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## No-tillage promotes C accumulation in soil and a slight increase in yield stability and profitability of rice in subtropical lowland ecosystems

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**No-tillage promotes C accumulation in soil but has only a slight effect on yield stability and profitability of rice in subtropical lowland ecosystems**

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**SUMMARY TEXT FOR THE TABLE OF CONTENTS**

A long-term (24-years) field experiment was assessed regarding the effect of no-tillage on organic C and rice yield in a subtropical lowland ecosystem in Southern Brazil. Based on our findings, no-tillage promotes C accumulation in subtropical paddy rice soils, but its effect on yield stability and profitability of rice crop is less prominent and only occur in long-term.

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1 **No-tillage promotes C accumulation in soil but has only a slight effect on yield stability and**  
2 **profitability of rice in subtropical lowland ecosystems**

3

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22 **No-tillage promotes C accumulation in soil but has only a slight effect on yield stability and**  
23 **profitability of rice in subtropical lowland ecosystems**

24

25 **Abstract**

26 **CONTEXT** No-tillage effect on soil organic C (SOC) and rice yield in lowland soils is poorly  
27 understood in subtropical ecosystems. **AIMS** We assessed, in a long-term (24-years) field experiment,  
28 the effect of no-tillage (NT) on SOC stocks in labile ( $> 53 \mu\text{m}$ , particulate) and stable ( $< 53 \mu\text{m}$ ,  
29 mineral associated) fractions of soil organic matter and on rice grain yield in comparison to  
30 conventional tillage (CT) and pre-germinated tillage (PG) systems in a lowland Gleysol in Southern  
31 Brazil. **METHOD** Soil from 8 different layers down to 40 cm under each tillage system was sampled  
32 in a field experiment, and additional soil samples were obtained from an adjacent area under native  
33 grassland (NG) as reference for SOC stocks. **KEY RESULTS** While PG and CT systems maintained  
34 similar SOC stocks in comparison to NG soil, NT increased SOC stocks at an annual rate of  $0.41 \text{ Mg}$   
35  $\text{ha}^{-1}$  in relation to the traditional CT soil, mainly in mineral-associated organic matter. Rice grain yield  
36 increased twice in the 24-years, amounting to  $12 \text{ Mg ha}^{-1}$  in the last crop season. Despite the lower  
37 rice yield in NT system than under CT and PG systems in the most crop seasons (mean of 7.7, 8.0 and  
38  $8.5 \text{ Mg ha}^{-1}$  for the whole experimental period, respectively), a slight effects on yield stability and  
39 profitability ( $<10\%$ ) were observed for NT. **CONCLUSIONS AND IMPLICATIONS** Based on our  
40 findings, no-tillage promotes C accumulation in subtropical paddy rice soils, but its effect on yield  
41 stability and profitability of rice crop is less prominent and only occur in long-term.

42

43 **Key words:** soil organic matter, C lability, paddy rice, profitability, southern Brazil.

## 44 **Introduction**

45 Rice (*Oryza sativa* L.) is the staple food for more than one-half of the world's population (Seck et al.,  
46 2012). Approximately 80 million ha of rice crop and 75% of the world's rice production are managed  
47 by flood irrigation at present (Irri, 2019). Irrigated rice in Brazil comes mainly from the subtropical  
48 region in the South. Rio Grande do Sul, which is the southernmost Brazilian state and borders on  
49 Argentina and Uruguay, is a massive rice granary with a cultivated area close to 1 Mha (Conab, 2018).  
50 Rice yield in this state has increased from 4.0 to 7.5 Mg ha<sup>-1</sup> over the last 20 years (Ibge, 2019) as a  
51 result mainly of the use of more productive plant varieties plus improvements in soil and crop  
52 management (Irga, 2018).

53 In Southern Brazil lowlands, rice is usually grown in tilled soil in order to standardize surfaces,  
54 and also to facilitate sowing and irrigation while minimizing competition from weeds (Sosbai, 2018).  
55 The most widely used soil tillage systems for rice cultivation in Southern Brazil are conventional  
56 tillage (CT) and minimum tillage (MT), both of which involve sowing in dry soil. However, pre-  
57 germinated tillage (PG) is traditionally adopted in small farms, in which soil is flooded for almost half  
58 of the year (i.e., about two months more than under CT; Sosbai, 2018).

59 Long-term field studies conducted in American, African and Asian Continents have shown  
60 irrigated rice cultivation in CT soils to have an adverse impact on soil organic carbon (SOC) (Buarach  
61 et al., 2014; Rosa et al., 2011; Zhou et al., 2009) despite the prevailing anoxic environment, where  
62 microbial activity is partially inhibited (Sarahwat, 2005). Because of the well-known impact of SOC  
63 on chemical, physical and biological properties of subtropical soils, a decline in SOC stocks can  
64 adversely affect soil quality and rice yield. The extended soil flooding period in PG may result in  
65 decreased microbial decomposition of soil organic matter (SOM) due to less efficient anaerobic  
66 metabolism (Rinklebe & Longer, 2006). These conditions may help maintain SOM status, but no  
67 scientific evidence from field studies exists as to whether this is indeed the case.

68 One of the current challenges posed by irrigated rice cultivation in the subtropical lowland  
69 ecosystems of Brazil arises from the adoption of no-tillage (NT), which may be an effective practical  
70 strategy for soil organic matter stabilization (Sahrawat, 2005, 2012), with potential benefits on SOC  
71 stocks and lability, which can provide a useful combined indicator of soil performance potential (Blair  
72 et al., 1995; Zanatta et al., 2019). However, some studies in lowland soils have shown frequent

73 flooding to decrease the stability of soil aggregates, thereby reducing physical protection of labile  
74 organic fractions such as particulate organic matter (Nascimento et al., 2009; Rosa et al., 2011) —it  
75 should be borne in mind that C of stable organic matter associated with minerals is the most strongly  
76 affected by tillage in flooded soils (Nascimento et al., 2009).

77 Despite the potential effect of soil quality on crop yields, the response of irrigated rice yield to  
78 NT remains unclear (Huang et al., 2015), with some studies suggesting a favorable effect (Buarach et  
79 al., 2014) and some an adverse influence (Singh et al., 2013; Alan et al., 2014). Rather than rice yield,  
80 the impact of tillage systems should be assessed in terms of between-season stability (Knapp & van  
81 der Heijden, 2018) and profitability (Page et al., 2019).

82 The starting hypothesis for this work was that no-tillage would have a more favorable long-  
83 term impact on SOC stocks and rice yield in subtropical lowland ecosystems than would tillage.  
84 Carbon stabilization in lowland soil under NT occurs mainly by association with minerals and has a  
85 minor effect on particulate SOM. Rather than grain yield, NT increases between-season yield stability  
86 and gross margin in rice crop. The main purpose of this work was to assess the long-term effect of  
87 three tillage systems on SOC stocks and lability, grain yield and its between-season stability, and gross  
88 margins of irrigated rice in a subtropical sandy loam Gleysol in Southern Brazil.

## 90 **Materials and Methods**

### 91 ***Site description, experiment design and conduction***

92 The study was based on a 24-yr old experiment at the Experimental Station of the Rio Grande do Sul  
93 Rice Institute in the municipality of Cachoeirinha (Rio Grande do Sul State, Southern Brazil;  
94 29°57'02" S, 51°05'02" W, 7 m a.s.l.). The climate as per Köppen's classification is Cfa, and the mean  
95 annual temperature and rainfall are 18.4 °C and 1412 mm, respectively. The soil was a sandy loam  
96 Gleysol (WRB/FAO) and had the following properties: clay content = 170 g kg<sup>-1</sup>, organic matter  
97 content = 17 g kg<sup>-1</sup>, p<sub>H2O</sub> = 5.3, available P (Mehlich-1) = 14.3 mg dm<sup>-3</sup> and available K (Mehlich-1)  
98 = 41.8 mg dm<sup>-3</sup>. The original vegetation was native grassland, which was preserved in a small area in  
99 the vicinity of the experiment site.

100 The experiment, established in 1994, involved a monoculture of irrigated rice (*Oryza sativa* L.)  
101 in the spring/summer in succession to a self-growing ryegrass (*Lolium multiflorum* Lam.) as a

102 nonirrigated cover crop in the autumn/winter. The soil was subjected to three tillage systems, namely:  
103 (a) conventional tillage (CT), where the soil was mobilized on a yearly basis through plowing (2  
104 months after rice harvest) and harrowing (first immediately after plowing and then 2 weeks after  
105 ryegrass desiccation) immediately before rice was sown in spring; (b) pre-germinated tillage (PG), by  
106 which soil was disturbed similarly to CT, but smoothing and levelling with a blade were performed  
107 under flooding (water layer of approximately 5 cm) prior to sowing previously germinated seeds; and  
108 (c) no-tillage (NT), with which the soil was not subjected to any tillage operation and the rice was  
109 sowed through the straw layer on the soil surface. The experimental design consisted of complete  
110 randomized blocks with three replications. Each plot was 28 × 40 m and separated from the others by  
111 levees.

112 A glyphosate-based herbicide was applied at 3 L ha<sup>-1</sup> to ryegrass at the flowering stage in early  
113 spring, followed by machine chopping of standing biomass in the three tillage systems. The rice sowing  
114 was performed until the first half of November. With CT and NT, rice was mechanically seeded at a  
115 rate of 125 kg ha<sup>-1</sup> with a seed drill along lines spaced 17.5 cm; also, the soil was irrigated by flooding  
116 when the plants were at developing stage V4 (Counce et al., 2000). With PG, the soil was flooded  
117 about 60 days before seeding and pre-germinated seeds were broadcast after the plots were leveled  
118 and flooded.

119 The rice varieties and rates of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O fertilization for each crop season were identical  
120 for all tillage systems (see Table S1 in Supplementary data).

### 122 ***Biomass and carbon input by rice and ryegrass***

123 An average annual C input was estimated for the whole experimental period as the combined biomass  
124 of ryegrass in winter and rice in summer. The shoot biomass of ryegrass was estimated by averaging  
125 biomass production in 2001, 2002, 2014 and 2015 as evaluated in six sub-samples collected from a  
126 0.5 m<sup>2</sup> area in each plot. The shoot biomass supplied by rice was estimated from rice grain yields,  
127 using an apparent (without roots) harvest index of 0.5 (Fageria et al., 2007). A dry matter contribution  
128 of roots equivalent to 30% of shoot biomass of rice (Holzschuh et al., 2009) and ryegrass (Assmann et  
129 al., 2014) was considered. Also, a mean concentration of 0.45 kg C kg<sup>-1</sup> dry matter of both residues  
130 was used to estimative of C input (Nascimento et al., 2009).



131

132 ***Soil sampling and analysis***

133 The soil profile was sampled at eight different depths (0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–15, 15–20,  
134 20–30 and 30–40 cm) after rice harvest in March 2015 (i.e., 21 years after the experiment was started).

135 An area under native grassland adjacent to the experimental site was also sampled at the same soil  
136 depths and used as reference for natural SOC stocks. A total of 20 soil sub-samples were collected  
137 with a cutting shovel and combined into a single composite sample from each plot that was air dried  
138 and ground to  $\leq 2$  mm in a Marconi 330 grinder. Approximately 20 g of soil was further ground to  $\leq$   
139 0.025 mm in a mortar and analyzed for C by dry combustion in a Fisher Scientific Flash 2000 CN  
140 analyzer.

141 SOC stocks were calculated by using the equivalent soil mass approach (Ellert & Bettany,  
142 1995) with CT soil mass as reference. Soil bulk density (BD, Table S2) was determined by using the  
143 volumetric ring method (Blake & Hartge, 1986) in duplicate at the same soil depths as for SOC.

144 Soil organic matter was subjected to granulometric fractionation in order to quantify the labile  
145 and stable C-pools in soil organic matter. Briefly, 20 g soil samples were shaken horizontally in 80  
146 mL of sodium hexametaphosphate solution ( $5 \text{ g L}^{-1}$ ) for 16 h (Cambardella & Elliott, 1992). The  
147 resulting suspensions were sieved through  $53 \mu\text{m}$  mesh and the retained fraction, which consisted of  
148 particulate SOM (POM, labile fraction), were washed, oven dried at  $50 \text{ }^\circ\text{C}$  and analyzed for C by dry  
149 combustion. The C content of mineral-associated organic matter (min-OM, stable fraction) was  
150 calculated as the difference between SOC and POM-C.

151 The carbon management index (CMI) for the 0–40 cm layer was calculated as originally  
152 proposed by Blair et al. (1995), and POM-C and Min-OM-C were used as proxies for the labile and  
153 stable C pools, respectively (Vieira et al., 2007). CMI was calculated from data for the native grassland  
154 (NG) soil used as reference (CMI = 100) as follows:

$$155 \text{ CMI} = \text{CLI} \times \text{CPI} \times 100 \quad (1)$$

$$156 \text{ CPI} = \text{C pool index} = \frac{\text{treatment Cstock}}{\text{reference Cstock}} \quad (2)$$

$$157 \text{ CLI} = \text{C lability index} = \frac{\text{C lability (L) in treatment}}{\text{C lability in the reference NG}} \quad (3)$$

158  $L = C \text{ lability} = \frac{C \text{ stock in labile SOM fraction } 0 - 40 \text{ cm}}{C \text{ stock in stable SOM fraction } 0 - 40 \text{ cm}}$  (4)

159  
160 ***Rice grain yield, between-season yield stability and gross margin***

161 Rice grain yield was determined by harvesting 6 subsamples from a 4 m<sup>2</sup> area in each plot and  
162 expressed at 130 g kg<sup>-1</sup> soil moisture. No rice was harvested in the 2001/02 season because the crop  
163 had been attacked by birds; also, soybean was grown from 2010/11 to 2013/14, so rice yield could  
164 only be assessed in 19 seasons.

165 Between-season stability in grain yield was assessed by calculating a relative stability ratio for  
166 each tillage system according to Knapp & van der Heijden (2018). Such ratio, which represents the  
167 variability per unit grain yield, was calculated by dividing the coefficient of variation (CV) for each  
168 tillage system by that for the CT system (the reference). Thus, ratio values of 1, < 1 or > 1 for a tillage  
169 system indicated that between-season stability in grain yield under it was identical with, lower than or  
170 higher than, respectively, that for CT system.

171 An economic analysis was conducted to compare the gross margins for the three tillage  
172 systems. Gross margin was calculated as the income from grain minus the operating costs involved in  
173 producing the summer cash crop each year. No fixed or overhead expenses such as rates, tax,  
174 insurance, interest or depreciation on machinery and buildings were considered. All costs and income  
175 were calculated on the basis of present-day values with provision for no fluctuations in input costs  
176 over time. The overall costs for rice under CT in the 2018/19 crop season estimated by the  
177 Riograndense Rice Institute were used as reference (1372 US\$ ha<sup>-1</sup>). With NT, the economy of diesel  
178 resulting from less tillage operations amounted to 96 US\$ ha<sup>-1</sup> and was subtracted from the costs of  
179 the CT system. With PG, the additional cost of irrigation for 2 further months (73 US\$ ha<sup>-1</sup>) was added  
180 to the CT costs. The gross income from the grain harvest was calculated on the assumption of a grain  
181 price of 221 US\$ Mg<sup>-1</sup> (viz., the current price rice paid to farmers). No fluctuations in grain price over  
182 time were considered.

183

## 184 ***Statistical analysis***

185 The results were checked for variance normality and homoscedasticity with the Kolmogorov–Smirnov  
186 and Levene test, respectively, and appropriated data transformation were performed when assumptions  
187 were violated. The analysis of the variance was performed and, when significant ( $p < 0.05$ ), the  
188 differences between treatment means were evaluated with Tukey's test at  $p < 0.05$ . The MIXED  
189 procedure was used to compare the effects of tillage method (T) and soil layer (L) on the response of  
190 the SOC and SOM pools. On the other hand, tillage methods (T) were compared in terms of their effect  
191 on rice yield and SOC stocks in the 0–40 cm layer. The results for NP were excluded from the statistical  
192 analysis because this tillage system did not follow the experimental design and was simply used as  
193 reference for the effects of tillage. The statistical procedures used tillage method as a fixed factor, and  
194 blocks and experimental errors as random variables. All analyses were performed with SAS® v. 9.4  
195 (Statistical Analysis System Institute, Cary, NC, USA).

196

## 197 **Results**

### 198 ***Carbon input by crops***

199 The average addition of biomass (shoots + roots) from winter ryegrass and summer rice for the three  
200 tillage systems was 15.09 Mg ha<sup>-1</sup> and rice biomass accounting for 64% of total biomass addition  
201 (Table 1). The annual C input ranged from 6.7 to 7.0 Mg ha<sup>-1</sup> across tillage systems.

202

### 203 ***Soil carbon content and stocks, and soil organic matter fractions***

204 Twenty-one years of contrasting tillage systems resulted in large differences in SOC stocks in the 0–40  
205 cm soil layer (Table 2). In fact, SOC stocks ranged from 46.5 Mg ha<sup>-1</sup> in CT soil to 55.6 Mg ha<sup>-1</sup> in  
206 NT soil (Table 2), with an intermediate value (47.8 Mg ha<sup>-1</sup>) in PG soil. However, the increased SOC  
207 level under NT was restricted to the surface soil layer (0–2.5 cm; Figure 1). The difference in SOC  
208 down to 40 cm between CT and NT (9.1 Mg ha<sup>-1</sup>) resulted in an annual C accumulation rate of 0.41  
209 Mg ha<sup>-1</sup>. SOC stocks under NT were similar to those in the reference soil (NG; Table 2).

210 POM-C and Min-C were affected by tillage system only in the surface soil layers (Figure 1).  
211 Thus, POM-C ranged from 4.9 to 24.4 g kg<sup>-1</sup> soil in the surface layer but dramatically decreased with  
212 depth (to 0.3–0.4 g kg<sup>-1</sup> soil in 30–40 cm layer; Figure 1). As with SOC, tillage had a substantial impact

213 on the surface soil layer, where POM-C was 100 and 110% greater with NT than it was with CT and  
214 PG, respectively. No differences in POM-C stocks in the 0–40 cm soil layer among tillage systems  
215 were observed, however (Table 2). NT also resulted in increased Min-C stocks in the surface soil layers  
216 (Figure 1), the increased levels reflecting in Min-C stocks down to 40 cm that were 16 and 19% greater  
217 than with CT and PG, respectively (Table 2).

218

### 219 *Carbon pool indices*

220 Relative to NG (CLI and CPI = 1, CMI = 100), CPI and CMI in the 0–40 cm layer were influenced by  
221 tillage system, whereas CLI was not (Table 2). Thus, CLI was 20–40% lower with the three systems  
222 than it was in the reference soil (NG). On the other hand, CPI was 10% lower with CT and PG, but  
223 10% higher with NT (Table 2). The combination of the previous CLI values and the respective  
224 quantitative changes in SOC stocks embodied in CPI led to a CMI value with CT, PG and NT  
225 corresponding to 59.6%, 79.9% and 81.4% that for NG.

226

### 227 *Rice yield, between-season yield stability and gross margin*

228 The average rice yield for the body of tillage systems increased from 5.0 Mg ha<sup>-1</sup> in the 1994/95 crop  
229 season to around 8.0 Mg ha<sup>-1</sup> in the 2000s and almost 12.0 Mg ha<sup>-1</sup> in the last season (Figure 2).  
230 Overall, rice grain yield under PG was higher than it was under CT and/or NT in 10 of the 19 crop  
231 seasons (Figure 2). In contrast, NT had slight lower yields than did CT and/or PG in 11 seasons (Figure  
232 2). The mean rice grain yield for the experimental period was 8.0 Mg ha<sup>-1</sup> with CT, 8.5 Mg ha<sup>-1</sup> with  
233 PG and 7.7 Mg ha<sup>-1</sup> with NT.

234

235 Yield stability, as measured in terms of the ratio of coefficient of variation for rice yield relative  
236 to the CT system, was higher with PG and NT. The yield stability ratio under NT exceeded 1.0 and  
237 was 15% higher on average than under CT and PG during the first 7 years, but decreased in the long  
238 term (Figure 3). In fact, the ratio was 4% and 10% lower under PG and NT, respectively, than it was  
239 under CT between the 8th and 14th year, and 13 % and 23% lower, respectively, between the 15th and  
240 24th year (Figure 3). These results indicate that PG and NT led to more stable yields in the long-term  
than did CT (Figure 3).

241 The absolute gross margins of rice production under NT and PG were slightly higher than they  
242 were under CT in 17 and 12 crop seasons, respectively (Figure 4a). Gross margins across seasons  
243 averaged at 428 US\$ ha<sup>-1</sup> with NT and 425 US\$ ha<sup>-1</sup> with PG, both figures being slightly higher than  
244 that for CT (392 US\$ ha<sup>-1</sup>). While the differential gross margin between PG and CT was similar  
245 throughout the experimental period, that between NT and CT increased from nearly -60 US\$ ha<sup>-1</sup> in  
246 the early years to around 131 US\$ ha<sup>-1</sup> in the latest (Figure 4b).

247

## 248 Discussion

### 249 *Soil organic carbon stocks*

250 Twenty-one years of contrasting tillage systems resulted in large differences (up to 9.1 Mg ha<sup>-1</sup>) in  
251 SOC stocks in the soil profile (0–40 cm; Table 2). Conversion from NG to irrigated rice in tilled soil  
252 decreased SOC stocks, with little difference between CT (2.8 Mg C ha<sup>-1</sup>) and PG (1.5 Mg C ha<sup>-1</sup>).  
253 This similarity in SOC down to 40 cm between NG, CT and PG was possibly a result of the combined  
254 effect of a high biomass input by summer rice (presumably higher than 9 Mg DM ha<sup>-1</sup>) and the slow  
255 decomposition of organic matter in flooded soil relative to NP even though the soil was annually  
256 perturbed by plowing and disking. Anaerobic organisms in flooded soil are less efficient in obtaining  
257 energy from organic matter than are aerobic organisms (Pett-Ridge et al., 2006)—and hence also less  
258 efficient in mineralizing plant residues incorporated into soil (Bellinger et al., 2012). The increased  
259 SOC levels of PG relative to CT may have resulted from the former system involving flooding for a  
260 longer time (approximately 2 months), thereby reducing the rate of mineralization of organic  
261 compounds in the soil.

262 On the other hand, increase of SOC stocks in NT relative to CT is consistent with previous  
263 reports (Liping and Erda, 2001; Nascimento et al., 2009; Rosa et al., 2011; Xu et al., 2013). The  
264 increased SOC stocks under NT resulted from enrichment with C of the soil surface layer by effect of  
265 the absence of perturbations and the presence of a high annual C input. Thus, NT increased SOC down  
266 to 40 cm at a rate of 0.41 Mg C ha<sup>-1</sup> yr<sup>-1</sup> relative to CT. These annual soil C accumulation rates in NT  
267 soil under irrigated rice are similar to those found by Zanatta et al. (2007) in uplands (0.44–0.60 Mg  
268 ha<sup>-1</sup>) under similar climatic conditions.

269 If annual C inputs are assumed to be similar among tillage systems, then the increase in SOC  
270 stocks was a result of more marked SOC stabilization in NT soil than in no-tilled soils. The SOC  
271 differences of NT with CT and PG can be ascribed to differences in Min-C stocks, which correspond  
272 to organomineral associations. The association of organic C with minerals has been deemed one of the  
273 main mechanisms for stabilizing organic C in soil, mainly in lowland rice soils where frequent flooding  
274 causes rupture of soil aggregates and low efficiency of physical protection to organic matter  
275 stabilization (Nascimento et al., 2009; Conceição et al., 2013).

276 Despite the marked residue input by rice, the increased C/N ratio and Si content (Devêvre &  
277 Horwáth, 2000; Pereira et al., 2004) relative to NG also probably diminished turnover of residual C  
278 and hindered its incorporation into the POM-C pool (or CLI). This was also probably why, even under  
279 NT, POM-C and CLI (0.7 in 0–40 cm soil layer) tended to decline and were never restored to the  
280 starting level in NG soil (Table 2). As a result, CMI was lower under CT and PG than it was under  
281 NG. On the other hand, NT allowed CMI in 0–40 cm soil layer to be partially recovered and CMI  
282 levels under this system to be similar to those in NG soil. Based on these results, NT provides an  
283 effective method for increasing SOC stocks in soil bearing irrigated rice in the long term, albeit with  
284 potentially adverse effects on the role played by labile carbon (POM-C) in soil.

285

### 286 ***Rice yield, between-season yield stability and gross margin***

287 A marked increase in rice yield (5–12 Mg ha<sup>-1</sup>) was observed over the 19 crop seasons irrespective of  
288 tillage system. This was mainly the result of improved soil and crop management, and of using shorter,  
289 soluble Fe-tolerant, resistant to diseases and, thus, more productive rice varieties. Especially prominent  
290 among soil and crop management conditions were the preferential time to sowing; better seed quality  
291 and treatment; a reduced sowing density; higher, more balanced fertilizer rates; earlier irrigation; and  
292 more efficient control of weeds, insect pests and diseases (Menezes et al., 2012).

293 The increased rice yield observed under PG in 11 of the 19 years can be ascribed to the fact  
294 that flooding was advanced by approximately 30 days before rice sowing. Earlier flooding led to also  
295 earlier electrochemical changes in the soil solution, and raised pH, reduced Al<sup>3+</sup> concentrations and  
296 increased nutrient availability in the soil solution as a result, thus boosting rice development

297 (Ponnamperuma, 1972). In addition, earlier flooding facilitated physical control of weeds (red rice  
298 mainly), thus reducing disease and damage to the crop (Sosbai, 2018).

299 On the other hand, NT exhibited lower rice yields in 11 of the 19 growing seasons.  
300 Interestingly, this system led to limited crop yield during the first ten seasons, possibly as a result of  
301 N being immobilized by soil microorganisms (Beri et al., 1992). This was probably not the case with  
302 CT and PG, which led to increased rice yields relative to NT. However, yields were similar with NT  
303 and PG in the last 5 crop seasons (except in penultimate season), and also with NT and CT in the last  
304 10 crop seasons (except in last season). The gradual increase in rice grain yield under NT in the long  
305 term may be associated to the increase in soil organic matter increasing the availability of N, which is  
306 the individual nutrient most markedly influencing rice development (Nadelhoffer et al., 1983).

307 NT and PG led to greater temporal yield stability than did CT (Page et al., 2019). Interestingly,  
308 relative yield stability ratio was negatively related to rice yield. Thus, as previously found by Knapp  
309 & Van Heijden (2018) and Page et al. (2019), such stability ratio decreased with increasing yield in  
310 long term. In relation to profitability, the reduced production costs of NT, and the yield advantage of  
311 NT and PG, resulted in higher gross margins with these two tillage systems than with CT. Although  
312 rice yield was substantially higher under PG than it was under NT, the former system was not more  
313 profitable than the latter owing to the increased cost derived from irrigation for 2 further months. Our  
314 results suggest that profits from conventional rice production systems in Southern Brazil could be  
315 increased by 33 US\$ ha<sup>-1</sup> by switching from CT to PG, and by 36 US\$ ha<sup>-1</sup> by replacing CT with NT.  
316

## 317 **Conclusions**

318 Tillage system is a key factor influencing soil organic matter and gross margins in irrigated rice. No-  
319 tillage appears to be a C-friendly system for lowland soils, mainly because it increases their contents  
320 in mineral associated organic matter. Despite lower rice yield than pre-germinated and conventional  
321 tillage systems, no-tillage determined a slight increase on yield stability and profitability, which effects  
322 are less obvious and only occur in the long term.

323

324

325 **Conflicts of Interest**

326 The authors declare no conflicts of interest

327

328 **Data Availability Statement**

329 The data that support this study will be shared upon reasonable request to the corresponding author.

330

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336

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## Figures Caption

**Figure 1.** Soil organic carbon (a), particulate organic C (b) and mineral associated organic C (c) in the 0–40 cm layer of a sandy loam Gleysol subjected to three different tillage systems for 21 years, or native grassland (NG), in Southern Brazil. Horizontal bars denote least significant differences as per Tukey's test at  $p < 0.05$ . ns not significant. CT conventional tillage, NT no-tillage, PG pre-germinated tillage.

**Figure 2.** Time course of irrigated rice grain yield from 1994/95 to 2017/18 in a sandy loam Gleysol subjected to three different tillage systems in Southern Brazil. Tillage means followed by same letter for each crop season were not significantly different as per Tukey's test at  $p < 0.05$ . In 2001/02, rice was attacked by birds, so it was not harvested. Also, in the growing seasons from 2010/11 to 2013/14, soybean was cultivated to control red rice. CT conventional tillage, NT no-tillage, PG pre-germinated tillage.

**Figure 3.** Between-season relative stability ratio of rice grain yield under pre-germinated tillage (PG) and no-tillage (NT) relative to the conventional tillage (CT) in the short (1–7 seasons), medium (8–15 seasons) and long-term (16–24 seasons) in a subtropical Gleysol in Southern Brazil. A ratio  $< 1$  or  $> 1$  for a tillage system indicate that between-season stability in grain yield under it was higher than or lower than, respectively, that for CT.

**Figure 4.** Gross margin of irrigated rice (US\$ ha<sup>-1</sup>) under three different tillage systems (CT, NT and PG) (a), and differential gross margin under NT and PG in relation to CT (reference) (b), on a sandy loam Gleysol in southern Brazil. The continuous line in the figure (b) represents the mean trend in differential gross margins across crop seasons in

differential gross margins between NT or PG in relation to CT system. No rice was harvested in 2001/02 because the crop was attacked by birds. Also, soybean was cultivated to control red rice in the growing seasons from 2010/11 to 2013/14. CT conventional tillage, NT no-tillage, PG pre-germinated tillage.

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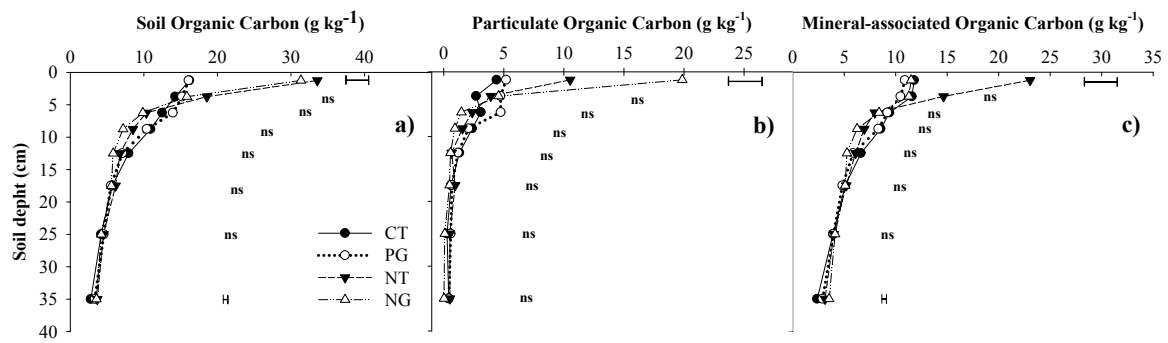


Fig. 1.

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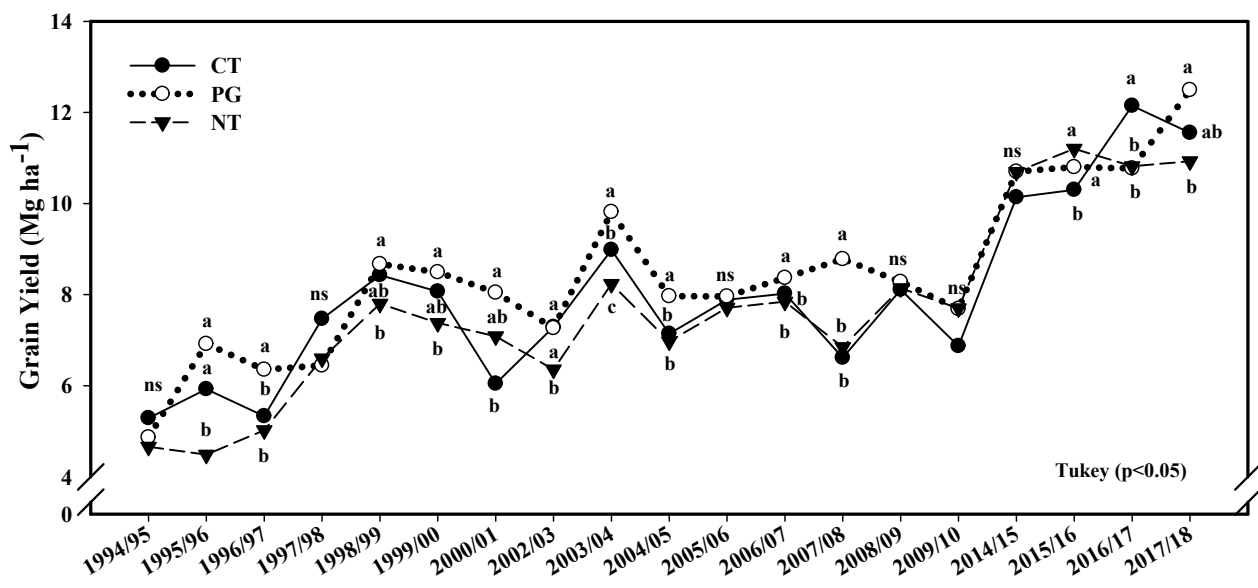
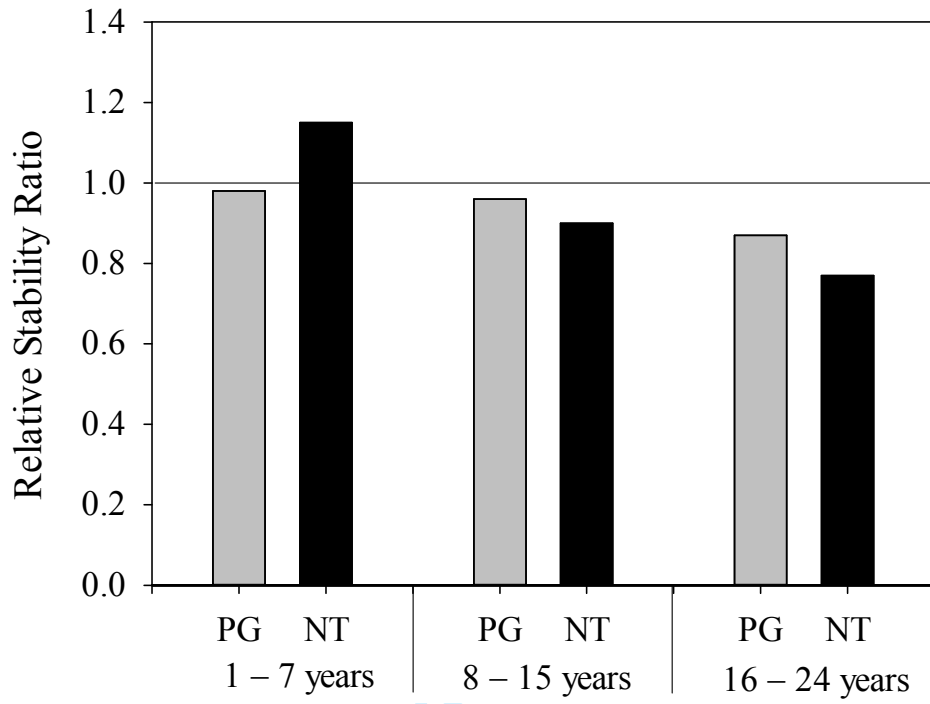


Fig. 2.

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**Fig. 3.**

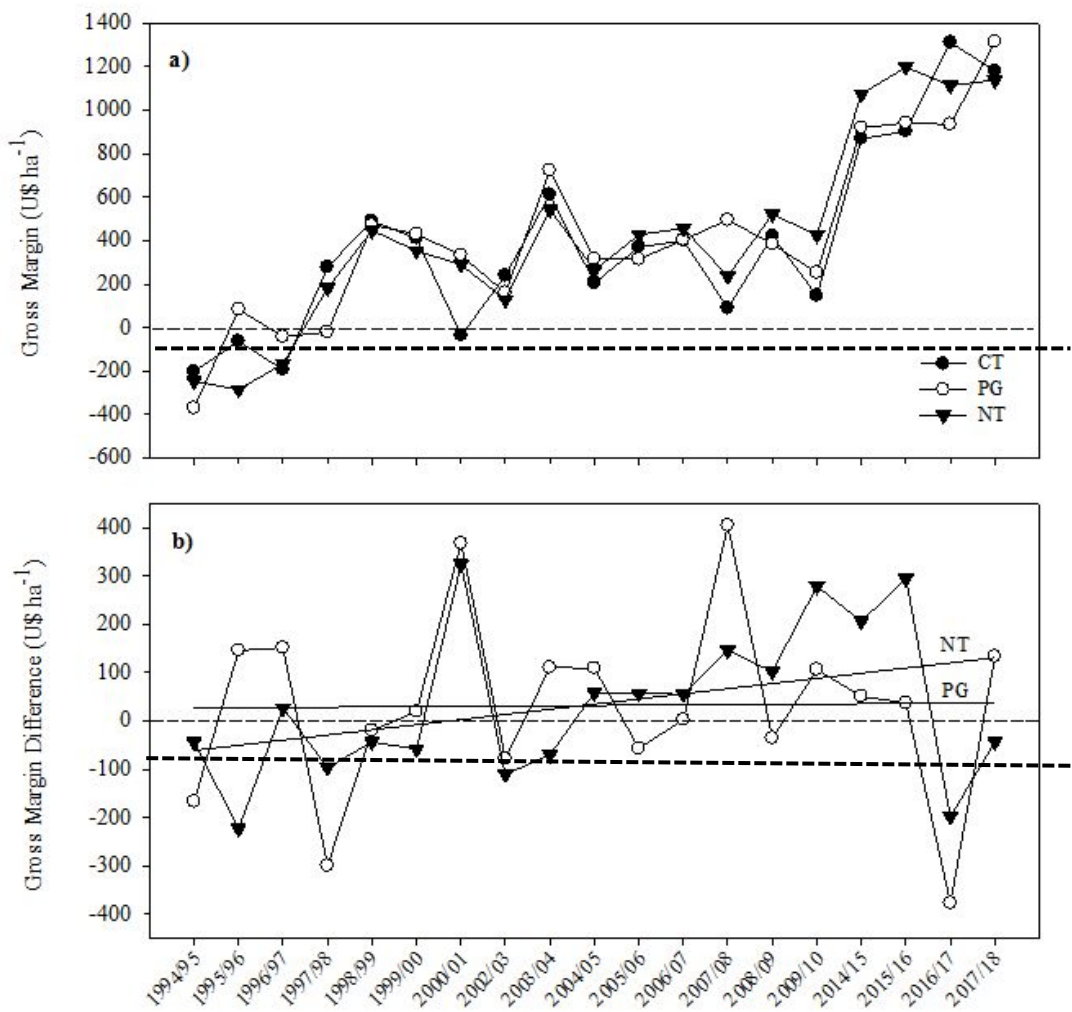


Fig. 4.

**Table 1**

Dry matter and C input (shoot + root) by ryegrass in winter and rice in summer in a subtropical Gleysol subjected to three different tillage systems in Southern Brazil.

Tillage system	Winter ryegrass	Summer rice	Annual
	----- Mg há <sup>-1</sup> -----		
<b><i>Dry matter</i></b>			
CT	5.43	9.55	15.0
PG	5.30	10.28	15.6
NT	5.49	9.22	14.7
<b><i>C input</i></b>			
CT	2.44	4.30	6.7
PG	2.39	4.63	7.0
NT	2.47	4.15	7.0

CT conventional tillage, PG pre-germinated tillage, NT no-tillage.

Rice root biomass was assumed to be 30% of shoot biomass of ryegrass (Assmann et al., 2014) and rice (Holzschuh et al., 2009).

Root shoot biomass for the whole experimental period (1994/95 to 2017/18) was estimated by using an apparent harvest index [grain/(grain + shoot)] of 0.5 (Fageria et al., 2007).

**Table 2**

Soil organic carbon (SOC) stock, particulate organic carbon (POM-C), mineral-associated carbon (Min-C), and C management index (CMI) components for the 0–40 cm layer of a subtropical Gleysol under native grassland and three tillage systems in Southern Brazil.

Soil attribute	Soil use and management system <sup>1</sup>			
	NG	CT	PG	NT
SOC stock, Mg C ha <sup>-1</sup>	49.3	46.5 b <sup>2</sup>	47.8 ab	55.6 a
POM-C stock, Mg C ha <sup>-1</sup>	12.0	8.1 b	10.3 a	10.8 a
Min-C stock, Mg C ha <sup>-1</sup>	37.3	38.5 b	37.5 b	44.8 a
<i>Carbon indices</i> <sup>3</sup>				
CPI	1.0	0.9 b	0.9 b	1.1 a
CLI	1.0	0.6 a	0.8 a	0.7 a
CMI	100.0	60 b	80 ab	81 a

CMI components: C pool index (CPI), C lability index (CLI).

<sup>1</sup> NG native grassland, CT conventional tillage, PG pre-germinated tillage, NT no-tillage.

<sup>2</sup> Means followed by the same letter in each row were not significantly different as per Tukey's test at  $p < 0.05$ . NG was not included in the statistical analysis because it do not follow any experimental design.

<sup>3</sup> C indices were calculated from eqs 1–3.

## SUPPLEMENTARY DATA

**Table S1.** Rice varieties and rates of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O used to establish rice each crop season from 1994/95 to 2017/18.

Season	Rice variety	Tillage system								
		CT			NT			PG		
		N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
----- kg há <sup>-1</sup> -----										
1994/95	BR IRGA 410	95	80	80	95	80	80	95	80	80
1995/96	BR IRGA 410	95	80	80	95	80	80	95	80	80
1996/97	BR IRGA 410	64	40	40	64	40	40	64	40	40
1997/98	BR IRGA 410	100	70	105	100	70	105	100	70	105
1998/99	BR IRGA 410	106	60	60	106	60	60	106	60	60
1999/00	BR IRGA 410	60	66	66	60	66	66	60	66	66
2000/01	BR IRGA 410	160	0	60	160	0	60	160	0	60
2001/02	IRGA 422 CL	140	60	90	140	60	90	140	60	90
2002/03	IRGA 422 CL	130	40	60	130	40	60	130	40	60
2003/04	IRGA 422 CL	145	60	90	145	60	90	120	60	90
2004/05	IRGA 417	135	60	90	135	60	90	135	60	90
2005/06	IRGA 417	135	60	90	135	60	90	135	60	90
2006/07	IRGA 422 CL	140	90	135	140	90	135	140	90	135
2007/08	IRGA 422 CL	120	40	90	120	40	90	120	40	90
2008/09	Puitá Inta CL	135	70	105	135	70	105	135	70	105
2009/10	Puitá Inta CL	150	50	75	150	50	75	150	50	75
2014/15	IRGA 424	166	68	108	166	68	108	166	68	108
2015/16	IRGA 424	166	68	108	166	68	108	166	68	108
2016/17	IRGA 424 RI	166	68	108	166	68	108	166	68	108
2017/18	IRGA 424 RI	166	68	108	166	68	108	166	68	108
Average		129	60	87	129	60	87	127	60	87

No rice was harvested in the 2001/02 season because the crop was attacked by birds. Also, soybean was cultivated to control red rice in the growing seasons from 2010/11 to 2013/14.

**Table S2.** Soil bulk density in different layers of an Entisol under three different tillage systems (CT, PG and NT) and native grassland in an adjacent reference area in a subtropical lowland ecosystem in Southern Brazil.

Soil layer (cm)	NT	PG	CT	NG
	-----Mg m <sup>-3</sup> -----			
0.0–2.5	1.37	1.38	1.62	1.32
2.5–5.0	1.37	1.38	1.62	1.32
5.0–7.5	1.71	1.55	1.64	1.46
7.5–10	1.71	1.55	1.64	1.46
10–15	1.80	1.65	1.80	1.62
15–20	1.79	1.73	1.84	1.68
20–30	1.79	1.73	1.84	1.68
30–40	1.79	1.73	1.84	1.68

NT no tillage, PG pre-germinated tillage,