

Soil aggregation and potential carbon and nitrogen mineralization with cover crops under tropical no-till

J.P.G. Rigon, A.J. Franzluebbbers, and J.C. Calonego

Abstract: Sustainability of agriculture with no-tillage (NT) production requires appropriate cover cropping and not a simple and random approach of any cover crop species. However, relatively little is known of the long-term soil surface impacts of different cover crops in rotation with soybean (*Glycine max*) under NT in the tropics. We evaluated the impacts of different cover crops on soil aggregation, soil organic carbon (C) and nitrogen (N) fractions, and soil microbial activity of a Typic Rhodudalf at the end of 12 years under NT in São Paulo state of Brazil. Treatments included main plots during the winter dry season (triticale [\times *Triticosecale*] and sunflower [*Helianthus annuus*]) and subplots during the spring cover crop season (pearl millet [*Pennisetum glaucum*], sunn hemp [*Crotalaria juncea*], forage sorghum [*Sorghum bicolor*], and fallow with occasional chiseling). All eight of these treatments followed soybean in summer. Winter crop treatments had no effect on soil aggregation, but total organic C and N concentrations were greater ($p < 0.05$) with triticale than with sunflower. Across depths, soil aggregation and soil C and N fractions were generally enhanced when sunn hemp was cover crop than with fallow in the spring. Triticale followed by sunn hemp provided soil cover and fresh mineralizable residue for improving soil quality in this unfertilized (N) soybean-based cropping system. Our results suggest that maintaining soil cover is important to improve soil aggregation and soil C and N fractions, but also the quality of organic inputs determined by cover crop species is an important factor controlling the dynamics of these soil responses.

Keywords: carbon and nitrogen fractions—diversified cropping systems—Oxisol—residue inputs

Soil health and soil quality, although not new concepts, have attracted considerable attention recently (Mitchell et al. 2017). Key functions of soil health and associated soil carbon (C) may be modified by improved management practices, including cover cropping and no-tillage (NT), and are often mediated by a diversity of microorganisms (Franzluebbbers 2016).

Minimizing soil disturbance is a critical component of conservation agriculture (CA) systems. The long-term beneficial effects of soil management with CA are difficult to dispute (Tiecher et al. 2017). However, CA does not mean only absence of soil disturbance or eliminating soil cultivation, and the simple adoption of NT itself does not guarantee the sustainability of agriculture, since

benefits of the approach are not universal across soils (Vandenbyngaert 2016; Reicosky 2015). Monoculture crop production with fallow periods, or lack of crop rotation and insufficient soil cover, violate the conservation concepts of CA (Derpsch et al. 2014). Differences in soil and crop yield responses among studies around the world can often be attributed to quantity and quality of biomass-C inputs and not only due to the absence of tillage (Franzluebbbers 2010).

Growing cover crops can be a key element of CA and play an essential role in achieving adequate surface residue cover in many regions. In addition, abundant and diverse cover crops can provide C and nitrogen (N) to the next crop managed with NT to enhance soil organic carbon (SOC) accu-

mulation (Franzluebbbers 2010; Derpsch et al. 2014). In tropical regions with low fertilizer inputs, the main factor controlling SOC accumulation under NT is the contribution of N_2 fixation by legume cover crops (Boddey et al. 2010), as well as greater microbial populations in the rhizosphere (Chen et al. 2008). However, irregular rainfall and dry winter in tropical regions make it more difficult to maintain soil cover throughout the year (Jantalia et al. 2007). Grass residues are characterized by a high C:N ratio and long persistence on the soil surface as a result of low decomposition rate (Teixeira et al. 2014), thereby fostering soil organic matter (SOM) accumulation and reducing mineralization (Shahbaz et al. 2017). Hence, crop rotation and the type of cover crop management system can influence SOC accumulation and its cascading effects on soil biological activity and aggregation.

Crop rotation has been suggested as a strategy to increase soil aggregation and SOC storage (Novelli et al. 2013, 2017; Lal 2015), mainly near the soil surface (<0.1 m depth) (Ruis and Blanco-Canqui 2017). However, effects are dependent on soil textural class, soil management duration, climatic conditions, and quantity and quality of crop residue.

Crop residue quality affects the extent and rate of reaction for soil microorganisms to release or immobilize inorganic N, according to C:N ratio and/or lignin:N ratio (Hadas et al. 2004; Millar and Baggs 2004; Berg and McLaugherty 2008). Knowledge of N mineralization from crop residues is important for planning supplemental quantity and timing of fertilizer N. According to Nivellet et al. (2016), increasing the quantity and quality of crop residues in diversified cropping systems without N fertilization under NT may enhance soil aggregation and soil C and N contents. Conservation cropping with NT management may confer beneficial impacts on soil aggregate stability and macroaggregate formation (Bossuyt et al. 2001; Six et al.

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Received October 24, 2019; Revised December 21, 2019; Accepted January 20, 2020.

2001), as well as stimulation of soil microbial activity (Franzluebbers and Brock 2007).

Protection of C in stable aggregates inhibits mineralization and increases C retention in soil (Cambardella and Elliott 1992). Accumulation of crop residues at the soil surface provides C and N inputs and fosters a zone of greater potential soil biological activity, which can be measured from soil C and N mineralization under standard conditions in the laboratory (Franzluebbers et al. 2018b). However, improved aggregation may also limit C and N mineralization in soil by protection of organic material within aggregates. We hypothesized that legume cover crops would enhance soil aggregation and reduce decomposition rates of organic matter as compared with grasses as cover crops under long-term NT. Our goal was to evaluate if choice of winter crop and spring cover crop in a NT soybean production system in the Brazilian tropics would affect soil aggregation and associated soil C and N fractions.

Materials and Methods

Site Description. The experiment was established in 2003 in Botucatu, São Paulo (22°49' S and 48°25' W), at an altitude of 780 m in a Typic Rhodudalf (Soil Survey Staff 2010). The tropical climate is mesothermal with a well-defined dry season from May to August (fall-winter in the Southern Hemisphere) and mean annual precipitation of 1,450 mm. Soil chemical (van Raij et al. 2001) and physical (Smith and Mullins 1991) characteristics of the experiment are shown in table 1.

Experimental Design. The experimental design was a randomized block with split-plot arrangement of eight treatments and four replications. Soybean (*Glycine max* L. Merr.) was grown in the summer in all plots (table 2). Treatments consisted of secondary-season grain crops (triticale [*x Triticosecale* Wittm.] or sunflower [*Helianthus annuus* L.]) grown in the fall-winter as main plots (256 m² each) followed by cover crops (pearl millet [*Pennisetum glaucum* L.], forage sorghum [*Sorghum bicolor* L. Moench], sunn hemp [*Crotalaria juncea* L.], or fallow) grown in the spring as subplots (40 m² each). This treatment arrangement was repeated annually from 2003 to 2015. Crop residues were maintained on the soil surface after each crop. Spring crops were chemically desiccated. Grain was harvested from main (soybean) and secondary-season crops (triticale and sunflower) and crop residues left on the field. Residue samples from each crop

Table 1
Chemical and physical properties of soil in August of 2014.

Soil properties	Depth (m)	
	0 to 0.1	0.1 to 0.2
pH (CaCl ₂)	5.1	4.5
Al (mmol _c dm ⁻³)	0.2	0.7
Ca (mmol _c dm ⁻³)	43	19
Mg (mmol _c dm ⁻³)	36	14
K (mmol _c dm ⁻³)	1.8	1.5
P (mg dm ⁻³)	33	17
Sand (g kg ⁻¹)	108	100
Silt (g kg ⁻¹)	237	245
Clay (g kg ⁻¹)	655	655
Microporosity (m ³ m ⁻³)	0.42	0.41
Macroporosity (m ³ m ⁻³)	0.12	0.09
Bulk density (Mg m ⁻³)	1.32	1.37

Notes: CaCl₂ = calcium chloride. Al = aluminum. Ca = calcium. Mg = magnesium. K = potassium. P = phosphorus.

species were analyzed for cellulose, hemicellulose, and lignin (Silva and Queiroz 2002) (table 3). The fallow treatment had no cover crop, and plots were chisel plowed in 2003, 2009, and 2013 just before soybean planting. The mechanical chiseling was carried out using a chisel plow with seven shanks set on two parallel bars and spaced 0.60 m from each other within a bar, resulting in effective 0.30 m shank spacing. Shanks were inclined forward at 25° angle, and the effective action depth was around 0.30 m (Calonego et al. 2017). Approximately 60 days after planting of spring cover crops, all plots were chemically desiccated with glyphosate at 2.5 kg

a.i. ha⁻¹, and then soybean as main cash crop was planted in 0.45 m wide rows targeting a population of 355,000 seeds ha⁻¹. Each year since 2003, soybean was inoculated with *Bradyrhizobium* sp. and fertilized with 50 kg ha⁻¹ of K₂O and 50 kg ha⁻¹ of P₂O₅ as potassium chloride and triple superphosphate, respectively. No N fertilizer was applied since the beginning of the experiment.

Soil Sampling. For soil C and N fractions, three cores (5 cm diameter at 0 to 0.1 and 0.1 to 0.2 m depths) were collected and composited diagonally across each subplot after soybean harvest in 2015. Soil was dried at 50°C and sieved to pass a screen with 2 mm openings. Another sampling for undisturbed soil was collected for soil characterization using a cylinder (5.0 cm high × 4.8 cm wide) to determine soil bulk density, total porosity, macroporosity, and microporosity. For soil aggregate analyses, soil monolith depths at 0 to 0.1 and 0.1 to 0.2 m were collected, gently broken into smaller fractions, and passed through a screen with 8 mm openings prior to drying at 50°C for 48 hours, and shipped to Raleigh, North Carolina, for further analyses. We oven-dried soil to standardize the procedure and avoid the confounding impacts of antecedent moisture content (Gollany et al. 1991).

Aggregate Fractionation. Dry-aggregate distribution of soil passing a screen with 4.75 mm openings was a necessary first step to characterize initial aggregate conditions prior to wet sieving. A 100 g portion of soil was placed on top of a nest of sieves (200 mm Ø with openings of 1, 0.25, and 0.053

Table 2
Crop rotation sequences from 2003 to 2015 and amount of crop residue in 2014.

Winter (main plot) crop	Spring (subplot) cover crop	Summer (entire area) main crop	Amount of crop residue in 2014 (Mg ha ⁻¹ y ⁻¹)†
Triticale	Sunn hemp	Soybean	13.14 ± 1.3
Triticale	Pearl millet	Soybean	11.02 ± 0.7
Triticale	Forage sorghum	Soybean	12.33 ± 0.9
Triticale	Fallow*	Soybean	9.20 ± 0.9
Sunflower	Sunn hemp	Soybean	11.70 ± 0.4
Sunflower	Pearl millet	Soybean	9.03 ± 0.8
Sunflower	Forage sorghum	Soybean	9.32 ± 1.1
Sunflower	Fallow	Soybean	8.58 ± 0.8

Notes: Triticale = *x Triticosecale*. Sunflower = *Helianthus annuus*. Sunn hemp = *Crotalaria juncea*. Pearl millet = *Pennisetum glaucum*. Forage sorghum = *Sorghum bicolor*. Soybean = *Glycine max*.

*Occasionally chisel plowed.

†Amount of residue in year 2014 (fall-winter 2014 + spring crops 2014 + soybean 2014/2015).

Table 3
Selected chemical characteristics of the crops in the crop rotation systems.

Crop	C/N	Lignin/N	N (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Triticale	96†	37	0.47	20.0	49.2	17.3
Sunflower	66	31	0.67	9.9	48.0	21.1
Soybean straw*	61	41	0.75	12.0	44.6	30.4
Forage sorghum	53	6	0.87	34.7	35.4	5.4
Pearl millet	34	4	2.44	27.5	24.0	10.1
Sunn hemp	16	5	2.86	11.9	33.8	14.9

Notes: C = carbon. N = nitrogen.

*After crop harvested.

†Data published in Raphael et al. (2016).

mm) and shaken for 1 minute on a vibrating shaker on Level 6 (CSC Scientific Co., Fairfax, Virginia). Soil retained on each sieve and that passing the 0.053 mm screen was weighed to determine dry-aggregate size distribution (Franzluebbers et al. 2000).

Sieve sizes were chosen based on the assumption that the >0.25 mm fraction was the most susceptible to changes in land use or management (Tisdall and Oades 1982). Water-stable aggregate distribution was determined from the reconstituted sample for dry-stable aggregation. Soil was placed on top of a nest of sieves (175 mm Ø with openings of 1.0 and 0.25 mm) and immersed directly in water with oscillation for 10 minutes (20 mm stroke length, 31 cycles min⁻¹). Water containing soil passing the 0.25 mm sieve was poured over a 0.053 mm sieve and gently washed with a stream of water. The three sieves were placed in an oven to dry at 55°C for 24 hours. Dry-stable and water-stable macroaggregates were defined by the fractions >0.25 mm (Elliott 1986). Mean-weight diameter of dry-stable aggregate distribution (MWD_{dry}) and mean-weight diameter of water-stable aggregate distribution (MWD_{wet}) were calculated by summing the products of mass fraction and mean diameter of each aggregate class, excluding the floating material (equation 1):

$$MWD_{wet\ or\ dry} = \sum_{i=1}^n F_i \times D_i \quad (1)$$

where F_i is the corrected mass proportion of the stable aggregate fraction i and D_i is the mean diameter of the stable aggregate fraction i . Stability of mean-weight diameter was calculated as water-stable mean-weight diameter divided by dry-stable mean-weight diameter. Stability of macroaggregates (>0.25 mm) was calculated from fraction of macroaggregates

following wet sieving divided by fraction of macroaggregates following dry sieving.

Soil Carbon and Nitrogen Fractions. Soil from sieved (2 mm) samples was ball-milled to a powder to determine SOC by dry combustion at 1,350°C (Leco CNS-2000, St. Joseph, Michigan). For mineralizable C, duplicate sieved soil samples of 50 g were wetted to 50% water-filled pore space (Franzluebbers and Brock 2007), placed into a 1 L canning jar along with vials containing 10 mL of 1 M sodium hydroxide (NaOH) to trap evolved carbon dioxide (CO₂) and water to maintain humidity, and incubated at 25°C for 24 days. Alkali traps were replaced at 3 and 10 days. Evolved CO₂ was calculated by titrating alkali with 1 M hydrogen chloride (HCl) to a phenolphthalein endpoint following precipitation of carbonate with excess barium chloride (BaCl₂) and vigorous stirring. At 10 days, one of the subsamples was removed from the incubation jar, fumigated with chloroform (CHCl₃) under vacuum for one day, vapors removed, placed into a separate canning jar along with vials of alkali and water, and incubated at 25°C for 10 days to determine soil microbial biomass C (SMBC). Potential C mineralization was calculated from the cumulative evolution of CO₂ during 24 days of incubation. Soil-test biological activity was defined as the flush of CO₂ during the first 3 days of incubation (Franzluebbers et al. 2018b). Inorganic N (ammonium [NH₄-N] + nitrite [NO₂-N] + nitrate [NO₃-N]) was determined from the filtered extract of a 10 g subsample of oven-dried (55°C, 48 hours) and sieved (<2 mm) soil shaken with 20 mL of 2 M KCl for 30 minutes using salicylate-nitroprusside and hydrazine auto-analyzer techniques (Bundy and Meisinger 1994).

Particulate organic matter was determined from the dried sample previously used for SMBC. The dried 50 g sample was shaken

in 100 mL of 0.1 M tetrasodium pyrophosphate (Na₄P₂O₇) for 16 hours, diluted to 1 L with distilled water, the suspension allowed to settle for 5 hours, and the particulate material caught on a 0.053 mm screen (Franzluebbers et al. 2000). Material retained on the screen was transferred to a drying bottle and weighed after oven-drying (55°C, 72 hours). Afterward, sand plus organic material was ground in a ball mill for 1 minute and analyzed for total C and N using dry combustion at 1,350°C (Leco CNS-2000, St. Joseph, Michigan).

Data Analysis. Data were tested for homogeneity of variance and normality. Data were subjected to analysis of variance using a randomized complete block design with four replicates by SAS version 9.2 (SAS Institute Inc 2009). Differences among means were considered significant at $p \leq 0.05$. Linear regression and correlation analysis were performed among variables, and Pearson's correlation coefficients obtained. Data were plotted with SigmaPlot v. 12 (Systat Software Inc., San Jose, California). Significant associations were declared at $p \leq 0.05$.

Results and Discussion

Soil Aggregation. Winter crops in the secondary season did not influence aggregation attributes, nor was an interaction with spring cover crops significant. Mean-weight diameter of dry aggregates was not affected by spring cover crop treatment, but MWD of water-stable aggregates was (table 4). Absence of a spring cover crop (fallow) resulted in lower MWD_{wet}, MWD stability, and macroaggregate stability than sunn hemp and forage sorghum as cover crop in both soil depths (table 4). With pearl millet as cover crop, MWD stability and macroaggregate stability were greater ($p < 0.05$) than with the fallow at a depth of 0 to 0.1 m, but not significantly different at a depth of 0.1 to 0.2 m. In general, the fallow treatment with occasional chisel tillage caused reductions in aggregation indices of at least 15% at soil surface compared with other treatments with cover crops.

Soil aggregation attributes in both soil depths were always greater ($p < 0.05$) with sunn hemp as spring cover crop, followed at the soil surface (0 to 0.1 m depth) by pearl millet and forage sorghum. In general, soil aggregation indices were always greater at 0 to 0.1 m depth than at 0.1 to 0.2 m depth, except for dry-stable aggregation, which

Table 4

Mean-weight diameter of dry-stable aggregates (MWD_{dry}), mean-weight diameter of water-stable aggregates (MWD_{wet}), mean-weight diameter stability (MWD Stability), and macroaggregate stability (MS) at 0 to 0.1 m (Sur) and 0.1 to 0.2 m (Sub) soil depths. Mean values followed by different letters in the same column are significantly different by t -test (LSD) at $p < 0.05$.

Treatment	MWD_{dry} (mm)		MWD_{wet} (mm)		MWD Stability (mm mm ⁻¹)		MS (g g ⁻¹)	
	Sur	Sub	Sur	Sub	Sur	Sub	Sur	Sub
Winter crops								
Sunflower	2.38	2.34	1.51	1.16	0.62	0.49	0.57	0.40
Triticale	2.43	2.34	1.59	1.14	0.65	0.48	0.60	0.39
Spring cover crops								
Fallow	2.38	2.33	1.34	1.07b	0.55b	0.45b	0.49b	0.35b
Sunn hemp	2.43	2.39	1.67	1.31a	0.68a	0.55a	0.64a	0.47a
Forage sorghum	2.39	2.36	1.56	1.24a	0.65ab	0.52a	0.59ab	0.43a
Pearl millet	2.42	2.27	1.63	0.99b	0.67a	0.43b	0.62a	0.32b
Source	p -value							
Winter (W)	0.48	0.99	0.62	0.73	0.65	0.70	0.63	0.73
Spring (S)	0.82	0.09	0.12	<0.01	0.04	<0.01	0.04	<0.01
W × S	0.93	0.24	0.73	0.61	0.69	0.78	0.73	0.73

was similar between depths. Dry-stable aggregation was considered a prerequisite normalization step to account for potential differences in handling of soil during sampling and processing, and therefore, was not expected to be affected by treatments. With sunn hemp as spring cover crop, MWD_{wet} , MWD stability, and macroaggregate stability at 0.1 to 0.2 m depth were nearly the same as values with fallow at 0 to 0.1 m depth.

Despite general improvements in soil aggregation attributes at the soil surface with all cover crops compared with fallow, aggregation with pearl millet as cover crop at the lower depth (0.1 to 0.2 m) declined, reaching a level comparable with fallow. In contrast, aggregation attributes with sunn hemp and forage sorghum remained greater than with fallow at 0.1 to 0.2 m depth.

Soil Carbon and Nitrogen Fractions. Overall, crop rotations under long-term NT influenced soil C and N fractions mainly at the soil surface (0 to 0.1 m). Soil organic C had a median value of 21.1 and 17.0 g kg⁻¹ at depths of 0 to 0.1 and 0.1 to 0.2 m, respectively. Total soil N (TN) averaged 1.91 and 1.58 g kg⁻¹ at depths of 0 to 0.1 and 0.1 to 0.2 m, respectively. Triticale as fall/winter crop had greater ($p < 0.001$) SOC and TN than sunflower at 0 to 0.1 m soil depth (table 5). No effect of winter crop was observed on particulate organic carbon (POC) and particulate organic nitrogen (PON), nor on any of these variables at a depth of 0.1 to 0.2 m. Among cover crop treatments, fallow with occasional chisel tillage had lower ($p <$

0.001) SOC, TN, POC, and PON, as well as the lability of soil C and N than other cover crop treatments at a depth of 0 to 0.1 m (table 5). However, an interesting result on SOC and TN was greater ($p < 0.05$) concentration with fallow than with pearl millet as cover crop at a depth of 0.1 to 0.2 m.

Unexpectedly, neither winter crop nor spring cover crop affected SMBC, net N mineralization, or residual inorganic N (data not shown). With an unprotected LSD, there was only a trend for lower net N mineralization and residual inorganic N with fallow compared with pearl millet at a depth of 0 to 0.1 m (table 5). Further soil sampling in spring and winter seasons are suggested to assess the SMBC.

Soil-test biological activity ($CMIN_{0-3d}$) and cumulative C mineralization ($CMIN_{0-24d}$) at a depth of 0 to 0.1 m had significant interaction between winter crop and spring cover crop treatments (table 6). Sunn hemp behaved differently depending on the preceding winter crop. This cover crop had the highest and lowest $CMIN_{0-3d}$ when cultivated after triticale and sunflower, respectively (85 and 46 mg kg⁻¹, $p < 0.05$). The same pattern was observed for $CMIN_{0-24d}$ (270 and 172 mg kg⁻¹, $p < 0.05$). Among the cover crops, fallow had the lowest $CMIN$ ($p < 0.05$), independently of the winter crops in both evaluations.

The proportion of SOC as $CMIN$ was greater with pearl millet and forage sorghum as spring cover crop than with fallow (data not shown). A similar trend occurred for the proportion of TN as $NMIN$ ($p = 0.08$).

Regression Analysis. Across soil depths, $CMIN_{0-3d}$ was most closely associated with $CMIN_{0-24d}$ (figure 1a; $r^2 = 0.94$; $p < 0.0001$). Other significant regressions ($p < 0.0001$) occurred between $CMIN_{0-3d}$ and $NMIN$ (figure 1b; $r^2 = 0.68$). Soil-test biological activity ($CMIN_{0-3d}$) was also highly related with TN and SOC (figure 1c; $r^2 = 0.73$ and 0.79, respectively), due mainly to the close association with each other (SOC and TN [$r^2 = 0.96$, data not shown]). Across winter crop and spring cover crop treatment, SMBC had highly significant association ($p < 0.0001$) with TN and SOC (figure 1c; $r^2 = 0.76$).

Soil aggregation indices also had positive associations ($p < 0.001$) with TN and SOC (figure 2a through 2d). The highest Pearson's correlation coefficients were observed for MWD_{wet} , MWD stability, and macroaggregate stability, ranging from $r = 0.67$ to 0.70. MWD_{dry} was also significantly associated ($p < 0.007$) with SOC, although the association was weak ($r^2 = 0.17$). In general, associations were slightly greater with TN than with SOC (figure 2).

Aggregate Fractions. We did not find treatment differences in MWD of dry-stable aggregates, and this is consistent with other observations in the literature (Blanco-Canqui and Ruis 2018). According to these authors, the improvement achieved through NT may be more effective on water-stable than dry-stable aggregates.

Absence of a cover crop in the fallow treatment during the spring season likely had a greater impact on aggregation indices than occasional chisel plowing to loosen soil. We base this interpretation on observations from other studies in tropical regions (Portella et al. 2012; Seben et al. 2014). Moreover, the chisel operation likely does not reduce aggregation in the surface layer (Nunes et al. 2015), because the shanks of chisel plow can break soil along natural fracture lines without damaging natural intra-aggregate structure. In general, growing crops with aggressive root systems improves soil structure (Calonego et al. 2017). In addition, our soil sampling occurred two years after the last chisel operation, and previous investigations at the study confirmed this tillage operation resulted in immediate impacts on soil compaction and structure, but did not last to the second year following the event (Calonego et al. 2017). Hence, as compared with reduced C input with fallow, the continuous flow of C in a diverse cropping system with springtime

Table 5

Soil organic carbon (SOC), total nitrogen (TN), particulate organic carbon (POC), particulate organic nitrogen (PON), net N mineralization during 24 days of incubation (NMIN), and residual inorganic N (RIN) at soil depths of 0 to 0.1 (Sur) and 0.1 to 0.2 m (Sub) as affected by winter crops and spring cover crops. Mean values followed by different letters in the same column are significantly different by *t*-test (LSD) at $p < 0.05$.

Treatment	SOC (mg kg ⁻¹)		TN (mg kg ⁻¹)		POC (mg g ⁻¹)		PON (mg g ⁻¹)		NMIN (mg kg ⁻¹)		RIN (mg kg ⁻¹)	
	Sur	Sub	Sur	Sub	Sur	Sub	Sur	Sub	Sur	Sub	Sur	Sub
Winter crops												
Sunflower	20.5b	17.0	1.87b	1.57	3.5	2.2	0.23	0.12	29.4	16.3	38.4	26.4
Triticale	21.6a	17.0	1.95a	1.59	3.7	1.7	0.24	0.09	30.3	15.9	38.7	27.3
Spring cover crops												
Fallow	19.4b	17.5ab	1.77b	1.61ab	2.9c	1.9	0.19c	0.11	24.0	14.5	32.8	27.6
Sunn hemp	21.5a	17.7a	1.95a	1.66a	3.5bc	2.5	0.23bc	0.14	28.5	17.8	38.3	27.4
Forage sorghum	21.6a	16.5bc	1.94a	1.55bc	3.8ab	1.7	0.25ab	0.09	32.2	16.0	40.1	25.4
Pearl millet	21.8a	16.3c	1.98a	1.51c	4.2a	1.8	0.28a	0.09	34.8	16.2	43.0	27.0
Source	p-value											
Winter (W)	0.03	0.89	0.04	0.74	0.13	0.18	0.17	0.21	0.54	0.73	0.71	0.60
Spring (S)	<0.01	0.04	0.03	0.02	<0.01	0.22	<0.01	0.24	0.07	0.35	0.09	0.82
W × S	0.21	0.66	0.30	0.17	0.06	0.18	0.06	0.14	0.24	0.19	0.14	0.78

cover crop leads to humification of organic compounds and close association of C with aggregates and soil particles, which is responsible for the formation of stable organic mineral complexes (Golchin et al. 1994).

Greatest aggregation indices in both soil depths from sunn hemp are in agreement with results reported by Hubbard et al. (2013) and Blanco-Canqui and Ruis (2018). According to these authors, sunn hemp in crop rotations using NT improves soil physical properties. This cover crop also increased macroporosity in a previous investigation from our long-term study (Calonego et al. 2017). Addition of readily mineralizable substrates causes an increase in aggregate stability (Schlecht-Pietsch et al. 1994; Roldan et al. 1994). Similarly, adding sunn hemp and late-maturing soybean cover crops to NT winter wheat (*Triticum aestivum* L.) reduced soil compaction and increased MWD_{wet} and SOC compared with NT without cover crops after 15 years in a temperate region (Blanco-Canqui et al. 2011). High-quality residues may increase the rate of soil aggregation (Six et al. 2001) and help to explain the result from sunn hemp. Sunn hemp as cover crop resulted in greater soybean yield (10-year average) at this site (Calonego et al. 2017) and led to greater concentration of SOC and other organic matter fractions (Raphael et al. 2016). Hence, this study supports the theory that crop diversity via rotation of grasses with legumes can improve aggregate stability, thereby confirming the results of other studies (Pérès et al. 2013; Lange et al. 2015; Comin et al. 2018).

Our results suggest that maintaining soil cover may not be enough to improve soil aggregation, but the quality of organic inputs can be an important factor in controlling aggregation and soil organic C and N dynamics. Although cover crop effects on soil aggregation and SOM have been observed before (Blanco-Canqui et al. 2011; Comin et al. 2018; Blanco-Canqui and Ruis 2018), few studies have emphasized these impacts under long-term NT in the tropics. As described by Fujisaki et al. (2018), a long period of evaluation is needed after a change in management practices to document the effectiveness of these practices on SOC stocks.

Soil Carbon and Nitrogen Fractions. Absence of crop residues with the spring fallow treatment that received occasional chisel tillage was a driving factor for the limited accumulation of soil C and N fractions (SOC, TN, POC, and PON), and these results are in agreement with other studies (Raphael et

al. 2016; Bayer et al. 2000). Elsewhere, fallow or bare soil has led to in situ SOC mineralization and a substantial decrease in SOC content (Paradelo et al. 2016). Our data suggest the presence of crop residues on the soil surface enhances soil responses under NT, not only the lack of soil disturbance. Hence, the amount and quality of residues covering soil appears to impact soil C and N attributes (Frasier et al. 2016), which are key features of CA systems (Derpsch et al. 2014). These results indicate that in a long-term NT system without N fertilization, legume cover crops may be necessary to enhance soil C and N fractions and to maintain soil microbial functional activities (Nivelle et al. 2016). Greater cover crop N inputs may be a reasonable substitute for synthetic N fertilizers, as also discussed in a recent review (Kaye and Quemada 2017).

Availability of N can be one of the main factors limiting SOC sequestration (Wieder

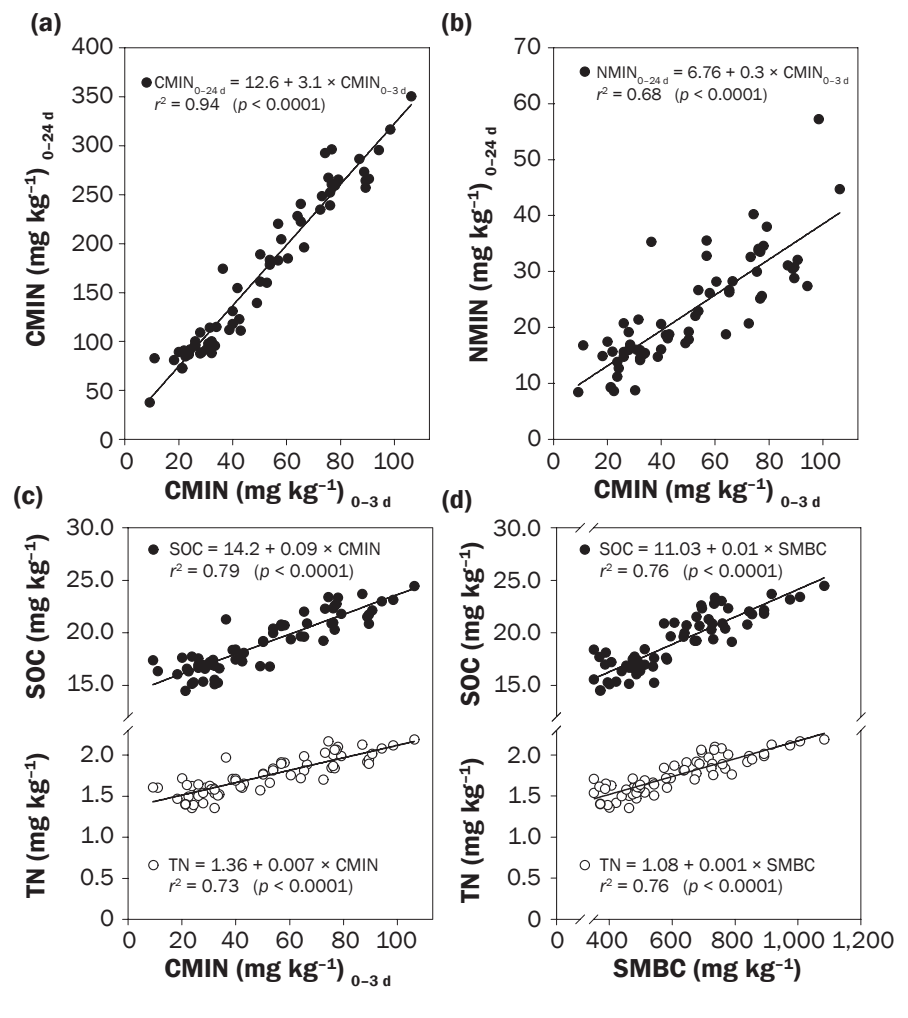
Table 6

Interaction between winter crops and spring cover crops on carbon mineralization (CMIN) at 0 to 3 days and 0 to 24 days. Means followed by different lowercase letters in a column are significantly different between winter crops and means followed by different uppercase letters in a row are significantly different among spring cover crops at $p < 0.05$. Least significant difference (LSD) is for all treatment combinations across winter crops and spring cover crops at $p < 0.05$.

Winter crops	CMIN 0 to 3 days (mg kg ⁻¹)				CMIN 0 to 24 days (mg kg ⁻¹)			
	Pearl millet	Forage sorghum	Sunn hemp	Fallow	Pearl millet	Forage sorghum	Sunn hemp	Fallow
Sunflower	77A	83A	46bB	48B	273A	268A	172bB	158B
Triticale	82A	76AB	85aA	63B	267	242	270a	213
LSD	19				73			

Figure 1

Association of soil-test biological activity ($CMIN_{0-3d}$) and soil microbial biomass carbon (SMBC) with cumulative carbon mineralization ($CMIN_{0-24d}$), net nitrogen mineralization (NMN), total nitrogen (TN), and soil organic carbon (SOC) across soil depths of 0 to 0.1 and 0.1 to 0.2 m.



et al. 2015). In this sense, root contributions could potentially explain the enhancement of TN and SOC at 0.1 to 0.2 m from sunn hemp cover cropping (table 5), which could have been made possible through changes in the intermediate fraction of POC and PON at this depth (Raphael et al. 2016). Crop residues (aboveground) are considered of high quality compared with root residues (belowground), which are relatively recalcitrant to decomposition (Bertrand et al. 2006). High-quality residues are the main source of microbial by-products, which can be considered precursors of stable SOM by promoting aggregation and strong chemical binding to the mineral soil matrix (Cotrufo et al. 2013; Lavelle et al. 2019). Therefore, we consider biologically fixed N from the legume cover crop as an important source of N for soil C and N accumulation (Frasier et al. 2017).

Sunn hemp residue is readily mineralizable during early stages of crop decomposition due to its low C:N ratio (Christensen 2001). Hence, its presence near the soil surface could reduce labile fractions of soil C and N. This demonstrated that high rates of residue mineralization reflect substrate C availability and is related to crop residue quality (Shahbaz et al. 2017). However, at 0.1 to 0.2 m depth, labile C inputs through root exudation can be enhanced while residues are decomposing in soil (Shahbaz et al. 2017). Therefore, a slight increase in subsurface POC and PON might have been a result of enhanced crop rooting (Franzluebbers and Stuedemann 2002).

The biochemical characteristics of crop residues from triticale and sunflower cropped before sunn hemp can also help explain our results. Greater lignin and C:N ratio from

triticale residue than from sunflower residue may pose a limitation to soil decomposers (table 3). Consequently, slower residue decomposition and higher residue persistence may enhance physical soil protection, as previously reported (Rigon et al. 2018). In contrast, fresh C available from sunflower resulted in greater soil-test biological activity than from triticale (table 6). In addition, the dry winter season in this region often limits sunflower's growth compared to triticale, leading to insufficient soil residue cover after spring crop seeding. Lack of residue cover could also help explain the lower SOC and TN under sunflower as winter crop (table 5). Additionally, cropping sunn hemp (high N residue content) after a limited amount of sunflower crop residue might intensify decomposition, known as the priming effect (Qiao et al. 2016). A similar behavior in the absence of cover crop with fallow supported this explanation of rapid mineralization. According to Shahbaz et al. (2018), some studies ignore interactions of labile C and fresh or decaying crop residues commonly present in field conditions.

The fallow treatment with absence of residue may have led to slightly lower SMBC (although not statistically significant). The observed trend is consistent with other observations that soil management has a significant impact on SMBC (Zhang et al. 2018). Sampling soil at shallower depth than 0 to 0.1 m may have detected a stronger effect.

Regression Analysis. The strong relationship between $CMIN_{0-3d}$ and $CMIN_{0-24d}$ provides further support for using a rapid assessment of potential soil biological activity (Franzluebbers 2016). It also confirms a similar relationship across 57 field trials on tall fescue pasture (Franzluebbers et al. 2018a). Soil-test biological activity ($CMIN_{0-3d}$) could be considered a good predictor of soil surface changes in soil C and N fractions (SOC, TN, POC, PON, and SMBC) under a variety of crop rotations under long-term NT. Soil C and N cycles are related to the processes of mineralization and immobilization, indicating a strong relationship between soil C and N transformations and soil CO_2 flux (Balota and Auler 2011). Several other studies have documented this close relationship of various soil C and N fractions with soil-test biological activity (Franzluebbers and Brock 2007; Franzluebbers et al. 2018b). According to these authors, soil-test biological activity is also more rapid and inexpensive than other procedures.

The relation between C stabilization and aggregation with C inputs from crop residue has been observed before (Lal 2015), and our data confirm this relationship with increases in soil aggregation indices and soil C and N fractions by winter cover crops under NT. Greater crop residue C inputs usually result in greater concentration of SOC. High-intensity cropping with three crops per year having different quantities and qualities of residue C and N inputs helps explain differences in SOC and TN after 12 years of NT soybean cropping systems. These results support other research that show cover crops have important potential to improve the sustainability of soybean cropping systems (Delgado and Gantzer 2015), with multiple ecosystem service benefits (Daryanto et al. 2018).

Summary and Conclusions

This study focused on establishing the impacts of different crop rotation under NT for 12 years on soil aggregation, soil organic C and N fractions, and soil microbial activity of a Typic Rhodudalf in a tropical region of Brazil. Our results provide a unique perspective on C and N fractions in response to different winter and spring crop options.

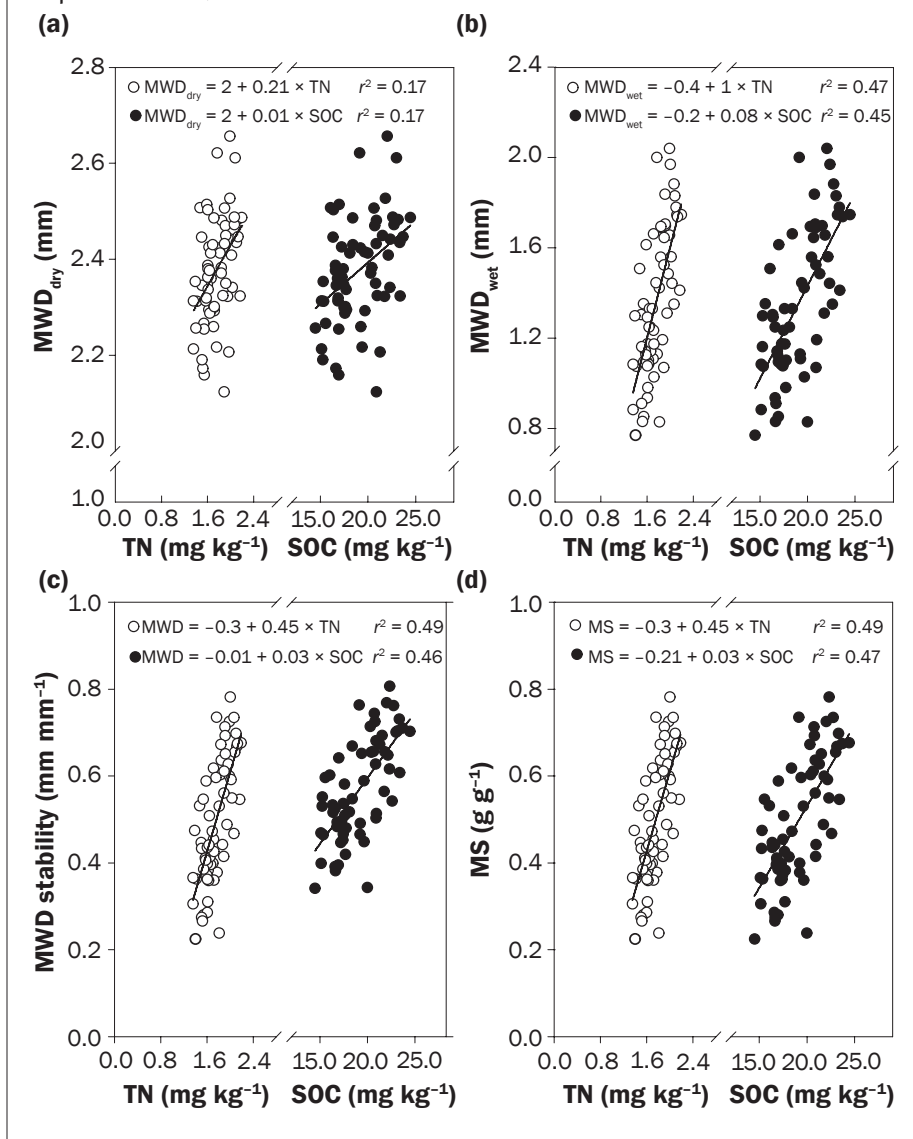
Cover crop residues are essential to increase SOC and TN in tropical soils under NT. However, our results suggest that not only is soil cover necessary to maintain or improve soil aggregation and C and N fractions, but the quality of organic inputs derived from cover crop species is also important to affect these changes. Low C:N ratio residues of sunn hemp confer both labile C and N inputs and stabilization to build soil resilience and quality physical protection when rotated with other high C:N ratio residues. In contrast, fallow with bare soil and occasional chisel tillage led to in situ SOC mineralization as a driving factor for reduced accumulation of soil C and N fractions. These results are important for enhancing the sustainability of agriculture in tropical regions.

Acknowledgements

The project was supported by the São Paulo State Foundation for Research Support (FAPESP) under grants 15/23389-6 and 13/23359-4. We thank North Carolina State University for hosting a portion of this research and express our gratitude to Ellen Leonard (research technician at North Carolina State University), Erin Silva (research technician with USDA Agricultural Research Service), and

Figure 2

Association of total nitrogen (TN) and soil organic carbon (SOC) with mean-weight diameter of dry-stable aggregates (MWD_{dry}), mean-weight diameter of wet-stable aggregates (MWD_{wet}), mean-weight diameter stability ($MWD_{Stability}$), and macroaggregate stability (MS) across soil depths of 0 to 0.1 and 0.1 to 0.2 m.



Isabela Gomes (graduate research assistant, University of Kentucky) for technical support.

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