

1 **TITLE PAGE**

2 **Greenhouse gas balance as affected by no-tillage and winter cover crops in a**  
3 **subtropical paddy rice ecosystem**

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24 **Greenhouse gas balance as affected by no-tillage and winter cover crops in a**  
25 **subtropical paddy rice ecosystem**

26

27 **Abstract**

28 Paddy rice production based on traditional soil management practices emits large  
29 amounts of methane (CH<sub>4</sub>) to ~~the~~ atmosphere. ~~Here, we assessed the~~The potential of no-  
30 tillage (NT) and winter cover crops (WCC) to mitigate net greenhouse gas (GHG)  
31 emissions and yield-scaled GHG emissions in a subtropical paddy rice ecosystem was  
32 investigated in a Gleysol of Southern Brazil. A long term (22-yrs) experiment was used  
33 to evaluated-evaluate regarding the effect of NT combined with winter fallow or three  
34 WCC (ryegrass, white oat and birdsfoot trefoil) on seasonal CH<sub>4</sub>-C and nitrous oxide  
35 (N<sub>2</sub>O-N) emissions and on soil organic C-carbon (SOC) stocks in comparison to  
36 conventional tillage (CT) under winter fallow, in a Gleysol of Southern Brazil. The  
37 changes in SOC were used as a proxy for annual net carbon dioxide (CO<sub>2</sub>) exchanges in  
38 the soil-atmosphere, taking tThe CT soil was taken as a reference, and the GHG balance  
39 (summation of CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> emissions multiplied by their global warming potential  
40 of 34, 298 and 1, respectively) and yield-scaled GHG emissions (GHG emissions per unit  
41 of grain yield) were calculated. ~~Aeross-Among~~ winter ~~managementsmanagement~~  
42 ~~practises~~, NT ~~decreased-reduced 25% of total~~ GHG emissions by 25% in comparison  
43 ~~to compared with the~~ CT system. This effect was mainly related to decrease of seasonal  
44 CH<sub>4</sub>-C emissions (31-113 kg ha<sup>-1</sup>) and C sequestration in soil (0.45-0.65 Mg ha<sup>-1</sup> yr<sup>-1</sup>) in  
45 ~~comparison to CT system~~, since soil N<sub>2</sub>O-N emission was not affected by management  
46 practices. The ~~sensible?~~ decrease of CH<sub>4</sub>-C emissions in NT, ~~even~~-under winter fallow  
47 with low biomass input, suggest that, in addition to the maintenance of crop residues on  
48 the soil surface, the lower emissions in NT soil were also related to the lower exposure of

**Commented [A1]:** This is a different abbreviation to first CH4 so wil hav eto be spelled out – methane carbon (CH<sub>4</sub>-C) and nitrous oxide nitrogen (N<sub>2</sub>O-N).

**Commented [A2]:** This is a very long sentence. Can it be re-written and broken up.

**Commented [A3]:** Is this CH<sub>4</sub>-C or CH<sub>4</sub>? Jumps between the two.

Commented [A4]: Can't start a sentence with an abbreviation

49 soil labile-C to methanogenic microorganisms. ~~WCC~~ did not ~~determine-provide an~~  
50 additional decrease ~~on in-~~ net GHG emissions ~~in-from~~ NT soil when compared ~~to-with~~  
51 fallow due to ~~due-to~~ the ~~increment-change~~ in soil C sequestration resulting from higher  
52 plant biomass input ~~was-being~~ partially off-set by increased soil CH<sub>4</sub>-C emissions. Based  
53 on our findings, NT mitigates net GHG emissions in subtropical paddy rice ecosystems,  
54 but no additional effect is observed if combined with WCC.

55

56 **Keywords:** nitrous oxide, methane, soil tillage, carbon sequestration, wetlands

## 57 Introduction

58 The development of agricultural production systems that ~~allow, at the same~~  
59 ~~time, simultaneously produce the production of~~ healthy food and ~~minimise mitigation of~~  
60 greenhouse ~~gases-gas~~ (GHG) emissions is one of the major challenges facing global  
61 warming and climate change (Princiotta 2009; Peters *et al.* 2013). Agriculture and land  
62 use change contribute to 25% of total GHG emission at ~~the~~ global level (Smith *et al.*  
63 2014), while in Brazil this contribution ~~increase-amounts~~ to 51% due to deforestation and  
64 ~~a~~ large agricultural ~~crop~~ area (Brasil 2016).

65 ~~The agriculture~~ Agricultural practises developed in ~~the Brazilian~~ lowlands,  
66 ~~especially-particularly the~~ irrigated rice, has ~~a-particular~~ characteristic~~ally-of~~ high soil  
67 methane (CH<sub>4</sub>) emissions, ~~which contribute-contributing to~~ approximately 18% of ~~the~~  
68 total GHG emissions from agriculture in southern Brazil (Observatório do Clima 2019).  
69 Since soil CH<sub>4</sub> fluxes are driven by the balance between methanogenesis and  
70 methanotrophy (oxidation of CH<sub>4</sub>) (Le Mer and Roger 2001), the anoxic soil condition of  
71 ~~the~~ lowlands favours ~~the~~ methanogenesis process and, consequently, CH<sub>4</sub> emission. The  
72 ~~strong-relatively high rate of~~ CH<sub>4</sub> emissions in the lowlands ~~at-of~~ this subtropical  
73 ecosystem ~~is generally considered to be caused by~~ ~~occurs-by~~ the wide adoption of

74 conventional tillage (CT) systems. ~~These practises with involve high- considerable~~ soil  
75 disturbance ~~by- through~~ ploughing, ~~disking- discing~~ and levelling, ~~and the which determine~~  
76 ~~incorporation- incorporation~~ of weeds and crop residues ~~into soil- which stimulating~~  
77 ~~stimulates~~ CH<sub>4</sub> production (Wang *et al.* 1998; Zschornack *et al.* 2011; Bayer *et al.* 2014).

78 In addition to CH<sub>4</sub>, soil losses of organic C under ~~conventional tillage- CT~~ and low  
79 C input ~~resulting off from~~ winter fallow (Rosa *et al.* 2011; Ghimire *et al.* 2011), contribute  
80 to net ~~net-~~ emissions of carbon dioxide (CO<sub>2</sub>) ~~to atmosphere- at- from~~ this subtropical  
81 ecosystem. While lowland soils under ~~conventional tillage- CT~~ are ~~great- significant~~  
82 sources of CH<sub>4</sub> and CO<sub>2</sub> ~~flux to the~~ atmosphere, nitrogen fertilization and soil N cycling  
83 also contribute to nitrous oxide (N<sub>2</sub>O) emissions ~~from in-~~ rice fields (Zhang *et al.* 2015;  
84 Islam *et al.* 2018), ~~but- Although its- N<sub>2</sub>O emission~~ rates ~~from lowland rice~~ are usually  
85 lower than those observed in uplands ~~due to....~~ (Bayer *et al.* 2014; Bayer *et al.* 2016).

86 A feasible strategy to GHG mitigation is the adoption of no-tillage (NT) that, due  
87 to ~~maintaining~~ crop residues on ~~the~~ soil surface, favour ~~a~~ decrease of soil CH<sub>4</sub> emissions  
88 (Bayer *et al.* 2014; Hao *et al.* 2016) and ~~retention of atmospheric CO<sub>2</sub>-C in soil organic~~  
89 ~~matter~~ in the less oxidative environment of NT soil (Nascimento *et al.* 2009; Rui and  
90 Zhang 2010). The combination of winter cover crops (WCC) can increase the potential  
91 of C sequestration in NT soil (Yagioka *et al.* 2015; Bayer *et al.* 2016), but an increase ~~in~~  
92 soil CH<sub>4</sub> emissions is also expected due to crop residues ~~represent- providing~~ a labile C  
93 source ~~to- for the~~ methanogenesis process ~~in soil~~ (Bayer *et al.* 2014; Haque *et al.* 2013;  
94 Zschornack *et al.* 2011). In the case of legume species of WCC, ~~N- of crop residues- residue~~  
95 ~~N~~ cycling in soil may increase mineral N forms and, as ~~a~~ consequence, favour N<sub>2</sub>O  
96 emission (Zschornack *et al.* 2011). However, in ~~the Brazilian~~ uplands, ~~this- the~~ impact of  
97 legume cover crops on N<sub>2</sub>O emissions is partially or totally off-set by C sequestration in  
98 soil (Bayer *et al.* 2016). For lowland soils ~~in subtropical ecosystems,- there is scant~~

**Commented [A5]:** Seems a bit contrary to say that cultivating the soil stimulates CH<sub>4</sub> production as subsequently it is stated that NT soil is less oxidative. Maybe it stimulates CH<sub>4</sub> release through disturbance, rather than actual production.

**Commented [A6]:** Usually residues on the soil surface are respired relatively quickly as CO<sub>2</sub> and are not retained in soil OM unless incorporated.

99 knowledge ~~on~~of the influence of conservation soil management systems comprising NT  
100 and WCC on GHG and C sequestration ~~is scant, mainly for subtropical ecosystems.~~

101 ~~Most~~Many GHG studies in agricultural systems have focused on only one of the  
102 three ~~GHG~~gases (CH<sub>4</sub>, N<sub>2</sub>O or CO<sub>2</sub>). ~~and, thus~~However, in order to assess, they are not  
103 ~~conclusive regarding~~ the real total-impact of management practices on net emissions of  
104 GHG, all three gas fluxes. ~~Aiming to have a real outline of the impact of soil management~~  
105 ~~practices on GHG emissions, the balance encompassing the three main GHG emissions~~  
106 must be determined (Robertson *et al.* 2001; Mosier *et al.* 2005; Bayer *et al.* 2016). For  
107 calculation of GHG balance, the annual or seasonal emissions of each gas ~~must be~~is  
108 multiplied by their respective radiative forcing index (1 for CO<sub>2</sub>, 34 for CH<sub>4</sub> and 298 for  
109 N<sub>2</sub>O), and then summed (Robertson *et al.* 2000; Mosier *et al.* 2005; Bayer *et al.* 2016).  
110 Paired to GHG balance per cultivated area, ~~the~~yield-scaled GHG emission measure  
111 provides the intensity of GHG per unit of grain yield (Mosier *et al.* 2006).

112 ~~Our starting hypothesis is that NT and WCC adoption decrease net GHG~~  
113 ~~emissions per area and per unit of yield in subtropical paddy rice ecosystems, what is~~  
114 ~~possibly related to increase of soil C sequestration and mitigation of soil CH<sub>4</sub> emission.~~  
115 The main objective of this study was to evaluate the potential of NT and WCC on GHG  
116 balance and yield-scaled GHG emissions in ~~a~~paddy rice fields in southern Brazil. The  
117 hypotheses were i) NT and WCC adoption decrease net GHG emissions per area and per  
118 unit of yield in subtropical paddy rice ecosystems. ii) A decrease in net GHG emissions  
119 in NT and WCC systems compared with CT is predominantly due to an increase in soil  
120 C sequestration and mitigation of soil CH<sub>4</sub> emissions.

121

## 122 **Material and methods**

### 123 *Site description and ~~e~~Experiment design ~~and~~ ~~conduction~~*

124 The study was based on a long-term field experiment (22 years) conducted in Rice  
125 Experimental Station of Rio Grandense Rice Institute, in Cachoeirinha, RS, Southern  
126 Brazil (29°55'30" S, 50°58'21" O, 7 m a.s.l). The climate is humid subtropical and  
127 classified as Cfa, ~~according to the~~ (Köppen classification), with a mean annual  
128 temperature and precipitation of 19.3 °C and 1434 mm, respectively (Climate data 2018).  
129 The loamy soil (140 g kg<sup>-1</sup> clay) is classified as Gleysol according to the FAO (1998).

130 Irrigated rice (*Oryza sativa* L.) was the summer crop in the ~~whole~~ experiment, and  
131 five treatments consisted of different combination of tillage system in spring and cover  
132 crops or fallow in winter, as follow: (i) conventional tillage (CT) and (ii) no-tillage (NT),  
133 both combined with winter fallow, and (iii-v) NT combined with three winter cover crops  
134 (WCC) - ryegrass (*Lolium multiflorum* L.), white oat (*Avena sativa* L.), and birdsfoot  
135 trefoil (*Lotus corniculatus* L.). The treatments were applied in plots of 5 m × 8.8 m, and  
136 ~~the distribution of~~ four replicates ~~at field followed were distributed as~~ a complete  
137 randomized block design.

138 The WCC were ~~sowed sown~~ in April through surface rice crop residues (no-till)  
139 and grown without irrigation. The seed rates for ryegrass, white oat and birdsfoot trefoil  
140 were 80, 20 and 8 kg ha<sup>-1</sup>, respectively. In treatments with fallow, only weeds and ryegrass  
141 developed in the plots.

142 Conventional tillage and NT systems were applied in spring. The CT consisted ~~in~~  
143 ~~of~~ ploughing and ~~dis~~cking at 25 and 7 cm, respectively. These operations buried the  
144 harvest residues of rice and weeds into soil. In ~~the~~ NT system, soil was ~~not-un~~disturbed  
145 and rice, weed and WCC residues were maintained on ~~the~~ soil surface. In ~~the~~ NT system,

146 weeds control and management of WCC were performed by desiccation with glyphosate  
147 (Roundup® 3.5 L p.c. ha<sup>-1</sup>) about 30 days before rice sowing.

148 The rice (cultivar IRGA 424 RI) was ~~sowed~~sown at a seed rate of 100 kg ha<sup>-1</sup> in  
149 the first half of October every year. The mean rates of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O applied at rice  
150 sowing were 108 and 68 kg ha<sup>-1</sup>. N fertilization rate was applied at 150 kg ha<sup>-1</sup> ~~splited~~ in  
151 two times, with 66% applied in V4 and 34% in R0 stages (CQFS-RS/SC 2004; SOSBAI  
152 2016). Weed control was carried out in the pre-emergence rice period using clomazone  
153 herbicide (Gamit® 1.2 L ha<sup>-1</sup>) and glyphosate (Roundup® 2.0 L ha<sup>-1</sup>). Post rice  
154 emergence, ~~cyhalophope~~cyhalofop herbicide (Clincher® 1.7 L ha<sup>-1</sup>) and ~~penoxulan~~  
155 penoxsulam (Ricer® 0.25 L ha<sup>-1</sup>) were used. Pest control was carried out with  
156 neonicotinoid-pyrethroid insecticide (Engeo Pleno® 0.2 L ha<sup>-1</sup>) in R0. Irrigation by  
157 flooding started in V4 ~~developing~~development stage of ~~the~~ plants just after the first N  
158 application, and a water ~~heigh-t~~height of 5-7 cm was maintained until rice maturation, when the  
159 water supply ~~has been cut~~was cut off.

160

#### 161 *Evaluation of biomass addition by cover crops, weeds and rice*

162 Aboveground biomass of WCC and weeds in winter (2017) and rice in summer  
163 (2017/18) were evaluated by sampling an area of 0.5 m<sup>2</sup> per plot at the beginning of  
164 flowering (R4 stage). Biomass samples were oven dried (forced air at 50 °C) until constant  
165 mass, and contribution of roots was considered as 30% of shoot addition by WCC or  
166 weeds (Zanatta *et al.* 2007) and rice (Insalud *et al.* 2006). Five subsamples were collected  
167 per plot.

168

#### 169 *Evaluation of rice yield*

170 Rice grain yield evaluation were performed in 2017/2018 crop seasons through a  
171 *Wintersteiger* mechanized harvester and were obtained by extrapolating the yield  
172 harvested in the useful area of each plot to one hectare and fitting the grain moisture to  
173 130 g kg<sup>-1</sup>.

174

#### 175 *Evaluation of CH<sub>4</sub> and N<sub>2</sub>O fluxes and calculation of seasonal emissions*

176 Air samples were collected by using the static chamber method (Parkin and  
177 Venterea 2010), in 2017/2018 growing season. Each chamber consisted of an aluminium  
178 base (50 cm × 100 cm) and an aluminium top of the same size. The bases were driven  
179 0.05 m into the soil before permanent flooding and were left fixed in the throughout the  
180 rice season.

181 Each base had an open bottom and sealable channels on the sides to facilitate free  
182 flowing of irrigation water in the rice season. The channels on the sides were sealed  
183 during air sampling events. Each base covered three rows of rice plants. During the rice  
184 season, an additional 0.20 or 0.30 m aluminium extensions were stacked on the bases as  
185 the plants grew up-taller and the respective chamber volume was applied for calculation  
186 of GHG fluxes. Internally within each chamber top had internally there was a rubber  
187 septum sampling port, and a stainless steel thermometer, and a battery operated three  
188 12V fans for mixing the headspace and homogenize air within the chamber (Bayer *et al.*  
189 2014).

190 Each air sampling session started at 09:00 h, when the aluminium chamber top  
191 were-was deployed on the aluminium base. Internal air samples were taken with  
192 polypropylene syringes (20 mL) at the chamber deployment (time zero) and 10, 20 and  
193 30 minutes later; and transferred into 12-ml vials previously evacuated (Exetainer®,  
194 Labco, UK). The N<sub>2</sub>O and CH<sub>4</sub> concentrations were measured in a Shimadzu GC 2014

**Commented [A7]:** How many replicate chambers? Was there 1 chamber per plot? N=4?

**Commented [A8]:** How often? How many observations were made?



195 chromatograph equipped with a flame ionization detector (FID) for CH<sub>4</sub>, and an electron  
196 capture detector (ECD) for N<sub>2</sub>O.

197 The N<sub>2</sub>O or CH<sub>4</sub> fluxes were calculated taking into account the: (i) linear increase  
198 of the internal gas concentration during the 30-min deployment, (ii) area covered by the  
199 chamber, (iii) volume, (iv) temperature and (v) pressure (assumed as 1 atm) inside the  
200 chamber (Parkin and Venterea 2010). Assuming that the gases flux from 09:00 to 12:00  
201 represents the average daily flux (Bayer *et al.* 2016), the seasonal emission was calculated  
202 by integrating N<sub>2</sub>O-N and CH<sub>4</sub>-C fluxes during the monitoring period.

**Commented [A9]:** Any info on quality control on the flux gradient?

203

#### 204 *Soil sampling, organic C analysis and calculation of stocks*

205 In September 2015, soil samples of the 0-2.5, 2.5-5.0, 5.0-7.5, 7.5-10, 10-15, 15-

**Commented [A10]:** Should this be 2005?

206 20, 20-30 and 30-40 cm layers were collected before rice sowing the rice (i.e., 18 years

**Commented [A11]:** How many soil samples from each plot, and were these integrated?

207 after the experiment was started). Trenches were dug manually to allow the assessment

**Commented [A12]:** Was the soil sampled in 2015 or 2005?

208 of soil bulk density using the volumetric ring method (Blake and Hartge 1986). In the

**Commented [A13]:** How many individual measurements were made? Can you provide SOC as a %, pH, TN %

209 same layers as those sampled for bulk density assessment, soil samples were collected

210 with a spatula and then air dried and ground to  $\leq 2$  mm in a Marconi 330 grinder.

211 Approximately 20 g of soil was further ground to  $\leq 0.025$  mm in an agate mortar and

212 analyzed for C by dry combustion in a Shimadzu VCSH analyzer. SOC stocks were

**Commented [A14]:** Shouldnt start a sentence with an abbreviation.

213 calculated for the whole-entire 0-40 cm profile, by using the equivalent soil mass

214 approach (Ellert and Bettany 1995) with CT soil mass as reference. The annual rate of

215 soil-organic-C-SOC accumulation was calculated as the ratio of difference between soil

216 organic-C-SOC stocks in the treatments and the reference system (CT fallow) and the

217 duration time-of the experiment when soil was sampled (18 years).

218

219 *Net balance of GHG emissions and yield-scaled GHG emission*

220 The net balance of GHG emissions for each management system was calculated  
221 according to the equation 1:

$$222 \quad \text{Net balance of GHG} = N_2O + CH_4 - \Delta SOC \quad (1),$$

223 where  $N_2O$  and  $CH_4$  are the cumulative annual emissions of  $N_2O$  and  $CH_4$  properly  
224 converted into  $CO_2$  equivalent ( $CO_{2eq}$ ) after considering the global warming potentials  
225 (298 for  $N_2O$ , 34 for  $CH_4$ ) and conversion factors; and  $\Delta SOC$  is the annual change in  
226 soil organic carbon (SOC) stock to 40 cm depth, which was assumed as a proxy of the  
227 net exchange of  $CO_2$  in the soil-atmosphere interface (Mosier *et al.* 2005).

228 The Yield-Scaled GHG was calculated by the ratio between the balance of net GHG  
229 emissions and the average grain yield of rice, to infer the intensity of GHG emission (in  
230  $Mg CO_2 eq ha^{-1}$ ) per unit (Mg) of rice grain yield.

231

232 *Statistical Analysis*

233 The results were checked for variance normality and homoscedasticity with the  
234 Shapiro-Wilk and Oneill & Matthews tests, respectively, and appropriated data  
235 transformation were performed when assumptions were violated. Seasonal  $CH_4$ -C and  
236  $N_2O$ -N emissions, balance of GHG, rice grain yield and yield-scaled GHG emissions  
237 were submitted to analysis of variance. When significant at the 5% level, the differences  
238 among treatments were subjected to Tukey's post-hoc test at the 5% significance level.

239 The MIXED procedure was used to compare the effects soil managements-in winter on  
240 GHG, soil organic C and rice yield. The statistical procedures used soil winter  
241 management as a fixed factor, and blocks and experimental errors as random variables.

242 All analyses were performed with SAS<sup>®</sup> v. 9.4 (Statistical Analysis System Institute,  
243 Cary, NC, USA).

244

## 245 **Results**

### 246 *Rice grain yield and annual input of biomass*

247 The rice grain yield was not influenced by tillage systems nor by WCC (Table 1),  
248 ranging from 8.2 Mg ha<sup>-1</sup> in CT-fallow to 8.7 Mg ha<sup>-1</sup> in NT- birdsfoot trefoil (Table 2). A  
249 mean rice yield of 8.4 Mg ha<sup>-1</sup> was attained across soil management systems (Table 2).

250 Annual biomass input ranged between 10.4 and 16.8 Mg ha<sup>-1</sup> (Table 2). In the  
251 winter, biomass input ranged from 0.7 (CT) to 1.6 Mg ha<sup>-1</sup> (NT) in the systems under  
252 fallow, while in the systems under cover crops the biomass input increased to 5.4 Mg ha<sup>-1</sup>  
253 on average, ranging from 3.9 to 6.8 Mg ha<sup>-1</sup> (Table 2). The highest biomass input was  
254 observed for the rice crop, ~~whose-which~~ accounted for 86-93% of total annual biomass  
255 input in the fallow systems in CT and NT, and for 60-72% for WCC systems.

256

### 257 *Seasonal CH<sub>4</sub> and N<sub>2</sub>O emissions*

258 Seasonal CH<sub>4</sub>-C emissions was affected by management systems (Table 1) and  
259 ranged from 359 to 472 kg ha<sup>-1</sup> (Table 2). Greater CH<sub>4</sub>-C emission was observed in CT  
260 with winter fallow compared to NT with winter fallow (a difference of 74 kg CH<sub>4</sub>-C ha<sup>-1</sup>  
261 ~~of-difference~~) (Figure 1a). Under NT, seasonal soil CH<sub>4</sub>-C emission was similar  
262 ~~comparing from~~ WCC and-fallow, with the exception of white oat ~~which that~~ presented an  
263 seasonal soil CH<sub>4</sub>-C emission slightly higher (441 kg ha<sup>-1</sup> ) ~~in-comparison-compared~~  
264 ~~with to~~ the others WCC (366 kg ha<sup>-1</sup>, on average) (Figure 1a).

265 Seasonal soil N<sub>2</sub>O-N emissions ranged from 0.25 to 0.87 kg ha<sup>-1</sup>, and were not  
266 influenced by tillage and WCC (Tables 1 and 2, Figure 1b).

267

268 *Soil organic C stocks and annual accumulation rates*

269 Soil organic ~~carbon~~C content was influenced by the management systems mainly  
270 in the surface soil layers (0-2.5 and 2.5-5.0 cm), where NT and WCC favored greater  
271 SOC content than CT and winter fallow (Figure 2a). Considering ~~the~~ 0-40 cm layer, lower  
272 SOC stocks were observed in CT (47.0 Mg ha<sup>-1</sup>) than NT (53.3 Mg ha<sup>-1</sup>), ~~when both were~~  
273 combined with winter fallow (Figure 2b). Under NT, the increase of biomass input by  
274 WCC did not ~~promoted-cause~~ an increase in SOC stocks in comparison to winter fallow.  
275 In comparison to CT, NT combined with fallow and WCC presented annual accumulation  
276 rates of SOC ranging from 0.45 to 0.65 Mg ha<sup>-1</sup> yr<sup>-1</sup> (i.e. 1.7-2.1 Mg CO<sub>2</sub>eq. ha<sup>-1</sup> yr<sup>-1</sup>;  
277 Table 3)

278

279 *Net balance of GHG and yield-scaled greenhouse gas emission*

280 Considering the three main GHG (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) emitted from the rice field,  
281 total GHG emissions ranged between 14.8 and 21.7 Mg CO<sub>2</sub>eq. ha<sup>-1</sup> among soil  
282 management systems (Table 3). In comparison to the traditional management system (CT  
283 combined with winter fallow), NT adoption decreased GHG emissions by 5.4 Mg CO<sub>2</sub>eq.  
284 ha<sup>-1</sup> when combined with winter fallow, and by 3.7-6.8 Mg CO<sub>2</sub>eq. ha<sup>-1</sup> when combined  
285 with WCC (Table 3).

286 Yield-scaled GHG emission was higher in CT (2.6 Mg CO<sub>2</sub> Mg<sup>-1</sup> grain rice) than  
287 in NT (1.9 Mg CO<sub>2</sub> Mg<sup>-1</sup> grain rice), both combined with winter fallow. No effect of  
288 WCC was observed on yield-scaled GHG emission in NT soil, ranging between 1.8 and  
289 2.2 Mg CO<sub>2</sub> Mg<sup>-1</sup> grain rice (Table 3).

290

291 **Discussion**

292 The lack of effect of NT and WCC on rice grain yield has been observed in other  
293 studies based in field experiments in Southern Brazil (Bayer *et al.* 2014; Zshornack *et al.*  
294 2016) and in other regions of the world (Biay-Singh *et al.* 2008; Huang *et al.* 2015). The  
295 reasonable explanation for ~~this the no effect of NT and WCC on lack of~~ rice yield ~~response~~  
296 ~~to management~~ is that all nutrient demand for the crop is supplied by fertilization, ~~and in~~  
297 ~~contrast to , different than observations occurs in Brazilian upland upland areas;~~ the  
298 impact of soil management practices on soil water retention and availability, soil  
299 compaction and Al toxicity, among others, ~~did was~~ not reflected in benefits ~~for to flooded~~  
300 rice ~~cultivated under flooding~~. This is a very different result than that observed in uplands,  
301 where water availability for crops ~~has been is~~ the main variable ~~related to the responsible~~  
302 ~~for the~~ increase of crop yields in rainfed no-till cropping systems (Franchini *et al.* 2012).

303 The strong impact of ~~the~~ NT system decreasing from 15 to 28% soil CH<sub>4</sub>  
304 emissions in comparison to the traditional CT combined with winter fallow is in  
305 agreement to previous studies, which accounted ~~for a~~ decrease ~~from of~~ 21% in Southern  
306 Brazil (Bayer *et al.* 2014) and from 22 to 27% in China (Zhang *et al.* 2015; Zhao *et al.*  
307 2016). ~~This lower CH<sub>4</sub> emission in NT has been attributed to the no soil incorporation of~~  
308 ~~biomass residues, a source of labile C readily available for methanogenic microorganisms~~  
309 ~~that are highly active in subsurface soil layers, considering that soil reduction degree is~~  
310 ~~larger (<150 to 200 mV) compared to surface layers (Zschornack *et al.* 2011; da Silva *et*~~  
311 ~~*al.* 2011; Wang *et al.* 1993).~~

312 The above mentioned explanation for higher soil CH<sub>4</sub> emissions under CT than  
313 under NT is reasonable for WCC treatments, where an ~~expressive~~ biomass input was  
314 observed (3.9-6.8 Mg ha<sup>-1</sup>) but ~~it is not to~~ ~~does not~~ explain the lower CH<sub>4</sub> emissions in  
315 NT for fallow system where biomass input by weed accounted 0.7 Mg ha<sup>-1</sup> in CT and 1.6

**Commented [A15]:** These were not measured in this study though so you can only presume this – needs to be written a little differently.

**Commented [A16]:** English needs to be rewritten.

**Commented [A17]:** English needs rewritten

**Commented [A18]:** Wrong word

316 Mg ha<sup>-1</sup> in NT system (Table 2). Higher CH<sub>4</sub>-C emissions in CT (472 kg ha<sup>-1</sup>) than NT  
317 (398 kg ha<sup>-1</sup>), even under winter fallow that determined a low amount of vegetable  
318 residues before soil tillage in spring, suggest that higher CH<sub>4</sub>-C emissions in CT soils are  
319 also related to the effect of ploughing and disking in the breakdown of soil aggregates  
320 and exposure of labile C for methanogenic microorganisms.

321 Despite the wide range of soil N<sub>2</sub>O-N emissions (0.25-0.86 kg ha<sup>-1</sup>) among soil  
322 management systems, due to high random variation, as evidenced by the high coefficient  
323 of variation, ranging from 55 to 149%, the observed difference of seasonal N<sub>2</sub>O emissions  
324 were not statistically significant ( $p > 0.05$ ) and cannot be attributed to the management  
325 practices (Table 1). High variation indexes are a common feature in studies on N<sub>2</sub>O  
326 emissions in agricultural soils, that have been attributed to the temporal and spatial  
327 variability that add up to the complex combination of the several soil variables  
328 encompassed in N<sub>2</sub>O production in soils (Hénault *et al.* 2012). The magnitude of N<sub>2</sub>O  
329 emissions is similar that observed in previous studies conducted in paddy rice fields  
330 (Bayer *et al.* 2014; Bayer *et al.* 2015; Zschornack *et al.* 2018), but lower (for the same  
331 time frame, 4 months) than usually observed in upland soils (Weller *et al.* 2015), which  
332 is possibly associated to the fact that emissions are restricted to the initial phase after  
333 flooding and after suspension of water supply, motivated by the presence of soil nitrate  
334 and by the activity of nitrifying and denitrifying microorganisms (Zou *et al.* 2005;  
335 Zschornack *et al.* 2011).

336 No-tillage increased SOC stocks in this lowland soil, a similar tendency observed  
337 in previous studies in Southern Brazil (Nascimento *et al.* 2009; Rosa *et al.* 2011), and  
338 other countries such as Uruguay (Terra *et al.* 2006) and China (Rui and Zhang 2010). The  
339 annual accumulation rates of SOC (0.45 to 0.65 Mg ha<sup>-1</sup>) in this lowland soil under NT  
340 system were similar than those observed in upland soils considering the time of NT

**Commented [A19]:** Needs to be rewritten due to English rather than content

341 adoption, clay content and soil depth (Bayer *et al.* 2006; Zanatta *et al.* 2007; Veloso *et al.*  
342 2018). Even these results suggest a similar impact of no soil disturbance on soil organic  
343 matter stabilization in lowland and upland soils, the importance of mechanisms  
344 encompassed on SOC stabilization may be different. In lowland soils cultivated with  
345 irrigated rice, flooding weakens and favors the disruption of aggregates, with lower  
346 impact of NT on physical protection of SOC (Nascimento *et al.* 2009). However, this  
347 effect must be partially off-set by the higher magnitude of organo-mineral interactions in  
348 flooded soils (Hanke and Dick 2017).

349 Winter cover crops had no effect on SOC stocks in NT soil, despite the higher  
350 annual biomass input in comparison to winter fallow. There is no reasonable explanation  
351 for the non-effect of WCC on SOC stocks, except for the spatial variability of the area  
352 and the fact that the production of biomass in winter is quite variable and, in several years  
353 a low crop development was observed either due to frequent rainfall and flooding of the  
354 soil or poor seed quality. The increase of biomass input in upland soils results in a linear  
355 increase of SOC stocks (Bayer *et al.* 2006; Veloso *et al.* 2018), despite in other studies a  
356 non-linear relationship has been verified, especially considering C stocks in surface layers  
357 with a low soil C saturation deficit, which determines a lower capacity of soil to  
358 stabilization of added C (Corbeels *et al.* 2016).

359 The GHG balance in rice production systems is represented by the net GHG,  
360 which is the sum of seasonal CH<sub>4</sub> and N<sub>2</sub>O emissions, and CO<sub>2</sub> emissions for which  
361 annual net change in SOC was used as a proxy (Piva *et al.* 2012). Across winter  
362 managements, NT-based rice production system had a net GHG lower than under CT  
363 combined with winter fallow. These results were mainly related to the atmospheric CO<sub>2</sub>-C  
364 sequestration in soil organic matter and to the decrease of seasonal soil CH<sub>4</sub>-C emissions.  
365 CH<sub>4</sub>-C was the stronger component of net GHG, representing more than 85% of total

**Commented [A20]:** Usually plant residues left on the soil surface are respired within a few months and lost as CO<sub>2</sub>, especially in humid or wet conditions. Only with incorporation and inputs of N and other nutrients is there a building of SOC stocks through microbial growth and the formation of humus or more resistant OM. What was the C:N ratio of the surface residues?

366 GHG emissions which is widely reported for flooded rice fields (Bayer *et al.* 2014, 2015;  
367 Kim *et al.* 2012). In contrast, SOC sequestration mitigated 12% of GHG emissions, in  
368 average of NT cropping systems.

**Commented [A21]:** Is it C sequestration per se, or mainly due to a lack of soil disturbance.

369 Higher yield-scaled GHG emission under CT with winter fallow than NT  
370 combined with the different winter managements indicated that NT has potential to  
371 decreased 26% of GHG emissions for each 1 Mg of grain rice produced. Thus, NT  
372 constitutes a technology with potential to increase the sustainability of irrigated rice  
373 production in subtropical ecosystems, which is usually very contested as to its  
374 environmental impact. In general, WCC had no effect on net balance of GHG and yield-  
375 scaled GHG emissions in NT soil, which is associated to the fact that although favoring  
376 soil organic carbon sequestration by increasing crop residues input, net GHG and yield-  
377 scaled GHG emissions was offset by also favoring CH<sub>4</sub>-C emissions. These opposite  
378 effects can be illustrated in the cropping system with white oat.

**Commented [A22]:** Can you put this in the context of other Summer crops such as sorghum or maize?

379

### 380 Conclusions

381 No-tillage mitigated net GHG emissions in comparison to ~~conventional tillage~~CT in  
382 lowland soils cultivated with flooded rice, which is mainly associated to decrease of soil  
383 CH<sub>4</sub>-C emissions and increase of soil C sequestration. In addition to maintenance of crop  
384 residues on soil surface, the results suggest that decrease of CH<sub>4</sub>-C emissions in no-tillage  
385 soils are also related to the lower exposure of labile C from soil to methanogenic  
386 microorganisms. Winter cover crops have no clear impact on net balance of GHG  
387 emissions and yield-scaled GHG emissions in NT soil, mainly because soil C  
388 sequestration due to higher aboveground biomass was partially off-set by increased CH<sub>4</sub>-  
389 C emissions.

**Commented [A23]:** And lack of soil disturbance. The maintenance of surface residues was not tested properly in this study. It would have had to have a design with paired CT-WC for each species for comparison with NT-WC.

390



391 **Conflicts of interest**

392 The authors declare no conflicts of interest.

393

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397 Grande do Sul State (Fapergs). The authors thank the staff at IRGA, whose help and  
398 support were essential.

399

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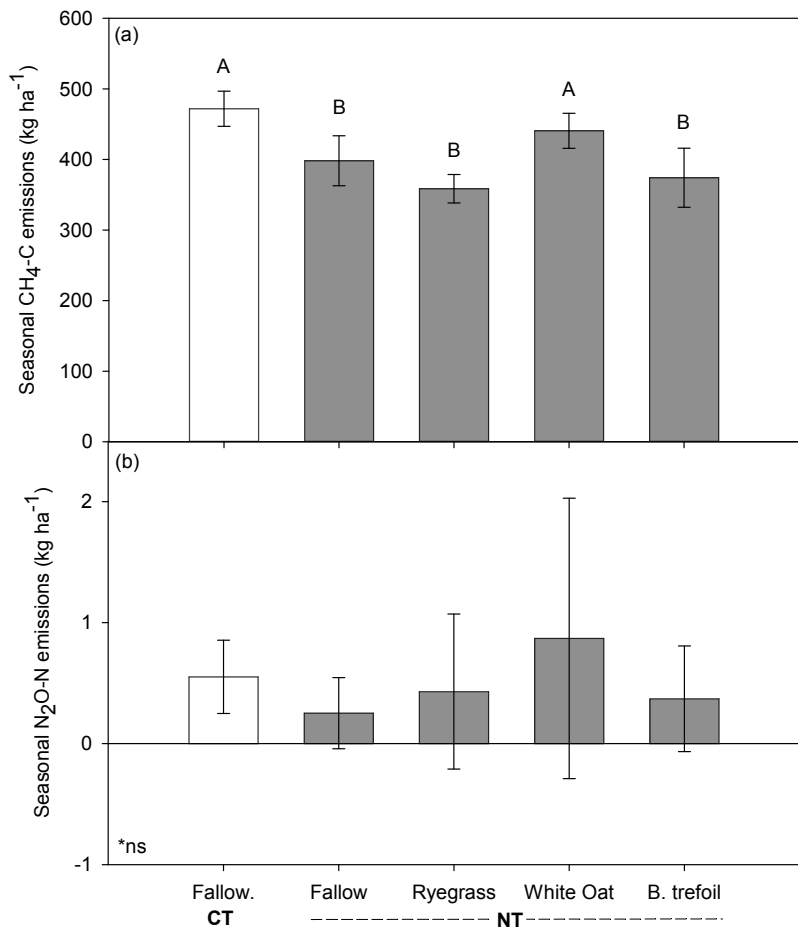
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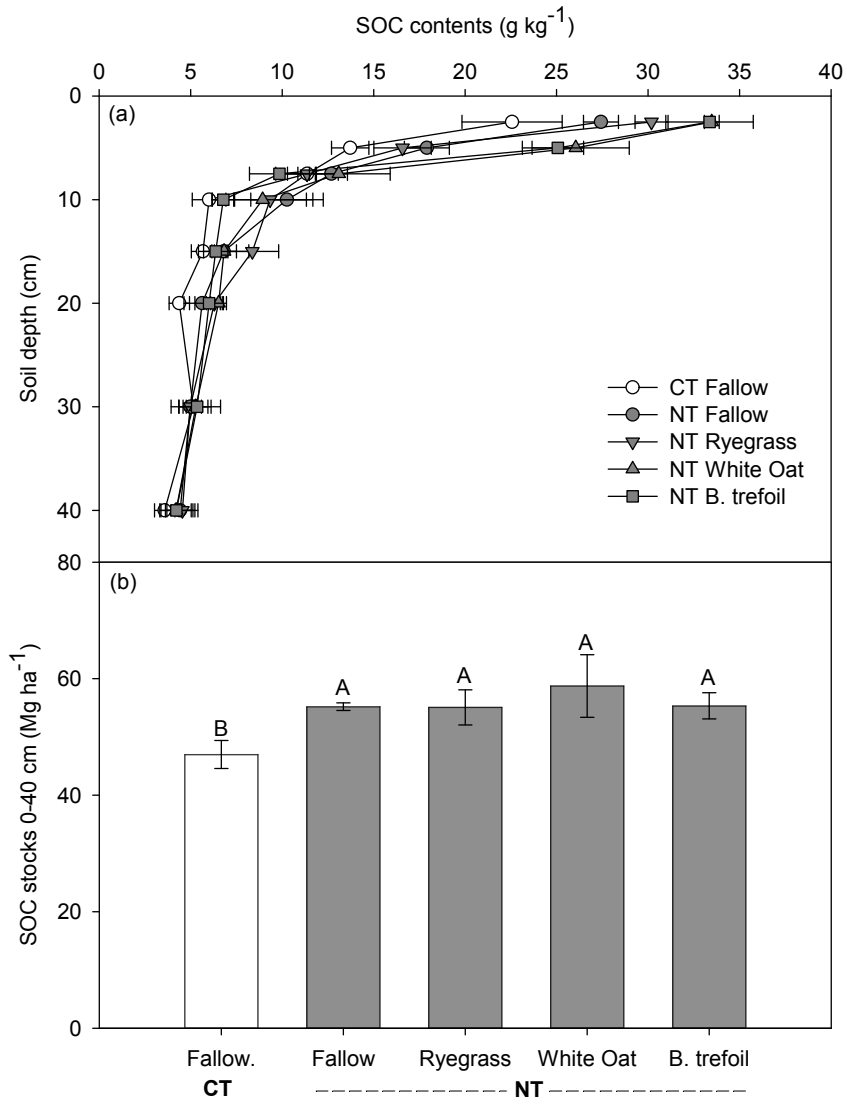
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572 **Figure 1.** Seasonal emissions of (a) methane (CH<sub>4</sub>) and (b) nitrous oxide (N<sub>2</sub>O) in a  
573 subtropical Gleysol subjected to different soil winter management (fallow or cover  
574 crops) combined with conventional tillage (CT) or no-tillage (NT) in Southern Brazil.  
575 Vertical lines denote the mean standard deviation. Different letters on the bars indicate  
576 significant difference between treatments by Test of Skott-Knott at 5% level. ns = ~~no~~  
577 insignificant.  
578

**Commented [A24]:** What is the *n*? No of observations?  
Was it a single measurement/plot?



580 **Figura 2.** Soil organic C (SOC) contents in soil profile (a) and stocks at 0-40 cm soil  
581 layer (b) of a Gleysol subjected to different soil winter managements (fallow or cover  
582 crops) combined with conventional tillage (CT) or no-tillage (NT) in southern Brazil.  
583 Vertical lines denote the mean standard deviation n=4?. Different letters on the bars  
584 indicate significant difference between treatments by Test of Skott-Knott at 5% level. ns  
585 =no insignificant.

586 **Table 1.** Analysis of variance data (values of calculated F and P of analyzed variables)  
587 for seasonal emissions of CH<sub>4</sub> ~~e-and~~ N<sub>2</sub>O, ~~stock and accumulation rate of~~ soil organic  
588 carbon (SOC) ~~stock and accumulation rate~~, net balance of GHG (net GHG), rice yield  
589 and yield-scaled GHG emissions in a subtropical Gleysol subjected to flooding rice production  
590 in Southern Brazil

<b>Dependent variable</b>	<b>F value</b>	<b>Pr &gt; Fc</b>
Rice Yield	1.45 <i>ns</i>	0.276
Seasonal CH <sub>4</sub> -C	11.87 *	<0.001
Seasonal N <sub>2</sub> O-N	0.44 <i>ns</i>	0.776
SOC stock	5.88 *	<0.001
SOC accumulation rate	9.25 #	<0.001
Net GHG	2.76 #	0.077
Yield-Scaled GWP	5.34 #	0.010

591  
592 \* indicates significant effect by the Skott-Knott test at 5% of probability and # indicates significant effect at 10% of  
593 probability; and ns indicates the lack of significant effect.

**Commented [A25]:** Is this according to management? Is it CT compared with NT or WCC? Need to explain this better in the legend or you could delete this table and include the data in Table 2 and the Figures

594 **Table 2.** Rice grain yield, biomass input by rice, cover crops and weeds in different  
 595 winter managements (fallow or cover crops) combined with conventional tillage (CT) or  
 596 no-tillage (NT) in a subtropical Gleysol subjected to flooding rice production in  
 597 Southern Brazil.

Tillage System	Winter management	Rice		Weeds / Cover crops	<b>Total</b>
		<b>Grain Yield</b>	<b>Biomass</b>	<b>Biomass</b>	
-----Mg ha <sup>-1</sup> -----					
CT	Fallow	8.2 ns	7.7	0.7	10.4
NT	Fallow	8.6	10.0	1.6	11.6
	Ryegrass	8.5	9.2	5.5	14.8
	White oat	8.2	10.0	6.8	16.8
	B. trefoil	8.7	9.8	3.9	13.7

598

599 ns= no significant by analysis of variance at 5% significance level.

600 Biomass input by rice, weed and cover crops were not statistically analyzed because they are only

601 support data for interpretation of *soil-organic-carbon* SOC and *greenhouse-GHG* gases results.

602 **Table 3.** Seasonal methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) emissions, annual accumulation rate  
 603 of SOC, net balance of GHG (net GHG) and yield-scaled GHG in a flooded rice field on a Gleysol  
 604 subjected to winter managements (fallow or cover crops) combined with conventional tillage (CT)  
 605 and no-tillage (NT) in Southern Brazil.

Tillage System	Winter management	CH <sub>4</sub>	N <sub>2</sub> O	SOC	Net GHG	Yield-Scaled GHG
		----- Mg CO <sub>2</sub> eq. ha <sup>-1</sup> yr <sup>-1</sup> -----				Mg CO <sub>2</sub> eq. Mg <sup>-1</sup> grain
CT	Fallow	21.4 a	0.3 a	0.0 b	21.7 a	2.6 a
NT	Fallow	18.1 b	0.1 a	1.7 a	16.5 b	1.9 b
	Ryegrass	16.3 b	0.2 a	1.6 a	14.8 c	1.8 b
	White oat	20.0 a	0.4 a	2.4 a	18.0 b	2.2 b
	B. trefoil	17.0 b	0.2 a	1.7 a	15.4 c	1.8 b

606

607 Different letters in column indicate significant difference (5%)