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1 **Legume cover crops under no-tillage favor organomineral association in**
2 **microaggregates and soil C accumulation**

3

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13

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23

- 24 **Abbreviations**
- 25 SOC, soil organic carbon
- 26 C, carbon
- 27 SOM, soil organic matter
- 28 CT, conventional tillage
- 29 NT, no-tillage
- 30 O/M, oat/maize
- 31 V/M, vetch/maize
- 32 OV/MC, oat+vetch/maize+cowpea
- 33 MWD, mean weight diameter
- 34 free-LF, free light fraction
- 35 occluded-LF, occluded light fraction
- 36 min-OM, mineral-associated fraction
- 37 SPT, polytungstate
- 38 CaCl₂, calcium chloride
- 39

40 **Legume cover crops under no-tillage favor organomineral association in**
41 **microaggregates and soil carbon accumulation**

42 **Abstract**

43 Both no-tillage and legume cover crops have been shown to increase soil organic carbon
44 (SOC) in subtropical soils. However, the mechanisms underpinning management
45 system effects on SOC accumulation are still not well understood. We used a
46 combination of aggregate size and density fractionation to elucidate these mechanisms
47 at a 30-year old experiment on an Acrisol in southern Brazil. The effects of two tillage
48 systems [conventional system (CT) and no-tillage (NT)] combined with three cropping
49 systems [oat/maize (O/M), vetch/maize (V/M) and oat+vetch/maize+cowpea (OV/MC)]
50 were evaluated in the top 20 cm soil layer. Overall, macroaggregation (> 0.25 mm) was
51 significantly influenced by tillage with NT showing values 14% greater than CT in the
52 0–5 cm soil depth. On average, the occluded light fraction-C content in
53 macroaggregates was more than twice as high under NT compared to CT (4.4 vs. 1.8 g
54 kg^{-1}). This effect was more pronounced when legume cover crops were grown.
55 However, the most significant effect of cover crops was observed in the organomineral
56 fraction of microaggregates, especially under NT (12.1 under O/M and 19.8 g kg^{-1}
57 under OV/MC). Our results suggest that, although NT increased the occluded light
58 fraction-C compared to CT, this effect was smaller than the gains that legume cover
59 crops offered in organomineral association.

60

61 **Keywords:** organic matter; soil aggregates; carbon stabilization; tillage systems;
62 cropping systems.

63

64

65 **1. Introduction**

66 Soil organic carbon is essential for more sustainable and resilient agricultural systems,
67 especially in tropical and subtropical soils, owing to its central role in determining soil
68 properties that strongly affect crop yield and the quality of the wider environment (Lal,
69 2009; Powlson et al., 2012).

70 Conservation management systems can make agricultural soils potential sinks of
71 atmospheric CO₂. An accumulation of SOC in agricultural systems of subtropical Brazil
72 can be achieved through management strategies such as reduced tillage or NT that
73 decrease the decomposition rate of soil organic matter (Bayer et al., 2006), and high C-
74 input intensive cropping systems (Dieckow et al., 2005; Zanatta et al., 2007). Switching
75 from CT to NT in Brazilian subtropical regions can result in C sequestration rates
76 ranging from 0.11 to 0.68 Mg ha⁻¹ yr⁻¹ depending on the intensity of cropping systems
77 adopted (Bayer et al., 2006). Intensifying cropping systems in Southern Brazil usually
78 involves the insertion of cover-crop species, especially legumes, for which a positive
79 effect on C input is usually related to soil N supply (Amado et al., 2006; Dieckow et al.,
80 2005b).

81 Physical fractionation (density or size) has been used to study the effect of
82 management systems on SOC stabilization (Dieckow et al., 2005a; Álvaro-Fuentes et al.,
83 2009; Andruschkewitsch et al., 2013; Conceição et al., 2013). Despite the wide range of
84 fractionation schemes used (Cambardella and Elliot, 1992; Dieckow et al., 2005a;
85 Conceição et al., 2008), three main fractions related to mechanisms of C stabilization
86 are usually assessed. The free light fraction (free-LF) which is considered a non-
87 protected C-pool, the occluded light fraction (occluded-LF) that is fraction located
88 inside of soil aggregates and assumed to be physically-protected against the attack of
89 soil microorganisms and their enzymes, and the mineral-associated fraction (min-OM)

90 that is a colloidal fraction interacting with mineral particles (Six et al., 1999; Rumpel et
91 al., 2010; Conceição et al., 2013). Carbon occlusion and organomineral association are
92 considered the main mechanisms of C stabilization in tropical and subtropical soils with
93 a predominance of Kaolinite (1:1 clay) and sesquioxides of Al and Fe (Conceição et al.,
94 2013; Deneff et al., 2007; Zotarelli et al., 2007).

95 It has been suggested that SOC stabilization in NT soils is closely linked to soil
96 macroaggregation (Six et al., 1999; Conceição et al., 2013). The slower turnover of
97 macroaggregates would favor the formation and stabilization of microaggregates within
98 macroaggregates (Six et al., 1999; Deneff et al., 2004; Du et al., 2015), and provide an
99 environment for C to be chemically stabilized by soil minerals (Conceição et al., 2013).
100 However, the mechanisms by which different cropping systems affect SOC stabilization
101 is still unclear, especially in tropical and subtropical soils.

102 The hypothesis of this work was that soil management systems involving no soil
103 disturbance (NT) and associated with large and diversified crop residue input, increase
104 the C occlusion in macroaggregates and, consequently, favour the formation of
105 organomineral associations and ultimately SOC stabilization. Our objective was to test
106 this hypothesis using a combination of aggregate size and organic matter density
107 fractionation, on a 30 year-old experiment of southern Brazil combining tillage and
108 cropping systems with legume cover crops.

109

110 **2. Materials and methods**

111 *2.1 Field experiment*

112 This study was based on a long-term field experiment (30 years) initiated in 1985 in an
113 area previously cultivated for approximately 16 years under conventional tillage, with
114 plowing and disking, at the Experimental Station of the Federal University of Rio

115 Grande do Sul, Eldorado do Sul, Rio Grande do Sul State, Brazil. Soil and climate
116 characteristics are presented in Table 1.

117 The ongoing experiment consists of the combination of two tillage [conventional
118 tillage (CT) and no tillage (NT)] systems with three cropping systems [oats (*Avena*
119 *strigosa* Schreb) / maize (*Zea mays* L.) (O/M), vetch (*Vicia sativa* L.) / maize (V/M)
120 and the consortium of oats + vetch / maize + cowpea (*Vigna unguiculata* (L.) Walp
121 (OV/MC)] distributed in a randomized block experimental design with split plots and
122 three replicates. Tillage systems were assigned to the main plots (15 × 20 m) and
123 cropping systems to the subplots (5 × 20 m). These tillage systems combined with
124 cropping systems were managed at two levels of fertilization: 0 and 180 kg ha⁻¹ of N-
125 urea (0N and 180N), applied manually in strips in the maize crop, characterized the sub-
126 subplots (5 × 10 m). Only the sub-subplots with no nitrogen fertilization were used in
127 this study.

128 Winter crops, managed as cover crops, were established in April–May of each
129 year using direct drilling in both CT and NT treatments. Oats, when grown alone, were
130 seeded at a rate of 80 kg ha⁻¹. When cultivated with vetch, oats were seeded at 30 kg
131 ha⁻¹ and vetch at 50 kg ha⁻¹. For vetch cultivated on its own, 80 kg ha⁻¹ was used. In the
132 OV/MC system, cowpea (summer cover crop) was manually sown 15 to 20 days after
133 maize, between the lines of this crop, in pits 40 cm apart, with 3 to 4 seeds per pit. After
134 the maize was harvested, cowpea was allowed to grow to its full development,
135 remaining until its mechanical management by knife roller before the winter crops were
136 planted (oats and vetch).

137 The CT plots were ploughed to a furrow-depth of 17 cm once a year in spring
138 before maize sowing using a three-disk plough and harrowed twice to a depth of 10 cm
139 using a disk harrow mixing the crops residues in this layer. At the same time,

140 glyphosate-based herbicide (Roundup, Monsanto) was applied in the NT plots at 1.4 kg
141 ha⁻¹ with respect to final glyphosate concentration, and two or three days later the
142 winter cover crops were managed with a knife-roller and the aboveground residues left
143 on the soil surface. In NT, soil disturbance occurred only in the sowing line, and the
144 residues of the cover crop were left on the soil surface.

145 Maize was planted with a NT planter in both tillage treatments in September–
146 October, with 90 cm spacing between rows and using a sowing rate adjusted to obtain
147 50–70 thousand plants per hectare. The fertilizers were applied at 8 cm soil depth at
148 maize sowing, at rates of 21.5 and 41.5 kg ha⁻¹ of P and K, respectively (50 kg ha⁻¹ of
149 P₂O₅ and K₂O each). The mean historic annual C input for both cover crops and maize
150 are provided in Table 1. The mean annual C input (aboveground and root, with roots
151 being assumed to account for 30% of the aboveground portion) was calculated from
152 cover crop data and the dry-matter maize yields compiled by Zanatta et al. (2007) for
153 the period 1985–2006, which were subsequently updated to 2014 (Fig. 1) (Veloso et al.,
154 2018).

155 2.2 Soil bulk density and total organic carbon determinations

156 Soil sampling was carried out in September 2014 in the 0–5, 5–10 and 10–20 cm soil
157 layers, prior to soil tillage for maize crop establishment. Trenches were opened,
158 perpendicular to the sowing lines, for sampling for bulk density which was measured in
159 duplicate using the volumetric ring method (volume of 101.5 cm³) (Blake and Hartge,
160 1986). Soil samples were collected from the different layers for SOC analysis. The
161 samples were air-dried, ground and passed through a 2-mm-mesh sieve, and then
162 ground in an agate mortar. The determination of SOC concentrations by dry combustion
163 was performed using an elemental analyzer (FlashEA 1112, Thermo, Electron
164 Corporation, Milan, Italy).

165 The SOC stocks up to a depth of 20 cm were calculated using the equivalent
166 mass approach (Ellert and Bettany, 1995), taking the soil mass of CT O/M management
167 system as the reference. The annual SOC accumulation rate was calculated as the
168 difference between the SOC stocks of the treatments and the reference system, divided
169 by the time elapsed since the implementation of the experiment, i.e. 30 years.

170

171 *2.3 Aggregate size distribution and physical fractionation of SOM*

172 At the same period, additional trenches were excavated with a cutting blade in order to
173 sample undisturbed soil in the 0–5, 5–10 and 10–20 cm layers for aggregate
174 fractionation. These undisturbed samples were manually ruptured at their weakness
175 points until the entire sample could be passed through a 9.51-mm mesh and dried in the
176 shade. Aggregates larger and smaller than 2 mm were weighed to determine the
177 proportion of aggregates making up the soil mass required to analyze the aggregate size
178 distribution in water and the physical density fractionation of SOM.

179 To determine the size distribution of aggregates in water, 50 g samples of soil
180 were weighed and moistened by capillarity for 12 hours on filter paper. Separations
181 were run in duplicate. Afterwards, they were transferred to 1000 mL plastic tubes
182 containing 500 ml of water and placed on a rotary shaker for 2 min at 16 rpm. The
183 samples were then transferred to the vertical oscillation shaker, following the procedure
184 of Carpenedo and Mielniczuk (1990). The samples were placed on a set of sieves with
185 mesh sizes 4.76, 2.00, 0.50, 0.25, and 0.053 mm, and shaken in water at 42 oscillations
186 per minute for 15 min, so that water at the lowest level reached the top of the aggregates
187 in the 4.76 mm sieve. The material that passed through the last sieve and was retained in
188 the bucket was flocculated with potassium aluminum (5 g L^{-1}).

189 The contents of the five sieves and the bottom were transferred to plastic
190 containers of known mass, dried for 48 h at 60 °C and then weighed. The mean weight
191 diameter (MWD) was calculated from the aggregates retained in the five sieves and the
192 bottom pan (equation 1):

$$193 \quad \text{MWD} = \frac{\sum m_{AGRi} \times c_i}{\sum m_{AGR}} \quad (1)$$

194 Where: MWD = mean weight diameter (mm); m_{AGRi} = mass of aggregates in
195 class i ; C_i = mean value of the class of aggregate class [(upper mesh diameter + lower
196 mesh diameter) / 2]; m_{AGR} = total mass of aggregates of the six classes. The soil
197 aggregate classes were pooled in large macroaggregates (> 2 mm), small
198 macroaggregates (2-0.250 mm) and microaggregates (<0.250 mm). The SOC
199 concentration of these aggregate size fractions was determined as previously described.

200 For the physical fractionation of SOM from large macroaggregates, small
201 macroaggregates and microaggregates, 10-g samples from each soil aggregate class
202 were placed in centrifuge tubes containing 80 ml of sodium polytungstate solution
203 (SPT) (2.0 g cm^{-3}) (Figure 1). After closure of the stopper tube and slow and manual
204 stirring, the free-LF of the OM was released (Conceição et al., 2008). The suspension
205 was centrifuged at 2000 g for 90 minutes. The supernatant, which contained the free-LF,
206 was filtered under vacuum. To remove excess SPT, the free-LF was washed with
207 distilled water and calcium chloride solution (CaCl_2) (0.01 mol L^{-1}). The SPT solution
208 (contained in the free-LF supernatant) was returned to the tube and the suspension was
209 subjected to ultrasonic vibration dispersion at the 240 J mL^{-1} level in order to disperse
210 the soil aggregates. This level of energy was previously defined as sufficient to disperse
211 organomineral complexes > 2 μm in order to obtain 99% of the total clay fraction (Inda
212 Junior et al., 2007).

213 After sample dispersion, the suspension was centrifuged again and the occluded-
214 LF fraction was separated by following the same procedure as for free-LF. The free-LF
215 and occluded-LF fractions were oven dried at 60 °C, ground in an agate mortar, and
216 analyzed for C as previously described. The C-min-OM was obtained by calculating the
217 difference between SOC and C of the C-free-LF + C-occluded-LF. The C recovery in
218 each soil aggregate class ranged from 85 to 96% of the SOC.

219

220 *2.4 Statistical analysis*

221 Data were subjected to analysis of variance (ANOVA) and, when significant (p
222 <0.05), the differences between means of treatments were evaluated by the Tukey test at
223 the 5% level. To compare the effects of the tillage (T) and cropping (CC) systems on the
224 SOC variables, the MIXED procedure was performed, which considered the main
225 factors and their interactions as fixed factors, and the block and the experimental errors
226 as random variables. All analyses were performed using SAS ® v.9.4 (Statistical
227 Analysis System Institute Cary, North Carolina).

228 The statistical model used in the analysis of variance to analyze SOC or C in the
229 classes of aggregates was as follows: $Y_{ijkl} = \mu + B_i + T_j + \text{Error}_{(ij)} + C_k + T_j C_k + \text{Error}_{(ijk)}$
230 $+ L_l + \text{Error}_{(lj)} + T_j L_l + C_k L_l + T_j C_k L_l \text{Error}_{(ijkl)}$, where μ = general mean of experiment;
231 B = block (i = 1, 2, 3); T = tillage systems (j = 1, 2); CC = cropping systems (k = 1, 2,
232 3); L = soil layer (l = 1, 2, 3) e Error = experimental error.

233 To compare the C concentration in the fractions of SOC within each soil
234 aggregate class, the statistical model used was follows: $Y_{ijkl} = \mu + B_i + T_j + \text{Error}_{(ij)} + C_k$
235 $+ T_j C_k + \text{Error}_{(ijk)}$, onde μ = general mean of experiment; B = block (i = 1, 2, 3); T =
236 tillage system (j = 1, 2); C = cropping systems (k = 1, 2, 3) e Error = error experimental.

237

238 **3 Results**

239 *3.1 Soil organic carbon*

240 The SOC concentration was higher in the superficial soil layer (0–5 cm) under NT
241 compared to CT, but this difference disappeared in the underlying layers (Figure 2a).
242 The SOC stocks down to a depth of 20 cm depth were therefore similar for the two
243 tillage systems (Table 2). However, cropping systems had a strong effect on SOC
244 stocks, which varied from 33.1 to 40.8 Mg C ha⁻¹, with a gradual increase due to
245 legume cover crop insertion in the cropping systems (Table 2). Consequently, on
246 average, legume-based cropping systems had the SOC accumulation rate 0.25 Mg C
247 ha⁻¹ yr⁻¹ higher than traditional cropping system involving non legume crops (O/M)
248 (Table 2).

249

250 *3.2 Soil aggregation*

251 The proportion of soil aggregates was strongly affected by tillage systems down
252 to a depth of 20 cm (Figure 3 a, b, c). In the three soil layers, NT (average of 34.6%)
253 resulted in a greater proportion of large macroaggregates compared to CT (average of
254 20.1%), yielding a MWD of 1.3 mm for CT and 2.2 mm for NT. The cropping systems
255 influenced the proportion of soil aggregates only in the 0–5 cm depth. In this soil layer,
256 the proportion of large macroaggregates was 40% greater and the MWD 24% greater,
257 under OV/MC compared to O/M (Figure 4 a).

258

259 *3.3 SOC concentration in soil aggregates*

260 Tillage and cropping systems differed in their effects on SOC concentrations in
261 the three aggregate size classes, and these effects were mostly visible in the 0–5 cm soil
262 layer (Figure 4). The SOC concentration in both macroaggregate fractions ranged from

263 10.3 to 19.5 g kg⁻¹, and was affected mainly by tillage systems (Figure 5 a, b). The SOC
264 concentration in the large and small macroaggregates in the superficial soil layer was
265 5.1 and 4.0 g C kg⁻¹ higher under NT than under CT, respectively (Figure 5 a, b). On
266 the other hand, cropping systems had a greater effect on the small macroaggregates
267 (Figure 5 e) and an especially noticeable effect on the microaggregates in the superficial
268 soil layer (Figure 4 f), with SOC concentrations in microaggregates varying between
269 15.3 and 21.3 g kg⁻¹. More noticeably, the system with two legume cover crops
270 (OV/MC) showed a strong C enrichment in the microaggregates of the superficial soil
271 layer compared to system with no legume cover crop (O/M) (Figure 5 f).

272

273 *3.4 Distribution of C in physical fractions of soil aggregates*

274 On average, the free-LF-C fraction of both macroaggregate fractions represented
275 9% of the total SOC, and was affected by both tillage and cropping systems (Table 3).
276 The proportion of free-LF was much smaller in the soil microaggregates. On average,
277 the C concentration in the macroaggregates was 2.4 times greater under NT compared to
278 CT (Table 3). A less pronounced but still noticeable effect was observed in the system
279 with two legume cover crops: the free-LF-C in small macroaggregates was 76% higher
280 with the legume crops compared to O/M (Table 3).

281 The occluded-LF-C concentration varied between 1.1 and 4.6 g kg⁻¹ among soil
282 management systems, accounting from 9 to 27% of SOC, and was strongly influenced
283 by soil tillage systems. The occluded-LF-C concentration was 2.2-, 2.8- and 2.6-fold
284 larger, in the large and small macroaggregates and the soil microaggregates,
285 respectively, under NT system compared to CT (Table 3). The highest C concentration
286 was found in the large macroaggregates under NT (4.9 g kg⁻¹) (Table 3). The occluded-
287 LF-C concentration was 70, 69 and 84% greater in large and small macroaggregates and

288 microaggregates, respectively, under OV/MC compared to O/M cropping system (Table
289 3).

290 The min-OM fraction accounted for the majority of the C contained in all
291 aggregate fractions (Table 3). The cropping systems had a strong effect on min-OM of
292 microaggregates, especially under NT (Table 3) where it was 64% greater under
293 OV/MC compared to O/M (Table 3). Although the effect was less pronounced, the main
294 effect of tillage system was also significant in large macroaggregates, where the min-
295 OM-C concentration was 28% greater in NT compared to CT (Table 3).

296 In all three aggregate fractions, in the 0–5 cm layer only, there was a significant
297 positive correlation between the concentration of occluded-LF-C and min-OM-C (Fig.
298 6).

299

300 4. Discussion

301 Overall, and as usually observed (Plaza-Bonilha et al., 2010; Conceição et al.,
302 2013), soil under NT presented a greater proportion of macroaggregates than CT. These
303 macroaggregates under NT were also enriched in C compared to CT, which is also
304 consistent with previous studies (Conceição et al., 2013; Plaza-Bonilha et al., 2010;
305 Plaza-Bonilha et al., 2013). Macroaggregates are complex structures, enriched in labile
306 organic material recently added to the soil (Angers and Giroux, 1996; Plaza-Bonilla et
307 al., 2013; Puget et al., 1999; Six et al., 1999). The implementation of NT on degraded
308 (or C depleted) soils results in the formation and stabilization of these macroaggregates
309 and the consequent preservation of LF-C in these aggregate structures
310 (Andruschkewitsch et al., 2013; Plaza et al., 2013). The higher proportion of soil
311 macroaggregates associated with higher occluded-LF-C concentration in superficial soil

312 layer led to greater C stocks under NT compared to CT in that layer (2.4 vs. 0.8 Mg
313 ha⁻¹, on average, data not shown).

314 The effect of tillage on C accumulation in macroaggregates was not apparent in
315 the 5–10 and 10–20 cm soil layers; hence, C stocks in the 0–20 cm layer were
316 statistically similar (Table 2; Figure 2). Plaza-Bonilla et al. (2010) and
317 Andruschkewitsch et al. (2014) likewise found that the higher SOC content within
318 macroaggregates under the NT treatment was restricted to the 0–5 cm soil depth.

319 As opposed to the effect of tillage, which was mostly observed in the
320 macroaggregate fractions, the effects of cover crops were essentially visible in the
321 organomineral associations (Min-OM-C) of soil microaggregates, particularly under the
322 NT system. Two factors probably contributed to this effect. First, there was more C
323 input in the OV/MC than O/M (8.9 Mg ha⁻¹ vs. 5.0 Mg ha⁻¹, Table 1). Second, the
324 greater lability of the legume cover crop residues (vetch and cowpea) likely contributed
325 to more efficient use of this material by microorganisms, and enhanced C stabilization
326 in soil organic matter by chemical bonding with the mineral soil matrix (Cotrufo et al.
327 2013; Cotrufo et al., 2015).

328 Overall, in all aggregate fractions, the min-OM-C fraction accounted for up to
329 75% of the SOC and was also the fraction most influenced by cropping systems (Table
330 3). The cropping system, including legume cover crops, was therefore the main factor
331 that affected SOC accumulation up to 20 cm depth. Although NT increased the
332 occluded-LF-C compared to CT, this effect was smaller than the benefits that legume
333 cover crops can offer through gains in organomineral association.

334 The significant relationship between occluded-LF-C and Min-OM-C (Figure 6 a,
335 b, c) suggests that the greater C occlusion favored the formation of organomineral
336 associations when NT and legume cover crops are combined. This may be because

337 aggregates “provide time” and environment for occluded LF to become associated with
338 the mineral soil matrix forming an organomineral association (Conceição et al., 2013;
339 Garcia-Franco et al., 2015). These results suggest that the reduced aggregate turnover
340 under NT relative to CT (Six et al., 1999) favoring the physical protection of particulate
341 OM as well as interactions between soil mineral particles and OM in the superficial soil
342 layer (Courier-Murias et al., 2013) is especially efficient **in stabilizing C when coupled**
343 **with legume cover crops.**

344

345 **5. Conclusions**

346 The mechanisms by which no-tillage and legume cover crops favor soil C
347 accumulation in a subtropical Acrisol differ. **No-tillage has a positive impact on C**
348 **stabilizing in soil macroaggregates, increasing the LF-C occlusion and leading to C**
349 **accumulation mainly in the top 5-cm soil layer. On the other hand, legume** cover crops
350 are associated with an increase in the amount and lability of residue inputs, which favor
351 organomineral associations in fine particles of soil, **especially when combined with NT.**

352

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460

1 **Table 1.** Geographic coordinates, regional climate parameters, characteristics and
 2 classification of the soil, and C input by cover crops and maize across the whole
 3 experimental period (30 years) in Southern Brazil. CT= conventional tillage, NT= no-
 4 tillage, O/M= oat/maize, V/M= vetch/maize, and OV/MC= oat+vetch/maize+cowpea.
 5

Characteristic			
County	Eldorado do Sul, RS		
Geographic coordinates	30°51'S, 51°38'W		
Altitude (m)	96		
Mean annual temperature (°C)	19.4		
Mean annual rainfall precipitation (mm)	1440		
Köppen Climate Classification	Cfa		
Soil group (WRB) ¹	Acrisol		
Soil type (US taxonomy)	Typic Paleudult		
Soil texture in 0–20 cm layer	Sandy clay loam		
Particle size distribution			
Clay	220		
Silt	240		
Sand	540		
Mineralogy ²			
Feo ³	0.9 g kg ⁻¹ soil		
Fed ⁴	11.8 g kg ⁻¹ soil		
Feo/Fed	0.1		
Gt/(Gt+Hm) ⁵	0.1		
Soil bulk density (Mg m ⁻³) ^f	CT	NT	
0–5 cm	1.5	1.3	
5–10 cm	1.7	1.6	
10–20 cm	1.7	1.7	
Annual C input (Mg ha⁻¹)^g	Maize	Cover crops	Total
O/M	2.3	2.7	5.0
V/M	2.6	3.8	6.4
OV/MC	5.2	3.7	8.9

6 ^a World Reference Base for Soil Resources (IUSS, 2006); ^b Original data presented by Inda-Junior et al.
 7 (2007); ^c Ammonium oxalate soluble Fe; ^d Dithionite-citrate-bicarbonate soluble Fe; ^e Gt: goethite; Hm:
 8 hematite; ^f NT, no-tillage with glyphosate desiccation prior to maize planting; CT, conventional tillage
 9 with plowing and two disking prior to maize planting; ^g Original data presented by Veloso et al. (2018),
 10 on average of two tillage systems; mean values across the whole experimental period (30 years).
 11

12 **Table 2.** Soil organic carbon stock in 0–20 cm layer of sandy clay loam Acrisol under
 13 conventional tillage (CT) and no-tillage (NT) in combination with three cropping
 14 systems [oat/maize (O/M), vetch/maize (V/M) and oat+vetch/maize+cowpea
 15 (OV/MC)].

16

Tillage	Cropping system	SOC stock (Mg ha ⁻¹)	SOC accumulation rate (Mg ha ⁻¹ year ⁻¹)
CT	O/M	32.4	-
	V/M	37.1	0.2
	OV/MC	39.8	0.3
NT	O/M	33.7	0.1
	V/M	38.0	0.2
	OV/MC	41.7	0.3
	Mean CT	36.4	0.1
	Mean NT	37.8	0.2
	Mean O/M	33.1 b	0.0 b
	Mean V/M	37.6 a	0.2 a
	Mean OV/MC	40.8 a	0.3 a
F value	Tillage (T)	0.69 ns	1.00 ns
	Crop (CS)	19.51 **	15.00 **
	T*CS	0.08 ns	0.56 ns

17 No significant differences ($p > 0.05$) between treatments are designated with (ns); Significant differences
 18 between treatments at 1% probability of error by test F are designated with (**); Lowercase letters compare
 19 averages of crop systems; SOC= soil organic carbon; SOC accumulation rate was calculated in relation to
 20 CT O/M management system.
 21

22 **Table 3.** Carbon concentration in free light fraction (free-LF), occluded light fraction (occluded-LF) and mineral-associated organic matter (min-
 23 OM) in aggregates **classes > 2 mm, 2–0.25 mm and <0.25 mm** of a sandy clay loam Acrisol **under two tillage systems (CT,**
 24 **conventional tillage and NT, no-tillage) and two cropping systems [oat/maize (O/M) and oat+vetch/maize+cowpea (OV/MC)].**

Tillage	Cropping	g kg ⁻¹ aggregate									
		> 2 mm			2-0.25 mm			< 0.25 mm			
system	Free-LF	Occluded-LF	Min-OM	Free-LF	Occluded-LF	Min-OM	Free-LF	Occluded-LF	Min-OM	Free-LF	Min-OM
CT	O/M	1.2	1.1	7.6	0.7	1.0 Ba	7.3	0.5	0.8	14.87	
	OV/MC	1.0	3.3	8.61	1.2	1.7 Ba	8.0	0.4	2.0	14.45	
	Mean CT	1.1	2.2	8.1 B	0.9 B	1.3	7.6	0.5	1.4	14.66	
NT	O/M	1.8	4.1	9.8	2.1	2.8 Ab	7.5	0.7	2.8	12.09	
	OV/MC	1.7	5.7	10.9	3.7	4.7 Aa	9.2	1.2	4.6	19.83	
	Mean NT	1.8	4.9	10.4 A	2.9 A	3.8	8.4	1.0	3.7	15.96	
F	O/M	1.5	2.6	8.7	1.4 b	1.9	7.4	0.6	1.8	13.48	
	OV/MC	1.4	4.5	9.8	2.5 a	3.2	8.6	0.8	3.3	17.14	
	Mean OV/MC	1.4	4.5	9.8	2.5 a	3.2	8.6	0.8	3.3	17.14	
Tillage	Crops	4.58 ns	9.05 ns	21.64 *	26.80 *	223.35 **	0.96 ns	2.60 ns	3.95 ns	0.61 ns	
	T*CS	0.94 ns	3.11 ns	0.01 ns	7.86 *	175.66 **	3.96 ns	1.44 ns	5.57 ns	23.60	
No significant differences (p > 0.05) between treatments are designated with (ns). Significant differences between treatments at 5% and 1% probability of error by test F are designated with (*) and (**), respectively; Uppercase letters compare tillage systems and lowercase letters compare cropping systems within tillage systems.											

25
 26
 27
 28
 29
 30

1 **Figure Caption**

2 **Figure 1.** Schematic map of density fractionation of soil organic matter in soil
3 aggregate classes.

4
5 **Figure 2.** Soil organic carbon concentration in 0–5, 5–10 and 10–20 cm of sandy clay
6 loam Acrisol subjected for long-term to conventional tillage (CT) and no-tillage (NT)
7 (a) in combination with three cropping systems [oat/maize (O/M), vetch/maize (V/M)
8 and oat+vetch/maize+cowpea (OV/MC)] (b).

9 The results were showed in average of cropping systems (a) and of tillage systems (b) because **the triple**
10 **interaction was not significant**. Uppercase letters compare soil layers within the tillage systems (a) or the
11 cropping systems (b). Lowercase letters compare tillage systems (a) or cropping systems (b) within soil
12 layer according to the Tukey test ($p < 0.05$).
13

14 **Figure 3.** Aggregate size-class distribution and mean weight diameter (MWD) in the (a)
15 0–5, (b) 5–10 and (c) 10–20 cm layers of a sandy clay loam Acrisol subjected to
16 conventional tillage (CT) **and** no-tillage (NT).

17 The results were showed in average of cropping systems because the triple interaction **was not significant**.
18 Uppercase letters compare soil layers within the tillage systems in each soil aggregate class. Lowercase
19 letters compare tillage systems within soil layer in each soil aggregate class according to Tukey test ($p <$
20 0.05).
21

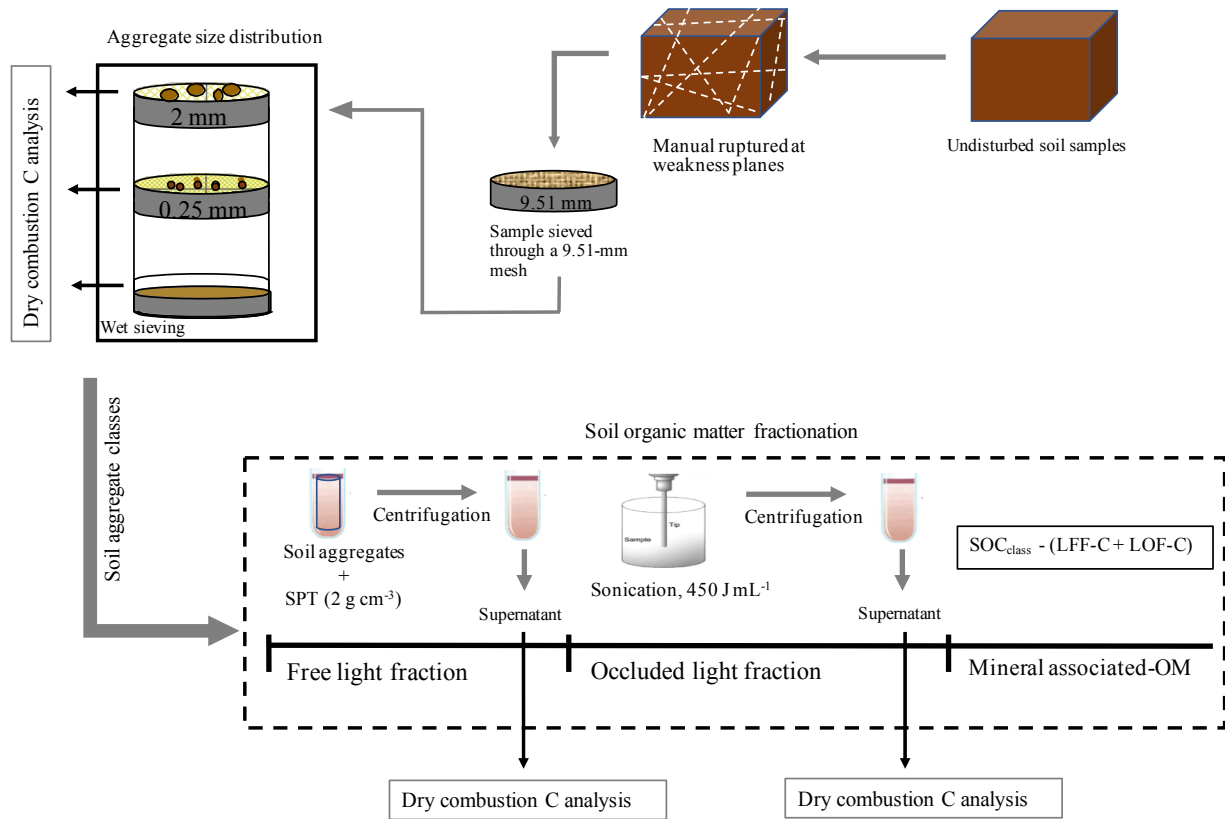
22 **Figure 4.** Aggregate size-class distribution and mean weight diameter (MWD) in the (a)
23 0–5, (b) 5–10 and (c) 10–20 cm layers of a sandy clay loam Acrisol subjected to
24 cropping systems oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea
25 (OV/MC) in average of tillage systems [conventional tillage (CT) and no-tillage (NT)].

26 The results were showed in average of tillage systems because only of cropping systems and soil layers
27 **effects were significant**. Uppercase letters compare soil layers within the cropping system in each soil
28 aggregate class. Lowercase letters compare cropping systems within soil layer in each class of aggregate
29 according to Tukey test ($p < 0.05$).
30

31 **Figure 5.** Concentration of organic carbon in aggregates > 2 mm (a, d), 2–0.25 mm (b,
32 e) and < 0.25 mm (c, f) in 0–5, 5–10 and 10–20 cm soil layers of a sandy clay loam
33 Acrisol subjected to conventional tillage (CT) and no-tillage (NT) or to three cropping
34 systems [oat/maize (O/M), vetch/maize (V/M) and oat+vetch/maize+cowpea
35 (OV/MC)].

36 As there **was** not triple interaction, the results were showed in average of cropping systems because the
37 **significant** interaction between tillage system and soil layer (a, b, c) and in average of tillage system
38 because the **significant** interaction between cropping system and soil layer (d, e, f). In (a, b, c); uppercase
39 letters compare soil layers within the tillage system in each aggregate class; lowercase letters compare
40 tillage systems within soil layer in each class of **soil** aggregate according to Tukey test ($P < 0.05$). In (d, e,
41 f), uppercase letters compare soil layers within the cropping system in each soil aggregate class;

42 lowercase letters compare cropping systems within soil layer in each class of soil aggregate according to
43 Tukey test ($p < 0.05$).
44



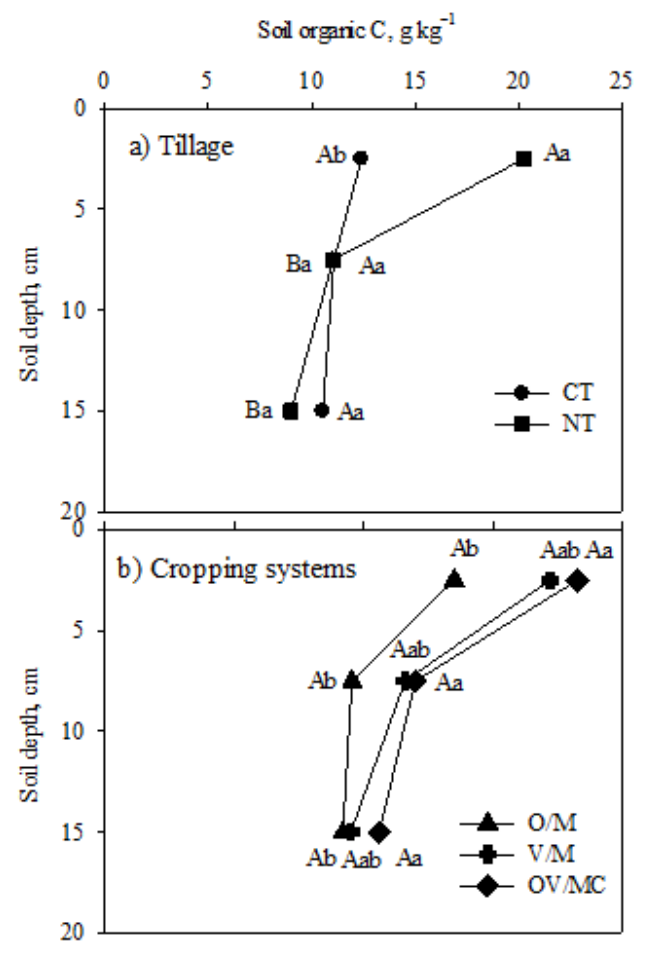
45

46 **Figure. 1.** Schematic map of density fractionation of soil organic matter in soil aggregate

47 classes.

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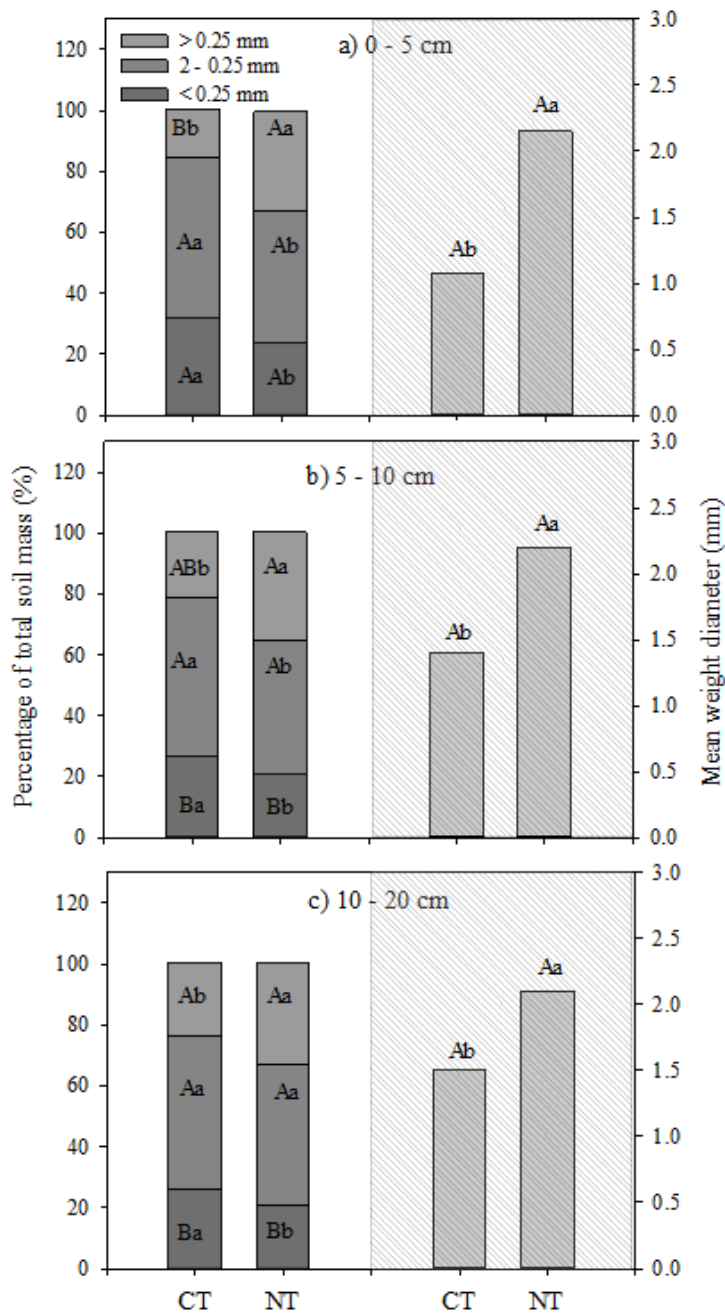
50

51 **Figure 2.** Soil organic carbon concentration in 0–5, 5–10 and 10–20 cm of sandy clay loam
 52 Acrisol subjected for long-term to conventional tillage (CT) and no-tillage (NT) (a) in
 53 combination with three cropping systems [oat/maize (O/M), vetch/maize (V/M) and
 54 oat+vetch/maize+cowpea (OV/MC)] (b).

55 The results were showed in average of cropping systems (a) and of tillage systems (b) because **the triple interaction**
 56 **was not significant**. Uppercase letters compare soil layers within the tillage systems (a) or the cropping systems (b).
 57 Lowercase letters compare tillage systems (a) or cropping systems (b) within soil layer according to the Tukey test
 58 ($p < 0.05$).

59

60

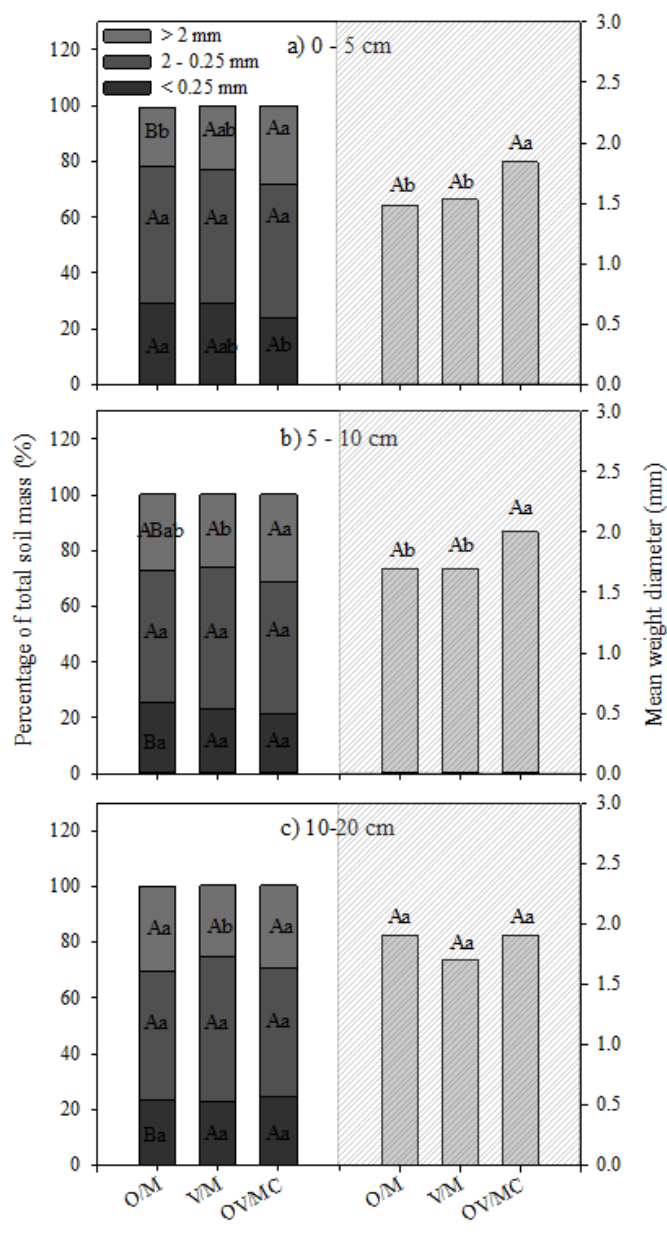


61

62 **Figure 3.** Soil aggregate size-class distribution and mean weight diameter (MWD) in the (a) 0–5,
 63 (b) 5–10 and (c) 10–20 cm layers of a sandy clay loam Acrisol subjected to conventional tillage
 64 (CT) and no-tillage (NT).

65 The results were showed in average of cropping systems because there was not triple interaction. Uppercase letters
 66 compare soil layers within the tillage systems in each soil aggregate class. Lowercase letters compare tillage systems
 67 within soil layer in each soil aggregate class according to Tukey test ($p < 0.05$).

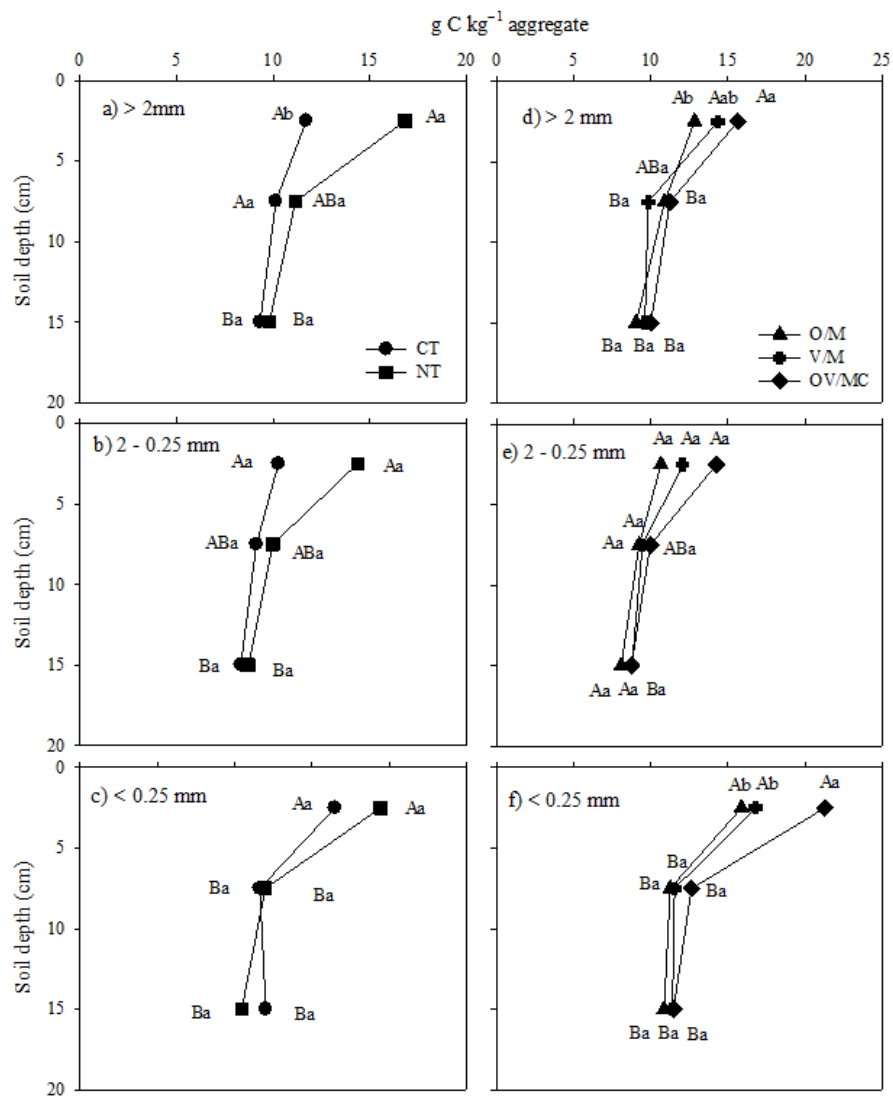
68



70

71 **Figure 4.** Aggregate size-class distribution and mean weight diameter (MWD) in the (a) 0–5, (b)
 72 5–10 and (c) 10–20 cm layers of a sandy clay loam Acrisol subjected to cropping systems
 73 oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC) in average of
 74 tillage systems [conventional tillage (CT) and no-tillage (NT)].

75 The results were showed in average of tillage systems because only of cropping systems and soil layers effects were
 76 significant. Uppercase letters compare soil layers within the cropping system in each soil aggregate class. Lowercase
 77 letters compare cropping systems within soil layer in each class of aggregate according to Tukey test ($p < 0.05$).
 78

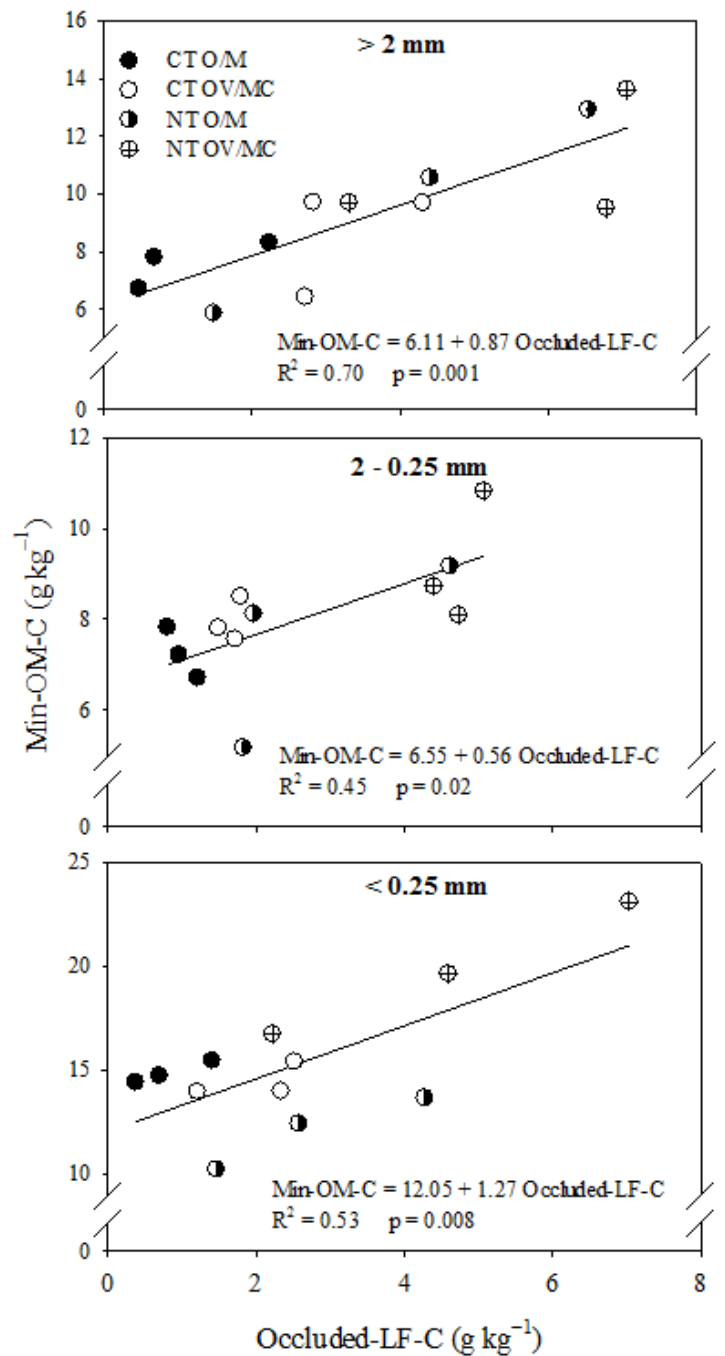


80

81 **Figure 5.** Concentration of organic carbon in aggregates > 2 mm (a, d), 2–0.25 mm (b, e) and <
 82 0.25 mm (c, f) in 0–5, 5–10 and 10–20 cm soil layers of a sandy clay loam Acrisol subjected to
 83 conventional tillage (CT) and no-tillage (NT) or to three cropping systems [oat/maize (O/M),
 84 vetch/maize (V/M) and oat+vetch/maize+cowpea (OV/MC)].

85 As there was not triple interaction, the results were showed in average of cropping systems because the significant
 86 interaction between tillage system and soil layer (a, b, c) and in average of tillage system because the significant
 87 interaction between cropping system and soil layer (d, e, f). In (a, b, c); uppercase letters compare soil layers within
 88 the tillage system in each aggregate class; lowercase letters compare tillage systems within soil layer in each class of
 89 soil aggregate according to Tukey test ($P < 0.05$). In (d, e, f), uppercase letters compare soil layers within the
 90 cropping system in each soil aggregate class; lowercase letters compare cropping systems within soil layer in each
 91 class of soil aggregate according to Tukey test ($p < 0.05$).

92



93

94 **Figure 6.** Regression of C concentration in the mineral associated organic matter (Min- MO-C)
 95 as a function of the concentration of C in the occluded light fraction (Ocluded-LF-C) in
 96 aggregates > 2 (a), 2–0.25 (b) and <0.25 mm (c) in 0–5 cm layer of sandy clay loam Acrisol
 97 under conventional tillage (CT) and no-tillage (NT) in combination with oat/corn (O/M) and
 98 oat+vetch /maize+cowpea (OV/MC).