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1 **High carbon storage in a previously degraded subtropical soil under no-tillage with**
2 **legume cover crops**

3

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17

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27 **High carbon storage in a previously degraded subtropical soil under no-tillage with**
28 **legume cover crops**

29 **Abstract**

30 The effect of no-tillage (NT) on soil organic carbon (SOC) storage may help Brazil meet its
31 37% greenhouse gas emissions reduction target by 2025. When combined with legume cover
32 crops, NT could result in even greater SOC storage than NT alone. The objective of this study
33 was to evaluate the SOC storage potential of NT and the contribution of legume cover crops
34 and nitrogen (N) fertilization to this potential in both the **surface** and **subsurface** soil layers of
35 a previously degraded subtropical Acrisol of Southern Brazil. Using a split-plot design, the
36 long-term field experiment compared the effect of NT and conventional tillage (CT), with or
37 without legume cover crops, and with or without mineral N fertilization. Thirty years of
38 contrasting management systems resulted in large differences (up to 35 Mg ha⁻¹) in SOC
39 stocks in the whole soil profile (0–100 cm). The combination that provided the greatest
40 increase in SOC was NT combined with two legume cover crops and N fertilization (1.15 Mg
41 ha⁻¹ year⁻¹ compared to CT, with no N fertilization or legume cover crop). Legume cover
42 crops were twice as efficient in storing SOC as N fertilization, with 1 kg of residue input
43 being converted to 0.15 kg of SOC. Overall, the variation in SOC stocks was explained
44 largely by plant carbon input ($R^2=80\%$) which varied with N fertilization and cropping
45 system. About half of the SOC storage that occurred in this 30-year-old NT system was
46 attributable to the increase in SOC stocks in the **subsurface** layer (30–100 cm), which was
47 confirmed by the contribution of C₃ cover crop residues using carbon isotope signature (from
48 14.8 to ~17.5‰ in the 75–100 cm layer). Thus, the legume cover crop made a strong
49 contribution to the potential of SOC storage in NT, and high rates of C storage occurred over
50 a longer period in **subsurface** soil layers than previously believed.

51

52 **Keywords:** Carbon sequestration, soil depth, conservation agriculture, cropping system,
53 climate change mitigation.

54

55 **1. Introduction**

56 The Paris climate agreement is aimed at holding global warming to below 2 °C by
57 2050 and to pursue efforts to limit it to 1.5 °C (Rogelj et al., 2016). Brazil made a voluntary
58 commitment to reduce greenhouse gas (GHG) emissions by 37% by 2025 and established a
59 climate plan, which encompasses a low carbon agriculture plan which includes NT farming as
60 one of five thrusts for mitigation of GHG emissions (Brazil Ministry of the Environment,
61 2015).

62 In the last century, Brazilian agriculture has revolved around conventional tillage
63 practices that have led to severe soil degradation problems including water erosion and the
64 loss of soil quality (Mielniczuk et al., 2003). In this context, the no-tillage (NT) system
65 emerged as a basis for conservation and sustainable agriculture (Paustian et al., 1997; Bayer et
66 al., 2000; Lal et al., 2007), with strong impact on chemical, physical and biological soil
67 quality (Mielniczuk et al., 2003). Studies conducted in tropical and subtropical environments
68 (Bayer et al., 2006) have highlighted mean annual SOC storage rates ranging from 0.35 to
69 0.48 Mg ha⁻¹yr⁻¹ when conventional tillage systems are converted into NT systems.

70 The effect of NT on SOC storage is also dependent on the amount and diversity of the
71 crops grown (Diekow et al., 2005; Martins et al., 2012; Raphael et al., 2016). Legume cover
72 crops play an essential role in SOC storage under NT, either through the biomass inputs
73 (shoot and root) associated with these plant species or through the symbiotically fixed N
74 which becomes available and increases the grain and biomass production of cash crops grown
75 in succession (Amado et al., 2006). The higher quality (N content and soluble fractions) of the
76 biomass of legume cover crops, whether cultivated alone or intercropped with other species,
77 may improve the efficiency of the microorganisms to accumulate C in the soil (Cotrufo,
78 2013). However, there is no consensus on the effect of nitrogen fertilization on soil SOC
79 stocks because, in spite of the positive effect it has on plant biomass addition and consequent

80 C input (Mack et al., 2004, Kirchmann et al., 2013), inorganic N can also cause accelerated
81 mineralization (Khan et al., 2007) although this conclusion is not universally accepted (e.g.,
82 Polwson et al., 2010).

83 Soil depth should be considered carefully in evaluating SOC storage under NT. In
84 temperate soils of North America and Europe, the gain of SOC in surface layer under NT may
85 be compensated by gains in subsurface layers under CT (Angers et al., 1997; Baker et al.,
86 2007; Blanco-Canqui et al., 2011; Dimassi et al., 2014). By contrast, some studies carried out
87 in tropical and subtropical soils in Brazil have shown that sampling of the surface soil can
88 lead to underestimation of the potential of SOC storage under NT (Boddey et al, 2010;
89 Albuquerque et al., 2015). In those studies, sampling of the 0–100 cm layer resulted in SOC
90 storage rates that were 59% (Boddey et al., 2010) and 100% (Albuquerque et al., 2015) higher
91 than for soil sampling done at a depth of 0–30 cm. The storage of C in subsurface layers
92 under NT may be significant in Brazilian tropical and subtropical soils, especially in the case
93 of cropping and rotation systems that incorporate legume cover crops (Boddey et al., 2010).
94 The high volume of rainfall may favor the percolation of organic compounds and contribute
95 to the potential for SOC storage in subsurface layers of tropical soils under conservation
96 management systems (Hobley et al., 2016). In addition, free-draining soils (Miranda et al.,
97 2016) with Bt horizons (Torres-Sallan et al., 2017) and functional groups on the surface of
98 iron and aluminum oxides that interact strongly and stabilize organic matter (Lawrence et al.,
99 2015) point to considerable potential for SOC storage in subsurface layers.

100 We hypothesized that over the long term, legume cover crops would make a strong
101 contribution to the potential of SOC storage under NT, and that C storage could occur at high
102 rates in subsurface layers of subtropical soils. Our objective was thus to evaluate the potential
103 that NT offers for SOC storage as well as the contribution of legume cover crops and N

104 fertilization to this potential in both **surface** and **subsurface** layers of a previously degraded
105 subtropical Acrisol.

106

107 **2. Materials and methods**

108 2.1. Description of the experiment

109 The study was conducted in a long-term experiment (30 years) at the Agronomic
110 Experimental Station of the Federal University of Rio Grande do Sul in the municipality of
111 Eldorado do Sul–RS (30°06'S, 51°40'W, elevation 96 m). The climate is subtropical (Cfa
112 according to the Köppen classification), with a mean temperature of 19.4 °C and annual
113 rainfall of 1440 mm. The soil was classified as a sandy clay loam granite-derived Acrisol
114 (FAO, 2002), with a loamy clay texture in the **surface** layer. The clay content in the soil
115 profile increases from 217 g kg⁻¹ in the 0–5 cm layer to 394 g kg⁻¹ in the 20–30 cm layer,
116 reaching 511 g kg⁻¹ in the 75–100 cm layer. The main minerals in the clay fraction are
117 kaolinite (720 g kg⁻¹) and iron oxides (109 g kg⁻¹) (Bayer et al., 2001).

118 Prior to this experiment, the field was a natural grassland (mainly *Paspalum* spp. and
119 *Andropogon* spp.), which was converted to cropland in 1969 and cultivated for 16 years by
120 using conventional tillage practices based on plowing and disking twice a year for winter and
121 summer annual crops with straw removal. When the experiment was started in 1985, the soil
122 showed serious physical degradation and water erosion (Bayer et al., 2000).

123 The experiment included two soil tillage systems (CT and NT) arranged in main plots
124 of 15 × 20 m. Each tillage system was composed of three cropping systems in subplots of 5 ×
125 20 m: black oats (*Avena strigosa* Schreb)/maize (*Zea mays* L.) (O/M), vetch (*Vicia sativa*
126 L.)/maize (V/M) and oats + vetch/maize + cowpea (*Vigna unguiculata* (L.) Wald) (OV/MC).
127 These combined tillages and cropping systems were managed with two levels of fertilization,
128 0 and 180 kg ha⁻¹ of N-urea (0 N and 180 N), applied in strips in the maize crop only,

129 consisting the sub-subplots (5×10 m). The experimental design consisted of randomized
130 blocks with split-split plots and three replicates.

131 Winter crops, managed as cover crops, were established in April–May of each year
132 using direct drilling in both CT and NT treatments. Oats, when grown alone, were seeded at a
133 rate of 80 kg ha^{-1} . When oats were grown with vetch, oats were seeded at 30 kg ha^{-1} , and
134 vetch at 50 kg ha^{-1} . For vetch cultivated alone, 80 kg ha^{-1} was used. In the OV/MC system,
135 cowpea was sown 15 to 20 days after the maize, between the lines of this crop which were 40
136 cm apart.

137 The CT plots were ploughed to a furrow-depth of 17 cm once a year in spring before
138 maize sowing by using a three-disk plough and harrowed twice to a depth of 10 cm by using a
139 disk harrow resulting in the incorporation of the crop residues in this layer. At the same time,
140 glyphosate-based herbicide (Roundup, Monsanto) was applied in the NT plots at a 1.4 kg ha^{-1}
141 rate relative to the final glyphosate concentration, and 2–3 days later the winter cover crops
142 were managed with a knife-roller and aboveground residues left on the soil surface. In NT,
143 soil disturbance occurred only in the sowing line and the residues of the cover crop were left
144 on the soil surface.

145 Maize was planted in September–October, with between-row spacing of 90 cm and a
146 sowing rate designed to obtain 50–70 thousand plants per hectare. The fertilizer rate applied
147 in maize was 21.5 and 41.5 kg ha^{-1} of P and K (50 and 50 kg ha^{-1} of P_2O_5 and K_2O),
148 respectively.

149 The mean annual C input (aboveground and root, with roots being assumed to account
150 for 30% of the aboveground portion) was calculated from cover crop data and the dry-matter
151 maize yields compiled by Zanatta et al. (2007) for the period 1985–2006, which were
152 subsequently updated to 2014 (Fig. 1). The annual aboveground maize dry matter yield was
153 estimated from grain yield, and aboveground C input calculated by assuming dry matter in

154 maize and the cover crops to contain 40% C. The values of CT and NT were averaged due to
155 similar C input in both tillage systems.

156

157 2.2. Soil bulk density and SOC concentrations and stocks

158 Soil samples from eight layers (0–5, 5–10, 10–15, 15–20, 20–30, 30–50, 50–75 and
159 75–100 cm) were collected in September 2014 prior to soil tillage for maize crop
160 establishment. Trenches were excavated with a backhoe to allow measurement of soil bulk
161 density in duplicate by the volumetric ring method (Blake and Hartge, 1986) only in sub-
162 subplots with no nitrogen fertilization, assuming that nitrogen fertilization did not modify soil
163 density.

164 Soil samples for determining SOC concentration were obtained from the 0 and 180 N
165 sub-subplots, using the same layers as for bulk density samples. Two sub-samples per plot
166 were collected with a spiral auger ($\Phi = 25$ cm) that were air dried and ground to ≤ 2 mm in a
167 Marconi 330 grinder, a subsample of ~ 2 g being further ground to ≤ 250 μm in an agate
168 mortar to determine SOC by dry combustion in a FlashEA 1112 instrument from Thermo
169 Electron Corp. (Milan, Italy).

170 SOC stocks down to depths of 30 and 100 cm were calculated using the equivalent soil
171 mass approach (Ellert and Bettany, 1995), which considers equal masses of soil between
172 treatments. The soil under CT O/M 0N was used as the reference for the equivalent mass. The
173 annual SOC storage rate was calculated as the difference between the SOC stocks of the
174 treatments and the reference system, divided by the time elapsed since the implementation of
175 the experiment, i.e. 30 years.

176 To assess the contribution of the cover crops to the SOC in the soil profile, isotopic
177 abundance (^{13}C) was determined for samples from the contrasting treatments in the sub-
178 subplots with no nitrogen fertilization: CT O/M, CT OV/MC, NT O/M and NT OV/MC. The

179 isotopic abundance values for the 0–5, 20–30 and 75–100 cm soil layers were determined
180 using an elemental analyzer (FlashEA 2000, Thermo Fisher Scientific, Bremen, Germany)
181 coupled to an isotope ratio mass spectrometer (IRMS) (Delta Advantage, Thermo Fisher
182 Scientific, Bremen, Germany).

183 The conversion rates of C input into SOC at 0–100 cm depth were calculated as the
184 ratio between the additional SOC stock at 0–100 cm depth and C input derived from legume
185 cover crops and N fertilization. For legume cover crops, the difference between SOC stocks
186 of legume cover crops treatments (V/M and OV/MC) and O/M was divided by the difference
187 of C input between the same treatments inside of each tillage system (NT or CT) for the 0 N
188 treatment. Values for V/M and OV/MC were then averaged. For N fertilization, the difference
189 between SOC stocks of 180 N and 0 N was divided by the difference of C input between the
190 same treatments inside of each tillage system (NT or CT) for the O/M system, only.

191

192 2.3. Historical evaluation of SOC stocks and storage rates

193 We compared our results obtained in 2014 with those obtained in five previous studies
194 conducted since the beginning of the field experiment to build a historical assessment of SOC
195 evolution, specifically for the 0–20 cm layer, and for the 0N treatment, which was the
196 sampling depth and N level common to all studies. Previous SOC measurements were carried
197 out in 1985 (Medeiros, 1988), in 1990 (Bayer and Mielniczuk, 1997), in 1994 (Bayer et al.,
198 2000), in 1998 (Lovato et al., 2004), and in 2003 (Zanatta et al., 2007). The methodological
199 approach for all these sampling dates was similar to that used in 2014, with the exception of
200 the analytical C method. Therefore, SOC data from previous studies using the Walkley-Black
201 analytical method (Nelson and Sommers, 1996) were corrected by a factor of 0.9422, which is
202 the slope of the linear equation obtained by fitting the data provided by the two analytical
203 methods (Walkley–Black and dry combustion) for more than 100 samples spanning a wide

204 range of SOC contents (Zanatta et al., 2007). The SOC dataset for previous years was also
205 recalculated from the original values using the equivalent soil mass method.

206

207 2.4. Statistical analyses

208 After normality was verified by means of the Kolmogorov-Smirnov test and
209 homogeneity of variance by the Levene test, the SOC data were subjected to analysis of
210 variance (ANOVA) and, significant results ($p < 0.05$) were compared using the Tukey test (p
211 < 0.05). The MIXED procedure was used to compare the effects of tillage methods (T), crop
212 systems (CC), nitrogen fertilization (N) and soil layers (L) on the response variables. This
213 procedure considers the main factors and their interactions as fixed factors and the block
214 variable and the experimental errors as random variables. Because the estimation of annual C
215 input was based on evaluations performed on most of years but not always for all treatments,
216 and in addition to the fact that C input from maize to have been indirectly estimated from
217 grain yield, the C input data was not statistically analyzed.

218 All analyses were performed using the SAS statistical package® v.9.4 (Statistical
219 Analysis System Institute, Cary, North Carolina). The statistical model used in the analysis of
220 variance to evaluate C concentration was as follows:

$$221 Y_{ijklm} = \mu + B_i + T_j + \text{Error}_{(ij)} + C_k + T_j C_k + \text{Error}_{(ijk)} + N_l + T_j N_l + C_k N_l + T_j C_k N_l + \text{Error}_{(ijkl)} + \\ 222 L_m + \text{Error}_{(im)} + T_j L_m + C_k L_m + N_l L_m + T_j C_k L_m + T_j N_l L_m + C_k N_l L_m + T_j C_k N_l L_m + \text{Error}_{(ijklm)}.$$

223 Where μ = general mean of the experiment; B = block ($i = 1, 2, 3$); T = tillage systems ($j = 1,$
224 2); C = cropping system ($k = 1, 2, 3$); N = nitrogen fertilization ($l = 1, 2$); L = soil layers ($m =$
225 $1, 2, 3, 4, 5, 6, 7, 8$), and Error = experimental error. To assess the SOC stocks and storage
226 rates, the variable soil layers and its associated errors were removed from the statistical
227 model.

228 Regression analyses were performed (SigmaPlot for Windows v. 12.0, Systat
229 Software, Inc., San Jose, CA) to explore the relationships between SOC stocks and C inputs
230 and the relationship between SOC storage rates in the 0–30 cm and 0–100 cm soil layers.

231 3. Results

232 3.1. Annual average of carbon input data

233 The inclusion of legume cover crops and nitrogen fertilization caused an
234 approximately twofold increase in the annual C input, which amounted to 4.98 and 10.0 Mg
235 $\text{ha}^{-1} \text{ year}^{-1}$ in the O/M 0 N and OV/MC 180 N systems (Fig. 1), respectively.

236 In systems where nitrogen fertilizer was applied to the maize crop, the impact of
237 legume cover crops on C input was smaller because C inputs from maize were similar for the
238 three cropping systems. In all cropping systems, maize made a significant contribution in
239 terms of C input, representing between 42 and 67% of the total C input (Fig. 1).

240

241 3.2. C storage in the surface soil layer over time

242 Thirty years of contrasting management systems resulted in a large difference in SOC
243 stocks in the 0–20 cm layer (Fig. 2a) of this previously degraded subtropical soil. From 1985
244 to 2014, in systems with no nitrogen fertilization, the conventional management system (CT
245 O/M) resulted in a decrease of 3.8 Mg ha^{-1} in SOC stocks of the 0–20 cm layer, with a greater
246 reduction in the first 5 years (Fig. 2a). In contrast, all systems with legume cover crops
247 showed a positive SOC balance over the 30 years. In NT, the OV/MC system increased SOC
248 stocks by 5.6 Mg ha^{-1} to 41.8 Mg ha^{-1} (Fig. 2a).

249 The SOC storage rate for the 20-cm soil layer reached its maximum level between five
250 and nine years after the experiment began, reaching 1 Mg $\text{ha}^{-1} \text{ year}^{-1}$ in the NT OV/MC
251 system (Fig 2b). After this peak in SOC storage, the rates decreased exponentially over time.
252 However, it is noteworthy that even after 30 years, all systems continued to accumulate SOC

253 in the 0–20 cm soil layer, at rates ranging from 0.05 to 0.32 Mg ha⁻¹ year⁻¹ according to the
254 tillage and cropping systems.

255

256 3.3. Whole profile (0–100 cm) soil C stocks after 30 years

257 3.3.1. Tillage systems

258 The increase in SOC under NT occurred mainly in the **surface** soil layer (0–5 cm) and
259 is also reflected in the higher SOC stocks observed in the 0–30 cm layer when compared to
260 CT (Table 1). SOC stocks between tillage systems ranged from 47.9 to 63.3 Mg ha⁻¹ in the
261 0–30 cm layer, and from 119.6 to 154.2 Mg ha⁻¹ in the 0–100 cm layer (Table 1). The largest
262 differences in the 0–100 cm layer between CT and NT were 13 and 11 Mg ha⁻¹ in the O/M
263 180 N and OV/MC 180 N systems, respectively, which led to significant increments of 0.42
264 and 0.36 Mg ha⁻¹ year⁻¹ in SOC storage rates **relative to the reference** (CT, O/M, ON, Table
265 2).

266

267 3.3.2. Cropping systems

268 The use of two legume cover crops (one in winter and the other in summer - OV/MC)
269 under NT increased SOC concentrations in the 0–10 cm layer by 39% compared to exclusive
270 grass-type crops (O/M), and this increase explained the difference in SOC stocks in the 0–30
271 cm layer (Table 1). In the 0–100 cm layer, OV/MC increased the SOC stocks by an average
272 of 14 Mg C ha⁻¹ relative to O/M (Table 1). When considering 180 N, and on average for CT
273 180 N and NT, the SOC storage rate was 0.38 Mg ha⁻¹ year⁻¹ greater under OV/MC than
274 under O/M for the 0–100 cm layer (Table 2).

275 The $\delta^{13}\text{C}/^{12}\text{C}$ of the soil under natural vegetation varied between -15.9 and -14.4‰
276 owing to the predominance of C4 plants. A decrease (more negative values) in the carbon
277 isotope signature was observed due to the contribution of C3 plants (oat, vetch and cowpea) to

278 SOC during the 30 years of experiment in all treatments (Table 3). The $\delta^{13}\text{C}/^{12}\text{C}$ values were
279 more negative under OV/MC than under O/M in the 0–5 and 75–100 cm soil layers.

280

281 3.3.3. Nitrogen fertilization

282 Nitrogen fertilization with urea at a rate of 180 kg ha^{-1} led to an average increase of
283 16% in SOC concentration in the first 10 cm of soil compared to unfertilized treatments
284 (Table 1). This significant difference in SOC in the **surface** layer translated into a significant
285 effect of fertilization on SOC stocks in both the 0–30 and 0–100-cm soil layers (Table 1). For
286 instance, in CT O/M, SOC stocks increased from $119.6 \text{ Mg C ha}^{-1}$ to $131.1 \text{ Mg C ha}^{-1}$ (Table
287 1) due to addition of 180 kg ha^{-1} of N-urea, leading to a SOC storage rate of 0.38 Mg ha^{-1}
288 year^{-1} (Table 2).

289 For both the 0–30 and 0–100 cm soil layers, annual C inputs explained between 68%
290 and 85% of the SOC stock variation under NT and CT (Fig. 3a and b). The SOC storage rate
291 was 62% higher when considering the whole soil profile (0–100 cm layer) compared to the
292 0–30 cm layer only (Fig. 4). Thirty years of NT and OV/MC with 180 kg ha^{-1} of N-urea
293 resulted in a SOC storage rate that was $1.15 \text{ Mg ha}^{-1} \text{ year}^{-1}$ higher in the 0–100 cm depth
294 compared to CT and O/M without nitrogen fertilization (Fig. 5). A marked effect on SOC was
295 observed in the **surface** layers but also throughout the soil profile down to a depth of 100 cm.

296 On average, the **additional** SOC storage attributable to the use of legume cover crops
297 was almost twice that induced by nitrogen fertilization (Fig. 6). In other words, each kg of C
298 input induced by the use of legume cover crops resulted in an average conversion rate of 0.15
299 kg of SOC per Kg of C input (range of 0.08–0.39), compared to only 0.08 kg SOC per kg C
300 input (range of 0.01–0.19) for nitrogen fertilization (Fig. 6).

301

302 4. Discussion

303 Thirty years of contrasting management systems resulted in large differences (up to 35
304 Mg ha^{-1}) in SOC stocks in the whole soil profile (0–100 cm) (Table 1) of this previously
305 degraded subtropical Acrisol. As observed in previous studies (Boddey et al., 2010;
306 Albuquerque et al., 2015; Miranda et al., 2016), the use of NT leads to significant SOC
307 storage in the Southern Brazil environment, both in the surface and subsurface soil layers. In
308 our study the tillage effect was not attributable to differences in C inputs as crop yields were
309 similar between the two tillage treatments. However, within each tillage system,
310 approximately 80% of the variation in SOC stocks was explained by C inputs as affected by
311 cropping systems and N fertilization (Fig. 3a and 3b). Cropping systems which included
312 legume cover crops increased SOC storage with the highest potential recorded when two
313 legumes (OV/MC) were cultivated. When averaged over the whole experimental period, vetch
314 and vetch+cowpea legume residues added 82 and 115 $\text{Kg N ha}^{-1}\text{yr}^{-1}$, respectively, which
315 improved grain yield and biomass production of maize in the crop sequence (Lovato et al.,
316 2004; Amado et al., 2006). In addition to favoring the supply of N and increasing C inputs
317 from maize or a subsequent crop, legume cover crops also added C from their own residues
318 (Lovato et al., 2004; Amado et al., 2006), which includes inputs from roots that likely affect
319 the SOC in deeper soil layers. Nitrogen fertilization (at a rate of 180 kg ha^{-1}) also had a
320 positive effect on SOC stocks due to the larger amount of residue (shoot + root) added by the
321 plants.

322 The SOC storage per unit of C input induced by legume cover crops was on average
323 about double of that induced by N fertilization (Fig. 6) which suggests a possible effect of
324 residue quality. Compared to grass species, the greater lability of the legume cover crop
325 residues may contribute more microbial residues, which are stabilized by chemical bonding
326 with the mineral soil matrix (Cotrufo et al., 2013, 2015). This difference could also be

327 attributable to mineral N fertilization inducing accelerated C mineralization (Khan et al.,
328 2007), although this mechanism is considered controversial (Powelson et al., 2010).

329 Considering only the surface soil layer (0–20 cm Fig. 2b), the pattern of high initial
330 rates of SOC storage (first 5–9 years) and the subsequent decline would at first have
331 suggested that the adoption of conservation management systems in a previously degraded
332 soil could be seen as a limited short-term strategy for removing CO₂ from the atmosphere.
333 However, more than half of the SOC storage after 30 years was attributable to the increase in
334 C stocks in the subsurface layer (30–100 cm) (Fig. 4). Based on the carbon isotope signature,
335 C storage in the deeper layers can be at least partly ascribed to the cover crop residues (shoot
336 and root). Indeed, the more negative values in the 75–100 cm soil layer (Table 3) of the
337 OV/MC system relative to O/M are due to the greater contribution of residues of C3 plants
338 (oats, vetch and cowpea) which favored SOC accumulation.

339 Our results showing substantial additional SOC storage in deep soil layers (below 30
340 cm) induced by conservation management agree with the findings of others studies conducted
341 in tropical (Miranda et al., 2016) and subtropical (Boddey et al., 2010; Albuquerque et al.,
342 2015) regions of Brazil. However, they contrast with the results of studies conducted in
343 temperate regions (Baker et al., 2007; Dimassi et al., 2014). This difference between regions
344 can be due to various factors, like differences in residue inputs (shoot and root), rainfall
345 patterns, and soil type. In European regions with rainfall levels varying between 350 and 800
346 mm year⁻¹, Dimassi et al. (2014) found a negative relationship between the increase in SOC
347 stocks in NT vs. CT and annual precipitation. This precipitation level is well below the mean
348 rainfall of the region where the present study was carried out (1440 mm year⁻¹). This higher
349 rainfall, taken together with the more labile material left on the NT surface, may favor the
350 percolation of dissolved organic carbon (DOC) into the soil profile (Sanderman et al., 2008;
351 Kaiser and Kalbitz, 2012).

352 In addition, the lighter texture of the soil in the **surface** layer may have promoted
353 rainwater percolation and, consequently, DOC translocation into the soil profile (Wang et al.,
354 2015). C sorption on minerals may be more efficient in the **subsurface** than in **surface** layers
355 because the greater C saturation deficit (Castellano et al., 2015). This also shows the
356 importance of cropping systems in terms of providing residues potentially contributing to
357 COD supply and resulting in their accumulation in deeper soil layers, especially in soils
358 whose clay content increases deeper in the soil profile (Torres-Sallan et al., 2017).
359 Furthermore, the oxide mineralogy of Acrisol is conducive to strong interactions with organic
360 matter, leading to C stabilization (Denef et al., 2004; Boddey et al., 2010).

361 Our results show that, under the humid subtropical climate conditions of southern
362 Brazil, a high potential exists for the storage of SOC along the soil profile. **Considering the**
363 **0–100 cm soil profile and compared to our reference system (CT, O/M, ON), 30 years of**
364 **alternative management systems resulted in high SOC storage rates (range from 0.38 to 1.15**
365 **Mg C ha⁻¹ year⁻¹, Table 2).** Thus, our results support the idea that soil carbon accounting
366 systems should consider soil layers deeper than 30 cm to fully account for management-
367 induced changes.

368 The potential effect of NT associated with legume crops on SOC storage is an avenue
369 that Brazil could pursue to meet its GHG emissions reduction target. At the Paris Convention
370 of the Parties (COP21) in December 2015, the French government proposed the “4 per 1000”
371 initiative “Soils for Food Security and Climate” (<https://www.4p1000.org/>). Considering
372 Brazil’s mean SOC stocks of 45 Mg C ha⁻¹ **in the 0–30 cm soil layer** (Fidalgo et al., 2007), its
373 target under the 4 per mille initiative would correspond to a SOC storage rate of 0.18 Mg C
374 ha⁻¹ year⁻¹. This target has been shown to be attainable with the adoption of conservation
375 management systems **compared to more conventional systems**, such as the adoption of two
376 legume cover crops in NT in our study (0.76 Mg C ha⁻¹ year⁻¹).

377 The proposed conversion of 8 million hectares from CT to NT over the next few years
378 in Brazil represents potential soil C storage of about 8 Tg C year⁻¹ (MAPA, 2012). Half of the
379 area under NT in Brazil (16 Mha) is currently devoted to monocultures. Since the adoption of
380 more diversified cropping systems could provide additional storage of 0.35 Mg C ha⁻¹ year⁻¹
381 (difference in SOC storage rate between NT O/M 180 N and NT OV/MC 180 N), the
382 resulting improvement in the management system could result in an additional SOC storage
383 of 5.6 Tg C year⁻¹. With the conversion of cropping areas to NT and the adoption of legume
384 cover crops under NT, a total soil carbon sequestration capacity of 50 Tg CO₂ year⁻¹ could
385 technically be attained in Brazil. Thus, if we consider improved soil management systems,
386 **relative to more traditional systems, in Brazil alone**, this would mean off-setting total CO₂
387 emissions by 11% [50 Tg CO₂ yr⁻¹ divided by 466 Tg CO₂ yr⁻¹ released from Brazilian
388 agriculture activities according to Lapola et al. (2014)], and hence contribute significantly to
389 the target set for Brazil 2025 (37% reduction by 2025) established at the Paris climate change
390 conference.

391 The net benefits of conservation management systems in terms of atmospheric C
392 removal in soil should be complemented with studies involving a global greenhouse gas
393 balance. In fact, some recent studies in Brazil have shown a limited impact of practices such
394 as no-tillage and legume cover crops on N₂O emissions, the effect of which was surpassed by
395 the resulting SOC storage (Bayer et al., 2016; Piva et al, 2012). However, the contribution of
396 N fertilization to GHG mitigation requires a more thorough assessment owing to its strong
397 impact on N₂O emissions.

398

399 **5. Conclusion**

400 Our study examined the effect of no-tillage, legume cover crops, and nitrogen (N)
401 fertilization from the perspective of potential SOC storage in **surface** and **subsurface** soil layer

402 of a previously degraded subtropical Acrisol. The results indicate that no-tillage with high and
403 diversified residue inputs from legume cover crops is an effective long-term measure for SOC
404 storage and potential mitigation of global warming in tropical and subtropical conditions in
405 comparison with traditionally adopted farming practices in Brazil (conventional tillage
406 without legume cover crops and N fertilization). Although the rate of SOC storage in **surface**
407 layers decreases over time, conservation management systems favored SOC accumulation in
408 **subsurface** layers in the studied subtropical soil, contributing to maintain high SOC storage
409 rates in the system during 30 years.

410

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