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# Mineral–organic associations are enriched in both microbial metabolites and plant residues in a subtropical soil profile under no-tillage and legume cover cropping

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1 **Mineral-organic associations are enriched in both microbial metabolites and plant residues**  
2 **in a subtropical soil profile under no-tillage and legume cover cropping**

3

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15

16 **SUMMARY TEXT FOR THE TABLE OF CONTENTS**

17

18 Long-term carbon stabilisation in tropical and subtropical soils rests on the formation of mineral–  
19 organic associations (MOAs), compounded by residues of plants and microorganisms. We  
20 sampled soil profiles in a long-term field experiment to evaluate if different soil managements  
21 affect these residues and consequent impact on the MOAs. Our findings highlight that the carbon  
22 accumulation is due to MOAs formation which is composed of both plant and microbial residues  
23 and is positively influenced by no-tillage and legume cover crops.

24

25 **Mineral-organic associations are enriched in both microbial metabolites and plant residues**  
26 **in a subtropical soil profile under no-tillage and legume cover cropping**

27

28 **Abstract**

29 The implementation of no-tillage (NT) coupled with legume cover cropping results in high  
30 carbon sequestration rates in soils of Southern Brazil. We hypothesized that this effect is due to  
31 the formation of mineral-organic associations (MOAs) through the enrichment in plant and  
32 microbial residues, arising from the absence of tillage and the presence of high-quality legume  
33 residues. We sampled soil profiles in a long-term field experiment, and used density and particle  
34 size fractionation in combination with carbohydrate analyses to compare the effect of conventional  
35 tillage (CT) vs. NT, combined or not with legume cover cropping, and combined or not with  
36 mineral N fertilization. Both NT and the presence of legume cover crops favoured the  
37 accumulation and enrichment in plant-derived carbohydrates in the surface soil layer (0–5 cm),  
38 due to the accumulation of plant residues. The ratio of microbe-derived carbohydrates (galactose,  
39 manose, fucose and rhamnose) to plant-derived carbohydrates (arabinose and xylose) increased  
40 with soil depth indicating that the soil C is more microbially processed at depth than at the surface.  
41 Conservation management systems also increased soil C at depth and this was most visible in the  
42 clay fraction, suggesting that additional C is relatively stable (mineral-organic associations). The  
43 additional clay-size C accumulating at depth under conservation treatments is of both microbial  
44 and plant origin. Our results support, with field-based data, the current hypothesis that mineral-  
45 associated C is composed of both plant and microbial residues, and is positively influenced by  
46 conservation management practices.

47 **Keywords:** organo-mineral associations, soil carbohydrates, soil organic carbon accumulation,  
48 no-till, cover crops, plant residues, microbial residues, soil management, legumes

49           **Introduction**

50           The Paris climate agreement aims at holding global warming below 2 °C with efforts to  
51 limit it to 1.5 °C (Rogelj et al., 2016). The soil organic carbon (SOC) pool might function as a  
52 significant sink for atmospheric carbon dioxide (CO<sub>2</sub>) and contribute to mitigate anthropogenic  
53 greenhouse gas emissions, provided that soil be properly managed (Lal, 2004). Changing  
54 agricultural practices represents one of the low-cost solutions proposed by Brazil to sequester  
55 carbon in soil and mitigate global warming (Brazil Ministry of the Environment, 2015).

56           Conversion from CT to NT is an important measure resulting in mean SOC sequestration  
57 rates of 0.32 and 0.48 Mg ha<sup>-1</sup> year<sup>-1</sup> in tropical and subtropical regions, respectively, in the top  
58 20 cm of soil (Bayer et al., 2006). Recently, it was demonstrated that combining legume cover  
59 cropping with NT and N fertilization may result in SOC sequestration rates up to 1.15 Mg ha<sup>-1</sup>  
60 year<sup>-1</sup> in subtropical soils down to 100 cm in the soil profile (Velooso et al., 2018).

61           Previous studies have shown that mineral-organic associations (MOAs) are composed  
62 largely of microbial residues (Puget et al., 1998; Kogel-Knabner et al., 2008; Rumpel et al., 2010).  
63 Moreover, labile plant residues can induce microbial growth and have high microbial use  
64 efficiency, thereby favoring C accumulation through the formation of stable MOAs (Rumpel et  
65 al., 2010; Cotrufo et al., 2013). Formation of MOAs appears to be an important mechanism of C  
66 stabilization in tropical and subtropical soils as 65 to 92% of SOC in these soils is associated with  
67 kaolinite and Fe/Al oxides as the major clay constituents (Amado et al., 2006). However, recent  
68 studies have also shown that the persistent SOC is not composed exclusively of compounds  
69 derived from microbial activity but that plant-derived compounds contribute directly to MOAs  
70 even after several decades of degradation (e.g. Barré et al., 2018; Angst et al., 2021).

71 Carbohydrate analysis can be used to investigate the contribution of microbial products to  
72 SOC in MOAs. Carbohydrates containing hexoses such as galactose (G), mannose (M), fucose (F)  
73 and rhamnose (R) are considered to be synthesized predominantly by microorganisms, whereas  
74 carbohydrates containing pentoses such as arabinose (A) and xylose (X) are synthesized  
75 predominantly by plants (Oades and Wagner, 1971; Chesire, 1979). Thus, the GMFR/AX and  
76 GM/AX ratios have been used to estimate the relative contribution of microbial vs. plant derived  
77 carbohydrates in soils ( Rumpel et al., 2010; Martins et al., 2012; Samson et al., 2020).

78 Our goal was to investigate the impact of cropping systems involving a cover crop of high  
79 residue lability associated with no-tillage (NT) on the accumulation of mineral-associated C and  
80 its associated carbohydrates, allowing to distinguish plant and microbe-derived residues. Using a  
81 long-term field experiment, we evaluated the effect of CT vs. NT, with or without legume cover  
82 cropping, on carbohydrate in SOC (in whole soil and MOAs), and in surface and sub-surface soil  
83 layers of a subtropical Acrisol in Southern Brazil.

84

## 85 **Material and methods**

### 86 *Description of the experiment*

87 The study was conducted in a long-term experiment (30 years) located at the Agronomic  
88 Experimental Station of the Federal University of Rio Grande do Sul, in Eldorado do Sul–RS  
89 (30°06'S, 51°40'W and altitude of 96 m). The climate is subtropical (Cfa according to the Köppen  
90 classification), with mean annual temperature and annual rainfall of 19.4 °C and 1440 mm,  
91 respectively. The soil of the experiment is classified as sandy clay loam granite-derived Acrisol  
92 (FAO, 2015). However, there is an increase in clay content down the soil profile, from 217 g kg<sup>-1</sup>  
93 at 0–5 cm, 394 g kg<sup>-1</sup> at 20–30 cm and reaching 511 g kg<sup>-1</sup> at 75–100 cm. The main clay minerals  
94 are kaolinite (720 g kg<sup>-1</sup>) and iron oxides (109 g kg<sup>-1</sup>) (Bayer et al., 2001).

95           Until 1969, the area was under native grassland (mainly *Paspalum* and *Andropogon*). In  
96 the following 16 years, the soil was under intensive plowing and disking operations with annual  
97 cropping, and was seriously degraded at the time of establishment of a factorial experiment in  
98 1985.

99           The experimental treatments comprised two tillage practices, CT and NT, applied in main  
100 plots of 15 × 20 m, which were divided in three 5 × 20 m subplots to compare three crop  
101 succession: black oats (*Avena strigosa* Schreb) – maize (*Zea mays* L.) (O/M); vetch (*Vicia sativa*  
102 L.) – maize (V/M); oats + vetch – maize + cowpea (*Vigna unguiculata* (L.) Walp) (OV/MC). Each  
103 subplot of the six tillage-cropping combinations were divided in two 5 × 10 m sub-subplots  
104 receiving either 0 or 180 kg ha<sup>-1</sup> of urea-N (0N and 180N), band-applied in the maize crop. The  
105 experimental design was a split-split plot with three replicates arranged in randomized complete  
106 blocks.

107           Winter cover crops were sown in April-May, at a seed rate of 80 kg ha<sup>-1</sup> for oats, 70 kg  
108 ha<sup>-1</sup> for vetch, and 30+40 kg ha<sup>-1</sup> for the oats+vetch combination. The cover crops were cut with  
109 a knife roller at the flowering stage (September-October). Tillage treatments and maize sowing  
110 were usually performed 1 to 3 weeks later. In CT, operations included plowing to 17-cm depth to  
111 incorporate cover crop residues, and two disking passes to 10 cm to prepare seedbed. In NT, soil  
112 was not disturbed and crop residues left on the surface, except on plant rows for seeding. Maize  
113 was planted at a row-spacing of 90 cm, with a targeted density of 50–70 thousand plants per  
114 hectare. Fifty kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 50 kg ha<sup>-1</sup> of K<sub>2</sub>O were applied at planting. In OV/MC, cowpea  
115 was sown 20–30 days after maize planting, in maize inter-rows with 40 cm between plants on the  
116 row. After maize harvesting, cowpea reached full development and was terminated with a knife  
117 roller immediately before the establishment of oats and vetch.

118 Annual C inputs in the cropping systems were calculated using a database compiling  
119 information since inception of the experiment in 1985 (Zanatta et al., 2007), and updated until year  
120 2014 (Veloso et al., 2018). Annual C input from roots was estimated to be 30% of C input from  
121 aboveground plant parts (Zanatta et al., 2007). The C input in CT O/M 0N, NT O/M 0N, NT  
122 OV/MC 0N and NT OV/MC 180N was 4.8, 5.2, 9.2 and 10.2 Mg ha<sup>-1</sup> yr<sup>-1</sup>.

123

#### 124 *Soil Neutral Carbohydrates*

125 Carbohydrates extractable with strong acid are considered to represent the total  
126 carbohydrates in the soil (Lowe, 1993). The method described by (Chantigny and Angers, 2008)  
127 was used for this determination. Specifically, 2 g of air-dried and finely ground (<0.15-mm) whole  
128 soil, and 1 g of fine silt and clay-sized MOAs were placed in 50-mL centrifuge tubes. Eight mL of  
129 12 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> were added to whole soil, and 4 mL to fine silt and clay fractions. The soil and  
130 acid were gently mixed with a glass rod to ensure that the soil was completely moistened with the  
131 acid. The tubes were loosely capped and left on the bench for 2 h. After this period, the slurry were  
132 transferred into a 250-mL centrifuge bottles by washing the tube with 184 mL of deionized water  
133 to bring the solution to 0.5 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. The bottles were sealed and placed in an oven set at  
134 85°C for 24 h. After cooling, the bottles were centrifuged at 16,000 × g for 10 min. A 15-mL  
135 aliquot of the supernatant was transferred to plastic vials and stored at -20 °C for subsequent  
136 analysis.

137 Soil carbohydrates were analyzed based on the methods of Martens and Loeffelmann  
138 (2002) and (Chantigny and Angers, 2008). Briefly, 5 mL of soil hydrolysate was transferred in a  
139 50-mL beaker and neutralized to pH 6.5-7.0 by dropwise addition of 0.5 mol L<sup>-1</sup> NaOH solution.  
140 The volume of NaOH solution used was recorded to adjust calculation of carbohydrate



141 concentrations. The neutralized solution was filtered using Whatman no. 42 filter paper.  
142 Approximately 2.5 mL of this filtrate was passed through a strong cation solid-phase exchange  
143 resin and collected in a 3-mL polypropylene Eppendorf vial. The cation-purified solution was then  
144 passed through a strong anion solid-phase exchange resin and collected in a new Eppendorf vial.

145 The monosaccharides present in the purified solutions were quantified using a liquid anion-  
146 exchange chromatograph with pulsed amperometric detection (Model Dionex ICS 5000+, Thermo  
147 Scientific, Sunnyvale, CA) equipped with automated sampler. The monosaccharide separation was  
148 performed with a CarboPac-PA1 pre-column (4 by 50 mm) in series with an analytical column (4  
149 by 250 mm) and 10  $\mu\text{L}$  injection loop. As eluents, we used 20 and 500  $\text{mmol L}^{-1}$  NaOH solutions  
150 for monosaccharide analyses (0.8  $\text{mL min}^{-1}$  flow rate) and conditioning of the column (1.0  $\text{mL}$   
151  $\text{min}^{-1}$  flow rate), respectively. Monosaccharides (fucose, rhamnose, galactose, glucose, mannose,  
152 arabinose, xylose) were identified based on their retention time and co-elution with spiked  
153 standards (Martens and Loeffelmann, 2002). Blank samples were included during the extraction  
154 and purification processes to correct for the small amounts of arabinose, xylose, and glucose  
155 yielded by filter papers.

156

### 157 *Statistical analyses*

158 After analysis for normality by the Kolmogorov-Smirnov test and homogeneity of variance  
159 by the Levene test, the data were submitted to analysis of variance (ANOVA). The statistical model  
160 used to evaluate the effect of treatments on soil neutral carbohydrate concentrations and SOC stock  
161 and accumulation was:  $Y_{ij} = \mu + B_i + T_j + \text{Erro}_{(ij)}$ , where  $Y$  = mean of treatment  $j$  in block  $i$ ;  $\mu$  =  
162 general average of the experiment;  $B$  = block ( $i = 1, 2, 3$ );  $T$  = treatments ( $j = 1, 2, 3, 4$ ); Error =

163 experimental error. All analyses were performed using SAS ® v.9.4 (Statistical Analysis System  
164 Institute, Cary, North Carolina).

165 When significant ( $p < 0.05$ ), treatment means were compared by contrast analysis using the  
166 Tukey's test ( $p < 0.05$ ):

167 i) No-tillage effect: CT O/M 0N vs. NT O/M 0N;

168 ii) Legume effect: NT O/M 0N vs. NT OV/MC 0N;

169 iii) Fertilization effect: NT OV/MC 0N vs. NT OV/MC 180N.

170

171 Concentrations of individual neutral carbohydrates were expressed on C basis. The sum of  
172 arabinose and xylose (AX) was taken as a proxy of plant-derived soil carbohydrate-C, whereas the  
173 sum of galactose, mannose rhamnose and fucose (GMRF) was taken as a proxy of microbe-derived  
174 soil carbohydrate-C. Regression analyses were performed (SigmaPlot for Windows v. 13.0, Systat  
175 Software, Inc., San Jose, CA) to explore the relationships between GMFR or AX and previously  
176 published C concentrations in whole soil and in fine silt- and clay-sized MOAs (Veloso et al.,  
177 2020).

178

## 179 **Results**

180 Plant-derived carbohydrates (AX values in Table 1) ranged from 658 to 2096 mg kg<sup>-1</sup> in  
181 whole soil and fractions, and markedly declined down the soil profile. In the surface soil layer (0-5  
182 cm), the concentration of plant-derived carbohydrates was 1.5 times greater under NT than CT in  
183 the fine silt fraction, and 1.3 times greater under NT in the clay fraction; the same trend (not  
184 significant) was found in the whole soil. Plant-derived carbohydrates were also 1.8 times more  
185 abundant, on average, in whole soil where legumes were included in the crop rotation, and 1.4

186 times more abundant with than without N fertilization. Similar effects of legumes and fertilization  
187 were also found in the clay and fine-silt fractions. The treatments did not influence plant-derived  
188 carbohydrates in the 20–30 cm depth, but a positive effect of legumes was found in the fine silt  
189 and clay fractions at the 75–100 cm depth.

190 As compared to the whole soil, concentrations of plant carbohydrates were generally  
191 greater in the clay fractions (1.26 to 1.88-fold), except in the surface layer of NT soils with legumes  
192 where concentrations were similar to whole soil (Table 1). The concentration of plant-derived  
193 carbohydrates in the fine silt fraction were generally similar to or lower than the whole soil at all  
194 soil depths.

195 Microbe-derived carbohydrates (GMFR values in Table 1) ranged from 880 to 3398 mg  
196  $\text{kg}^{-1}$  in whole soil and fractions in the surface soil layer, and declined more gradually than plant-  
197 derived carbohydrates down the soil profile, with values ranging from 554 to 1845  $\text{mg kg}^{-1}$  at  
198 20–30 cm depth, and from 131 to 1034  $\text{mg kg}^{-1}$  at 75–100 cm depth. In the surface soil layer,  
199 microbe-derived carbohydrates were more abundant under NT than CT in the fine silt and clay  
200 fractions; a similar trend (not significant) was found for the whole soil. Microbe-derived  
201 carbohydrates were also more abundant in the presence of legumes and with N fertilization in  
202 whole soil and fractions of the surface soil layer. A positive effect of NT and a negative effect of  
203 N fertilization were found in the 20–30 cm soil layer in the clay fraction only. A negative effect  
204 of NT was found in the whole soil and the clay fraction at the 75–100 cm depth, whereas a positive  
205 effect of legumes was found in whole soil and both fractions, and a positive effect of fertilization  
206 was found in whole soil. As compared to the whole soil, the clay fraction was enriched in microbe-  
207 derived carbohydrates in all soil layers, but this relative enrichment was greater in surface soil and  
208 declined at depth (Table 1). The fine silt fraction was enriched in microbial carbohydrates in the

209 surface soil layer compared to the whole soil, especially in NT systems, but was clearly  
210 impoverished at greater depths (Table 1).

211 The microbe-to-plant-derived carbohydrate ratio (GMFR/AX values in Table 1) ranged  
212 from 0.90 to 2.85 and was generally narrower in surface than in deeper soil layers in all fractions.  
213 In the clay fraction of the surface soil layer, the ratio was narrower with than without legumes; the  
214 same trend (not statistically significant) was found in the whole soil and the fine-silt fraction. The  
215 ratio was not significantly influenced by treatments at greater depths. While the GMFR/AX ratio  
216 was wider in the clay and fine-silt fraction than in whole soil in the surface layer, the ratio was in  
217 the order: whole soil > clay fraction > fine-silt fraction at greater depths.

218 Significant and positive relationships were found between the concentration of neutral-  
219 sugars and whole soil C, fine silt-C and clay-C (Figs. 1-3) concentrations. The concentration of  
220 GMFR was positively related to whole soil C concentration in the 0–5 and 75–100 cm layer (Fig.  
221 1 a,c), and to fine silt-C (Fig. 2 a,b,c) and clay-C at all depths (Fig. 3 a,b,c). A positive relationship  
222 was also found between the concentration of AX and whole soil C (Fig. 1 d) and clay-C in the top  
223 soil layer only (Fig. 3d), and fine silt-C at all depths (Fig. 2d, e, f).

224

## 225 **Discussion**

### 226 *Surface soil layer*

227 Our previous report at this site showed that implementing NT and legumes cover  
228 cropping significantly increased SOC storage in Southern Brazil (Veloso et al., 2018). After 30 y,  
229 this effect was visible in the whole soil profile (100 cm) with SOC stock being 20 (NT alone) to  
230 35 (NT + legumes) Mg ha<sup>-1</sup> greater than in the CT system.

231 In NT soils, the narrower microbe-to-plant carbohydrate ratio in systems including  
232 legumes (with and without N fertilizer) reflected the large increase in LF-C in the surface soil layer  
233 (Veloso et al., 2020) that led to a dominant contribution of plant-derived carbohydrates. As  
234 compared to whole soil, the clay and fine-silt fractions were slightly enriched or had comparable  
235 plant-derived carbohydrate concentration in the surface soil layer. In contrast, both fractions were  
236 generally highly enriched in microbe-derived carbohydrates, resulting in wider microbe-to-plant  
237 carbohydrate ratio in fractions than in whole soil, especially with the clay fraction. These findings  
238 support our previous results that plant-derived light-fraction (LF) C is a dominant SOC source in  
239 the surface soil layer (Veloso et al., 2020), and further indicate that part of this LF-C was heavily  
240 colonized with microbes and had initiated decomposition and encrustation with soil particles,  
241 resulting in enrichment of SOC with microbial carbohydrates in fine silt- and clay-sized MOAs.

242

### 243 *Subsurface soil layers*

244 Deeper in the soil profile, the direct influence of crop residues on MOA formation was  
245 much smaller (much less LF-C as observed in Veloso et al., 2020). In these soil layers, it is likely  
246 that microbial activity was stimulated by percolation of soluble substrates from surface crop  
247 residues and/or diffusion of root exudates, leading to direct formation of fine MOAs. This  
248 hypothesis is supported by the markedly wider microbe-to-plant carbohydrate ratio in whole-soil  
249 and clay-sized MOA ( $> 2$ ), in subsurface than in surface soil layers (Table 1), suggesting that the  
250 contribution of microbially-derived compounds to SOM increases with soil depth (Angst et al.,  
251 2018). Our results further suggest that the contribution of microbial-derived compounds to  
252 stabilized MOAs may also increase with soil depth.

253           The positive effect of NT on SOC accumulation was more visible in clay fraction in the  
254 20–30 cm depth (Fig. 3b), where this fraction represented 87-91% of SOC (Veloso et al., 2020),  
255 showing that SOC accumulation is more directly associated with the clay fraction in deeper soil  
256 layers. This suggests these systems also stimulated the accumulation of microbial metabolites at  
257 depth and the formation of presumably stable fine MOAs. This C accumulation at depth can be  
258 hypothesized to be mediated by percolation of soluble compounds through the biopores, as  
259 discussed earlier, or by exudation and decay of deep roots, which stimulated microbial activity and  
260 formation of fine MOAs at depth (Hobley et al., 2016). This hypothesis would also explain that  
261 while both plant-derived and microbe-derived carbohydrates were closely related to SOC, fine-  
262 silt-C and clay-C concentrations in the surface soil layer and to fine-silt-C at greater depths,  
263 microbe-derived but not plant-derived carbohydrate concentration explained variations in clay-C  
264 concentration in the 20–30 and 75–100 cm depths (Fig. 3). Our results are in agreement with Angst  
265 et al. (2018) who suggested that subsoil SOC accumulation was highly influenced by OM input  
266 and its retention by the soil mineral matrix through the contribution of microbial-derived  
267 compounds.

268           The lower concentration of microbial residue in the NT O/M treatment in whole soil, fine  
269 silt and clay fraction of the 75–100 cm soil layer compared to CT O/M was not completely  
270 understood, as this trend was not observed for C concentration (Veloso et al., 2020). However, the  
271 inclusion of the legume cover crop under NT (NT OV/MC) increased the concentration of both  
272 microbial and plant carbohydrates at that depth suggesting that legume cover crops are a greater  
273 driver of carbohydrate accumulation at depth than NT alone. Both plant- and microbial-derived  
274 carbohydrates could be leached from the topsoil layer or deposited directly at depth by the roots,  
275 and then colonized by microbes. These two mechanisms correspond to the “ex vivo modification”

276 and “in vivo turnover” pathways described by Liang et al. (2017) and evidenced by Angst et al.  
277 (2017) and Córdova et al. (2018), and likely drive MOA formation and accumulation at depth. In  
278 the ex vivo modification pathway, extracellular enzymes release depolymerized products of plant  
279 compounds that are not readily assimilated by microbes and thus susceptible to rapid adsorption  
280 to mineral surfaces and stabilization in MOAs. In contrast, in the in vivo turnover pathway,  
281 microbes assimilate plant-derived organic matter and synthesize new compounds that  
282 subsequently interact with the mineral soil matrix to form MOAs. Overall, our results are in  
283 agreement with Barré et al. (2018) and Angst et al. (2021), who demonstrated that persistent SOC  
284 is not composed exclusively of compounds derived from microbial activity but that plant-derived  
285 compounds also directly contribute to SOC even after several decades of degradation. Our results  
286 contribute to the current debate on the composition of persistent SOC related to substrate quality  
287 and microbial turnover (Kallenbach et al. 2016; Liang et al. 2017; Barré et al., 2018; Córdova et  
288 al., 2018), suggesting that MOAs are enriched in both microbial metabolites and plant residues in  
289 a subtropical soil profile under no-tillage and legume cover cropping.

290

## 291 **Conclusions**

292 Using a long-term field experiment, we found a dominant effect of accumulation of crop residues  
293 as influenced by conservation management systems, that led to a dominant contribution of plant-  
294 derived carbohydrates. in surface soil layer. The widening in microbe-to-plant carbohydrate ratio  
295 with depth in whole soil and the clay fraction indicate that the soil C is more microbially processed  
296 at depth than at surface. The conservation management systems increased soil C at depth and this  
297 was most visible in the clay fraction, suggesting that the additional C accumulated in these systems  
298 is fairly stable (mineral-organic associations). However, the additional clay-C accumulating at

299 depth under conservation treatment comprised both microbe- and plant-derived materials. These  
300 results strengthen our understanding of how carbon accumulates and MOAs are formed at different  
301 depths in subtropical soils after adoption of no-tillage and legume cover cropping.

302

### 303 **Conflict of interest**

304 The authors declare no conflicts of interest.

305

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314

315

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## **SUMMARY TEXT FOR THE TABLE OF CONTENTS**

Long-term carbon stabilisation in subtropical soils rests on the formation of mineral–organic associations (MOAs), that is compounded by residues of plants and microorganisms. We sampled soil profiles in a long-term field experiment to evaluate if different soil managements affect these residues and consequent impact on the MOAs. Our findings highlight that the carbon accumulation is due to MOAs formation which is composed of both plant and microbial residues and is positively influenced by no-tillage and legume cover crops.

1 **Figures**

2

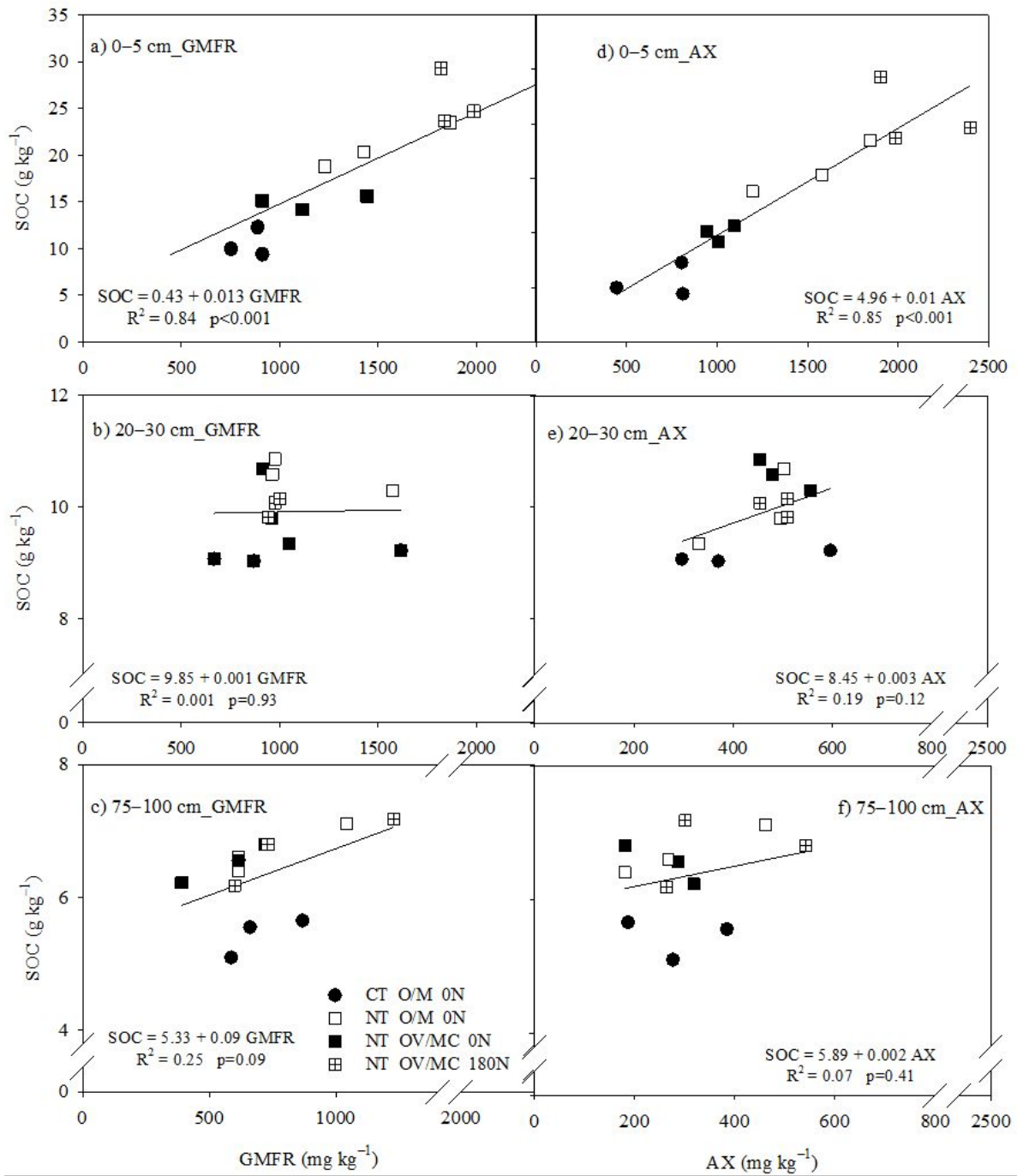
3 **Figure 1.** Regression of GMFR concentration (a,b,c) and AX concentration (d,e,f) as function of  
4 SOC concentration of whole-soil in 0–5, 20–30 and 75–100 cm soil layer the from Acrisol  
5 submitted to conventional tillage (CT) and no tillage (NT) with oat/maize (O/M) and  
6 oat+vetch/maize+cowpea (OV/MC); and two nitrogen fertilization rates, 0 e 180 kg of urea ha<sup>-1</sup>  
7 (0N and 180N).

8

9 **Figure 2.** Regression of GMFR concentration (a,b,c) and AX concentration (d,e,f) as function of  
10 carbon concentration in fine-silt fraction in 0–5, 20–30 and 75–100 cm soil layer the from Acrisol  
11 submitted to conventional tillage (CT) and no tillage (NT) with oat/maize (O/M) and  
12 oat+vetch/maize+cowpea (OV/MC); and two nitrogen fertilization rates, 0 e 180 kg of urea ha<sup>-1</sup>  
13 (0N and 180N).

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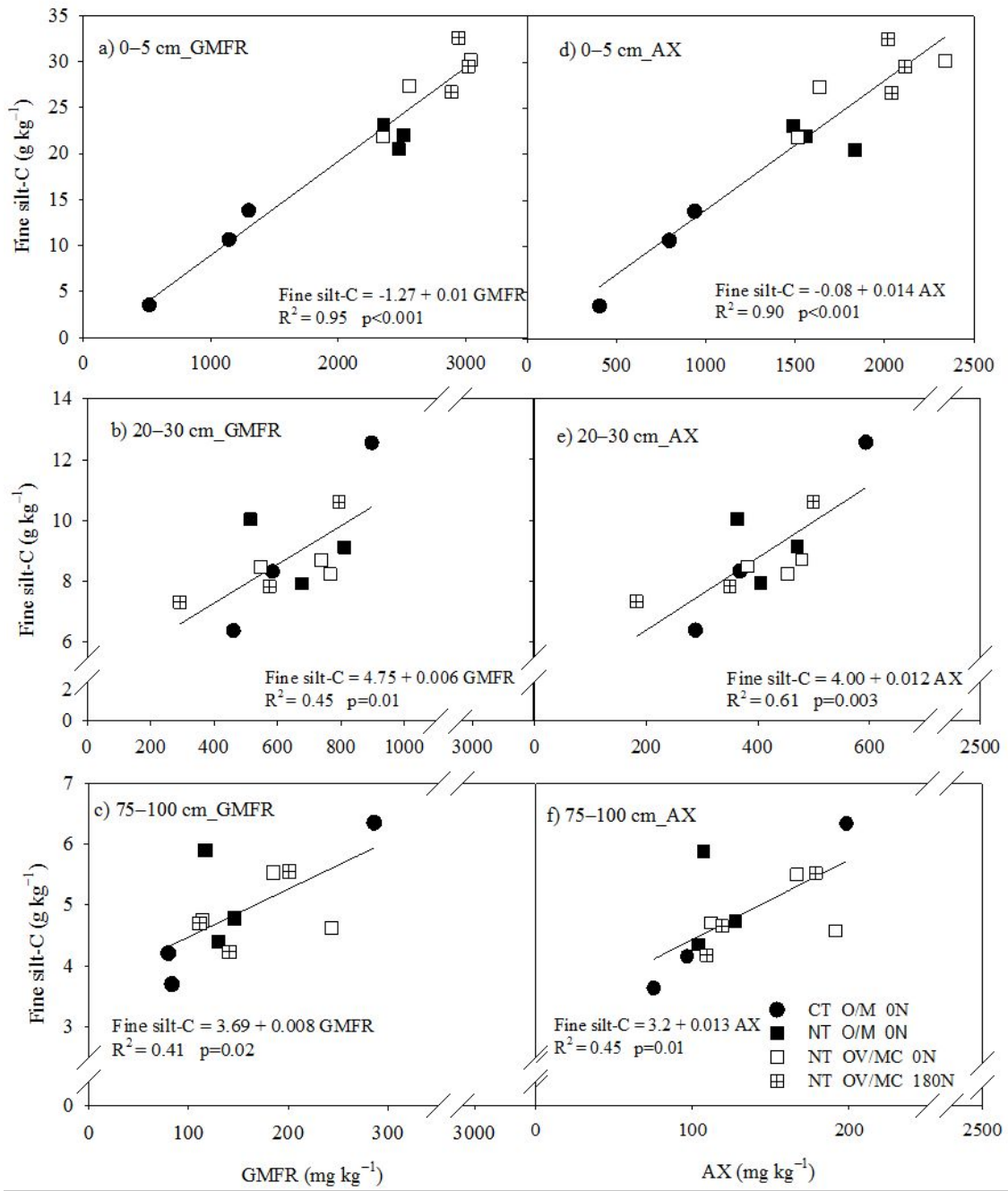
15 **Figure 3.** Regression of GMFR concentration (a,b,c) and AX concentration (d,e,f) as function of  
16 carbon concentration in clay fraction in 0–5, 20–30 and 75–100 cm soil layer the from Acrisol  
17 submitted to conventional tillage (CT) and no tillage (NT) with oat/maize (O/M) and  
18 oat+vetch/maize+cowpea (OV/MC); and two nitrogen fertilization rates, 0 e 180 kg of urea ha<sup>-1</sup>  
19 (0N and 180N).



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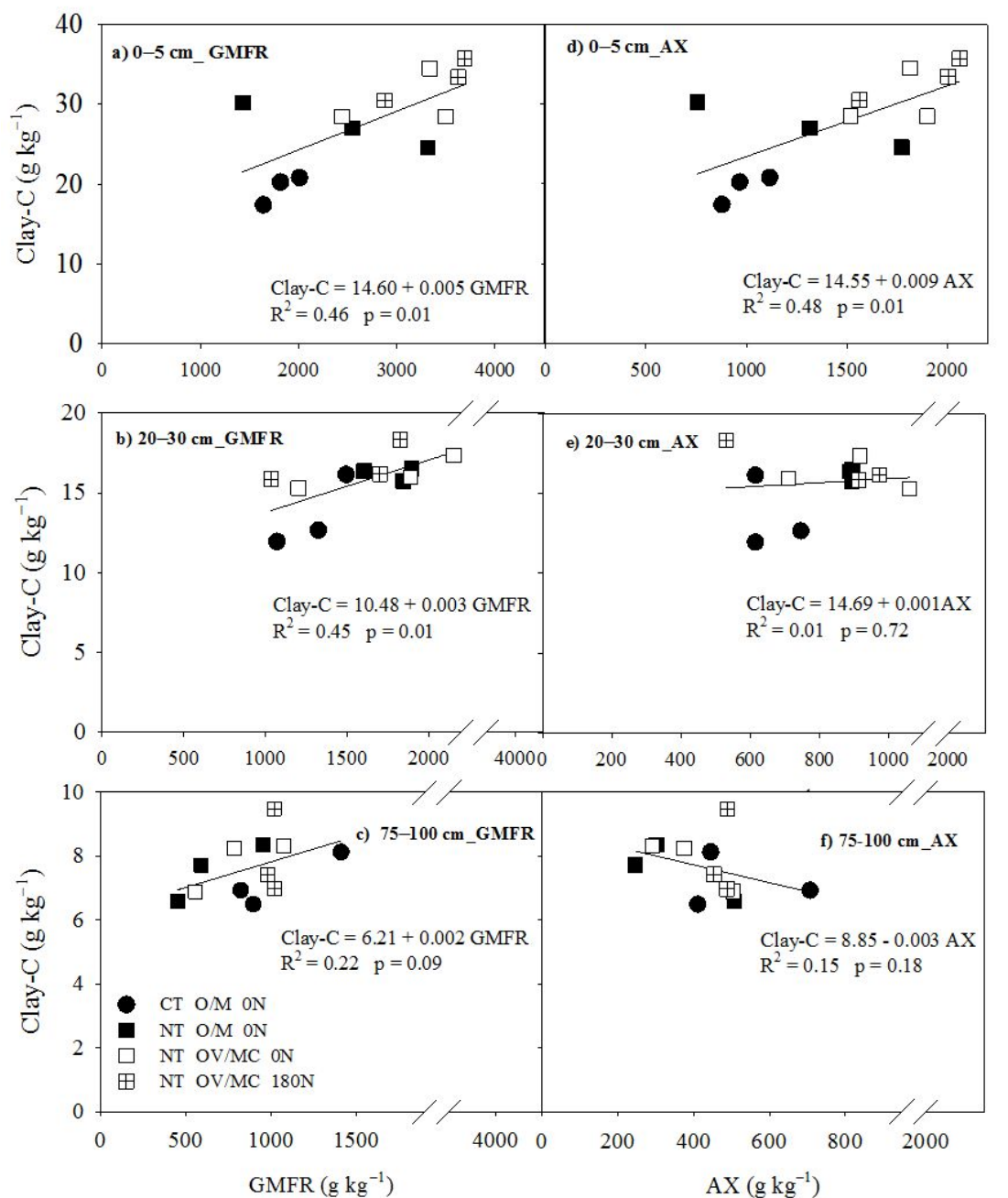
23 **Figure 1.**



25

26

27 **Figure 2.**



**Figure 3.**



**Table 1.** Total plant- (AX) and microbe-derived (GMRF) carbohydrates and GMFR/AX ratio in whole soil (WS) and clay- and fine silt-sized fractions in 0–5, 20–30 and 75–100 cm layers of an Acrisol submitted to conventional tillage (CT) and no tillage (NT), with oat/maize (O/M) or oat+vetch/maize+cowpea (OV/MC) succession, and with (180 kg of urea ha<sup>-1</sup> [180N]) or without (0N) fertilization.

Tillage	Crop succession	Fertilization	AX‡			GMFR‡			GMRF/AX ratio		
			WS	Clay	FS	WS	Clay	FS	WS	Clay	FS
----- mg kg <sup>-1</sup> -----											
<b>0 – 5 cm</b>											
CT	O/M	0	657.6	988.8	709.6	880.1	1813.2	981.6	1.39	1.83	1.38
NT	O/M	0	1015.4	1283.3	1628.1	1156.5	2443.2	2445.3	1.13	1.90	1.50
	OV/MC	0	1541.2	1744.1	1833.8	1506.5	3100.4	2655.0	0.98	1.78	1.45
	OV/MC	180	2096.0	1875.7	2059.0	1880.5	3398.2	2949.9	0.90	1.81	1.43
Contrast†	No-tillage		0.09	0.006	<0.001	0.10	0.002	<0.001	0.14	0.19	0.08
	Legumes		0.04	<0.001	0.01	0.05	0.001	0.07	0.34	0.04	0.37
	Fertilization		0.03	0.03	0.007	0.04	0.04	0.02	0.63	0.49	0.82
<b>20 – 30 cm</b>											
CT	O/M	0	421.0	690.9	416.4	1051.6	1317.6	647.4	2.44	1.92	1.52
NT	O/M	0	473.2	891.7	413.1	909.6	1686.7	667.8	1.93	1.89	1.60
	OV/MC	0	506.0	917.2	438.0	1184.9	1845.4	683.1	2.33	2.05	1.55
	OV/MC	180	491.2	805.9	347.1	972.4	1551.7	553.6	1.98	2.02	1.54
Contrast	No-tillage		0.42	0.19	0.92	0.54	0.02	0.91	0.12	0.51	0.52
	Legumes		0.59	0.86	0.70	0.25	0.22	0.93	0.14	0.20	0.85
	Fertilization		0.81	0.44	0.19	0.37	0.05	0.49	0.19	0.90	0.94
<b>75 – 100 cm</b>											
CT	O/M	0	282.6	393.4	125.2	704.7	823.9	149.6	2.65	2.17	1.11
NT	O/M	0	262.3	351.6	112.7	575.0	666.5	130.9	2.28	2.01	1.16
	OV/MC	0	303.4	484.1	159.7	767.4	1034.3	180.7	2.85	2.17	1.12
	OV/MC	180	369.5	476.9	138.6	853.9	1008.6	150.6	2.49	2.12	1.09
Contrast	No-tillage		0.72	0.43	0.48	0.01	0.001	0.75	0.38	0.23	0.87
	Legumes		0.47	0.03	0.02	0.001	<0.001	0.41	0.20	0.24	0.91
	Fertilization		0.26	0.89	0.24	0.03	0.50	0.61	0.41	0.58	0.90

† Contrast analysis was used to test the effects of no-tillage (CT O/M 0 vs. NT O/M 0), legume cover cropping (NT O/M 0 vs. NT OV/MC 0) and nitrogen fertilization (NT OV/MC 0 vs. NT OV/MC 180) with their respective *P* value.

‡ AX, sum of arabinose and xylose concentrations; GMFR, sum of galactose, mannose, fucose and rhamnose concentrations.