No-tillage and crop-livestock with silage production impact little on carbon and nitrogen in the short-term in a subtropical Ferralsol

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ABSTRACT: We assessed the short-term (3.5 years) contributions of no-tillage (NT) relative to conventional tillage (CT), and of integrated crop-livestock systems combined with no-tillage (ICL-NT) relative to NT, on soil carbon (C) and nitrogen (N) and their physical fractions (free-light, occluded-light and heavy), in condition of maize silage production. An Umbric Ferralsol (Latossolo Bruno) was cultivated with ryegrass (*Lolium multiflorum*), for cover-crop in CT and NT, pasture in ICL-NT; and maize for silage. No-tillage, relative to CT, improved C and N stocks in 0-5 cm and mainly in the heavy fraction, but not deeper, so that stocks in whole 0-20 cm were (statistically) similar in both systems (~67 Mg C ha^{-1} and ~4.8 Mg N ha^{-1}). Likewise, the ICL-NT did not affect C and N stocks to 20 cm relative to NT, not even in the top layer or in some physical fraction. The short 3.5-year period, the originally high C and N contents of this Umbric Ferralsol and the removal of silage have possibly limited the effects of NT or ICL-NT. However, the adoption of those two conservation systems have at least no adverse effects on C and N stocks, without detriment to their already known productive and economic benefits.

Key words: carbon sequestration; conservationist agriculture; densimetric fractionation; soil organic matter

Plantio direto e lavoura-pecuária com produção de silagem tem pouco impacto no carbono e nitrogênio no curto prazo em um Latossolo subtropical

RESUMO: Foram avaliadas as contribuições de curto prazo (3,5 anos) do plantio direto (PD) em relação ao preparo convencional (PC) e da integração lavoura-pecuária sob PD (ILP-PD) em relação à lavoura contínua em PD sobre os estoques de carbono (C) e nitrogênio (N) do solo e frações físicas (leve livre, leve oclusa e pesada), em condições de produção de milho para silagem. Um Latossolo Bruno foi cultivado com azevém (*Lolium multiflorum*) no inverno, para cobertura em PD e PC pastagem em ILP-PD, e milho para silagem no verão. O solo sob PD, em relação ao PC, melhorou os estoques de C e N na camada de 0-5 cm e principalmente na fração pesada, mas não em profundidade, de modo que os estoques na camada de 0-20 cm foram (estatisticamente) similar em ambos os sistemas (~67 Mg C ha^{-1} e ~4,8 Mg N ha^{-1}). Da mesma forma, a ILP-PD também não alterou os estoques de C e N do solo até 20 cm em relação ao PD, e nem mesmo na camada superior ou em alguma das frações físicas. O curto período de 3,5 anos, e os conteúdos originalmente elevados de C e N deste Latossolo Bruno e a remoção da fitomassa para silagem, possivelmente limitaram os efeitos do PD ou ILP-PD. No entanto, a adoção desses dois sistemas conservacionistas não teve nenhum efeito prejudicial nos estoques de C e N, sem prejuízo aos seus já conhecidos benefícios produtivos e econômicos.

Palavras-chave: sequestro de carbono; agricultura conservacionista; fracionamento densimétrico; matéria orgânica do solo

Introduction

The less oxidative soil environment under no-tillage (NT) may promote organic matter (OM) accumulation and, consequently, improvements in soil quality (Laroca et al., 2018). The annual rates of carbon (C) accumulation in the 0 to 20 cm of soil under NT vary from almost zero to approximately 1 Mg C ha^{-1} yr^{-1}, with an average rate of 0.48 Mg C ha^{-1} yr^{-1} in the subtropical Southern Brazil (Bayer et al., 2006b).

Nevertheless, soil C and nitrogen (N) accumulation, improvement in soil quality and crop yields with NT are closely dependent on crop rotation plans that support adequate shoot and root residue input, both in winter and summer seasons. In this respect, winter fallow and or the cultivation of low-input cash or cover crops may reduce or even make null the rates of soil C and N accumulation, leading some authors to contest the potential of NT soil to serve as C or N sink (Haddaway et al., 2017).

The combination of NT crop production with livestock production through the integrated crop-livestock system (ICL) is a further and important step towards conservationist agriculture, with environmental and productive benefits being reported (Carvalho et al., 2018). With adequate grazing, pasture net primary production may be even higher compared with no grazing, as observed in black oat or ryegrass (Sandini et al., 2011) and in brachiaria pastures (Debiasi & Franchini, 2012). Despite that, there are divergent results in studies on the contribution of ICL to soil C and N storage. Some studies present increase in C stocks with ICL (Salton et al., 2011), while others present its maintenance (Salvo et al., 2010; Franzluebbers & Stuedemann, 2013) or even depletion (George et al., 2013).

Progress on understanding the impacts of soil use and tillage systems on soil quality and OM accumulation and stabilization have been obtained by physical fraction approaches, which allows the isolation and quantification of structurally and functionally distinct compartments of the OM (Christensen, 2001). Densimetric fractionation allows the separation of three fractions: the free light (FL) fraction, which consists of inter-aggregate particulate OM (not protected); the occluded light (OL) fraction, which consists of intra-aggregate particulate OM (physically protected); and the heavy fraction, which consists of humified OM interacting with mineral surfaces via organo-mineral associations (Christensen, 2001). The light fractions, due to their particulate inherent features and fast turnover rates, are supposedly the most sensitive fraction to soil use and tillage systems, and so might be very useful in the assessment of short-term effects that those systems cause or will cause on soil OM of the soil (Conceição et al., 2008).

The Campos Gerais region of Paraná State, where the Brazilian NT has one of its birthplaces, is also a major milk producer of the country. One of the common soil use systems adopted in crop-dairy farms of the region is based on winter ryegrass for grazing and summer maize for silage, characterizing a sort of ICL system. That raises the question: how this soil use system with aboveground biomass removal with silage and grazing affects C and N stocks of the whole soil and its physical fractions, even under the conservationist NT management? Taking into account this question related to a particular condition of NT and ICL-NT, and the divergent results among studies related to ICL and soil OM, we noticed a need for additional information about effects of NT and ICL on soil C and N stocks.

The hypothesis of this study is that, in the short term and under removal of the aboveground maize for silage, the conservationist management systems of NT, relative to CT, and ICL-NT, relative to NT, have little impact to improve the whole C and N stocks of subtropical Umbric Ferralsol (Latossolo Bruno); yet, they do increment the stocks in the light (particulate) fractions, which are probably more sensitive to soil management changes than the heavy (humified) fraction. Therefore, our objective was to evaluate the short-term (3.5 years) impact of NT and ICL-NT conservationist systems with maize growing for silage on C and N stocks of a subtropical Umbric Ferralsol and its physical fractions.

Materials and Methods

Field experiment

The study was conducted in a field experiment initiated in the winter of 2005, at the experimental station of the ABC Foundation, in Castro-PR, Brazil (24º 47' 53'' S, 49º 57' 42'' W, and 996 m of altitude). The soil was a clayey Umbric Ferralsol (Latossolo Bruno) according to a local survey, with 439 g kg^{-1} clay, 177 g kg^{-1} silt, and 384 g kg^{-1} sand. The slope was less than 5%. Climate was humid subtropical, Cfb (Köppen), with average monthly temperature varying from 12.5 ºC (July) to 20.6 ºC (February), and with average annual precipitation of 1.656 mm. The local native vegetation was short grassland named "Campos Gerais", which in the 1960s was converted into annual cropland based on conventional tillage, with disc plowing, a management system that lasted until no-tillage was adopted at the experiment establishment in 2005.

The experiment comprised three soil use systems and seven tillage system, and was cultivated with annual ryegrass (*Lolium multiflorum* L.) in winter and maize (*Zea mays* L.) for silage in summer. Maize was harvested at the R5.5 growth stage, at a cutting height of 10-15 cm. The ryegrass sowing in a row, with a seeder, with a spacing of 17 cm and density of 100 kg ha^{-1} of seed, usually in the month of April. Three combinations of soil use and tillage systems were selected and evaluated in this study: two where ryegrass was used as winter cover crop and soil was subjected either to conventional tillage (CT) or no-tillage (NT); and a third system where ryegrass was cattle-grazed, corresponding to the integrated crop-livestock combined with no-tillage (ICL-NT). Therefore, the treatments were as follows: (i) conventional tillage (CT) with ryegrass for coverage; (ii) no-tillage (NT) with ryegrass for coverage; (iii) integrated crop-livestock under no-tillage (ICL-NT). The choice of these treatments is due to the fact that NT or ICL-NT is widely used in farms of the region;

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and CT served as a control treatment. The experiment was arranged in a complete randomized block design with four replicates. In CT, operations included one a heavy disc harrow up to approximately 15 cm depth followed by two leveling disc harrow up to approximately 10 cm depth; and they were carried out twice a year, in autumn, before ryegrass sowing, and in spring, before maize seeding. In NT, ryegrass was desiccated with glyphosate herbicide at its flowering stage, in the spring. In ICL-NT, ryegrass was grazed by Jersey or Holstein heifers (10-18 months old), in three or four grazing cycles per season, each cycle starting when ryegrass was 25 cm high and finishing two or three days later when ryegrass was lowered to 10 cm high, whit a variable stocking rate. The lasting ryegrass residue after the last grazing cycle of the season was desiccated with glyphosate herbicide.

Data of aboveground biomass production and addition of ryegrass were obtained in the archives of the experiment; while aboveground biomass addition of maize was assumed as zero, considering the biomass was exported for silage. For ryegrass in ICL-NT, the biomass addition was that of dung, and assumed as being 30% of the produced biomass, considering a digestibility of 70% (Miguel et al., 2012).

Soil sampling, physical fractionation and C and N analysis

Soil samples of the 0-5, 5-10 and 10-20 cm layers were collected in December 2008, when the experiment was 3.5 years old. Samples were collected with spatula in two pits 20 cm wide and 40 cm long located in the central region of each plot of 10 × 10 m. Two sub-samples were obtained in each pit, four per plot. After air-drying, samples were ground to ≤ 2 mm and an aliquot was further ground to ≤ 250 μm to be analyzed for C and N by dry combustion (Elementar Vario EL Analyzer).

Soil samples were subjected to density fractionation to obtain the free light (FL), occluded light (OL) and heavy fractions of OM, using a method adapted from Conceição et al. (2008). In a 100 mL centrifuge tube containing 20 g of soil (≤ 2 mm) we added 60 mL of sodium polytungstate solution adjusted to a density of 2.0 Mg m^{-3}. Each tube was sealed with a rubber stopper and manually and slowly inverted five times, so that the FL fraction could be released from between the soil aggregates. After centrifugation (90 min, 2000 ×g), the supernatant was vacuum filtered and the FL fraction retained in the glass fiber filter (Whatman GF/C) was washed with distilled water to remove the excess sodium polytungstate and then dried at 60 °C. To obtain the OL fraction, the tube was refilled with 60 mL of sodium polytungstate solution, the soil pellet was dispersed by ultrasonic energy (410 J mL^{-1}, previous test was carried out) and the suspension centrifuged (90 min, 2000 ×g). The OL fraction in the supernatant was recovered by filtering and washing, as described for the FL fraction. After being weighed, the FL and OL fractions were ground to ≤ 250 μm and analyzed for C and N by dry combustion (Elementar Vario EL Analyzer). The C concentration of the heavy fraction was assumed as being the difference between the C concentration in the whole soil sample and the C concentration in the FL plus OL fractions (all in g kg^{-1} of soil). Same approach was used for N concentration.

The C and N stocks in the whole soil sample and in physical fractions were calculated according to the equivalent soil mass approach (Alburquerque et al., 2015), considering the mass of the CT soil as reference. The mass of soil was calculated for each layer taking into account its bulk density, which was measured with a metal cylinder (Ø 56.3 mm; h 29.8 mm) placed vertically in the middle of each layer, according to the core method (Embrapa, 2011).

The annual C and N accumulation rates over the 3.5 years of the experiment was calculated for NT, relative to CT, and for ICL-NT, relative to NT. This is referred to as the relative sequestration rate rather than the absolute sequestration rate because it does not consider the OC stock at the beginning of the experiment, information on which was not available; according to the equations below, as exemplified for C (Equations 1 and 2).

$$C \text{ accumulation rate in NT} = \frac{(\text{soil C stock in NT}) - (\text{soil C stock in CT})}{3.5 \text{ yr}} \quad (1)$$

$$C \text{ accumulation rate in ICL-NT} = \frac{(\text{soil C stock in ICL-NT}) - (\text{soil C stock in NT})}{3.5 \text{ yr}} \quad (2)$$

where: C accumulation rate in NT (Mg ha^{-1} yr^{-1}); soil C stock in NT, soil C stock in CT and soil C stock in ICL-NT (Mg ha^{-1}).

Statistical analysis

Data were submitted to analysis of variance and the means were compared by the Tukey test ($p \leq 0.10$); according to the complete randomized block design of the field experiment, through the statistical program Statistica 7.0 (Statsoft, 2007).

Results and Discussion

No-tillage improved C and N concentrations and stocks relative to CT, but that improvement was restricted only to the first 5 cm of soil (Tables 1 and 2). In that layer, NT accumulated 0.61 Mg C ha^{-1} yr^{-1} and 0.06 Mg N ha^{-1} yr^{-1}, relative to CT (Table 2).

Nearly all the accumulation from C and N occurred in the heavy fraction (0.59 Mg C ha^{-1} yr^{-1} and 0.05 Mg N ha^{-1} yr^{-1}), but not in the light fractions (FL and OL), where stocks remained similar in NT and CT (Table 2). The fact that C and N accumulated in the heavy fraction but not significantly in the light fractions is intriguing and contradicts our hypothesis that such accumulation in short-term conservation systems takes place in the light (particulate) fractions, which are supposedly more sensitive to soil management changes than the heavy (humified) fraction. One explanation to this contradictory result could be that at soil sampling, 70-75 days after ryegrass desiccation and during maize vegetative growth, much of the light fraction derived from the latest biomass residue input (ryegrass desiccation) was decomposed and partially incorporated and accumulated into the increasing C and N pools of the heavy fraction of NT soil. Thus, no difference for the light fraction level between NT and CT. Thus, some studies

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Table 1. Carbon (C) and nitrogen (N) concentrations and C:N ratio of a subtropical Umbric Ferralsol (Latossolo Bruno) and its physical fractions (FL, free-light; OL, ocluded-light; and heavy) subjected to conventional tillage (CT), no-tillage (NT) or integrated-crop livestock under no-tillage (ICL-NT). Castro-PR, Brazil.

	C (g kg⁻¹)				N (g kg⁻¹)				C:N ratio				Fraction mass:soil mas			Bulk density (Mg m⁻³)
	Soil	FL	OL	Heavy	Soil	FL	OL	Heavy	Soil	FL	OL	Heavy	FL	OL	Heavy	
								0-5 cm								
CT	31.0 b	87.4 a	90.2 a	27.4 b	2.30 b	5.03 a	5.81 a	2.11 b	13.5 a	17.4 a	15.5 a	13.0 a	0.027 a	0.031 a	0.942 a	1.09 a
NT	34.9 a	75.4 ab	72.7 a	31.9 a	2.67 a	5.20 a	5.14 a	2.48 a	13.1 ab	14.5 b	14.1 b	12.9 a	0.024 a	0.048 a	0.928 a	1.17 a
ICL-NT	35.5 a	71.5 b	73.4 a	32.9 a	2.82 a	4.75 a	5.27 a	2.66 a	12.6 b	15.1 b	13.9 b	12.4 a	0.028 a	0.037 a	0.935 a	1.16 a
								5-10 cm								
CT	29.2 a	68.1 a	125.0 a	26.7 a	2.22 a	4.37 a	8.95 a	2.06 a	13.1 b	15.6 a	14.0 b	13.0 b	0.020 a	0.017 a	0.963 a	1.20 a
NT	27.6 a	46.3 b	137.2 a	25.7 a	2.12 ab	2.85 b	8.21 a	2.02 a	13.0 b	16.2 a	16.7 a	12.8 b	0.012 a	0.015 a	0.973 a	1.23 a
ICL-NT	27.7 a	46.1 b	92.4 a	26.2 a	1.99 a	3.34 b	5.89 a	1.89 a	13.9 a	13.8 b	15.7 a	13.9 a	0.025 a	0.016 a	0.959 a	1.19 a
								10-20 cm								
CT	26.7 a	55.3 a	119.4 a	25.4 a	1.78 a	3.25 a	6.76 a	1.71 a	15.0 a	17.0 b	17.7 b	14.8 a	0.008 a	0.011 a	0.981 a	1.22 a
NT	25.4 a	68.5 a	73.1 b	24.8 a	1.71 a	3.64 a	3.74 b	1.68 a	14.9 a	18.8 a	19.5 a	14.7 a	0.002 a	0.011 a	0.987 a	1.24 a
ICL-NT	25.8 a	46.3 a	87.2 b	24.8 a	1.68 a	2.92 a	4.94 ab	1.63 a	15.4 a	15.9 b	17.7 b	15.3 a	0.006 a	0.014 a	0.980 a	1.17 a

Means followed by the same letter, in the same column and soil layer, do not differ significantly according to the Tukey test ($p \geq 0.10$).

Table 2. Carbon (C) and nitrogen (N) stocks and annual accumulation rates (ΔC and ΔN) of a subtropical Umbric Ferralsol (Latossolo Bruno) and its physical fractions (FL, free-light; OL, ocluded-light; and heavy) subjected to conventional tillage (CT), no-tillage (NT) or integrated-crop livestock under no-tillage (ICL-NT). Castro-PR, Brazil.

	Soil	FL	OL	Heavy	Soil	FL	OL	Heavy
	C (Mg ha⁻¹)				N (Mg ha⁻¹)			
				0-5 cm				
CT	16.90 b	1.29 a [8]	1.52 a [9]	14.09 b [83]	1.25 b	0.07 a [6]	0.10 a [8]	1.08 b [86]
NT	19.03 a	0.99 a [5]	1.90 a [10]	16.14 a [85]	1.46 a	0.07 a [5]	0.13 a [9]	1.26 a [86]
ICL-NT	19.34 a	1.08 a [6]	1.48 a [8]	16.78 a [86]	1.54 a	0.07 a [5]	0.11 a [7]	1.36 a [88]
				5-10 cm				
CT	17.51 a	0.82 a [5]	1.28 a [7]	15.41 a [88]	1.33 a	0.05 a [4]	0.09 a [7]	1.19 a [89]
NT	16.87 a	0.38 a [2]	1.29 a [8]	15.20 [90]	1.29 a	0.02 a [2]	0.08 a [6]	1.19 a [92]
ICL-NT	16.91 a	0.97 a [6]	0.93 a [5]	15.01 a [89]	1.22 a	0.07 a [6]	0.06 a [5]	1.09 a [89]
				10-20 cm				
CT	32.52 a	0.54 a [2]	1.60 a [5]	30.38 a [93]	2.17 a	0.03 a [1]	0.09 a [4]	2.05 a [95]
NT	31.13 a	0.19 a [1]	1.05 a [3]	29.89 a [96]	2.11 a	0.01 a [0]	0.05 a [3]	2.05 a [97]
ICL-NT	31.56 a	0.37 a [1]	1.50 a [5]	29.69 a [94]	2.06 a	0.02 a [1]	0.09 a [4]	1.95 a [95]
				0-20 cm				
CT	66.93 a	2.64 a [4]	4.40 a [7]	59.89 a [89]	4.76 a	0.16 a [3]	0.28 a [6]	4.32 a [91]
NT	67.03 a	1.56 a [2]	4.24 a [6]	61.23 a [91]	4.86 a	0.10 a [2]	0.27 a [6]	4.49 a [92]
ICL-NT	67.81 a	2.42 a [3]	3.91 a [6]	61.48 a [91]	4.82 a	0.16 a [3]	0.26 a [5]	4.40 a [91]
	ΔC (Mg ha⁻¹ yr⁻¹)				ΔN (Mg ha⁻¹ yr⁻¹)			
				0-5 cm				
NT	0.61 *	-0.09 ns	0.11 ns	0.59 *	0.06 *	0.00 ns	0.01 ns	0.05 *
ICL-NT	0.09 ns	0.03 ns	-0.12 ns	0.18 ns	0.02 ns	0.00 ns	-0.01 ns	0.03 ns
				5-10 cm				
NT	-0.18 ns	-0.12 ns	0.00 ns	-0.06 ns	-0.01 ns	-0.01 ns	0.00 ns	0.00 ns
ICL-NT	0.01 ns	0.16 ns	-0.10 ns	-0.05 ns	-0.02 ns	0.01 ns	0.00 ns	-0.03 ns
				10-20 cm				
NT	-0.40 ns	-0.10 ns	-0.16 ns	-0.14 ns	-0.02 ns	-0.01 ns	-0.01 ns	0.00 ns
ICL-NT	0.12 ns	0.05 ns	0.12 ns	-0.05 ns	-0.01 ns	0.00 ns	0.01 ns	-0.02 ns
				0-20 cm				
NT	0.03 ns	-0.31 ns	-0.04 ns	0.39 ns	0.03 ns	-0.02 ns	0.00 ns	0.05 ns
ICL-NT	0.22 ns	0.24 ns	-0.09 ns	0.07 ns	-0.01 ns	0.02 ns	0.00 ns	-0.03 ns

Means followed by the same letter, in the same column and soil layer, do not differ significantly according to the Tukey test ($p \geq 0.10$).
Value in brackets denotes the percentage relative to the total C or N stocks.
Annual accumulation rates (ΔC or ΔN) are significant (*) or not significant (ns) according to the Tukey test ($p < 0.10$; $p \geq 0.10$). Accumulation rates for NT were calculated relative to CT, and for ICL-NT they were calculated relative to NT; considering the 3.5-year age of the experiment.

show that there is a higher rate of decomposition of residues and release of nutrients in the first 15 days, and close to 75 days, approximately 50% of the material, has already been decomposed and incorporated into the soil (Aita & Giacomini, 2003; Medrado et al., 2011).

Since C and N accumulations were not observed in the 5-10 and 10-20 cm layers, the incremental effect of the 0-5 cm was not noticed in the whole 0-20 cm layer, so that no differences occurred between NT and CT for C and N stocks in this layer, and the corresponding C and N accumulation rates for NT

became null (0.03 Mg C ha^{-1} yr^{-1} and 0.03 Mg N ha^{-1} yr^{-1}, not significant, Table 2). In the top 5 cm of NT soil, the minimum soil disturbance and therefore the maintenance of the surface and root residues were possibly the main driving factors of the significant C and N accumulations, a well-known process (Bayer & Mielniczuk, 1997). However, the non-significant rates of C and N accumulation to the top 20 cm under NT differed from results of Bayer et al. (2000) and Sá et al. (2001), who also worked in subtropical Brazilian experiments but observed rates up to 0.81 Mg C ha^{-1} in the first 20 cm, using crop rotation and including legumes in the system, promoting greater C addition to the soil. Yet, compared to the 9 years of the experiment of Bayer et al. (2000), on an Acrisol (Argissolo Vermelho), and to the 22 years of that of Sá et al. (2001), on a Rhodic Ferralsol (Latossolo Vermelho), both without removal of crop aboveground residues, the field experiment of the current study differed from theirs on three main aspects: the young age, 3.5 years; the soil class, Umbric Ferralsol (Latossolo Bruno); and the removal of maize aboveground for silage.

In the current study, 3.5 years was not enough for NT to accumulate significant C and N stocks to 20 cm. The originally high C stock of this Umbric Ferralsol (> 65 Mg C ha^{-1} in the first 20 cm, Table 2) might have also contributed to limit increments to C and N, a supposition supported by Campbell et al. (1991), who found no OM response to 31 years of crop rotations in soil with high OM content (about 64 Mg C ha^{-1} in the top 15 cm).

Nonetheless, the low biomass input due to maize removal for silage was also an important, if not the most important, cause of the lack of C and N accumulation in the 0-20 cm of NT soil. In both tillage systems, the overall aboveground dry matter yield averaged approximately 21 Mg DM ha^{-1} yr^{-1}, which is quite high (4 of ryegrass and 17 of maize) (Table 3). But with silage removal, the effective aboveground input in the two tillage systems was only the about 4 Mg DM ha^{-1} yr^{-1} of ryegrass, which is surely little to improve C and N in subtropical NT soil (Bayer et al., 2006a; Assmann et al., 2014; Alburquerque et al., 2015). As reported by Ferreira et al. (2012), the minimal required maintaining SOC stocks; which in Brazilian subtropical clayey soils under NT, must be above 6.8 Mg DM ha^{-1} yr^{-1}.

Regarding to the ICL-NT, there was no effect of this system on soil C and N concentrations (Table 1) or stocks (Table 2) relative to NT system, not even in the 0-5 cm. As discussed for tillage systems, it is likely that the relatively short 3.5-year experimental period, the originally high C and N contents of this Umbric Ferralsol, and the removal of the aboveground

biomass of maize for silage have limited the responses of soil C and N to ICL-NT. In this system the aboveground biomass yield was 23.4 Mg DM ha^{-1} yr^{-1} (4.9 for grazed ryegrass and 18.4 for silage maize, Table 3). But, taking into account that maize was removed for silage and that ryegrass was grazed to have only about 30% of its biomass returned as dung pats [considering 70% digestibility (Miguel et al., 2012)], the effective aboveground input was just the approximately 1.5 Mg DM ha^{-1} yr^{-1} as manure (Table 3). That was even lower than the aboveground input of about 4 Mg DM ha^{-1} yr^{-1} in NT. Yet, regardless of the very low aboveground input in ICL-NT, the soil C and N stocks were not depleted in ICL-NT relative to NT, but maintained. We argue that, in this ICL system with aboveground silage removal, roots are indeed playing the vital role of adding biomass to keep soil C and N levels. This argument is supported by studies that suggest that roots are indeed important C and N contributors to the soil OM pool, once they might interact closely with mineral surfaces, be physically protected within aggregates, and be more recalcitrant than the aboveground residue (Kong & Six, 2010).

Other studies have also reported no changes in soil C and N stock in areas under ICL (Salvo et al., 2010; Franzluebbers & Stuedemann, 2013). The cited studies and ours demonstrate that although not increasing soil C and N stocks, the adoption of ICL had at least no adverse effects on those stocks, without compromising the already known productive and economic advantages of this system.

Like in the whole soil, C and N stocks in physical fractions did not respond to ICL-NT, relative to NT, neither in fractions of the top 5 cm. Once more, our hypothesis that short-term accumulation should take place in the light fractions was not confirmed. Part of the same explanation given for the lack of response of the light fractions to NT relative to CT can be used here, i.e. that soil sampling was too late relative to the latest biomass input (ryegrass desiccation), so that much of the light fraction derived from ryegrass, in this case ryegrass root, was already decomposed. Nonetheless, the lower aboveground input in ICL-NT (about 1.5 Mg DM ha^{-1} as dung) compared to that in NT (approximately 4 Mg DM ha^{-1} as ryegrass) might also be an important reason for that lack of response in the light fractions.

Effects of ICL-NT on C and N stocks of physical fractions diverge between studies, just as the effects on total C and N stocks. Studies in the Brazilian or Uruguayan subtropics, including either annual or multi-year pasture cycles, report a decrease of the C stock in the particulate fraction with ICL-NT

Table 3. Production and addition of aboveground biomass of ryegrass and silage maize in a subtropical Umbric Ferralsol (Latossolo Bruno) subjected to conventional tillage (CT), no-tillage (NT) or integrated-crop livestock under no-tillage (ICL-NT). Castro-PR, Brazil.

	Biomass production (Mg DM ha^{-1})				Biomass addition (Mg DM ha^{-1})		
	Ryegrass	Maize	Total		Ryegrass	Maize	Total
CT	3.94 ±1.54	16.80 ±2.37	20.75 ±5.30		3.94 ±1.54	0.0	3.94 ±1.54
NT	4.05 ±1.51	17.42 ±1.91	21.47 ±5.15		4.05 ±1.51	0.0	4.05 ±1.51
ICL-NT	4.94 ±2.14	18.46 ±1.86	23.40 ±5.63		1.48 ±0.64	0.0	1.48 ±0.64

± standard deviation.

(Assmann et al., 2015; Salvo et al., 2010). On the contrary, in a study in the Brazilian Cerrado and under multi-year cycles showed that ICL-NT increased the C stock in that fraction (Salton et al., 2011). Such differences of results among studies just indicate that there is no standard or strict ICL system, with similar effects on soil, but systems sensitive to all sorts of environmental and anthropic influences that lead to distinct effects on soil OM, mainly due to the quantity of residues input and animal management.

With respect to the influence of physical fractions, regardless of soil use, tillage or layer, the OL fraction tended to store more C and N than the FL, in short term (Table 2), possibly due to the physical protection (occlusion) offered in stable aggregates (Conceição et al., 2008; Reis et al., 2014). Compared to the heavy fraction, the light fractions had higher C and N concentrations and higher C:N ratios (Table 1), what is consistent with the presence of particulate OM derived from plant residues in the light fractions and with the composition of the more humified and stable OM in the heavy fraction (Balabane & Plante, 2004). The greater stability of C and N in heavy fraction is mainly due to the organo-mineral associations resulting from ligand exchange bonding between the functional groups of OM (carboxylic and phenolic) and metals of the crystalline structure of minerals (Von Lützow et al., 2008). The importance of the heavy fraction as the principal C and N sink in subtropical soils under NT was already reported by Conceição et al. (2013), in agreement with the stabilization of the OM by organo-mineral association and microaggregate physical occlusion that occur in the heavy fraction (Balabane & Plante, 2004).

However, some studies indicate that under certain conditions of climate and soil, stabilization of the particulate fraction of OM (labile fraction) associated with minerals can also occur (Reis et al., 2014; Salvo et al., 2010), mainly due to the greater capacity that clay soils under no-tillage has to perform organo-mineral interactions and sorption with these labile fractions, the effects being more pronounced in the soil surface (0-3 cm), diluting in the arable layer (Salvo et al., 2010).

Conclusions

Short-term (3.5-year) no-tillage had little effect on total C and N accrual of subtropical Umbric Ferralsol (Latossolo Bruno), relative to conventional tillage management; in system under removal of the aboveground maize for silage. Increments of C and N stocks were restricted only to the first 5 cm of soil and to the heavy fraction.

Under the same condition of silage removal, short-term integrated crop-livestock combined with no-tillage did not increase the soil C and N stocks, in any layer or fraction, relative to crop no-tillage; but did maintain them.

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