiment was implemented in April 2007, in the municipality of Ituporanga, SC, Brazil, at the Experimental Station of the Agricultural Research and Rural Extension Corporation of Santa Catarina. The soil of the area was classified as a Humic Dystrudepts (Soil Survey Staff [2010](#page5)), and presented in the 0–10 cm layer the following physical and chemical attributes, evaluated according to Embrapa (1997):

1. g kg–1 of sand, 264 g kg–1 of silt, and 326 g kg–1 of clay; pHH2O of 6.1; exchangeable Ca, Mg, and Al of 6.4, 2.7, and
2. 0 cmolc dm–3 (extracted with 1 M KCl) respectively; available P and K of 42 and 208 mg dm–3 (Mehlich-1 extraction) respectively; and TOC of 23.08 g kg–1.

Carbon and nitrogen in aggregates and bulk soil

The climate of the region is wet mesothermal (Cfa) with hot summers, according to the Köppen classification, with no defined dry season, presenting an average annual temperature of 17.68C and average annual rainfall of 1400 mm. A randomised block experimental design was used, with eight treatments and four replications. The area of each plot was 8.7 m2, consisting of seven rows with 30 onion plants each. The treatments included crop systems for onion production, based on different soil cover crops used for mulching in NTS.

When the experiment was planted in 2007, the oat (*Avena* *strigosa* Schreb), vetch (*Vicia villosa* Roth), and oilseed radish(*Raphanus raphanistrum* subsp. *sativus* (L.) Domin) soil cover crops were sown in the whole area; subsequently, the eight treatments (T1–T8) were implemented, with the soil cover crops and crop sequences (Table [1](#page5)). The rotation or succession systems and the sequence of soil cover plants were modified after 2011, and a soil CTS treatment was implemented for comparison with the other NTS treatments (Table [2](#page5)). The soil preparation in T7 was carried out with one ploughing and two harrowing processes. The sequence of treatments was repeated after 2014 (Table [2](#page5) ) for the years 2011, 2012, and 2013, i.e. the sequence of rotation or succession restarted every three years.

Weed, pest, and disease controls were carried out using chemical products approved by the Brazilian Ministry of Agriculture, Livestock, and Food Supply for onion crops. Approximately 14 days before onion planting, the plants were killed with the herbicide glyphosate (360 g L –1) at

1. L ha–1. Weed control during the onion cycle was carried out with three applications of herbicides: two with ioxynil (250 g L–1) at 1 L ha–1 at 35 and 65 days after seedling transplantation (DAT), and one with clethodim (240 g L–1) at 0.4 L ha–1 at 85 DAT. The control of pests, especially *Thrips* *tabaci* Lind, was carried out using three insecticideapplications: one with imidacloprid (700 g L–1) at 0.1 kg ha–1 at 30 DAT, and two with lambdacyhalothrin (50 g L–1) at 0.1 L ha–1 at ~60 and 81 DAT. The control of fungal diseases, mainly mildew (*Peronospora destructor*) and *Alternaria solani*, was carried out with six applications offungicides: four with metalaxyl (40 g L–1) + mancozeb (640 g L–1) at 35, 50, 65, and 80 DAT; and two with tebuconazole (200 ml L–1) + trifloxystrobin (100 ml L–1) at 80 and 94 DAT. All applications were performed using personal protective equipment.

The species chosen for the experiment (Tables [1](#page5) and [2](#page5)) are commercial species that are frequently used in the study region, which present good adaptation, availability of seeds in the market, easy handling, and good dry matter production. The commercial and technical factors of the work were combined by adding treatments that were possible for farmers to adopt, while being useful to elucidate questions related to chemical aspects of the adoption of NTS for onion crops.

The experimental area had been cultivated in a conservation production system since 1995, when the soil pH was last corrected to 6.0 by liming. The crops in the experiment site had been under NTS, except T7, which had been under CTS since 2011 to compare it to the other treatments in NTS.

During the experiment, the soil was fertilised only for the onion and maize crops, according to the recommendations for

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| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | *Soil Research* |  | C |  |
| **Table 1. Species used in rotation or succession with onion crops in different soil tillage systems for 2007–2010, Ituporanga, SC, Brazil**Speciesareoat(*Avenastrigosa*Schreb),onion(*Alliumcepa*L.),rye(*Secalecereale*L.),showyrattlebox(*Crotalariaspectabilis*Roth),vetch(*Viciavillosa*Roth),common bean (*Phaseolus vulgaris*L.),jackbean(*Canavaliaensiformis*(L.)DC.),sunflower(*Helianthusannuus*L.),maize(*Zeamays*L.),pearlmillet(*Pennisetumglaucum*(L.)R.Br.),velvetbean(*Mucunapruriens*var.*utilis*(Wall.exWight)L.H.Bailey),oilseedradish(*Raphanusraphanistrum*subsp.*sativus*(L.)Domin),andbarley(*Hordeumvulgare*L.).T1,successionofonionandmaizeinno-tillagesystem(NTS);T2,rotationofsoilcovercrops(winter)andbiennialonioninNTS;T3,rotationofmaize,wintergrasses,andonioninNTS;T4,successionofsummerlegumeandannualonioninNTS;T5,rotationofsummergrass,wintergrasses,andannualonioninNTS;T6,successionofsummerlegume,wintergrass,andannualonioninNTS;T7,successionofmaizeandonioninconventionaltillagesystem(CTS);T8,successionofintercropsofsoilcovercrops(summer)andannualonioninNTS |  | T 2007 2008 2009 2010WinterSummerWinterSummerWinterSummerWinter Summer |  | T1 Oat + vetch + oilseed radish Maize Fallow Onion Maize Fallow onion Maize Fallow Onion MaizeT2Oat+vetch+oilseedradishMaizeOat+oilseedOnionSunflowerOat+vetch+CommonbeanRye+oilseedOnionMaizeradish+ryeoilseedradishradishT3Oat+vetch+oilseedradishMaizeOat+oilseedradishOnionMaizeVetchMaizeRyeOnionMaizeT4Oat+vetch+oilseedradishMaizeOat+oilseedOnionVelvetbeanRyeMaizeOilseedradishOnionVelvetbeanradish+ryeT5Oat+vetch+oilseedradishOnionPearlmilletOilseedradishOnionPearlmilletOat+vetch+MaizeBarleyOnion Pearl milletoilseedradishT6Oat+vetch+oilseedradishOnionJackbeanRyeOnionVelvetbeanOnionVelvetbeanRyeOnionVelvetbeanT7Oat+vetch+oilseedradishOnionJackbean+OatOnionShowyrattleboxRyeMaizeOatOnionShowyrattleboxPearlmilletT8Oat+vetch+oilseedradishOnionSunflowerOat+ryeOnionSunflower+velvetVetchMaizeRye+oat+OnionPearlmillet+velvet | bean + pearl millet oilseed radish bean + sunflower |  |  |
|  |  |  |  |



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**Table 2. Species used in rotation or succession with onion crops during 2011–2013, Ituporanga, SC, Brazil**

Oat (*Avena strigosa* Schreb), onion (*Allium cepa* L.), rye (*Secale cereale* L.), vetch (*Vicia villosa* Roth), common bean (*Phaseolus vulgaris* L.), sunflower (*Helianthus annuus* L.), maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* (L.) R.Br.), velvet bean (*Mucuna pruriens* var. *utilis* (Wall. ex Wight) L. H. Bailey), and oilseed radish (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin). T1, succession of onion and maize in no-tillage system (NTS); T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in conventional tillage system; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| T |  | 2011 |  |  | 2012 |  |  | 2013 |  |
|  | Winter |  | Summer | Winter |  | Summer | Winter |  | Summer |
|  |  |  |  |  |  |  |  |  |  |
| T1 | Fallow | Onion | Maize | Fallow | Onion | Maize | Fallow | Onion | Maize |
| T2 | Vetch |  | Maize | Rye + | Onion | Maize | Oilseed |  | Common bean |
|  |  |  |  | oilseed radish |  |  | radish + rye | |  |
| T3 | Rye | Onion | Maize | Oat | Onion | Maize | Rye | Onion | Maize |
| T4 | Fallow onion | | Velvet bean | Fallow onion |  | Velvet bean | Fallow onion | | Velvet bean |
| T5 | Rye | Onion | Pearl millet | Oat | Onion | Pearl millet | Rye | Onion | Pearl millet |
| T6 | Rye | Onion | Velvet bean | Rye | Onion | Velvet bean | Rye | Onion | Velvet bean |
| T7 | Fallow | Onion | Maize | Fallow | Onion | Maize | Fallow | Onion | Maize |
| T8 | Fallow | Onion | Pearl millet + | Fallow | Onion | Pearl millet + | Fallow | Onion | Pearl millet + |
|  |  |  | velvet bean + |  |  | velvet bean + |  |  | velvet bean + |
|  |  |  | sunflower |  |  | sunflower |  |  | sunflower |
|  |  |  |  |  |  |  |  |  |  |

**Table 3. Shoot dry matter (SDM) yield of the soil cover crops, spontaneous vegetation, and maize in rotation or succession with onion crops under no-tillage and conventional tillage systems in 2016**

T1, succession of onion and maize in no-tillage system (NTS); T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in conventional tillage system; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 |
|  |  |  |  |  | (Mg ha–1) |  |  |  |
|  |  |  |  |  |  |  |  |  |
| SDM yield | 5.97 | 9.73 | 10.5 | 4.01 | 11.6 | 7.58 | 4.76 | 11.0 |
| SDM yield of maize | 8.82 | 8.20 | 7.64 | – | – | – | 8.50 | – |
|  |  |  |  |  |  |  |  |  |

these crops (CQFS-RS/SC [2004](#page5)). Annual soil fertilisation for onion crops consisted of 75 kg ha–1 of N, 120 kg ha–1 of P2O5, and 60 kg ha–1 of K2O, with all phosphorus (P) and potassium

1. and 15 kg ha–1 N applied at planting; the remaining N was applied at 45, 65, and 85 DAT. The P contents in the area in 2010 were very high, thus soil fertilisation consisted of 50 kg ha–1 of P, and in the following onion crop consisted of 80 kg ha–1. Soil fertilisation with P and K was not performed for maize crops due to the high contents of these nutrients in the soil; N fertilisation consisted of 90 kg ha–1 of N (urea) when the maize plants had six to eight leaves.

Before the onion seedlings were manually transplanted, the soil cover plants were killed and furrows were opened with a machine adapted for onion planting in NTS. The onion cultivar used was Empasc 352 (Bola Precoce); the spacing used was 0.40 m between rows and 0.10 m between plants, with seven onion rows per plot.

*Dry matter of soil cover crops and onion yield*

The shoot dry matter (SDM) yield of the soil cover plants was evaluated in the same year that the soil samples were collected (i.e. 2016). The SDM yield of spontaneous vegetation was evaluated together with the soil cover plants; the predominant

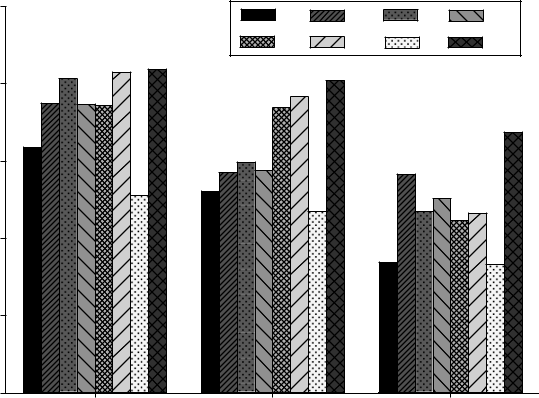
species of these spontaneous plants were from the following botanical families: Amaranthaceae (10%); Asteraceae, Caryophyllaceae, and Compositae (10%); Convolvulaceae, Cruciferae, and Cyperaceae (25%); Euphorbiaceae, Fabaceae, and Lamiaceae (10%); Leguminosae, Liliaceae, Malvaceae, and Oxalidaceae (10%); and Plantaginaceae, Poaceae, and Polygonaceae (20%). The SDM yield of maize (Table [3](#page5)) in the treatments containing this species (T1, T2, T3, and T7) was also evaluated. The average onion bulb yields of each treatment for 2011–16 are shown in Fig. [1](#page5).

*Soil sampling and analyses*

In September 2016, nine years after the beginning of the experiment, undisturbed and disturbed soil samples were collected in each plot. A 40 cm 40 cm 40 cm hole was opened between the onion rows in each plot using a spade, and samples were collected from the 0–5, 5–10, and 10–20 cm soil layers. The samples were placed in plastic bags and sent to the Laboratory of Soil Management and Classification of the Federal University of Santa Catarina, where they were air-dried. Undeformed samples were manually disaggregated following cracks or weak points, and passed through

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|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 5.00 |  |  |  |  |  | T1 | T2 | T3 | T4 |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | A | T5 | T6 | T7 | T8 |  |
|  |  |  | A | A |  |  | A |  |  |  |
|  | 4.00 |  |  |  |  |  |  |  |  |
|  | A |  | A A |  |  | A A |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  | B |  |  |  |  |  |  |  | A |  |
| (mm) |  |  |  | C | B | B B |  | B |  |  |
| 3.00 |  |  |  |  |  |  |
|  |  |  |  |  | B |  | B |  |  |
| GMD |  |  |  |  |  |  | B |  | B | B B |  |
| 2.00 |  |  |  |  |  |  |  | C | C |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  | 1.00 |  |  |  |  |  |  |  |  |  |  |
|  | 0.00 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 0−5 |  |  | 5−10 |  | 10−20 | |  |



Layer (cm)

**Fig. 1.** Geometric mean diameter (GMD) of aggregates of soilssubjected to different managements and soil cover plants, Ituporanga, SC, Brazil. T1, succession of onion and maize in no-tillage system (NTS); T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in conventional tillage system; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS. Bars with different letters within each soil layer differ by Skott–Knott test at *P* < 0.05.

8.00-mm and 4.00-mm mesh sieves to obtain soil aggregates (Embrapa 1997). Deformed samples were passed through a 2.00-mm mesh sieve to obtain the air-dried fine earth (bulk soil). The following analyses were performed on soil aggregates and bulk soil.

*Physical analyses*

*Aggregate stability* A 25-g sample of the aggregatesretained on the 4.00-mm mesh sieve was sieved though a set of sieves with decreasing mesh diameter: 2.00, 1.00, 0.50, 0.25, 0.105, and 0.053 mm (Embrapa 1997). The aggregates were placed on the 2.00-mm mesh sieve and moistened with water spray, and subjected to vertical wet sieving for 15 min in

1. Yoder apparatus (Yoder [1936](#page5)). Subsequently, the material retained in each sieve was removed, separated by water jet, placed in identified and weighed aluminium crucibles, and placed in an oven until constant weight to obtain their dry weight.

The geometric mean diameter (GMD) of the aggregates was calculated using the dry weight of the aggregates (Embrapa 1997), which was also used to evaluate their distribution in the following mean diameter classes: 8.00 > Ø 2.0 mm (macroaggregates), 2.0 > Ø 0.25 mm (mesoaggregates), and Ø < 0.25 mm (microaggregates) (Costa Junior *et al*. [2012](#page5)).

*Chemical analyses*

The dry weight of macroaggregates and mesoaggregates was included for the chemical analysis to obtain a larger quantity of material. Therefore, the chemical parameters were evaluated considering the macroaggregates (8.00 > Ø

0.25 mm), microaggregates (Ø < 0.25 mm), and bulk soil (Ø < 2.0 mm). These aggregates were gently ground with a mortar and pestle, homogenised, and used in the following analyses.

*TOC and TN* The TOC and TN contents in the bulk soil,macroaggregates, and microaggregates were determined in an elemental dry combustion analyser (FlashEA 1112, Thermo Finnigan, Bremen, Germany) at the Laboratory of Research in Biotransformation of Carbon and Nitrogen (LABCEN), in Santa Maria, RS, Brazil.

*Natural abundance of 13C and 15N* The isotopic abundanceof d15N and d13C was determined in aliquots of ~300 mg of each sample of bulk soil, macroaggregates, and microaggregates (milled and passed through a 100-mm mesh sieve), with precision of 0.0001. The samples were then packed in tin capsules and evaluated in a continuous flow isotopic mass spectrometer coupled to a total C and N analyser (DeltaPlus; Carlo Erba EA 1108, Finnigan MAT, Bremen, Germany) of the LABCEN. The isotopic variation of C was expressed as d13C (‰) in relation to the PDB (Pee Dee Belemnite) international standard, and for N, as d15N (‰) in relation to atmospheric air (0.3663%).

*Statistical analyses*

The results were analysed for normality and homogeneity of the data by the Lilliefors (Lilliefors [1967](#page5)) and Bartlett (Bartlett [1937](#page5)) tests respectively. The data were subjected to ANOVA (F test) and, when the effects were significant, the means were compared by the Scott–Knott test at *P* < 0.05 using Sisvar 5.6 software. Statistical analyses were performed for the eight treatments, soil aggregates (macro and micro), and bulk soil. Subsequently, the data of each treatment were subjected to statistical analysis independently, and the results of the soil aggregates and bulk soil were compared by least significant difference of *t*-test at *P* < 0.05.

**Results and discussion**

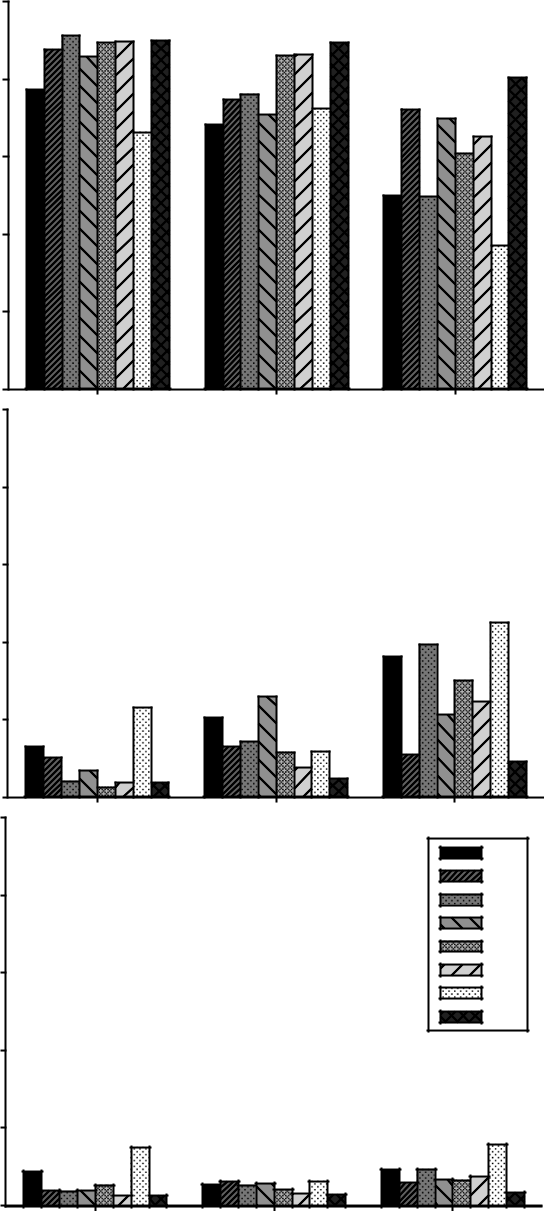
*Soil aggregate stability*

The highest GMDs in the soil surface layer (0–5 cm) (3.718–4.193 mm) were found in the T2–T6 and T8 treatments; and the lowest GMDs were in T1 and T7, with the lowest of 2.551 mm for T7. The highest GMDs in the 5–10 cm soil layer were in T5, T6, and T8. The highest GMD in the 10–20 cm layer (3.376 mm) was in T8, and the lowest in T7 (1.667 mm) and T1 (1.691 mm) (Fig. [2](#page5)).

The lowest GMD in T1 and T7, which had succession of maize and onion, were related to the absence of summer or winter soil cover crops, reflected in lower TOC contents, and probably lower root system activity in these treatments compared to the others that had soil cover plants. Ladoni *et al*. ([2016](#page5))and Comin *et al*. ([2018](#page5))demonstrated theimportance of cover crops roots on the C dynamics in the soil. The lower TOC found in T1 and T7 (Table [4](#page5)) is also related to the lower plant diversity in these two treatments. This correlation was also found by Steinbeiss *et al*. ([2008](#page5)), who investigated the link between plant diversity and soil C storage. Low TOC contents generate less water-stable aggregates, which results in the disruption of larger

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|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  | Macroaggregates | | | | |  |  |  |  |  |
|  | 25 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | A | A | A A | |  | A |  |  |  |  | A |  |  |  |  |  |
|  |  |  | A |  |  |  | A A |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 20 | B |  |  |  |  |  |  |  | B B |  |  |  |  |  |  | A |  |
|  |  |  |  |  |  |  |  |  | B |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | B |  |  |  |
|  |  |  |  |  |  |  | C |  | B | B |  |  |  | B |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | B |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | B |  |
|  | 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | C |  | C |  |  |
|  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | D |  |
|  | 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 25 |  |  |  |  |  |  |  | Mesoaggregates | | | | |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| mass (g) | 20 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Aggregates |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | A |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 10 |  |  |  |  |  |  |  |  |  |  |  |  | B |  | B |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  | A |  |  |  |  |  | C |  |  |
|  |  |  |  |  |  |  | A |  |  |  |  |  |  |  | D | D |  |
|  |  |  |  |  |  |  |  | B |  |  |  |  |  |  |  |
|  | 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | B |  |  |  |  |  |  |  | C C |  |  |  |  |  |  |  |  |
|  |  | C |  |  |  |  |  |  | C | C |  |  | E |  | E |  |
|  |  |  |  | DD DD | | |  | D |  |  | D |  | D |  |  |  |  |
|  | 0 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 25 |  |  |  |  |  |  |  | Microaggregates | | | | |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T1 |  |
|  | 20 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T2 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T3 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T4 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T5 |  |
|  | 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T6 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T7 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | T8 |  |
|  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 5 |  |  |  |  |  | A |  |  |  |  |  |  |  |  |  | A |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | B | C C C | | C | C |  | C | A | A A A | B B | A | B | B | D | B D D | C |  |
|  | 0 |  |  |  |  |  |  |  |  |  | E |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 0−5 | |  |  |  |  | 5−10 | |  |  |  |  | 10−20 | |  |



Layer (cm)

**Fig. 2.** Distribution of aggregates by diameter classes (macroaggregates,mesoaggregates, and microaggregates) in soils cultivated with onion crops under no-tillage system (NTS) and conventional tillage system (CTS) including crop rotation or succession and use of soil cover crops, Ituporanga, SC, Brazil. T1, succession of onion and maize in NTS; T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in CTS; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS. Bars with different letters within each soil layer differ by Skott–Knott test at *P* < 0.05.

(macroaggregates) into smaller aggregates (microaggregates) (Fig. [3](#page5)), mainly in the soil surface layer, in which T7 and T1 had the lowest amounts of macroaggregates and highest amounts of mesoaggregates and microaggregates. The predominance of smaller aggregates and lower TOC generated a lower aggregation index in T1 and T7, since there is generally a positive correlation between organic matter and aggregate stability, as demonstrated by Six *et al*. ([2004](#page5)) and Tivet *et al*. ([2013](#page5)), with *r* = 0.98 (*P* < 0.001).

The GMD is an estimate of the size of the aggregates of highest occurrence in a soil (Soares *et al*. [2018](#page5)). Thus, soils with more stable aggregates of larger diameter have higher GMD, as found for soils in T2–T6 and T8, mainly in the 0–5 and 10–20 cm soil layers (Fig. [2](#page5)), which had more macroaggregates and so lower mesoaggregates and microaggregates compared to T1 and T7 (Fig. [3](#page5)).

A low plant diversity generates less root activity and less release of root exudates (Lange *et al*. [2015](#page5)). Thus, there is lower root penetration by mechanical force, and lower cementing agents in the soil, reducing the stability of larger diameter aggregates, as found in T1 and T7 treatments. The T7 had lower GMD than T1 in the 0–5 and 10–20 cm layers (Fig. [2](#page5)) and, consequently, less macroaggregates and higher mesoaggregates and microaggregates (Fig. [3](#page5)). These differences between T1 and T7 are related to the soil use systems. The NTS in T1 was more efficient in increasing soil TOC (Table [4](#page5)) and aggregation (Fig. [2](#page5)) than the CTS in T7, in which ploughing and harrowing were used for soil tillage, accelerating the SOM oxidation and reducing aggregate stability in water.

Comparing the effect of CTS (maize under monoculture and maize in succession with cotton (*Gossypium hirsutum* L.)) and NTS (maize under two- and three-year rotation with cotton and crotalaria (*Crotalaria juncea*)) over four years, Thierfelder and Wall ([2010](#page5)) found that treatments under NTS had greater TOC and aggregate stability than those under CTS. Costa Junior *et al*. ([2012](#page5)) evaluated GMD of aggregates in the soil surface layer (0–5 cm) and found more macroaggregates in soils under NTS than under CTS. According to these authors, GMDs of soils under NTS were ~40% higher than for soils under CTS.

The highest GMD in the 10–20 cm soil layer was found in T8; this may be because of the intercrop of three soil cover crop species (sunflower, velvet bean, and pearl millet). This increases the addition of C in the soil via rhizodeposition, generating the higher TOC in macroaggregates in the 10–20 cm layer in T8 (Table [4](#page5)). This can also favour plant root development for water and nutrient absorption due to the presence of different root systems. This explains the better aggregation and aggregate stability in deeper layers in T8 compared to the other treatments.

The highest and lowest macroaggregate and microaggregate contents in the 10–20 cm soil layer in T8 confirmed the higher GMD found in this treatment. The higher macroaggregate contents in deeper layers in T8 can be attributed to the use of the intercrop of soil cover crops, which generated a greater area of root development, and high biomass production (Table [3](#page5)) and deposition of root

Carbon and nitrogen in aggregates and bulk soil *Soil Research* G

**Table 4. Total organic carbon (TOC) and total nitrogen (TN) in soils cultivated with onion crops under no-tillage and conventional tillage systems including crop rotation or succession and use of soil cover crops, Ituporanga, SC, Brazil**

Means followed by a different uppercase letter in columns differ by Scott–Knott test at *P* < 0.05, and means followed by a different lowercase letter in rows differ by *t*-test at *P* < 0.05. Macro, macroaggregates; Micro, microaggregates; CV, coefficient of variation; T1, succession of onion and maize in no-tillage system (NTS); T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in conventional tillage system; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment |  | TOC (g kg–1) | |  |  | TN (g kg–1) | |  |  |
|  | Bulk soil | Macro | Micro | CV (%) | Bulk soil | Macro | Micro | CV (%) | |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 0–5 cm |  |  |  |  |  |
| T1 | 31.34 Db | 34.70 Aa | 22.71 Dc | 3.81 | 2.71 Da | 2.83 Ba | 2.55 Ca | 8.58 |  |
| T2 | 33.57 Cb | 38.72 Aa | 31.05 Cc | 3.41 | 2.97 Ca | 3.16 Aa | 2.83 Ba | 6.21 |  |
| T3 | 38.46 Ba | 30.77 Bb | 24.95 Dc | 2.69 | 3.30 Ba | 2.86 Ba | 2.26 Db | 8.20 |  |
| T4 | 42.85 Aa | 37.15 Ab | 32.80 Bc | 2.53 | 3.98 Aa | 3.46 Ab | 3.17 Ac | 3.01 |  |
| T5 | 40.77 Aa | 41.37 Aa | 35.76 Ab | 5.16 | 3.55 Ba | 3.47 Aa | 3.22 Aa | 6.34 |  |
| T6 | 41.03 Aa | 39.24 Aa | 29.27 Cb | 5.18 | 3.55 Ba | 3.74 Aa | 2.85 Bb | 3.99 |  |
| T7 | 28.48 Ea | 26.88 Cb | 22.65 Dc | 2.32 | 2.31 Ea | 2.28 Ca | 1.97 Eb | 4.16 |  |
| T8 | 39.12 Ba | 38.33 Aa | 30.49 Cb | 8.05 | 3.45 Bb | 3.74 Aa | 2.76 Bc | 4.33 |  |
| CV (%) | 3.33 | 5.61 | 5.16 |  | 3.58 | 7.55 | 5.35 |  |  |
|  |  |  |  | 5–10 cm |  |  |  |  |  |
| T1 | 24.93 Db | 25.32 Ba | 21.17 Bc | 2.02 | 2.06 Ca | 2.14 Aa | 1.95 Ba | 4.60 |  |
| T2 | 29.02 Ba | 29.56 Aa | 22.60 Ab | 3.80 | 2.52 Ba | 2.35 Aa | 1.94 Bb | 6.35 |  |
| T3 | 30.15 Ba | 25.90 Bb | 23.66 Ac | 2.60 | 2.64 Ba | 2.24 Aab | 2.08 Ab | 8.84 |  |
| T4 | 33.21 Aa | 28.80 Ab | 22.90 Ac | 3.53 | 2.95 Aa | 2.30 Ab | 2.01 Ac | 5.21 |  |
| T5 | 30.57 Ba | 30.39 Aa | 22.26 Ab | 3.05 | 2.65 Ba | 2.49 Aa | 2.14 Ab | 5.56 |  |
| T6 | 29.88 Ba | 24.30 Bb | 20.15 Bc | 2.46 | 2.75 Ba | 2.38 Ab | 1.87 Bc | 6.74 |  |
| T7 | 27.91 Ca | 25.63 Bb | 19.02 Cc | 4.44 | 2.22 Ca | 2.19 Aa | 1.72 Cb | 5.20 |  |
| T8 | 31.70 Aa | 30.14 Ab | 23.54 Ac | 1.18 | 2.72 Ba | 2.37 Ab | 2.16 Ab | 6.97 |  |
| CV (%) | 3.3 | 2.92 | 3.47 |  | 6.20 | 5.37 | 3.85 |  |  |
|  |  |  |  | 10–20 cm | |  |  |  |  |
| T1 | 21.79 Cb | 22.93 Ca | 20.86 Ac | 0.89 | 1.70 Cb | 1.86 Aa | 1.80 Aa | 2.02 |  |
| T2 | 24.44 Ba | 21.16 Db | 17.54 Cc | 3.54 | 1.91 Ba | 1.68 Ab | 1.40 Bc | 3.39 |  |
| T3 | 24.74 Ba | 22.49 Cb | 18.59 Bc | 4.57 | 1.89 Ba | 1.78 Aab | 1.54 Bb | 7.12 |  |
| T4 | 26.09 Aa | 22.53 Cb | 21.43 Ab | 4.46 | 2.06 Aa | 1.74 Ab | 1.88 Aab | 4.75 |  |
| T5 | 22.80 Ca | 22.12 Ca | 20.25 Ab | 3.49 | 1.84 Ba | 1.82 Aa | 1.66 Bb | 3.39 |  |
| T6 | 26.07 Aa | 23.51 Bb | 18.61 Bc | 3.63 | 1.98 Aa | 1.77 Aa | 1.58 Ba | 8.47 |  |
| T7 | 21.62 Ca | 20.23 Da | 17.66 Cb | 4.43 | 1.45 Da | 1.66 Aa | 1.59 Ba | 8.34 |  |
| T8 | 26.63 Aa | 25.25 Aa | 20.14 Ab | 3.56 | 2.10 Aa | 1.75 Ab | 1.63 Bb | 5.38 |  |
| CV (%) | 3.89 | 3.88 | 3.2 |  | 3.13 | 7.48 | 6.01 |  |  |
|  |  |  |  |  |  |  |  |  |  |

exudates. Thus, the intercrop of soil cover plants in T8 favoured the formation of larger and more stable aggregates in deeper layers, generating lower mesoaggregate and microaggregate contents in this treatment (Fig. [3](#page5)).

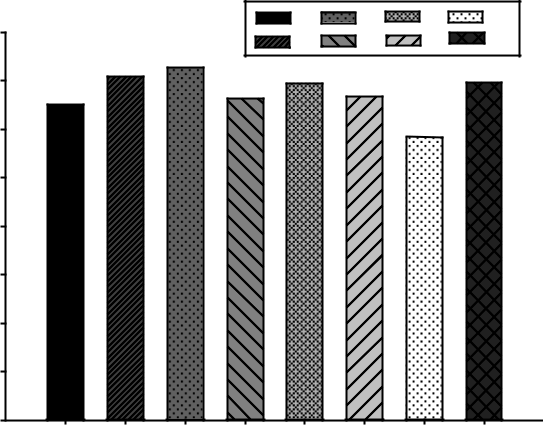
The T5, T6, and T8 treatments had the highest GMD values in the 5–10 cm layer (Fig. [2](#page5)), consistent with their higher macroaggregate and lower microaggregate contents compared to the other treatments (Fig. [3](#page5)). These results may be due to the constant presence of soil cover crops in T5 and T6, since these treatments had soil cover crops in winter and summer, differing from the other treatments, which had soil cover plants only in winter (T2 and T3) or summer (T4), or had no soil cover plants (T1 and T7). A greater plant diversity generates more stable and larger aggregates, as demonstrated by Comin *et al*. ([2018](#page5)) in an evaluation of the effect of different cover crops on soil TOC. Treatment T8 showed the positive effect of the intercrop of soil cover plants in summer with the highest GMD and macroaggregate contents.

The treatments with soil cover crops conducted in NTS generally had more stable aggregates and lower mesoaggregate contents compared to T1 and T7. Treatment T4 had the highest mesoaggregate contents in the 5–10 cm layer, indicating that velvet bean residues were preferentially consumed by microorganisms, probably due to their higher N content and lower C/N ratio, reducing the amount of SOM for macroaggregation. Only T4 had legumes as a soil cover crop, and these plants have a lower efficiency for increasing or maintaining aggregate stability than grasses (Gould *et al*. [2016](#page5)). Gould *et al*. ([2016](#page5)) evaluated soil aggregate stability in a long-term field experiment with grass species and one legume species, and intercropping of these species, and found that treatments with grasses (which have more fine roots) had a stronger correlation with soil aggregate stability than legumes.

The intercropping of plants with different root systems (fasciculate and pivotal) generates a favourable environment for soil aggregation, especially for macroaggregates (Vezzani

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|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 40 |  |  |  | T1 | T3 | T5 | T7 |  |
|  |  |  |  | T2 | T4 | T6 | T8 |  |
|  |  |  |  |  |  |
|  | 35 |  |  |  |  |  |  |  |  |
| (Mg/ha) | 30 |  |  |  |  |  |  |  |  |
| 25 |  |  |  |  |  |  |  |  |
| bulbs |  |  |  |  |  |  |  |  |
| 20 |  |  |  |  |  |  |  |  |
| onion | 15 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| Yield | 10 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  | 5 |  |  |  |  |  |  |  |  |
|  | 0 |  |  |  |  |  |  |  |  |
|  | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 |  |
|  |  |  |  | Treatments | |  |  |  |  |



**Fig. 3.** Average yield of onion bulbs between the 2011 and 2016 cropseasons, in crops under no-tillage system (NTS) and conventional tillage system (CTS), including crop rotation or succession. T1, succession of onion and maize in NTS; T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in CTS; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS.

and Mielniczuk [2011](#page5); Costa Junior *et al*. [2012](#page5); Gould *et al*. [2016](#page5)). According to Tivet *et al*. ([2013](#page5)), depending on the plant species used in NTS, aggregate formation and C and N distribution in aggregate classes are affected by the biomass production and root system development area, and this effect increases over time of soil use.

*TOC and TN in soil*

*TOC*

The highest TOC in bulk soil was in T4–T6 in the 0–5 cm layer; in T4 and T8 in the 5–10 cm layer; and in T4, T6, and T8 in the 10–20 cm layer. The lowest TOC was in T1 and T7; for the 10–20 cm layer, T5 did not differ from T1 and T7 (Table [4](#page5)).

The lowest TOC in macroaggregates in the 0–5 cm layer was in T7. Considering only the NT treatments, T3 had the lowest TOC. Treatments T2, T4, T5, and T8 had the highest TOC in the 5–10 cm layer. However, T6 and T8 had the highest TOC in macroaggregates in the 10–20 cm layer. Thus, the lowest TOC was in T2 and T7.

The highest TOC in microaggregates in the soil surface layer (0–5 cm) was in T5, and the lowest in T1, T3, and T7. Treatment T7 had the lowest TOC in the 5–10 cm soil layer. Considering only the NT treatments, T1 and T6 had the lowest TOC. Treatments T1, T4, T5, and T8 had the highest TOC in the 10–20 cm layer, whereas T2 and T7 had the lowest.

The treatments conducted under NTS tended to have higher TOC than the treatment under CTS (T7). Over the years, the use of NTS with soil cover crops in succession or rotation with onion (except T1) and using minimum soil turning favoured

the build-up of SOM, which is the main source of organic C to the soil. However, SOM can be quickly lost when the soil is subjected to intensive tillage systems, such as CTS, because it increases the soil surface temperature and buries residues of previous crops, increasing their contact with the soil biota, accelerating their mineralisation (Silva *et al*. [1994](#page5); Loss *et al*. [2015](#page5)).

The plant residues and rhizodeposition from the soil cover plants of different botanical families in the NTS treatments are materials rich in C that are added to the soil, and these contributions of organic material are used by the established soil microbial community (Li *et al*. [2015](#page5)). In addition, the different types of root systems (grasses and legumes) of the crops and soil cover crops used in the intercrop (T2 and T8) or single (T3–T6) treatments in NTS may have increased the soil TOC due to their exudates and contribution to the soil microbial biomass, which act as cementing agents, binding soil particles and resulting in the protection of C in the interior of aggregates (Somasundaram *et al*. [2017](#page5)).

The lower TOC in bulk soil and in macroaggregates and microaggregates of T7 are due to the CTS, in which soil is turned through plough and harrowing practices, disrupting its structure and favouring the mineralisation of plant residues, which causes loss of C to the atmosphere as CO2 (Pulleman *et al*.[2005](#page5)). However, TOC in bulk soil in T7 (10–20 cm) didnot differ from T5; the macroaggregate contents in T7 did not differ from those in T1, T3, and T6 in the 5–10 cm layer, and from that in T2 in the 10–20 cm layer; and the microaggregates in T7 did not differ from those in T1 and T3 (5–10 cm) and T2 (10–20 cm). These results are due to successive incorporations of maize crop residues, which have high C/N ratio, favouring C immobilisation and increase in the 5–10 and 10–20 cm layers. Similar results were found by Pulleman *et al*. ([2005](#page5)), who evaluated the distribution of particulate organic matter in permanent pasture areas, with soil turning and conventional or organic management; they found that ploughing causes a considerable input of organic residues below the rooting zone.

Through evaluation of soil management (CTS, reduced soil preparation, and NTS) effects on fertility parameters (TOC and humic substances), in a five-year experiment in a vegetable production area, Lima *et al*. ([2017](#page5), [2018](#page5)) found that the addition of plant residues by soil cover crops, combined with low soil disturbance, led to a more efficient formation of humic substances and, therefore, increased fertility and stable organic matter and TOC contents in the 0–10 and 10–30 cm soil layers.

The higher TOC in macroaggregates in all evaluated layers in T8 was due to the combination of different soil cover plants, which added C through the plant residues covering the soil surface, and due to the diversity of root systems and root exudates released in the soil subsurface. Ladoni *et al*. ([2016](#page5)) evaluated the contribution of soil cover crops (*Trifolium* *pratense* L. and rye (*Secale cereale*)) in soybean and maizecrops under CTS and NTS, with low chemical or organic inputs, to soil soluble particulate fractions of soil C. They found a higher contribution of soil cover crop roots to the particulate C added to the soil compared to aboveground biomass, and lower amounts of soil C in the treatment under CTS compared to NTS.

Carbon and nitrogen in aggregates and bulk soil *Soil Research* I

The use of three grasses (black oats, rye, and pearl millet) in different periods as soil cover plants (T5) led to a high accumulation of TOC in the microaggregates. This is because grasses have a dense root system that emit organic exudates, and have frequent renewal of fine roots, generating more residues that can be protected in smaller aggregates. Grasses present higher C/N and lignin/N ratios, which results in slower decomposition of their residue, favouring the increase of C contents bonded to clay and silt and providing better conditions for the formation of microaggregates, which generates a higher amount of protected C in this aggregate diameter class (Gazolla *et al*. [2015](#page5)).

Bulk soil and macroaggregates had higher TOC than microaggregates in all evaluated layers and treatments (Table [4](#page5)). Macroaggregates are formed by the union of microaggregates, thus, they tend to require more C compounds for their formation, which generates more C associated with minerals protected in their interior (Seben Junior *et al*. [2016](#page5)). Some treatments under NTS presented higher TOC in bulk soil than in macroaggregates. This may be due to the constant presence of soil cover crops in the crop succession or rotation, which results in constant deposition of plant residues in the soil and at different stages of decomposition throughout the bulk soil and all aggregate fractions (Loss *et al*. [2009*c*](#page5)). This is corroborated by the results for T1, in which no soil cover plants were used; this treatment had lower TOC in bulk soil than in macroaggregates at all depths.

The T1 had higher TOC in macroaggregates than in bulk soil at all depths. Treatment T7 had higher TOC in bulk soil than in macroaggregates in the 0–5 and 5–10 cm layers, and no differences between bulk soil and macroaggregates in the 10–20 cm layer; however, it showed an increase in TOC of 7% in bulk soil. Since there was no use of soil cover pants in T1 and T7, the differences between these treatments were due to the soil management system – NTS had more stable aggregates (Fig. [3](#page5)) and higher GMD (Fig. [2](#page5)) than the CTS. Thus, the higher TOC in T1 compared to T7 indicates that TOC was more protected in NTS.

*TN*

Treatment T4 had the highest TN in bulk soil in the 0–5 and 5–10 cm layers. Treatments T4, T6, and T8 had the highest TN in the 10–20 cm layer; and the lowest TN was in T1 and T7, but was higher in T1 than T7 (Table [4](#page5)). The highest TN in macroaggregates in the 0–5 cm layer was in T2, T4–T6, and T8, and the lowest in T7; but there were no differences in TN between treatments in the other layers. The highest TN in microaggregates in the 0–5 cm layer was in T4 and T5, and the lowest in T7, followed by T3 and T1; the highest TN in the 5–10 cm layer was in T3–T5 and T8, and the lowest in T7; and the highest TN in the 10–20 cm layer was in T1 and T4, with the other treatments all having similar TN.

Velvet bean was used as a single cover plant in T4, and was intercropped with grasses in T6 and T8; this led to higher TN in the bulk soil in these treatments at all depths. Velvet bean was planted immediately after the onion crop, and remained in

the field during all of summer. Thus, the association and multiplication of rhizobacteria were favoured, increasing soil N contents, which can be protected in aggregates, or be released to plants from the mineralisation of SOM and death of the microorganisms. The biological N fixation promoted by legumes is affected by soil pH and temperature, SOM, rhizobia abundance, and their interaction with the soil physical, chemical, and biological properties. Thus, in treatments under NTS, in which these factors are favoured, SOM accumulation increases and, consequently, there is multiplication of the biota of the soil (Kihara *et al*. [2012](#page5); Torabian *et al*. [2019](#page5)).

The highest TN in macroaggregates for the 0–5 cm layer was in T2, T4–T6, and T8 and was due to the use of vetch and oilseed radish (T2) and velvet bean (T4, T6, and T8); oilseed radish is efficient in assimilating nitrate in the soil surface layer (Wang and Weil [2018](#page5)), and legumes favour symbiotic atmospheric N2 fixation (Amado *et al*. [1999](#page5)). Thus, plant residues that were protected within the aggregates were rich in N in these treatments. However, only grasses were used in rotation in T5, and this treatment had similar N contents in macroaggregates to those of the treatments with legumes. This may be due to the dry matter production of grasses, rye, and millet, which immobilised the N during its decomposition. The slower mineralisation of N in the soil is also due to the formation of organic matter and the minimum soil turning, which leads to lower temperature, higher moisture, and preservation of aggregates in the surface layer (De Neve [2017](#page5); Miller and Geisseler [2018](#page5)).

The highest TN in microaggregates was in T4 and T8 and may be attributed to the presence of legumes and the higher N contents in the light organic matter in these treatments (Udom and Omovbude [2019](#page5)). Ryegrass, pearl millet, and oat grasses immobilised N in their tissues in the T3 and T5 treatments, thus, after the plants were killed, the plant residues in the soil were likely to have high N contents. Moreover, grasses are more efficient in increasing or maintaining aggregate stability than legumes (Gould *et al*. [2016](#page5)), thus the N in plant residues is more protected in microaggregates. The highest TN in microaggregates in T1 in the 10–20 cm layer may be due to the presence of N-fixing plants of the Fabaceae family.

The lower TN in bulk soil and in macroaggregates and microaggregates in T7 was due to the absence of soil cover crops, which resulted in less input of plant material, and also to the soil turning, which accelerated losses of N, which were mainly added by chemical fertilisation in this treatment. This is shown by the lower light organic matter in T7 (Nath and Lal [2017](#page5)), which is one of the soil N stocks. The N applied via urea was lost more quickly in CTS than NTS, in which the SOM combined with microorganisms and soil aggregates can retain and slowly release N and other nutrients. Uribe *et al*. ([2018](#page5)) compared the impact of CTS and NTS on N and K losses in potato (*Solanum tuberosum*) crops and found that practices, such as the use of minimum soil turning, green manure, and permanent soil cover, resulted in greater N accumulation in the soil.

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*Natural abundance of C (*d*13C) and N (*d*15N) in soil*

d*13C*

The least negative values of d13C, i.e. the higher isotopic enrichment, in the 0–5 cm layer were found in T5, T1, and T3, and the most negative in T6, T2, and T4 in bulk soil, macroaggregates, and microaggregates respectively (Table [5](#page5)). The least negative values of d13C in the 5–10 cm layer were found in T1, T3, T5, and T7 in bulk soil; in T5 in microaggregates; and in T1, T3, T5, and T7 in macroaggregates. Treatment T6 had the most negative values of d13C in bulk soil, macroaggregates, and microaggregates. The least negative values of d13C in bulk soil in the 10–20 cm layer were in T7, followed by T1 and T3. The T1, T5, and T7 had the least negative values of d13C in microaggregates; T7 and T8 had the most negative d13C values in macroaggregates.

The isotopic values were due to the plant species used in each treatment. Plants with C3 photosynthetic cycle fix

atmospheric CO2 through the rubisco enzyme, whereas C4 plants fix CO2 through the PEP carboxylase enzyme, which has a high affinity for CO2. Due to the lower affinity for CO2 of rubisco, this enzyme has a preference for the light isotope of C (12C) compared to the heavy isotope (13C). This leads to a lower accumulation of 13C in C3 plants compared to C4 plants (Alves *et al*. [2005](#page5); Mendonca¸ *et al*. [2010](#page5)). The d13C fixed by C3 plants varies from –24‰ to –34‰, whereas in C4 plants it varies from –6‰ to –19‰ (Smith and Epstein [1971](#page5)).

Thus, the less-negative d13C values found in T1, T3, T5, and T7 were mainly due to the use of C4 plants in these treatments, such as millet in T5 and T8, and maize in T1–T3 and T7. The treatment that most resembled the values of C4 plants (d13C of –6‰ to –19‰) was T5, which had soil cover plants only of grasses, despite only millet being a C4 plant. Contrastingly, because T6 contained only C3 plants it had the most negative values in the surface layer, and closer to –24‰.

The NTS in T1–T3, T5, and T8 favoured the SOM accumulation over the crop cycles; this may also be due to

**Table 5. Natural abundance of 13C and 15N in soils cultivated with onion crops under no-tillage and conventional tillage systems including crop rotation or succession and use of soil cover crops, Ituporanga, SC, Brazil**

Means followed by a different uppercase letter in columns differ by the Scott–Knott test at *P* < 0.05, and means followed by a different lowercase letter in rows differ by *t*-test at *P* < 0.05. CV, coefficient of variation; Macro, macroaggregates; Micro, microaggregates; T1, succession of onion and maize in no-tillage system (NTS); T2, rotation of soil cover crops (winter) and biennial onion in NTS; T3, rotation of maize, winter grasses, and onion in NTS; T4, succession of summer legume and annual onion in NTS; T5, rotation of summer grass, winter grasses, and annual onion in NTS; T6, succession of summer legume, winter grass, and annual onion in NTS; T7, succession of maize and onion in conventional tillage system; T8, succession of intercrops of soil cover crops (summer) and annual onion in NTS

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| T |  | 13C (‰) |  |  |  | 15N (‰) |  |  |
|  | Bulk soil | Macro | Micro | CV (%) | Bulk soil | Macro | Micro | CV (%) |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  | 0–5 cm |  |  |  |  |
| T1 | –19.57 Ba | –19.82 Ba | –20.13 Ba | –2.25 | 7.26 Aa | 7.87 Aa | 5.73 Ab | 6.84 |
| T2 | –21.08 Da | –21.49 Da | –21.33 Da | –0.81 | 6.86 Aa | 7.55 Aa | 5.68 Ab | 6.36 |
| T3 | –19.35 Ba | –19.80 Ba | –20.09 Ba | –1.61 | 7.22 Aa | 7.38 Aa | 5.89 Ab | 3.51 |
| T4 | –21.85 Ea | –21.49 Da | –21.84 Da | –1.20 | 6.35 Ba | 6.37 Ba | 5.31 Ab | 5.45 |
| T5 | –18.64 Aa | –18.99 Aa | –19.07 Aa | –3.46 | 6.64 Ba | 7.35 Aa | 5.97 Aa | 5.94 |
| T6 | –23.08 Fa | –22.97 Ea | –23.08 Ea | –1.67 | 6.24 Ba | 6.44 Ba | 5.19 Ab | 4.36 |
| T7 | –20.13 Ca | –20.61 Ca | –20.69 Ca | –1.22 | 6.97 Aa | 7.40 Aa | 6.06 Ab | 7.36 |
| T8 | –20.68 Ca | –20.81 Ca | –20.89 Ca | –1.43 | 6.09 Ba | 6.61 Ba | 5.57 Aa | 6.87 |
| CV (%) | –1.85 | –1.88 | –1.69 |  | 5.88 | 5.35 | 6.99 |  |
|  |  |  |  | 5–10 cm |  |  |  |  |
| T1 | –20.51 Aa | –20.95 Ba | –20.90 Aa | –1.90 | 7.65 Aa | 8.11 Aa | 5.93 Ab | 4.23 |
| T2 | –21.10 Ba | –21.49 Ca | –21.52 Ba | –0.96 | 7.64 Aa | 7.22 Aa | 5.46 Ab | 5.80 |
| T3 | –20.15 Aa | –20.59 Ba | –20.52 Aa | –1.25 | 7.85 Aa | 7.40 Aa | 5.43 Ab | 3.35 |
| T4 | –21.52 Ba | –21.44 Ca | –21.63 Ba | –1.51 | 7.07 Ba | 7.07 Aa | 5.16 Ab | 1.88 |
| T5 | –20.09 Aa | –19.96 Aa | –20.42 Aa | –1.57 | 7.28 Ba | 7.72 Aa | 5.62 Ab | 7.63 |
| T6 | –22.24 Ca | –22.10 Da | –22.10 Ca | –1.04 | 7.07 Ba | 7.43 Aa | 5.76 Ab | 4.90 |
| T7 | –20.47 Aa | –20.69 Ba | –20.69 Aa | –0.61 | 7.17 Ba | 7.53 Aa | 4.66 Bb | 4.57 |
| T8 | –21.07 Ba | –20.94 Ba | –21.25 Ba | –0.59 | 7.14 Ba | 7.37 Aa | 5.63 Ab | 4.40 |
| CV (%) | –1.32 | –1.06 | –1.31 |  | 3.68 | 4.54 | 6.85 |  |
|  |  |  |  | 10–20 cm |  |  |  |  |
| T1 | –20.31 Ba | –20.43 Aa | –20.62 Ba | –2.39 | 7.53 Aa | 7.70 Aa | 6.13 Ab | 3.80 |
| T2 | –20.60 Ca | –21.02 Ba | –20.00 Ba | –3.24 | 7.66 Aa | 7.36 Aa | 5.20 Bb | 6.59 |
| T3 | –20.33 Ba | –21.02 Bb | –20.18 Ba | –1.65 | 7.61 Aa | 6.90 Ab | 4.74 Bc | 4.80 |
| T4 | –20.83 Ca | –20.92 Ba | –20.79 Ba | –2.05 | 7.27 Aa | 7.25 Aa | 5.25 Bb | 4.56 |
| T5 | –20.65 Ca | –20.41 Aa | –20.79 Ba | –1.58 | 7.24 Aa | 7.75 Aa | 5.82 Ab | 3.72 |
| T6 | –21.19 Ca | –21.44 Ba | –20.51 Ba | –2.42 | 7.21 Aa | 7.32 Aa | 6.03 Ab | 3.95 |
| T7 | –19.68 Ab | –20.01 Ab | –18.34 Aa | –0.98 | 6.40 Ba | 7.57 Aa | 4.80 Bb | 4.72 |
| T8 | –20.77 Cb | 20.84 Bb | –19.33 Aa | –2.56 | 7.37 Aa | 7.58 Aa | 5.88 Ab | 3.93 |
| CV (%) | –1.43 | –2.05 | –2.96 |  | 3.21 | 4.40 | 6.38 |  |
|  |  |  |  |  |  |  |  |  |

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their higher d13C contents (less-negative values) compared to the other treatments, because the longer the SOM remains in the soil, the more C12 tends to be decomposed into CO2, while 13C remains in the soil (Liu *et al*. [2018](#page5)). Although T4 and T6 were under NTS, favouring soil cover, they generally had lower d13C values (more-negative values) in all evaluated layers; this may be due to the presence of C3 plants in the

rotation – velvet bean (T4 and T6) and rye (T6) – incorporating less soil 13C.

The effect of the soil management systems on 13C is shown by the d13C results in T1 and T7. These treatments had the same crop sequence (maize and onion); T1 had higher d13C (less negative) in the soil surface layer, and the deeper layers presented higher values (less negative) in T7, in bulk soil, macroaggregates, and microaggregates. These results were due to the soil turning, which incorporated maize plant residues into deeper layers, resulting in less-negative 13C values in the 10–20 cm layer.

There were practically no differences in the d13C found in bulk soil, macroaggregates, and microaggregates in each treatment. This denotes a favourable environment for C protection; however, the d13C in bulk soil, macroaggregates, and microaggregates differed in the deepest layer in T7 and T8. In these treatments, less-negative 13C values were found in microaggregates compared to macroaggregates and bulk soil.

The C contents in microaggregates are more protected than in macroaggregates or bulk soil, mainly from oxidative processes, which can increase the decomposition of C (Six *et al*.[2002](#page5);Del Galdo *et al*.[2003](#page5)). Therefore, the less-negativevalues in the microaggregates may be due to their formation in association with C recently incorporated in the macroaggregates. Maize residues were incorporated into T7, thus more residues of C4 plants were associated with microaggregates; and rhizodeposition of pearl millet in T8 (NTS) may have favoured C4 plant materials that were incorporated into microaggregates, resulting in less-negative values.

d*N15*

The T1–T3 and T7 treatments had the highest 15N contents in bulk soil in the 0–5 cm layer. The T1–T3 had the highest values of 15N in the 5–10 cm layer. Only T7 differed from the other treatments, with the lowest values of 15N in the 10–20 cm layer. Differences in values of 15N in macroaggregates between treatments were found only in the 0–5 cm layer; the highest values of 15N were in T1–T3, T5, and T7. No differences in 15N values in microaggregates were found between treatments in the 0–5 cm layer. Only T7 differed from the other treatments in the 5–10 cm layer, presenting the lowest values of 15N. Treatments T1, T5, T6, and T8 had the highest 15N values in microaggregates in the 10–20 cm layer (Table [5](#page5)).

The lowest values of 15N, mainly in macroaggregates (0–5 cm) and in bulk soil (0–5 and 5–10 cm), in treatments with velvet bean as a cover plant (T4, T6, and T8) may be due to the atmospheric N2-fixing plants, whose 15N contents were low due to a dilution effect caused by N2, since atmospheric 15N excess is zero. However, soil usually contains a little 15N

due to the isotopic fractionation between 14N and 15N that occurs in physical, chemical, and biological processes that involve N from organic matter and soil; thus the 15N content in non-N-fixing plants will be similar to that available in soil (Miranda *et al*. [2003](#page5)).

The T7 had the lowest values of 15N in microaggregates in the 5–10 cm layer, and in bulk soil in the 10–20 cm layer due to the plough and harrowing processes. According to Szpak ([2014](#page5)), soil turning redistributes N and SOM along the soil profile and changes the 15N variation in deeper layers, compromising the N transformations. These results corroborate those of Loss *et al*. ([2016](#page5)), who found lower 15N values in deeper layers in treatments under soil turning (CTS) compared to those under NTS and forest areas.

Higher values of 15N were found in macroaggregates and bulk soil compared to microaggregates, in all evaluated layers. This was because SOM protection is greater in microaggregates, making microbial oxidation more difficult. Costa Junior *et al*. ([2011](#page5)) also found similar results, with higher N and 15N accumulation in aggregates of size >2.00 mm.

**Conclusions**

The greater plant diversity in the treatments with soil cover plants in rotation or succession with onion crops (T2–T6 and T8) resulted in higher GMD of aggregates in the 0–5 and 10–20 cm soil layers compared to treatments without the use of soil cover plants (T1 and T7).

Treatment T8 (succession of intercrops of soil cover crops in the summer and annual onion) was more efficient in increasing the GMD of aggregates in the 10–20 cm layer than the other treatments under NTS.

The treatments with succession of maize and onion under NTS (T1) and CTS (T7) showed differences in almost all evaluated attributes; the NTS was more efficient at improving the evaluated soil physical and chemical attributes.

The use of NTS with plants of the Poaceae and Fabaceae families in single or intercrop systems for onion production resulted in higher TOC and TN contents in the 0–5 and 5–10 cm soil layers compared to CTS.

The use of the natural abundance of 15N showed that C and N were more protected in microaggregates than in macroaggregates and bulk soil in all evaluated treatments and soil layers. Macroaggregates had more TOC and TN than microaggregates.

**Conflicts of interest**

We have no conflict of interest to declare.

**Acknowledgments**

The authors express their thanks to the National Council for Scientific and Technological Development – CNPq (Process No 302603/2015–8 and 403949/2016–5) and the Experimental Station of Ituporanga, SC, Brazil, for the availability of the experimental area. This study was financed in part

by the Coordenacão¸ de Aperfeicoamento¸ de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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