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# **Tillage and nitrogen fertilization in irrigated maize: key practices to reduce soil CO<sub>2</sub> and CH<sub>4</sub> emissions.**

**Evangelina Pareja-Sánchez<sup>a\*</sup>, Carlos Cantero-Martínez<sup>a</sup>, Jorge Álvaro-Fuentes<sup>b</sup> and Daniel Plaza-Bonilla<sup>a</sup>**

<sup>a</sup>Crop and Forest Sciences Dpt., Associated Unit EEAD-CSIC, Agrotecnio Center. University of Lleida, Av. Alcalde Rovira Roure, 191, 25198 Lleida, Spain.

<sup>b</sup>Departamento de Suelo y Agua, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), POB 13034, 50080 Zaragoza, Spain.

\*Corresponding author: [e.parejasanchez@gmail.com](mailto:e.parejasanchez@gmail.com)

## **1 Abstract**

2 In newly irrigated Mediterranean agroecosystems, the combined effect of tillage  
3 and N fertilization on soil carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) fluxes is at present  
4 poorly understood. The goal of this study was to quantify both soil CO<sub>2</sub> and CH<sub>4</sub>  
5 emissions as well as crop performance under different tillage systems and N fertilization  
6 rates during three maize (*Zea mays* L.) growing seasons (2015-2017) in a semiarid area  
7 converted to irrigated. Three types of tillage (conventional tillage, CT, reduced tillage,  
8 RT, and no-tillage, NT) and three mineral N fertilization rates (0, 200, and 400 kg N ha<sup>-1</sup>  
9 <sup>1</sup>) were compared in a randomized block design with three replications. Weekly soil CO<sub>2</sub>  
10 and CH<sub>4</sub> emissions, soil temperature and gravimetric moisture were measured. Moreover,  
11 maize above-ground biomass, grain yield, and above-ground C-inputs were quantified.  
12 Carbon dioxide emissions ranged from 173 to 4378 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>. No-tillage showed  
13 a greater mean soil CO<sub>2</sub> flux than CT when applying the highest rate of N (400 kg N ha<sup>-1</sup>  
14 <sup>1</sup>). Although some emissions of CH<sub>4</sub> were observed, all treatments acted as net CH<sub>4</sub> sinks

15 during most of the experimental period. A linear multiple relationship between soil CO<sub>2</sub>  
16 fluxes and soil gravimetric moisture (0-5 cm depth) and temperature (10 cm depth) were  
17 found. In the 2015 growing season, greater cumulative CO<sub>2</sub> emissions were found under  
18 NT and RT compared with CT, while in 2016 NT showed the highest values compared  
19 to CT with intermediate values in RT. Differently, in 2017 no differences between tillage  
20 systems were found. When applying N fertilizer, NT and RT increased maize grain  
21 production and above-ground C-inputs compared to CT, since a severe soil crusting  
22 occurred in this last, which caused crop water deficit. The results suggest that tillage  
23 intensity and N fertilization rate reduction can increase maize biomass production and  
24 yield which leads to greater C-input that returns to the soil.

25 **Keywords**

26 Soil greenhouse gases; Irrigated maize; N fertilization; tillage systems; Management  
27 practices; Mediterranean agroecosystem.

## 28 **1. Introduction**

29 Global atmospheric concentrations of CO<sub>2</sub> and CH<sub>4</sub> have markedly increased in  
30 the last decades. Carbon dioxide primarily increased due to fossil fuel burning and land  
31 use change. Meanwhile, CH<sub>4</sub> increased as a result of the production and transport of fossil  
32 fuel as well as the enteric fermentation in livestock, manure management, rice cultivation,  
33 biomass burning, and waste management in the agricultural sector (U.S. EPA, 2012).  
34 About 10–12% of global anthropogenic emissions of greenhouse gases (GHG) are  
35 generated by the agricultural sector (IPCC, 2014). This effect could be greatly mitigated  
36 through a proper choice of crop and land management systems.

37 Tillage practices can affect soil biochemical and physical properties, consequently  
38 influencing the release of CO<sub>2</sub>. In the last decades carbon loss from soils to the  
39 atmosphere as CO<sub>2</sub> has increased due to inappropriate tillage practices (Rakotovao *et al.*,  
40 2017). During intensive tillage operations, like moldboard plowing, soil structure is  
41 greatly disturbed and the CO<sub>2</sub> contained in the soil pore system is lost while releasing  
42 organic C protected within aggregates making it more accessible to decomposers. In  
43 addition, tillage practices can affect soil temperature and soil moisture conditions which  
44 are strongly related to soil CO<sub>2</sub> fluxes (Ren *et al.*, 2007). Meanwhile, the use of  
45 conservation tillage systems, such as reduced tillage (RT) and no-tillage (NT), has been  
46 promoted as a practice to enhance soil carbon levels. The increase in soil organic carbon  
47 (SOC) pool under conservation tillage can be attributed to either an increase in C inputs  
48 from crop residues (Stewart *et al.*, 2016) or a decrease in CO<sub>2</sub> efflux from the soil.  
49 Therefore, the effect of soil C sequestration is particularly crucial for predicting the future  
50 trend of CO<sub>2</sub> concentration in the atmosphere (DeLuca and Zabinski, 2011). This  
51 importance is reflected in recent international initiatives such as the 4 per 1000 which was

52 launched during the COP21 in Paris and promotes the adoption of recommended  
53 management practices aimed to increase SOC sequestration.

54         Soils can act as a source or sink of atmospheric CH<sub>4</sub>, depending on the amount of  
55 moisture, N level and soil microbial community (Gregorich *et al.*, 2005). Tillage  
56 influences the processes that regulate the emission or oxidation of CH<sub>4</sub> through its impact  
57 on the soil water regime. Moisture usually exerts strong control over CH<sub>4</sub> uptake rates,  
58 through soil structure. Therefore, structural degradation, particularly through compaction,  
59 which is a common problem in intensively tilled soils, can adversely affect CH<sub>4</sub>  
60 consumption (Ball *et al.*, 1999).

61         The emissions of CO<sub>2</sub> and CH<sub>4</sub> from soils are also affected by the application of  
62 nitrogen fertilizer (Lee *et al.*, 2007) which may also play a significant role in soil C  
63 sequestration (Lal, 2004). The application of N fertilizer can increase above-ground  
64 biomass production and root respiration, which can lead to soil C storage by increasing  
65 crop residue C input (Al-Kaisi and Yin, 2005). Moreover, it has been reported that the  
66 use of N fertilizer can reduce the uptake of CH<sub>4</sub> by soils (Hütsch, 2001; Bodelier and  
67 Laanbroek, 2004) as a result of the inhibition of CH<sub>4</sub> oxidation activity of methanotrophs  
68 (Reay and Nedwell, 2004).

69         In different rainfed Mediterranean areas, RT and NT techniques have been  
70 introduced during the last two to three decades aimed at reducing costs, saving time and  
71 increase soil water conservation (Lampurlanes *et al.*, 2016). Currently, a significant  
72 fraction of the Mediterranean rainfed areas are being transformed into irrigation, allowing  
73 farmers the cultivation of more productive summer crops such as maize, which require  
74 greater nitrogen fertilizer rates to maximize yields. However, in these newly transformed  
75 irrigated cropping systems, farmers have been induced to return to intensive tillage

76 systems, which are the most common in irrigated Mediterranean cropping systems. In  
77 these areas, the limited previous experience about the implementation of RT or NT  
78 systems constrains the adoption by farmers and puts at risk the soil quality benefits  
79 achieved with the long-term use of NT under former rainfed conditions. Moreover,  
80 conversion from rainfed to irrigation may lead to increases of GHG emissions (Aguilera  
81 *et al.*, 2013) by creating more favorable soil conditions for microbial activity (Calderon  
82 and Jackson, 2002) which may counterbalance the increase in C inputs returned to the  
83 soil negatively affecting the overall C budget.

84         There are many studies measuring soil GHG emissions in both rainfed and  
85 irrigated systems (Meijide *et al.*, 2010; Anapalli *et al.*, 2019), but to our knowledge no  
86 information exists nothing is known about changes in GHG emission due to the  
87 transformation to irrigated after long-term rainfed cropping. Previous research in irrigated  
88 maize production under Mediterranean conditions showed that GHG emissions can be  
89 influenced by N fertilization rates (Álvaro-Fuentes *et al.*, 2016) and tillage systems (Forte  
90 *et al.*, 2017). However, little is known about the combined effects of both management  
91 practices on CO<sub>2</sub> and CH<sub>4</sub> fluxes in soils recently transformed into irrigation. Therefore,  
92 the goal of the present study was to quantify the interactive effects of tillage and N  
93 fertilization rate on soil CO<sub>2</sub> and CH<sub>4</sub> emissions and on the performance of irrigated maize  
94 to identify environmentally sustainable practices.

95

## 96 2. Materials and methods

### 97 2.1 Experimental site and treatments.

98 This study was conducted during three consecutive maize growing seasons (2015,  
99 2016, and 2017) at Agramunt, NE Spain (41°48' N, 1°07' E, 330 m asl). The climate is  
100 semiarid Mediterranean with a continental trend. Mean annual precipitation and  
101 temperature are 401 mm and 14.1°C respectively (data from 1985 to 2015). Mean annual  
102 potential evapotranspiration is 855 mm (data from 1985 to 2015).

103 The study was carried out on the same plots used since 1996 as a rainfed long-  
104 term field experiment which compared three tillage systems (conventional tillage, CT;  
105 reduced tillage, RT; no-tillage, NT) and three increasing rates of mineral N under barley  
106 monoculture (Angás *et al.*, 2006). In 2015, this rainfed experiment was transformed into  
107 irrigation with solid set sprinklers and maize (*Zea mays* L.) monoculture as cropping  
108 system. After the shift from rainfed to irrigation, the field experiment maintained the same  
109 tillage treatments (CT, RT, and NT) while N fertilization rates were adapted to maize (0,  
110 200, and 400 kg N ha<sup>-1</sup>). A total of 27 plots (50x6 m) were arranged in a randomized  
111 complete block design with three replications. The soil was classified as Typic  
112 Xerofluvent (Soil Survey Staff, 2014) and presented a silt loam texture (sand, 30.8%; silt,  
113 57.3%; clay, 11.9%) in the upper (0-28 cm) horizon. The main physico-chemical  
114 properties (0-28 cm soil depth) at the beginning of the experiment (1996) were as follows:  
115 pH (H<sub>2</sub>O, 1:2.5): 8.5; electrical conductivity (1:5): 0.15 dS m<sup>-1</sup>; P Olsen: 35 ppm; K  
116 (Amm. Ac.): 194 ppm; water retention (-33 kPa): 16 g g<sup>-1</sup>; water retention (-1500 kPa):  
117 5 g g<sup>-1</sup>. While soil organic carbon concentration (0-30 cm) was 7, 9 and 9 g kg<sup>-1</sup> under  
118 CT, RT and NT, respectively in 2015.

119           The CT treatment was implemented according to the traditional practices of maize  
120 cultivation in the area. Tillage was performed before planting during March or April,  
121 consisting of one pass of a rototiller to 15 cm depth, followed by one pass of a subsoiler  
122 to 35 cm depth and finally one pass of a disk plough to 20 cm depth with almost 100% of  
123 the crop residues incorporated into the soil. The RT consisted of one pass of a strip-till  
124 implement during April. Finally, in the NT treatment no tillage practices were carried out.  
125 Weeds were controlled by applying a non-selective herbicide (i.e. glyphosate) at 1.5 L ha<sup>-1</sup>  
126 before planting. Planting was performed in April with the use of a pneumatic row direct  
127 drilling machine equipped with double disc furrow openers (model Prosem K, Solà,  
128 Calaf, Spain) in the three tillage systems. Planting depth was adapted to each tillage  
129 treatment. Rotary residue row cleaners were installed to clear the path for the row unit  
130 openers. Maize (cv. Kopias) was planted during the last week of April, in rows 73 cm  
131 apart at a planting density of 90,000 plants ha<sup>-1</sup> in the three years. Nitrogen fertilization  
132 treatments were split in one pre-planting application with urea (46% N) (April) and two  
133 top-dressing applications at the V5 to V10 stages (May and July, respectively) with  
134 calcium ammonium nitrate (27% N) with 50, 75 and 75 kg N ha<sup>-1</sup> applied in each split in  
135 the 200 kg N ha<sup>-1</sup> rate, being doubled in the 400 kg N ha<sup>-1</sup> rate. Mineral P and K  
136 applications were oriented to satisfy crop needs at the same rate for all tillage treatments:  
137 ca. 154 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> yr<sup>-1</sup> and 322 kg K<sub>2</sub>O ha<sup>-1</sup> yr<sup>-1</sup>, in the first two years. In the third year  
138 the levels of available P and K in the soil were appropriate for the crop, making  
139 unnecessary further P and K applications. Irrigation was supplied to meet the estimated  
140 evapotranspiration (ET) of the crop minus the effective precipitation, which was  
141 estimated as 75% of precipitation (when precipitation > 5 mm) (Dastane 1978). Maize  
142 evapotranspiration (ET<sub>c</sub>) was calculated with the corresponding weekly ET values  
143 multiplied by the crop coefficient. Crop coefficients were estimated in accordance with



144 crop development (ranging between 0.3-1.2). The ET was calculated using the Penman-  
145 Monteith equation. Meteorological data were obtained from an automated weather station  
146 located near the experimental site. The amount of water applied by irrigation was 631,  
147 672 and 696 mm in 2015, 2016 and 2017, respectively, during the maize growing period.  
148 Maize grain was harvested at the beginning of November using a commercial combine.  
149 Afterwards, crop residues were chopped and spread over the soil. Weed and pest control  
150 were carried out according to the standard practices in the area.

## 151 *2.2 Measurements of soil CO<sub>2</sub> and CH<sub>4</sub> emissions.*

152 Soil CO<sub>2</sub> and CH<sub>4</sub> emissions were simultaneously measured with the non-steady-  
153 state chamber method (Hutchinson and Mosier, 1981), following the procedure of Plaza-  
154 Bonilla *et al.* (2014). In each plot, two polyvinylchloride rings with a 315-mm-diameter  
155 were inserted 5 cm into the soil. The rings were only removed during tillage, planting and  
156 harvesting operations, allowing a minimum lapse of 24 h following ring rearrangement  
157 at the initial location before any gas sampling to avoid the concomitant effects of soil  
158 disturbance on gas emissions. For the two top-dressing applications, the rings were not  
159 removed since the fertilizer was not incorporated. When the measurements were  
160 performed, polyvinylchloride chambers 20-cm high were fitted into the rings. The  
161 chambers were covered with a reflective insulation layer (model Aislatermic, Arelux,  
162 Zaragoza, Spain). As a sampling port, a metal fitting was attached in the center of the top  
163 of the chamber and was lined with two silicon-Teflon septa. Soil CO<sub>2</sub> and CH<sub>4</sub> fluxes  
164 were measured weekly during the growing season (April to November), with more  
165 intensive samplings during N fertilizer applications (i.e. 24 h. prior and 3 h. and 72 h.  
166 after) and every 21 days in the period between crops in winter (November to March) in  
167 order to quantify the emissions for the entire year. This process was repeated for 3  
168 consecutive years (from April 2015 to November 2017). Gas samples were collected at

169 regular intervals of 0, 20 and 40 minutes after closure of the chamber, using 20 mL  
170 polypropylene syringes and stored into 15 mL borosilicate vials (Exetainer, model 038  
171 W, Labco, High Wycombe, UK). A total of 162 gas samples were collected on every  
172 sampling date (27 plots x 2 observations per plot x 3 sampling times per chamber). The  
173 concentration of CO<sub>2</sub> and CH<sub>4</sub> in the samples was determined using an Agilent 7890A  
174 gas chromatography system equipped with a flame ionization detector (FID) coupled to  
175 a methanizer and three valves in order to obtain the gases of interest for each gas injection.  
176 A HP-Plot Q column (30 m long, 0.32 mm in section and 20 µm thick) was used, with a  
177 15-m long pre-column of the same characteristics. The injector and the oven temperatures  
178 were set to 50°C. The FID and the methanizer were set to 250 and 375°C, respectively.  
179 For the detector, H<sub>2</sub> was used as a carrier gas and N<sub>2</sub> as a make-up gas at a flow of 35 and  
180 25 mL min<sup>-1</sup>, respectively. Soil fluxes of CO<sub>2</sub> and CH<sub>4</sub> were calculated taking into  
181 account the linear accumulation of the gases in the chamber headspace over the closure  
182 period (40 min) and correcting the values for air temperature.

### 183 *2.3 Soil and crop measurements.*

184 During each gas flux sampling, soil temperature was measured using a hand-held  
185 probe (TM65, Crison, Barcelona, Spain) at 10 cm depth. Moreover, soil samples (0-5 cm  
186 depth) were obtained near each chamber. Gravimetric soil moisture was determined by  
187 oven drying each soil sample at 105°C until constant weight. Daily average air  
188 temperature and total rainfall during the study period were collected from an automated  
189 weather station located within 50 m of study site.

190 At harvest, maize plants were cut at the soil level along 2-5 m (depending on plant  
191 density) from the three central rows of each plot. The number of plants and ears was  
192 counted and registered. Afterwards, a sub-sample of two entire plants and five ears was

193 dried at 65°C, weighed and ground. Grain moisture was adjusted to 14% moisture content.  
194 The C content of maize grain and above-ground biomass was determined by dry  
195 combustion (model Truspec CN, LECO, St Joseph, MI, USA).

#### 196 *2.4 Data analysis.*

197 Cumulative emissions of CO<sub>2</sub> and CH<sub>4</sub> were quantified on a mass basis (i.e., kg C  
198 ha<sup>-1</sup>) using the trapezoid rule, differentiating between maize growing seasons (April-  
199 November) and periods between crops in winter (November-March) for the three years.

200 Data analysis was performed with the statistical package JMP 13 (SAS Institute  
201 Inc 2018). Data were checked for normality by plotting a normal quantile plot. A  
202 logarithmic transformation was carried out to normalize soil CO<sub>2</sub> fluxes. An analysis of  
203 variance (ANOVA) was performed with tillage, N fertilization rate, sampling date or year  
204 or period (depending on the variable) and their interaction as effects. Sampling date was  
205 used for analyzing gravimetric soil moisture, daily CO<sub>2</sub> and CH<sub>4</sub> emissions; period was  
206 used for cumulative CO<sub>2</sub> and CH<sub>4</sub> emissions and year was used for above-ground  
207 biomass, grain yield and above-ground biomass C-inputs returned to the soil. When  
208 significant, differences among treatments were identified at 0.05 probability level of  
209 significance with a Tukey HSD test. The possible impact of different predictive variables  
210 on the emission of each of the two gases was tested with the use of multiple regressions,  
211 identifying the variables with a significant effect. To do so, soil CO<sub>2</sub> emissions were  
212 logarithmically transformed.

### 213 **3. Results**

#### 214 *3.1 Weather characteristics during the experimental period.*

215 Air temperature, precipitation and irrigation water during the three years are  
216 presented in Table 1. During the maize growing seasons studied, daily mean air  
217 temperature ranged from 6.5 to 29.1 °C (average of 19.3 °C), from 8.7 to 27.5 °C (average  
218 of 18.8 °C) and 3.1 to 28.8 °C (average of 18.8 °C) in 2015, 2016 and 2017, respectively.  
219 Meanwhile, in the 2015-2016 and 2016-2017 periods between crops in winter, air  
220 temperature ranged from 0.1 to 14.8 °C (average of 7.7 °C) and -3.6 to 16.4 °C (average  
221 of 7.1 °C), respectively. Cumulative rainfall was 226, 151 and 78 mm for the 2015, 2016  
222 and 2017 growing seasons, respectively (Table 1). During the periods between crops in  
223 winter in 2015-2016 and 2016-2017 rainfall was 108 mm and 106 mm, respectively.  
224 Irrigation water applied was 631, 672 and 696 mm for 2015, 2016 and 2017, respectively  
225 (Table 1).

#### 226 *3.2 Soil environment during the experimental period.*

227 Over the experimental period, soil temperature ranged from 1.1 to 27.3°C. Mean  
228 soil temperature during the maize growing seasons 2015, 2016 and 2017 were 18.6, 17.1  
229 and 19.8 °C, respectively. Meanwhile, in the periods between crops in winter 2015-16  
230 and 2016-17 mean soil temperature were 6.9 and 8.7 °C, respectively. During the study,  
231 gravimetric soil moisture was significantly influenced by the interaction between tillage  
232 system and period (Table 2). In the 2015 and 2017 maize growing seasons, mean of  
233 gravimetric soil moisture showed greater values in NT compared to RT and CT, while in  
234 2016 and in the period between crops in winter 2015-2016 gravimetric soil moisture  
235 followed the order NT>RT>CT. In addition, in the period between crops in winter 2016-  
236 2017 NT and RT showed greater gravimetric soil moisture than CT (Fig 1).

### 237 3.3 Soil carbon dioxide and methane emissions.

238 The interaction between tillage and sampling date had a significant effect on soil  
239 CO<sub>2</sub> emissions (Table 2). Greater CO<sub>2</sub> fluxes were observed under NT and RT than under  
240 CT on most sampling dates during maize growing seasons. There was an increase in CO<sub>2</sub>  
241 emissions in the months of July and August, coinciding with the period of maximum  
242 maize growth (Fig. 2a). In the three years of study, the largest differences between tillage  
243 systems occurred just after the second top-dressing N application. Conversely, in the two  
244 periods between crops in winter (2015-2016 and 2016-2017), no differences between  
245 tillage treatments on CO<sub>2</sub> fluxes were found, with an average emission of 420 mg CO<sub>2</sub>-C  
246 m<sup>-2</sup> d<sup>-1</sup> (Fig. 2a). In the control treatment, greater soil CO<sub>2</sub> emissions were observed under  
247 NT (1557 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) compared to CT (855 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) with intermediate  
248 values in RT (1372 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) as an average of the three years covered by the  
249 experiment. When applying 200 kg N ha<sup>-1</sup> greater soil CO<sub>2</sub> emissions were observed in  
250 NT and RT (1401 and 1287 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>, respectively) than in CT (1041 mg CO<sub>2</sub>-  
251 C m<sup>-2</sup> d<sup>-1</sup>). Finally, greater soil CO<sub>2</sub> emissions were observed under NT when applying  
252 400 kg N ha<sup>-1</sup> (1559 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) compared to CT under the same rate application  
253 (1007 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) with intermediate values in RT (1328 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) as an  
254 average of the three years covered by the experiment.

255 Methane fluxes ranged from -1.60 to 1.24 mg CH<sub>4</sub>-C m<sup>-2</sup> d<sup>-1</sup> (Fig. 2b). Although  
256 some emissions to the atmosphere were observed, all treatments acted as net CH<sub>4</sub> sinks  
257 during most of the experimental period. Only tillage and sampling date single effects  
258 significantly influenced CH<sub>4</sub> fluxes (Table 2). Regarding to this, greater uptake of CH<sub>4</sub>  
259 was observed in the three tillage systems compared following the order RT>NT>CT (-  
260 0.29, -0.23 and -0.14 mg CH<sub>4</sub>-C m<sup>-2</sup> d<sup>-1</sup>, respectively). Soil uptake of CH<sub>4</sub> tended to  
261 decrease with increasing fertilizer N rates.

262 *3.4 Soil temperature and gravimetric soil moisture effects on CO<sub>2</sub> emissions.*

263 Soil CO<sub>2</sub> emissions were regulated by the combination of soil temperature (°C) at  
264 10 cm soil depth and gravimetric soil moisture (g 100 g<sup>-1</sup>) at 0-5 cm soil depth ( $P < 0.001$ ,  
265  $r^2=0.53$ , RMSE=0.55), being the two variables significant predictors. The resulting model  
266 was:

267  $\text{Log (soil CO}_2 \text{ emissions)} = \text{Exp} (4.30 + 0.10 * \text{Soil temperature} + 0.06 * \text{Gravimetric soil}$   
268  $\text{moisture})$ .

269 High soil CO<sub>2</sub> fluxes ( $> 1500 \text{ mg CO}_2\text{-C m}^{-2} \text{ d}^{-1}$ ) were observed when soil  
270 temperature at 10 cm depth exceeded 15 °C and gravimetric soil moisture (0-5 cm) was  
271 greater than 20 g 100<sup>-1</sup> (Fig. 3).

272 *3.5 Above-ground biomass and maize grain yield.*

273 Above-ground biomass was significantly affected by the interaction between  
274 tillage system and N fertilization and the interaction between N fertilization and year  
275 (Table 2). In this regard, NT and RT led to greater above-ground biomass when applying  
276 200 kg N ha<sup>-1</sup> compared to the use of the same rate under CT. Meanwhile, the use of 400  
277 kg N ha<sup>-1</sup> led to greater values in NT followed by RT and CT (Fig. 4a). Moreover, in 2016  
278 and 2017, the rate of 200 (18785 and 19230 kg ha<sup>-1</sup>, respectively) and 400 kg N ha<sup>-1</sup>  
279 (20632 and 19348 kg ha<sup>-1</sup>, respectively) showed greater above-ground biomass compared  
280 to the control (12256 and 10005 kg ha<sup>-1</sup>, respectively) without significant differences  
281 between N rates in 2015.

282 The interaction between tillage and N fertilization and their interaction with the  
283 year had a significant effect on maize grain yields (Table 2). In 2015 and 2017, grain  
284 yields were higher under NT (11406 and 9844 kg ha<sup>-1</sup>, respectively) and RT (9548 and

285 9278 kg ha<sup>-1</sup>, respectively) than under CT (5594 and 6478 kg ha<sup>-1</sup>, respectively), without  
286 differences between tillage treatments in 2016. As an average of the three seasons, the  
287 rate of 200 kg N ha<sup>-1</sup> showed greater grain yield under NT compared to CT with  
288 intermediate values in RT. Meanwhile, when applying 400 kg N ha<sup>-1</sup>, NT and RT led to  
289 greater grain yield compared to CT (Fig. 4b).

### 290 *3.6 Cumulative soil CO<sub>2</sub> and CH<sub>4</sub> emissions and above-ground biomass C inputs*

291 Cumulative CO<sub>2</sub> emissions were significantly affected by the interaction between  
292 tillage and period (Table 2). In the 2015 growing season, greater cumulative CO<sub>2</sub>  
293 emissions were found under NT and RT compared with CT. In 2016 NT showed the  
294 highest values compared to CT with intermediate values in RT. Differently, in 2017 no  
295 differences between tillage systems were observed (Fig 5a). Contrarily to CO<sub>2</sub>,  
296 cumulative CH<sub>4</sub> emissions were not significantly affected by any effect (Table 2),  
297 although a trend of greater uptake of CH<sub>4</sub> was observed under RT compared with CT and  
298 NT (Fig 5b).

299 The interaction between tillage and N fertilization and between N fertilization and  
300 year had a significant effect on above-ground biomass C-input (Table 2). In 2016 and  
301 2017 the fertilizer rates of 200 and 400 kg N ha<sup>-1</sup> showed greater values compared to the  
302 control, while in 2015 no significant differences were observed between N rates (Fig 5c).  
303 Moreover, the rates of 200 and 400 kg N ha<sup>-1</sup> led to greater above-ground biomass C-  
304 input in NT (10159 and 11124 kg C ha<sup>-1</sup>, respectively) and RT (9210 and 9003 kg C ha<sup>-1</sup>,  
305 respectively) than in CT (6978 and 6131 kg C ha<sup>-1</sup>, respectively), as an average of the  
306 three years covered by the experiment. Contrarily, no significant differences between  
307 tillage systems on above-ground biomass C-inputs were found in the control treatment.

308

## 309 **4. Discussion**

### 310 *4.1 Tillage and N fertilization rate effects on soil CO<sub>2</sub> emissions.*

311           The combined effects of tillage and N fertilization on soil CO<sub>2</sub> emissions observed  
312 in this study would be related to the impact of these practices on soil environmental  
313 conditions such as soil temperature and soil moisture and on the availability of organic C  
314 due to changes in crop residue production. No-tillage showed higher CO<sub>2</sub> emissions when  
315 applying high rates of N (400 kg N ha<sup>-1</sup>) compared to CT. Although irrigation water  
316 supply was the same for all treatments, NT led to greater soil moisture (being 60% higher  
317 NT than CT). The presence of crop residues on the soil surface under NT enhances soil  
318 water availability compared to CT by reducing soil water evaporation and increasing  
319 infiltration (Lampurlanés *et al.*, 2016). The increase in soil moisture observed in NT was  
320 accompanied by higher CO<sub>2</sub> emissions denoting greater enzymatic activity stimulated by  
321 the greater levels of SOC concentration in NT (being 58% greater in NT compared to CT  
322 (42.2 vs. 17.8 g kg<sup>-1</sup>, respectively in the 0–10 cm layer)) and root respiration in the soil  
323 in this treatment compared to CT. These data show that in spite of the higher CO<sub>2</sub>  
324 emissions in NT, these are compensated by its high capacity for C sequestration. Authors  
325 like Manzoni *et al.* (2012) showed that as soil water availability increases, the metabolic  
326 activity of soil fauna is enhanced, resulting in higher soil heterotrophic respiration,  
327 impacting soil carbon content. The low emissions in CT, even when using high rates of  
328 mineral N, could be due to long-term CT leads to a deterioration of the soil physical  
329 properties. This degradation was due to a lower structural stability, causing soil surface  
330 crusting, which resulted in lower water infiltration (1.70, 2.40 and 3.14 mm h<sup>-1</sup> for CT,  
331 RT and NT, respectively) into the soil profile, compromising plantlet establishment (with  
332 a density of plants 19% and 22% lower in CT compared to RT and NT, respectively) and  
333 leading to plant water stress (Pareja-Sánchez *et al.*, 2017). Under these circumstances, the



334 impact of tillage systems on soil structure played a major role on crop productivity.  
335 Therefore, soil respiration and the amount of C returned to the soil as crop residues were  
336 reduced under CT.

337         The combined effect of increased soil water and a more stable soil structure under  
338 NT and RT had a positive response on maize above-ground biomass and grain yield  
339 production. Accordingly, increased crop residue production may have led to increased  
340 availability of C and increased soil microbial activity impacting soil CO<sub>2</sub> emissions  
341 (Tenesaca and Al-Kaisi, 2015). In the previous barley production under rainfed  
342 conditions in the same field, there was a reduction greater than 50% in microbial biomass  
343 and enzymatic activities in CT compared to NT (Álvaro-Fuentes *et al.*, 2013), which in  
344 turn led to higher SOC sequestration under long-term NT (Morell *et al.*, 2011). This  
345 increase of SOC sequestration in NT was due to the higher C inputs as well as the absence  
346 of soil physical disturbances that benefited the physical storage of carbon in soil  
347 aggregates (Álvaro-Fuentes *et al.*, 2008).

348         Previously, in the same experimental field, in rainfed NT barley conditions  
349 cumulative CO<sub>2</sub> emissions were 37% lower compared to the values found in our study in  
350 irrigated conditions and in CT this difference dropped to 46% (Plaza-Bonilla *et al.*, 2014).  
351 The difference between these studies could probably be attributed to the increase in  
352 heterotrophic and autotrophic respiration taking place under irrigated conditions. In  
353 particular, the N fertilization applied in our experiment led to higher above-ground  
354 biomass and therefore higher heterotrophic respiration rate under NT and RT than in CT.  
355 On the other hand, the increase in autotrophic respiration under NT and RT was caused  
356 by an increase in root respiration which led to significant increase in crop production  
357 (Kou *et al.*, 2008) as well as more organic C available to decomposers. This fact would  
358 explain the higher cumulative CO<sub>2</sub> emissions observed under NT and RT in comparison

359 with CT (38% and 28% higher, respectively). Contrarily, the application of N did not  
360 enhance soil CO<sub>2</sub> emissions under CT. There is no consensus about the impact of N  
361 availability on soil C mineralization with studies showing either a stimulatory or a  
362 suppressive effect (Al-Kaisi and Guzman, 2013). In this line, although the application of  
363 mineral nitrogen generally stimulates CO<sub>2</sub> emissions (Iqbal *et al.*, 2009) throughout the  
364 rise in C inputs, some authors have pointed out that CO<sub>2</sub> fluxes can decrease when using  
365 high N rates, as a consequence of a lower activity of enzymes (DeForest *et al.*, 2004). A  
366 higher soil mineral N availability under CT could have decreased the need for soil  
367 microorganisms to mineralize soil organic matter to obtain the necessary N for growth  
368 and reproduction (Smith *et al.*, 2012) and, as a consequence, reduce soil respiration.

#### 369 *4.2 Temporal dynamics of soil CO<sub>2</sub> emissions.*

370 During the maize growing season, mean annual cumulative CO<sub>2</sub> values obtained  
371 in our study (2256-3255 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>) were within the range of the values reported  
372 by Forte *et al.* (2017) and Guardia *et al.* (2017) in irrigated maize under similar  
373 Mediterranean conditions. However, during the period between crops in winter, soil CO<sub>2</sub>  
374 emissions were reduced (with an average of 655 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>) as a consequence  
375 of the lower air temperatures (Al-Kaisi and Yin, 2005) as well as the absence of root  
376 respiration during this phase.

377 The highest cumulative emissions in 2016 coincided with an increase in above-  
378 ground biomass C-inputs to the soil from the previous campaign. Therefore, cumulative  
379 values would be closely related to the amount of C available for microbial decomposition.  
380 Moreover, an increase in soil CO<sub>2</sub> emissions was observed during July and August in the  
381 three maize growing seasons studied (2015, 2016 and 2017), when the maximum maize  
382 growth rate occurs. During this period (July and August) around 50% of soil respiration

383 is the result of root respiration plus the decomposition of root exudates (Rochette *et al.*,  
384 1999). Additionally, these results during July and August could also be attributed to an  
385 enhancement in soil organic matter mineralization as a result of greater soil surface  
386 temperature and moisture as the multiple linear relationship obtained between these  
387 variables corroborates. The highest CO<sub>2</sub> fluxes occurred when soil temperatures were  
388 greater than 15 °C and gravimetric soil moisture was greater than 20%. This demonstrates  
389 that soil temperature and gravimetric soil moisture regulated CO<sub>2</sub> emissions interactively  
390 (Almagro *et al.*, 2009).

#### 391 *4.3 Tillage and N fertilization effects on CH<sub>4</sub> emissions and temporal dynamics.*

392 In the experiment, significant differences in CH<sub>4</sub> fluxes were only found between  
393 tillage systems. Data showed that CH<sub>4</sub> uptake was lower in CT compared with RT and  
394 NT, as observed by Plaza-Bonilla *et al.* (2014) in the same experiment under previous  
395 rainfed conditions. In this line, Hütsch, (2001) suggested that CH<sub>4</sub> oxidation can be  
396 reduced by tillage due to its effects on gas diffusivity and to long-term damage of  
397 methanotrophic bacterial community. Low CH<sub>4</sub> emissions to the atmosphere were  
398 observed only few days after N fertilization. Respect to this, Hütsch *et al.* (1993) showed  
399 that the addition of mineral N reduces the uptake of atmospheric CH<sub>4</sub> by soils.

400 Soil properties such as temperature, water content, bulk density, and SOC have an  
401 important role in the activity of methanogens and methanotrophs, affecting the sign of  
402 CH<sub>4</sub> emissions (Mitra *et al.*, 2002). In the present study, the soil acted as a net sink of  
403 CH<sub>4</sub> in all treatments, indicating a greater proportion of methanotrophic activity. Other  
404 authors have also reported that CH<sub>4</sub> is primarily consumed by soils under upland field  
405 crops cultivation (Kessavalou *et al.*, 1998; Venterea *et al.*, 2005). About 42% and 49%  
406 higher uptake of CH<sub>4</sub> occurred during the 2015 maize growing season compared to the  
407 2016 and 2017 seasons, respectively. These results could be explained by the lower soil

408 water contents during the previous rainfed management, closer in time in 2015 compared  
409 to 2016 and 2017, which could have favored the methanotrophic communities (von  
410 Fischer and Hedin, 2007). This hypothesis would be supported by the greater amount of  
411 methane uptake by soil found during a barley rainfed season in the same experiment,  
412 which reached average values  $-0.78$  and  $-1.72$  kg CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> under CT and NT,  
413 respectively (Plaza-Bonilla *et al.*, 2014) while in irrigated maize were  $-0.55$  and  $-0.65$  kg  
414 CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> under CT and NT, respectively.

415

416 **Conclusion**

417           This experiment highlights the extent of changes on the dynamics of soil CO<sub>2</sub> and  
418 CH<sub>4</sub> emission in Mediterranean agroecosystems recently transformed into irrigation. In  
419 this study, soil CO<sub>2</sub> emissions were greater when increasing the rate of N in combination  
420 with a tillage reduction as result of enhanced maize growth promoting root respiration  
421 and greater soil organic C availability to decomposers. Differently, under CT an increase  
422 in N fertilization reduced soil respiration. The higher soil mineral N availability under CT  
423 could have decreased the need for soil microorganisms to mineralize soil organic matter.  
424 In general the soil acted as a CH<sub>4</sub> sink in all treatments. However, CH<sub>4</sub> oxidation was  
425 lower in CT compared with RT and NT, which could be the result of the effect of tillage  
426 on gas diffusivity. Compared to a previous period under rainfed barley production, annual  
427 soil CO<sub>2</sub> emissions were 37 to 46% greater, depending on the type of tillage. Contrarily,  
428 CH<sub>4</sub> uptake decreased after the transformation into irrigation, as a result of the wetter soil  
429 conditions which could have affected the methanotrophic communities by reducing soil  
430 CH<sub>4</sub> oxidation. Our study suggests that, in maize-based Mediterranean agroecosystems  
431 recently transformed to irrigation, the reduction in tillage intensity and N fertilization  
432 rates increases soil CO<sub>2</sub> emissions and CH<sub>4</sub> uptake. The higher maize growth and organic  
433 C production under these management practices could lead to soil C storage, reducing the  
434 C footprint of the cropping system.

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567

568 **Figure captions**

569 **Fig. 1** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage)  
570 effects on gravimetric soil moisture at 0-5 cm depth. Values correspond to three  
571 consecutive maize growing seasons (2015, 2016 and 2017) and periods between crops in  
572 winter (PC 2015-2016 and PC 2016-2017). Different lowercase letters indicate significant  
573 differences between tillage treatments for a given period at  $P < 0.05$ . Vertical bars indicate  
574 standard deviation.

575 **Fig. 2** Soil CO<sub>2</sub> (a) and CH<sub>4</sub> (b) emissions as affected by tillage (CT, conventional tillage;  
576 RT, reduced tillage; NT, no-tillage) during the 2015, 2016 and 2017 maize growing  
577 seasons and two periods between crops in winter (2015-2016 and 2016-2017). Arrows  
578 indicate dates of N fertilizer application. For a given date, asterisks indicate significant  
579 differences between tillage systems at  $P < 0.05$ .

580 **Fig. 3** Effects of soil temperature at 10 cm depth and gravimetric soil moisture (0-5 cm  
581 depth) on CO<sub>2</sub> fluxes. Values correspond to three consecutive maize growing seasons  
582 (2015, 2016 and 2017) and two periods between crops in winter (2015-2016 and 2016-  
583 2017). Each point corresponds to the average of the different treatments for a given  
584 sampling date ( $n = 102$ ).

585 **Fig. 4** Above-ground maize biomass (a) and grain yield (b) as affected by tillage system  
586 (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and N fertilization rate (0,  
587 200 and 400 kg N ha<sup>-1</sup>). Values correspond to the means of three consecutive maize  
588 growing seasons (2015, 2016 and 2017). Different lowercase letters indicate significant  
589 differences between tillage systems for a given N fertilization rate at  $P < 0.05$ . Vertical  
590 bars indicate standard deviation.

591 **Fig. 5** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage)  
592 effects on cumulative soil CO<sub>2</sub> (a), and CH<sub>4</sub> emissions (b) and year (2015, 2016 and 2017)  
593 effects on above-ground biomass C-inputs (c). Values correspond to three consecutive  
594 maize growing seasons (2015, 2016 and 2017) and two periods between crops in winter  
595 (PC 2015-2016 and PC 2016-2017). Different lowercase letters indicate significant  
596 differences between tillage treatments for a given period (a and b) and significant  
597 differences between years for a given N fertilization rate (c) at P< 0.05. Vertical bars  
598 indicate standard deviation.

599

600 **Table 1** Mean monthly air temperature (T) (°C) monthly precipitation (P) (mm) and monthly irrigation (I) (mm) in the field experiment during three consecutive maize growing  
 601 seasons (2015, 2016 and 2017) and periods between crops in winter (PC 2015-2016 and PC 2016-2017).

602

Month	Maize 2015			PC 15-16			Maize 2016			PC 16-17			Maize 2017		
	T	P	I	T	P	I	T	P	I	T	P	I	T	P	I
April	13.4	15.0	9.8	-	-	-	12	78.0	5.2	-	-	-	12.5	0.0	0.0
May	18	1.0	119.2	-	-	-	15.4	60.0	55.8	-	-	-	17.7	0.0	42.6
June	21.7	24.0	72.7	-	-	-	20.6	1.0	134.1	-	-	-	22.6	0.0	151.8
July	25.4	25.0	232.6	-	-	-	23.7	0.0	222.3	-	-	-	24	3.0	204.8
August	22.9	36.0	178.1	-	-	-	22.8	5.0	246.8	-	-	-	23.6	12.0	244.1
September	17.5	98.0	20.3	-	-	-	19.9	4.0	52.0	-	-	-	17.3	22.0	52.6
October	14	2.0	0.0	-	-	-	14.4	39.0	0.0	-	-	-	15	26.0	0.0
November	8.9	26.0	0.0	-	-	-	8.4	41.0	0.0	-	-	-	8.8	15.0	0.0
December	-	-	-	5.8	0.0	0.0	-	-	-	3.6	3.0	0.0	-	-	-
January	-	-	-	6.5	2.0	0.0	-	-	-	2.9	14.0	0.0	-	-	-
February	-	-	-	7.1	37.0	0.0	-	-	-	7.6	12.0	0.0	-	-	-
March	-	-	-	8.4	30.0	0.0	-	-	-	10.4	44.0	0.0	-	-	-

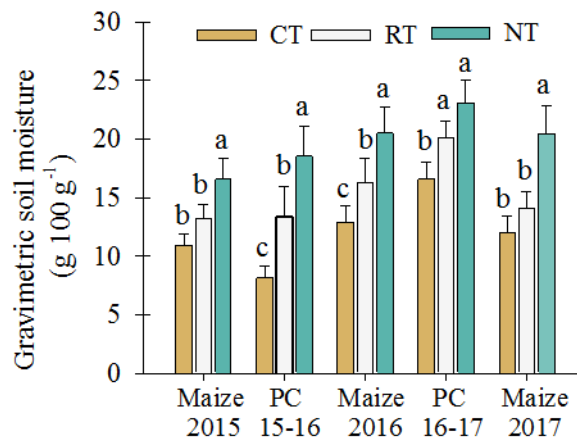
603 **Table 2** Analysis of variance (*P*-values) of gravimetric soil moisture, soil CO<sub>2</sub> flux, soil CH<sub>4</sub> flux and cumulative CO<sub>2</sub> and CH<sub>4</sub> emissions, above-ground biomass, grain yield  
604 and above-ground biomass C inputs for each maize growing season (2015, 2016 and 2017) and periods between crops in winter (2015-2016 and 2016-2017), as affected by  
605 tillage, N fertilization, date/year/period and their interaction.

Source of variation	Gravimetric soil moisture	CO <sub>2</sub> flux	CH <sub>4</sub> flux	Cumulative CO <sub>2</sub> emissions	Cumulative CH <sub>4</sub> emissions	Above-ground biomass	Grain yield	Above-ground biomass C inputs
<b>Tillage (Till)</b>	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.001	<0.001
<b>N fertilization (Fert)</b>	ns	ns	ns	ns	ns	<0.001	<0.001	<0.001
<b>Date/Year/Period</b>	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.001	<0.001
<b>Till*Fert</b>	ns	ns	ns	ns	ns	0.009	<0.001	0.01
<b>Till*Date/Year/Period</b>	<0.001	<0.001	ns	<0.001	ns	ns	0.01	ns
<b>Fert*Date/Year/Period</b>	ns	ns	ns	ns	ns	0.04	<0.001	<0.001
<b>Till*Date/Year/Period*Fert</b>	ns	ns	ns	ns	ns	ns	ns	ns

ns, non-significant



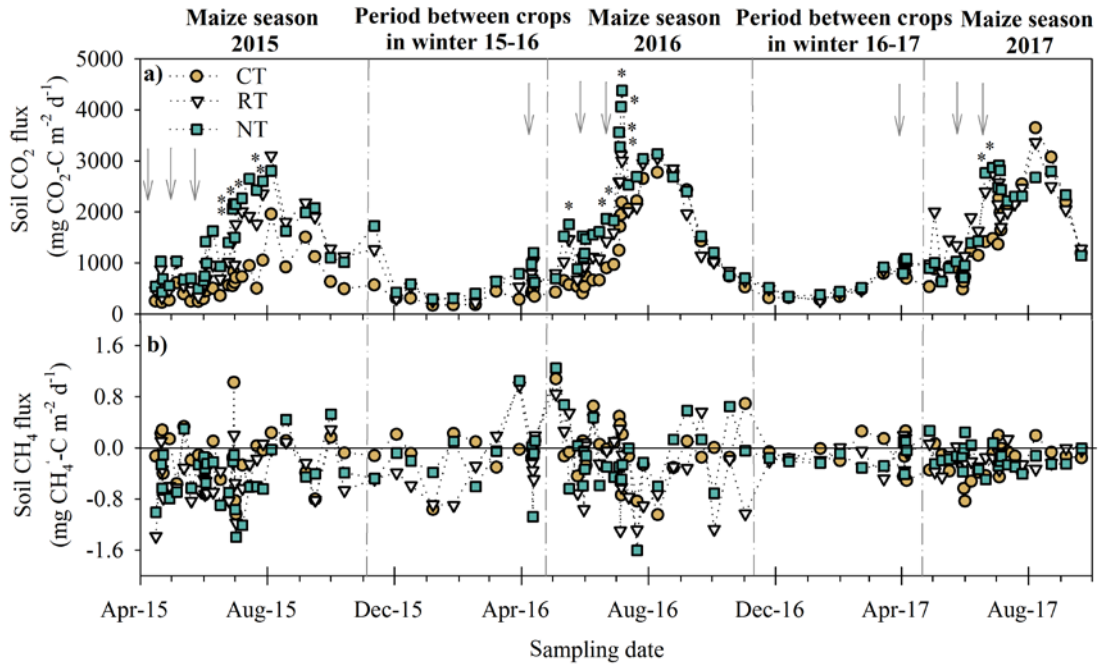
606 **Fig. 1** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) effects on gravimetric  
 607 soil moisture at 0-5 cm depth. Values correspond to three consecutive maize growing seasons (2015, 2016  
 608 and 2017) and periods between crops in winter (PC 2015-2016 and PC 2016-2017). Different lowercase  
 609 letters indicate significant differences between tillage treatments for a given period at  $P < 0.05$ . Vertical bars  
 610 indicate standard deviation.



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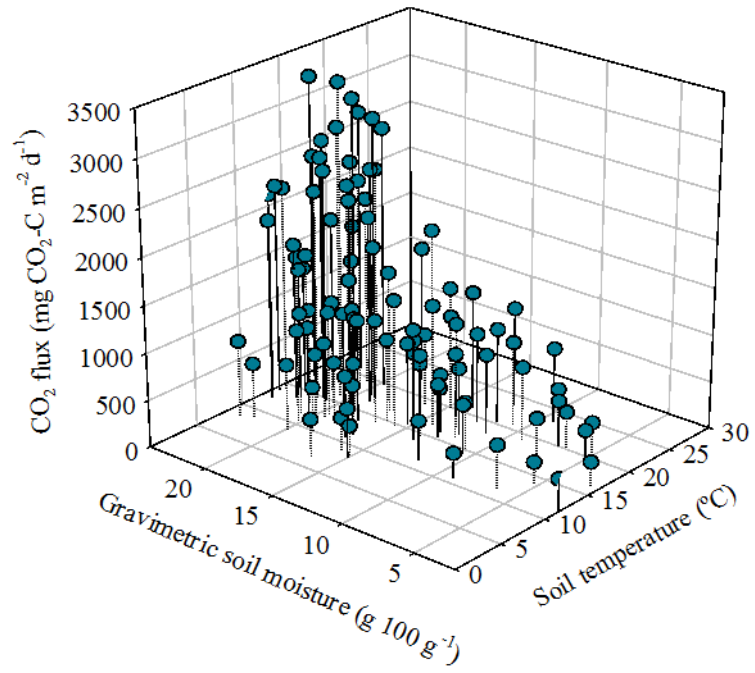
613 **Fig. 2** Soil CO<sub>2</sub> (a) and CH<sub>4</sub> (b) emissions as affected by tillage (CT, conventional tillage; RT, reduced  
 614 tillage; NT, no-tillage) during the 2015, 2016 and 2017 maize growing seasons and two periods between  
 615 crops in winter (2015-2016 and 2016-2017). Arrows indicate dates of N fertilizer application. For a given  
 616 date, asterisks indicate significant differences between tillage systems at  $P < 0.05$ .



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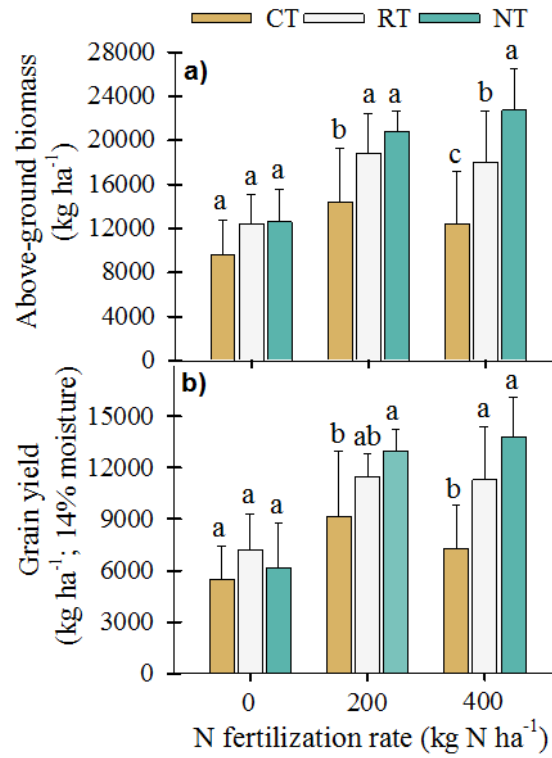
619 **Fig. 3** Effects of soil temperature at 10 cm depth and gravimetric soil moisture (0-5 cm depth) on CO<sub>2</sub>  
620 fluxes. Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and two  
621 periods between crops in winter (2015-2016 and 2016-2017). Each point corresponds to the average of the  
622 different treatments for a given sampling date (n = 102).



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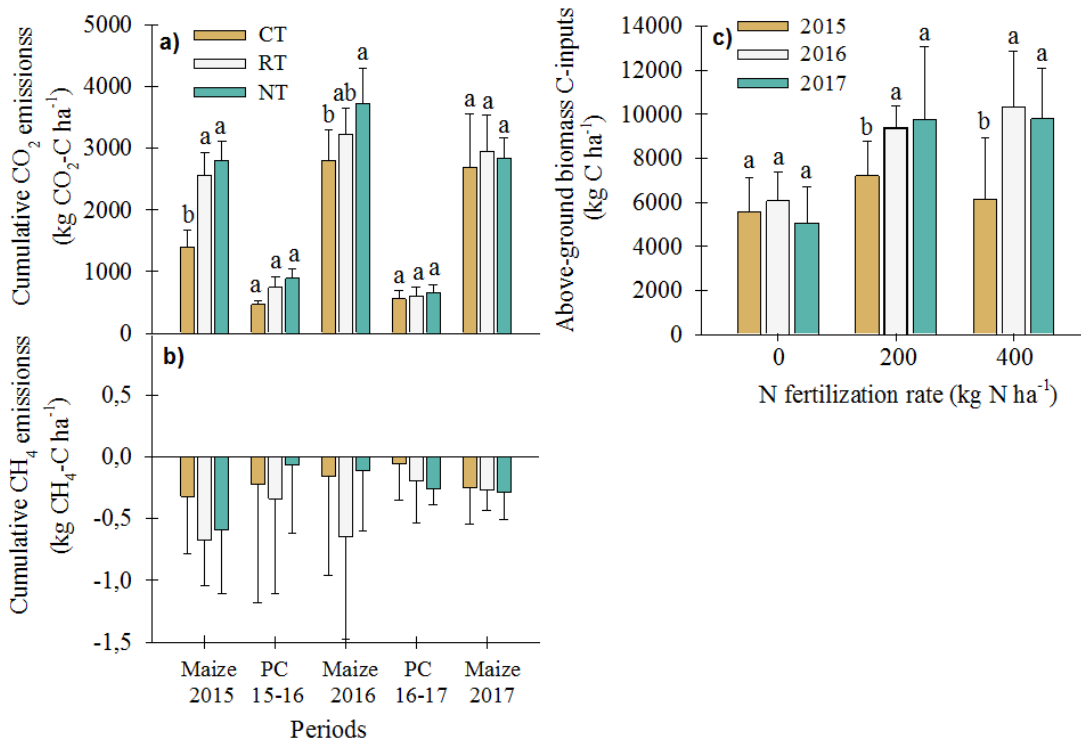
625 **Fig. 4** Above-ground maize biomass (a) and grain yield (b) as affected by tillage system (CT, conventional  
 626 tillage; RT, reduced tillage; NT, no-tillage) and N fertilization rate (0, 200 and 400 kg N ha<sup>-1</sup>). Values  
 627 correspond to the means of three consecutive maize growing seasons (2015, 2016 and 2017). Different  
 628 lowercase letters indicate significant differences between tillage systems for a given N fertilization rate at  
 629  $P < 0.05$ . Vertical bars indicate standard deviation.



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631

632 **Fig. 5** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) effects on cumulative  
 633 soil CO<sub>2</sub> (a), and CH<sub>4</sub> emissions (b) and year (2015, 2016 and 2017) effects on above-ground biomass C-  
 634 inputs (c). Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and two  
 635 periods between crops in winter (PC 2015-2016 and PC 2016-2017). Different lowercase letters indicate  
 636 significant differences between tillage treatments for a given period (a and b) and significant differences  
 637 between years for a given N fertilization rate (c) at  $P < 0.05$ . Vertical bars indicate standard deviation.



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