Soil organic carbon sequestration when converting a rainfed Mediterranean barley-based cropping system to irrigated corn under different tillage systems and N fertilizer rates

Evangelina Pareja-Sánchez^{1*}, Carlos Cantero-Martínez¹, Jorge Álvaro-Fuentes², Daniel Plaza-Bonilla¹

¹Crop and Forest Sciences Dpt., Associated Unit EEAD-CSIC, Agrotecnio Center. University of Lleida, Av. Alcalde Rovira Roure, 191, 25198 Lleida, Spain.

²Departamento de Suelo y Agua, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), POB 13034, 50080 Zaragoza, Spain.

*Corresponding author <u>e.parejasanchez@gmail.com</u>

1 Core Ideas

- 2[•] C-inputs are an important factor explaining soil organic carbon (SOC) sequestration rates.
- **3** Reduced and no-tillage increased C-inputs at the highest N-fertilizer rate.
- 4• No-tillage significantly increased SOC sequestration at the highest N-fertilizer rate.
- 5 Conversion from a rainfed to an irrigated cropping system resulted in an increase SOC
- 6 sequestration rate from 222 to 969 kg C ha⁻¹ year⁻¹.
- 7 Particulate organic carbon was the indicator that best explained SOC increase after the
- 8 conversion from a rainfed to an irrigated cropping system.

9 Abstract

10 The aim of this study was to evaluate the impact of 21 years of tillage and N fertilization and the conversion from a rainfed to an irrigated cropping system on soil organic C (SOC). 11 The study was carried out in NE Spain in a long-term tillage and N rate field experiment 12 established in 1996 under barley rainfed conditions which in 2015 was converted into 13 14 irrigation with corn. Three types of tillage (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) and three mineral N fertilization rates (0, 60 and 120 kg N ha⁻¹ under barley, 15 and 0, 200, and 400 kg N ha⁻¹ under corn) were compared. Annual C-inputs as above-16 ground crop residues and annual SOC sequestration rate (Δ SOC_{rate}) (0-40 cm depth) were 17 18 calculated in three different periods (P1, P2 and P3) under rainfed (-R) and irrigated (-I) 19 conditions (P1-R, from 1996 to 2009; P2-R, from 2009 to 2015; P3-I, from 2015 to 2017). At the end of P3-I, particulate organic C (POC) was measured from the 0-5, 5-10, 10-20, 20 20-30 and 30-40 cm depths. Averaged over all treatments, Δ SOC_{rate} was 492, 222 and 969 21 kg C ha⁻¹ year⁻¹ for P1-R, P2-R and P3-I, respectively. In P1-R and P3-I, C-input explained 22 70% of the variability of Δ SOC_{rate}. In P1-R, Δ SOC_{rate} followed the order NT > RT > CT, 23 while for N rate, order was high > medium > 0. In P3-I at the highest N rate, ΔSOC_{rate} 24 followed the order NT>RT>CT. In P2-R, ΔSOC_{rate} did not show differences between tillage 25 and/or N rate treatments. The increase in SOC after conversion from a rainfed to an 26 irrigation system was mainly explained by POC, which was increased by 75% compared 27 28 to the previous rainfed period. The modification of the cropping system through the introduction of irrigation and adequate crop management practices under no-tillage and 29 adjusted N fertilizer rates can contribute to the sequestration of large amounts of 30 31 atmospheric CO₂.

32 Abbreviations

- $\Delta SOC_{rate,}$ annual soil organic carbon sequestration rate; CT, conventional tillage; NT, no
- tillage; P1-R, P2-R, P3-I, periods under rainfed (-R) and irrigated (-I) conditions; RT,
- 35 reduced tillage.

36 1 Introduction

37 In arid and semiarid regions, as is the case in Mediterranean areas, crop productivity is severely limited by water availability (Apesteguía et al., 2015; Cantero-Martínez, Angás, 38 & Lampurlanés, 2007). The main production system in the rainfed Mediterranean areas has 39 40 been the monoculture cropping of winter cereals (primarily barley and wheat), whose yield is highly dependent on seasonal rainfall and water storage capacity of the soil (Austin et 41 al., 1998). During the last three decades, in many areas these rainfed cropping systems are 42 43 characterized by the use of conservation agriculture, which involves reduced and no-tillage (RT and NT, respectively), as well as a lower application of fertilizer N than in irrigated 44 cropping systems. Conversely, corn is one of the most important field crops in irrigated 45 46 cropping systems, characterized by the use of high rates of N fertilization and intensive tillage (CT) systems. Currently, in the Mediterranean region, there is an increasing 47 conversion from rainfed to irrigated cropping systems. This conversion generates 48 49 significant consequences in agroecosystems, since the possibility of irrigation leads farmers to modify crop rotations and management practices, opting for more productive 50 crops and changing the management of the cropping system (type of tillage, fertilization 51 rates, control of biotic stresses, etc.). The conversion from rainfed to irrigated cropping 52 systems could directly affect soil organic carbon sequestration (SOC). Different 53 mechanisms could affect SOC via the modification of the cropping system, as for example, 54 55 changing water availability in agricultural soils would lead to greater microbial activity enhancing C decomposition (Gillabel et al., 2007). Soil organic carbon could also be 56 57 influenced by the amount of crop residues returned to the soil, being more abundant in irrigated than in rainfed cropping systems. The greater contribution of crop residues would 58 increase the SOC, since it is known that both variables are directly related (Dimassi et al., 59 60 2014). In addition, changing the type of crop could also modify the quality of crop residues

and their composition (e.g. ratios C:N, lignin:N). It is widely accepted that the C:N ratio 61 62 influences the decomposition rate of organic materials. Thus, a material with lower C:N ratio (such as legumes residues) being known as a more labile material, is easily 63 decomposed by the microorganisms than higher C:N ratio organic material (such as cereal 64 residues) (Manzoni, Jackson, Trofymow, & Porporato, 2008). After the transformation of 65 a cropping system, changes in N fertilization rates may also affect SOC dynamics. In this 66 67 regard, Mulvaney, Khan, & Ellsworth, (2009) showed that high fertilizer N rates accelerates SOC mineralization. However, Russell, Laird, Parkin, & Mallarino (2005) 68 found no difference in SOC with N fertilization rate. In addition, N fertilization may also 69 70 increase SOC stocks, since an increase in crop productivity can lead to greater C-inputs 71 returned to the soil (Luo, Wang, & Sun, 2010). However, N fertilizer addition can also 72 increase C losses to the environment by reducing the soil C:N ratio, enhancing microbial 73 activity and C mineralization (Li et al., 2009; Patrick et al., 2013; Mahal et al., 2019). Likewise, the type and intensity of the tillage operation used by farmers varies in irrigated 74 75 cropping systems compared to rainfed ones, influencing the stability of soil aggregates and the release of organic matter (Grandy & Robertson, 2006). 76

77 Changes in SOC levels are not immediately detectable after a change in agricultural management has occurred. For this reason, long-term experiments are essential to study the 78 impact of agricultural management on SOC changes. Indeed, the identification and 79 80 quantification of key C fractions sensitive to changes in management practices and land uses may help to elucidate the mechanisms of C sequestration in agroecosystems (Plaza-81 82 Bonilla, Álvaro-Fuentes, & Cantero-Martínez, 2014). For example, Álvaro-Fuentes, Cantero-Martinez, & Arrue (2008) found that under semiarid conditions, particulate 83 organic matter (POM) and mineral-associated C (C-Min) increased under NT compared to 84 85 CT. They argued that the increase in soil surface (0-5cm) SOC under NT compared with

CT was due to the more humified and recalcitrant nature of the C-Min fraction. Greater 86 87 SOC accumulation as C-Min implies its stabilization in the long term in NT compared with CT. Therefore, an optimal use of different agronomic practices (e.g. soil tillage, N 88 fertilization, etc.) may boost C sequestration in the soil (West & Post, 2002). In semiarid 89 Mediterranean rainfed conditions, several studies have shown significant increases of SOC 90 stocks after the adoption of conservation tillage. For example, Álvaro-Fuentes *et al.* (2009) 91 92 and Hernanz, Sánchez-Girón, & Navarrete (2009) demonstrated that continuous NT leads to SOC sequestration with rates ranging from 0.40 to 0.50 t C ha⁻¹ yr⁻¹. 93

94 Irrigated systems in combination with conservation agriculture practices like NT or
95 RT are assumed to have a larger potential to increase SOC contents than irrigation in
96 combination with CT (Martens, Emmerich, Mclain, & Johnsen, 2005). For instance,
97 Halvorson, Reule, & Mosier (2004) observed an increase in SOC under NT in irrigated
98 continuous corn systems compared to CT although both tillage treatments (NT and CT)
99 produced similar levels of crop residues. Greater SOC decomposition under CT would
100 explain that result.

Currently, long-term NT and other conservation practices such as the maintenance 101 102 of permanent soil cover, crop rotation or the diversification of plant species, are recommended to maintain or increase SOC stocks in rainfed areas (Plaza-Bonilla et al., 103 104 2015) but less is known in irrigated cropping systems, particularly in Mediterranean areas. 105 In addition, there is little information about the change that could occur in carbon 106 sequestration rates when converting from rainfed cropping systems to irrigated. Halvorson, 107 Reule, & Mosier (2004) reported that the annual rate of SOC sequestration increased (1.4 Mg SOC ha⁻¹ yr⁻¹, 0-15 cm depth) with increasing N rate under NT in irrigated continuous 108 corn in comparison to CT which had a rate of 0.2 Mg C ha⁻¹ yr⁻¹ in Colorado (USA). 109 Therefore, there is a need to identify the most beneficial combined management practices 110

to enhance SOC sequestration when converting rainfed cropping systems into irrigatedones.

113	This study was conducted using a long-term tillage and N fertilization experiment
114	established in 1996 in rainfed conditions which was converted in 2015 into irrigation. In a
115	first rainfed period, Morell et al. (2011) already observed a positive effect of NT in SOC.
116	This study is a step forward in which we evaluated (i) 21 years impact of tillage and N
117	fertilization and (ii) the conversion of rainfed into irrigated systems on SOC.

118 2 Materials and methods

119 *2.1. Site and treatments description.*

An ongoing long-term experiment on soil tillage and N fertilization rates was established in 1996 under rainfed conditions at Agramunt, Catalonia, NE Spain (41°48' N, 1°07' E, 330 m asl). The extended description of the field experiment including soil and climate conditions is described in Cantero-Martínez, Angás & Lampurlanés (2003). The Mediterranean field site is located in a semi-arid continental climate. Mean annual precipitation, potential evapotranspiration, and temperature are 401 mm, 855 mm, and 14.1°C, respectively (data from 1985 to 2015).

127 The experiment compared three tillage systems (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) and three increasing rates of mineral N (0, 60 and 120 kg N ha⁻ 128 ¹) under barley (*Hordeum vulgare* L.) monoculture. In 2015, the rainfed experiment was 129 130 converted to sprinkler irrigation and monoculture corn (Zea mays L.) monoculture as cropping system. After the shift from rainfed to irrigation, the field experiment maintained 131 the same tillage intensities (CT, RT, and NT) while N fertilization rates were adapted to 132 corn needs (0, 200, and 400 kg N ha⁻¹) (Table 1). A total of 27 plots (50x6 m) were arranged 133 in a randomized complete block design with three replications. The soil was classified as 134 Typic Xerofluvent (Soil Survey Staff, 2014) and had a silty clay loam texture (sand, 30.8%; 135 silt, 57.3%; clay, 11.9%) in the upper (0-28 cm) horizon. Other main physico-chemical 136 properties (0-28 cm soil depth) at the beginning of the experiment (1996) were as follows: 137 pH (H₂O, 1:2.5): 8.5; electrical conductivity (1:5): 0.15 dS m⁻¹; soil organic carbon 138 concentration 9 g kg⁻¹; Olsen P: 35 mg kg⁻¹; K (ammonium acetate): 194 mg kg⁻¹; water 139 retention (-33 kPa): 16 kg kg⁻¹; water retention (-1500 kPa): 5 kg kg⁻¹. Crop management 140 practices were conducted following the local traditional practices (Table 1). After 2015, 141

irrigation was supplied to meet the estimated evapotranspiration (ET) of corn minus the effective precipitation, which was estimated as 75% of precipitation (when precipitation > 5 mm) (Dastane, 1978). Corn evapotranspiration (ETc) was calculated with the corresponding weekly reference ET values multiplied by the crop coefficient. Crop coefficients were estimated in accordance with crop development (ranging between 0.3 and 1.2). The ET was calculated using the Penman-Monteith equation. Meteorological data were obtained from an automated weather station located near the experimental site.

149 2.2 Soil sampling and analysis.

150 During the rainfed period (1996 to 2015), soil samples were collected in September 1996, after crop harvest in July 2009, and before the conversion from rainfed to irrigated 151 152 conditions and corn planting in March 2015. The last sampling was carried out in 153 November 2017 after corn harvest. All samples were analyzed in the corresponding year 154 of extraction. However, the samples from 2009, 2015 and 2017 were analyzed by the same 155 technician, while those from 1996 were analyzed by a different technician. The same methodology and the same laboratory were used in all samples. There was an exception to 156 the same methodology as the 1996 samples were treated differently. Nevertheless, in 1996 157 158 the Walkley and Black (Nelson & Sommers, 1982) method was followed without external heating and later a coefficient of 1.33 was applied according to a previous calibration. Soil 159 160 samples were always collected at 0-5, 5-10, 10-20, 20-30, and 30-40 cm soil depths in 161 two samples per plot for the dates described. In each plot and depth, soil bulk density was 162 measured in 2009 (Morell et al., 2011). For C analyses, the soil was air dried and ground to pass a 2-mm sieve. The total SOC content was measured by the wet oxidation method 163 164 of Walkley and Black with a 1 g subsample (Nelson & Sommers, 1982). SOC contents 165 were calculated on a mass per unit area basis by multiplying the C concentration values 166 obtained from the oxidation method by the corresponding soil bulk density values.

Moreover, the SOC stock (kg C ha⁻¹) was corrected in terms of equivalent soil mass 167 168 following the procedure of Ellert & Bettany (1995) for the 0 to 40 cm soil depth interval. The annual SOC sequestration rate (Δ SOC_{rate}) (kg C ha⁻¹ year⁻¹) (0-40 cm soil depth) was 169 calculated for each treatment in three different periods: from 1996 to 2009 (P1-R), from 170 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I). The period 1996-2009 (P1-R) was 171 previously reported by Morell et al. (2011). Our intention was to compare these data 172 173 corresponding to rainfed conditions with the new data obtained under irrigated conditions. 174 Furthermore, at the beginning and at the end of the period under irrigation (2015 and 2017), 175 permanganate-oxidizable organic C (POxC), particulate organic carbon (POC) and mineral-associated organic carbon (C-Min) were also measured (0-5, 5-10, 10-20, 20-30, 176 and 30–40 cm depths). These C fractions were chosen since they were identified as highly 177 sensitive to changes in management practices in our semiarid areas (Plaza-Bonilla, Álvaro-178 Fuentes, & Cantero-Martínez, 2014). C-Min and POC were isolated using a physical 179 180 fractionation method adapted from Cambardella & Elliot (1992). Briefly, twenty-gram subsamples of soil from each depth and plot were dispersed in 100 mL of 5 g L^{-1} sodium 181 hexametaphosphate for 15 h on a reciprocal shaker. Then, the samples were passed through 182 183 a 50-µm sieve to separate the POC and C-Min. The material passing through the sieve (C-184 Min) was collected in aluminium pans and oven dried at 50°C. The Walkley and Black wet oxidation method was then used to measure the C concentration in the C-Min fraction. The 185 186 POC content was determined as the difference between total SOC content and C-Min content. POxC was quantified according to the method of Weil et al. (2003). Briefly, 2.5 g 187 of air-dried soil were weighed into polypropylene 50 mL centrifuge tubes. To each tube, 188 18 mL of deionized water and 2 mL⁻¹ of 0.2 M KMnO₄ stock solution were added and tubes 189 were shaken for exactly 2 min on an oscillating shaker. Tubes were removed from the 190 shaker and allowed to settle for exactly 10 min. After 10 min, 0.5 mL of the supernatant 191

were transferred into a second 50 mL centrifuge tube and mixed with 49.5 mL of deionized water. An aliquot (200 μ L) of each sample was loaded into a well plate containing a set of internal standards, including a blank of deionized water, four standard stock solutions (0.005, 0.01, 0.015, and 0.02 M L⁻¹ KMnO₄), a soil standard and a solution standard (laboratory reference samples). All internal standards were analytically replicated on each plate. Sample absorbance was read with a spectrophotometer at 550 nm. POxC was calculated as Weil *et al.* (2003):

199 POxC (mg kg⁻¹soil) = $(0.02 \text{ mol } \text{L}^{-1} - (a + b \text{ x } (\text{Abs})) \text{ x } (9000 \text{ mg } \text{C mol}^{-1}) (0.02 \text{ L solution}$ 200 x W⁻¹)

where a is the intercept and b is the slope of the calibration obtained with the standards,Abs is the absorbance of the sample and W is the weight (kg) of the soil used.

203 *2.3 Carbon inputs.*

In the rainfed period, barley above-ground biomass was determined just before 204 205 harvest (end of June – beginning of July). Three samples per plot were taken by cutting the plants at the soil surface level on 50 cm along the rows. In the irrigated period, corn above-206 207 ground biomass was determined in late-October right before harvest. Samples were taken by collecting plants of two central rows 2-5 m long, depending on plant density, in three 208 209 sampling areas per plot. In 2015, a 5 m sampling length was taken in all treatments due to 210 the low plant density in CT. In 2016 and 2017, the length of the sampling row was 2 m. 211 Barley and corn above-ground biomass was oven-dried at 60°C for 48 h, threshed and weighed excluding the grain, hereafter referred as crop residues. The C content of barley 212 213 and corn crop residues was determined by dry combustion (model Truspec CN, LECO, St Joseph, MI, USA). Afterwards, C inputs were calculated by multiplying the crop residues 214 215 biomass by their C concentration.

217 Statistical analyses were performed with the statistical package JMP 13 (SAS 218 Institute Inc, 2018). Data were checked for normality with the Shapiro-Wilk Test. All data 219 complied with normality. Measured $\triangle SOC_{rate}$ and C-input were statistically tested with 220 analysis of variance (ANOVA), which was performed for each period (P1-R, P2-R and P3-I) with tillage, N fertilization and their interaction as fixed effects and block as random 221 222 effect. Meanwhile, an ANOVA of POxC, POC and C-Min was performed with block and 223 soil depth as the repeated measure with tillage, N fertilization, year, and their interaction as fixed effects. Since the interaction of year with the rest of effects was non-significant, a 224 225 separate ANOVA was finally carried out for each year (2015 and 2017). Statistical analysis 226 of these fractions was performed on C-Min and POC concentration in table 5 and figure 4 whereas analysis on equivalent mass was included in table 4. When significant, differences 227 228 among treatments were identified at 0.05 probability level of significance with a Tukey HSD test. Simple regression analyses were performed with the statistical package JMP 13 229 (SAS Institute Inc, 2018) to test the presence of relationships between ΔSOC_{rate} and C-230 input for each period (P1-R, P2-R and P3-I). 231

233 **3 Results**

3.1. Weather characteristics during the experimental period.

235 Monthly rainfall, potential evapotranspiration, air temperature and irrigation for 236 each year are presented in Fig. 1. The annual precipitation was highly variable during the 237 study period, ranging between 183 and 645 mm in 2017 and 1997, respectively. Years 1998, 2001, 2004, 2005, 2006, 2007, 2011 and 2012 were characterized by a long drought 238 which affected the autumn soil water recharge period (September-December). Conversely, 239 240 1996, 1997, 2002, 2003, 2009 and 2010 were characterized by 25 to 60% more rainfall 241 than the 30-yr average (i.e. 401 mm). During the irrigated phase, the water deficit was 242 offset by irrigation, with a supplemental irrigation water of 631, 672 and 696 mm in 2015, 243 2016 and 2017, respectively, during the corn growing season (April to September).

The yearly air temperature followed the typical Mediterranean pattern with hot summers, with air temperatures above 35 °C, and the winters mild to cold.

246 *3.2. Crop C-inputs and soil organic carbon stock in each period.*

Crop residue C-inputs were significantly affected by the interaction between 247 tillage system and N fertilization under rainfed conditions (P1-R and P2-R). However, in 248 249 P3-I only tillage and N fertilization main effects (without interaction) significantly 250 influenced C-inputs (Table 2). According to the interaction between tillage systems and N 251 fertilization, in P1-R and P2-R, C-inputs did not show differences among tillage systems 252 when no N fertilizer was applied, while NT showed large C-inputs compared to RT and CT when applying medium N fertilization rates. Moreover, in P1-R, NT showed greater C-253 254 inputs compared to RT and CT when applying the high rate of N fertilization. In P2-R, NT and RT showed larger C-inputs compared to CT when applying the high rate of N 255 256 fertilization (Table 2). In P3-I, averaged across rates of N fertilization, greater C-inputs

were observed under NT compared to RT and CT (3255, 2630 and 2213 kg C ha⁻¹ yr⁻¹,
respectively) (Table 2). Furthermore, in P3-I C- inputs (averaged across tillage systems)
were greater for the high N fertilization rate (2968 C ha⁻¹ yr⁻¹) than the control (2378 C ha⁻¹ yr⁻¹) in whereas medium rate had intermediate values (2752 C ha⁻¹ yr⁻¹) (Table 2).

261 Soil organic carbon sequestration rate (Δ SOC_{rate}) (0–40 cm) significantly varied among periods, with mean values across all treatments of 492, 222 and 969 kg C ha⁻¹ yr⁻¹, 262 in P1-R, P2-R and P3-I, respectively (Table 3). In P1-R (1996 to 2009), ΔSOC_{rate} was 263 significantly affected by tillage and N fertilization main effects (without interaction) while 264 in P3-I (2015 to 2017), Δ SOC_{rate} was affected by the interaction among tillage and N 265 fertilization (Table 3). Differently, in P2-R (2009 to 2015), ΔSOC_{rate} did not show 266 differences between treatments (Table 3). In contrast, in P1-R, NT presented the highest 267 268 Δ SOC_{rate} compared to CT with intermediate values in RT (Table 3), while high N rate showed greater \triangle SOC_{rate} compared to the control with intermediate values in the medium 269 N rate (Table 3). In P3-I, the high rate of N fertilizer in NT led to greater \triangle SOC_{rate} compared 270 to CT with intermediate values in RT (1959, 731, and 1380, kg C ha⁻¹ yr⁻¹, respectively), 271 272 while under the control and medium N rates \triangle SOC_{rate} did not show differences among tillage systems (Fig. 2). 273

In P1-R and P3-I, a significant positive linear relationship was found between ΔSOC_{rate} and annual C-inputs, explaining 70% of the variance of ΔSOC_{rate} ($r^2=0.70$; p < 0.001) (Fig. 3). In contrast, in P2-R, ΔSOC_{rate} was not correlated with C-inputs.

277 *3.3 Soil C fractions influenced by the conversion of rainfed to irrigated cropping system.*

In 2015 and 2017, POC was significantly affected by N fertilization at the 0–40cm
depth, with greater values under high N rate compared to the control and medium N rate.

280 (Table 4). Also, in 2017 POC was significantly affected by tillage system as one of the 281 main effects with NT having significantly higher POC than CT whereas RT had 282 intermediate values. However, C-Min did not show differences between treatments for any of the years (Table 4). At the beginning and at the end of P3-I (2015 and 2017), POxC, 283 POC and C-Min were significantly affected by the interaction between tillage system and 284 285 soil depth (Table 5). Also, in 2015 POC was significantly affected by N fertilization and 286 tillage system as main effects. In 2017, C-Min were significantly affected by the interaction 287 between N fertilization, tillage system and soil depth while POC was affected by the 288 interaction between tillage system and N fertilization (Table 5). In 2015 and 2017 POxC 289 was significantly affected by tillage systems at the soil surface (0-5 cm depth), with greater 290 values under NT than CT and intermediate values for RT (Fig. 4a and 4b). Similarly, POC 291 was significantly affected by tillage systems at 0-5 and 5-10 cm soil depths in 2015 with a significant decreasing trend of POC when increasing tillage intensity (NT>RT>CT) (Fig. 292 293 4c). Meanwhile in 2017, POC was significantly affected by tillage systems at the soil surface (0–5 cm depth), with greater values under NT than CT and intermediate values for 294 RT (Fig. 4d). In 2015, C-Min was higher in NT and RT compared to CT at the soil surface 295 296 (0-5 cm), while the contrary occurred at the deepest soil layer (30-40 cm) (Fig. 4e). 297 Interestingly, in 2017 C-Min concentration only showed differences among tillage systems in the surface layer (0-5 cm), with greater C-Min under NT than CT and intermediate values 298 299 in RT (Fig. 4f).

Results of this study indicated that in Mediterranean conditions, the conversion from a rainfed to an irrigated cropping system generates major changes on the soil organic carbon (SOC) dynamics.

304 It is well-known that the amount of C inputs returned to the soil is a key factor driving the changes in SOC stocks (Virto, Barré, Burlot, & Chenu, 2012), since SOC results 305 306 from the decomposition of biotic residues. Our results are fully consistent with this conclusion since C-inputs explained 70% (Fig. 3) of the variation in SOC sequestration 307 308 rates both in P1-R and in P3-I. These two periods (P1-R and P3-I) faced major changes in the cropping system (in P1-R the adoption of RT or NT vs. the traditional CT and in P3-I 309 310 the conversion from a rainfed to an irrigated cropping system). According to the C 311 saturation concept, the response of SOC in relation to C-inputs depends on the amount of 312 C that can be retained with clay and silt particles (Hassink, 1996; Hassink, 1997). Consequently, as long as the finer particles are not saturated with C, they will have the 313 capacity to hold more C, protecting it in aggregates, provided that appropriate agricultural 314 practices are carried out. In our study, SOC content at the beginning of the experiment 315 316 (1996) was low since the traditional practices of the area consisted of intensive tillage and crop residue removal. The maintenance of crop residue in the soil surface under NT and its 317 318 incorporation in RT and CT after the start of the experiment, would have favoured the increase of SOC in all three tillage systems during P1-R. In our study, in P1-R, ΔSOC_{rate} 319 320 was greater in NT (46%) compared to CT, while ΔSOC_{rate} was greater under the high N application rate with respect to the control, as previously reported by Morell et al. (2011). 321 322 These authors showed that soil water conservation under NT and RT treatments during dry seasons combined with adequate N fertilization allowed for higher C-inputs returned to the 323 324 soil compared to CT.

In P2-R, \triangle SOC_{rate} was 55% lower than in P1-R with an average value of all 325 treatments of 222 kg C ha⁻¹ yr⁻¹ and did not show differences among treatments, despite the 326 fact that C-inputs were similar to P1-R. It is known that SOC sequestration is a finite 327 328 process (Powlson, Whitmore, & Goulding, 2011). In our experiment, although decomposition rates were not measured, the similar C inputs and the reduction in the SOC 329 change rate observed between P1-R and P2-R would be indicating that SOC levels were 330 331 reaching a steady state (von Lützow et al., 2006). For instance, in a NT chronosequence experiment formerly managed under CT in Mediterranean conditions, Álvaro-Fuentes et 332 al.,(2014), observed that more than 75% of the total SOC sequestered was gained during 333 334 the first 11 years after NT adoption, with the highest SOC sequestration rate during the first 5 years after NT adoption. From the 11th year onwards, the change in the annual rate of 335 SOC sequestration decreased significantly and SOC reached a new equilibrium. Therefore, 336 337 the greatest differences in SOC sequestration rates occur at the first years after NT adoption, while rates tend to decrease after the first decade. That process would explain the 338 339 strong decrease in SOC sequestration rate under NT and RT observed in our experiment in 340 P2-R.

In P3-I, the conversion from rainfed to irrigated land allowed us to modify the 341 342 cropping system, opting for a more productive summer crop such as corn, as well as the management practices, which directly affected SOC sequestration. In this study, the 343 344 cultivation of corn resulted in greater amounts of crop residues returned to the soil (in P3-I, C-input increased by 65% and 88% compared to P1-R and P2-R, respectively) and a 345 mean SOC increase about 969 kg C ha-1 year-1 when averaged over tillage treatments and 346 N rates. In this regard, an 88% increase in SOC (0-40 cm depth) was found with the 347 introduction of irrigated corn in NT in comparison with the total rainfed period (sum of P1-348 R and P2-R). This increase of SOC in P3-I was the result of the modification of the 349

350 cropping system, which entails a range of different mechanisms that affect SOC dynamics. 351 For example, there was an increased amount of C-inputs as a result of greater crop productivity under irrigation, which increased the SOC (Powlson, Glendining, Coleman, 352 353 & Whitmore, 2011). Adviento-Borbe et al. (2007) showed an increase in SOC sequestration (440 and 662 kg ha⁻¹ yr⁻¹ for RT and CT, respectively, at 0-30 cm soil depth) 354 355 due to increased crop biomass inputs in two continuous corn systems, six years after the 356 introduction of changes in the intensity of management in irrigated agroecosystems in Nebraska. The conversion of a cropping system to irrigation with summer crop cultivation 357 358 could also influence SOC dynamics by modifying water availability in agricultural soils, 359 when temperatures are higher. The increase in moisture can lead to anoxic conditions which 360 slow down the decomposition of organic matter (krogh et al., 2003), leading to further C 361 sequestration. Another mechanism which could explain the increase of SOC when 362 changing from a rainfed barley to a irrigated corn-based cropping system would be the lignin:N ratio of crop residues which plays an important role in their decomposition 363 364 (Paustian, Collis, & Paul, 1997). The value of lignin:N can be considered as an index of biodegradability (Nourbakhsh, 2006). For instance, SOC is usually greater in cropping 365 system with corn than with barley since barley produces less residue that has lower lignin:N 366 367 ratios than corn. Equally, the stability of aggregates and the release of the organic matter can be influenced by the type and intensity of tillage which can vary in irrigated crop 368 rotations compared to rainfed ones. 369

Interestingly, our study showed that the conversion from a rainfed to an irrigated cropping system led to a strong increase of a labile SOC fraction such as POC. Particulate organic carbon was extremely sensitive to the conversion of a rainfed to an irrigated cropping system with a 75% increase as an average of tillage system (0-40 cm soil depth). In this regard, this fraction was previously identified as one of the most sensitive to changes 375 in tillage and N fertilization management practices in Mediterranean soils (Plaza-Bonilla, 376 Álvaro-Fuentes, & Cantero-Martínez, 2014). Labile soil C fractions such as POC play a 377 key role in the formation of microaggregates and macroaggregates acting as binding agents 378 (Elliott, 1986), allowing the persistence of SOC (Puget, Chenu, & Balesdent, 2000). Conversion of rainfed into irrigated cropping systems may have important effects on soil 379 aggregation, given the feedback mechanism between soil aggregation and SOC dynamics 380 381 (Six, Bossuyt, Degryze, & Denef, 2004). These effects are mainly linked to changes in the organic carbon cycle, caused by the increase in crop residues (Adviento-Borbe et al., 2007), 382 383 and the increase in soil microbial activity (Denef, Stewart, Brenner, & Paustian, 2008). In 384 addition, POC can be affected by tillage, with an improvement in C-physical protection 385 within aggregates when NT is used. In this study the main driver of the increase in POC in 386 soils would be the contribution of crop residue (Golchin, Oades, Skjemstad, & Clarke, 387 1994). Differently to POC, POxC showed a small increase (6%) after the conversion from a rainfed to an irrigated cropping system. This last fraction is usually related to a more 388 389 active C cycle, which in turn, favours microbial activity (Haynes, 2005). Opposite to the 390 more labile fractions, C-min remained stable given its recalcitrant nature. Moreover, the C fractions studied were affected by tillage, with a greater accumulation of POC, POxC and 391 392 C-Min at the soil surface under NT compared to CT. The results suggest that the increase of SOC when decreasing tillage intensity (NT > RT > CT) would mainly be a consequence 393 394 of an enhancement of POC. Tillage disrupts soil aggregation, reducing physical protection of POC occluded within aggregates (Grandy & Robertson, 2006) reducing the opportunity 395 for SOC sequestration. 396

In the irrigated period (P3-I), N fertilization rate also played an important role on
SOC sequestration, since high N rates combined with NT led to increase SOC sequestration
due to increased C-inputs as a result of the greater crop productivity of an irrigated cropping

400 system devoted to corn production. Our data showed that under NT and RT the crop made 401 a more efficient use of the nitrogen fertilizer applied compared to CT. The lower response to N application under CT would be due to poor soil structure and surface crusting which 402 403 reduced water availability. The occurrence of soil crusting under CT reduced the infiltration of water into the soil profile compromising plant establishment (Pareja-Sánchez 404 405 et al., 2017). Crop growth under CT was limited because of reduced water infiltration and 406 C-inputs as crop residues were 32% lower than under NT at the highest N rate. In this line, 407 Follett, Jantalia, & Halvorson (2013) suggested that increasing N fertilization rates may increase SOC stocks depending on soil depth considered and tillage practice. For example, 408 409 in irrigated continuous corn systems, Halvorson, Reule, & Mosier (2004) showed that the rate of SOC sequestration (0-15 cm depth) under NT with a high N fertilization rate was 410 1.4–2.0 Mg C ha⁻¹ yr⁻¹, while the CT treatment showed a rate of 0.2 Mg C ha⁻¹ yr⁻¹ even 411 412 with about the same level of crop residues as under NT. Similarly, Follett, Castellanos, & 413 Buenger (2005) observed an increase in SOC under NT systems with high N fertilization rate (1 and 1.9 Mg C ha⁻¹yr⁻¹ in the 0–15- and 15–30-cm depths, respectively), compared 414 to CT with the same N rate (0.2 and 0.6 Mg C ha⁻¹ yr⁻¹ in the 0–15- and 15–30-cm depths, 415 respectively) under wheat-corn rotations in irrigated conditions in Mexico. They suggested 416 417 that the greater SOC sequestration under NT was due to higher above-ground crop residue. As shown by our study under Mediterranean conditions, the use of no-till in irrigated 418 cropping systems based on corn increases SOC sequestration compared to the former 419 rainfed cropping systems based on winter cereal production. 420

421 Conversion from rainfed land to irrigation also may lead to other environmental 422 threats as for example an increase of soil greenhouse emissions, which may be 423 compensated with soil C sequestration. The magnitude of soil C sequestration is crucial for 424 the future trend of CO₂ and N₂O concentration in the atmosphere (DeLuca & Zabinski,

2011). In our study, the greater levels of SOC found under NT and, particularly, with the 425 426 application of N fertilizers could also boost soil N₂O emissions, by nitrification and denitrification, and soil CO₂ emissions, by the increase in the amount of crop residues 427 428 incorporated to the soil. In this regard, in the previous barley rainfed period (P2-R), cumulative annual N₂O emissions were 34% lower (Plaza-Bonilla, Álvaro-Fuentes, Arrúe, 429 & Cantero-Martínez, 2014), than the values found in the irrigated period (P3-I) (Pareja-430 Sánchez, Cantero-Martínez, Álvaro-Fuentes, & Plaza-Bonilla, 2020). These data suggest 431 that the increase in soil moisture and the use of higher N fertilization rates when 432 transforming to irrigation were the main causes behind the increase in N₂O emissions. 433 434 However, the increase in N₂O emissions can be compensated with SOC sequestration. In P3-I, N₂O emissions corresponded to 167 kg CO₂ equivalent ha⁻¹ year⁻¹, as an average of 435 treatments and years (Pareja-Sánchez, Cantero-Martínez, Álvaro-Fuentes, & Plaza-436 437 Bonilla, 2020), while SOC sequestration amounted 3553 kg CO₂ equivalent ha⁻¹ year⁻¹. Therefore, our data showed that the greater emission of N₂O would probably be 438 439 compensated with the SOC sequestration at least during the first years after the change to 440 irrigation. However, soil N₂O and CO₂ emissions would continue over time according to crop needs, as CO₂ emissions related to the energy needed for pumping irrigation water. 441

442

444 5. Conclusion

This study shows that the modification of the cropping system due to the conversion
of Mediterranean rainfed agroecosystems into irrigation, exerted a significant impact on
soil organic carbon sequestration rates.

448 The amount of C-inputs as crop residues is an important factor explaining soil carbon sequestration rates. In the rainfed Mediterranean conditions the use of NT increases 449 450 soil water conservation compared to conventional tillage leading to higher C-inputs and allowing the response of the crop to N application increasing soil carbon sequestration 451 452 rates. However, 13 years after the implementation of these practices, SOC sequestration rates were reduced from 492 to 222 kg C ha⁻¹ yr⁻¹, which suggests that an equilibrium phase 453 454 was almost reached for SOC. When Mediterranean rainfed cropping systems based on 455 winter cereal production are converted into irrigated cropping systems based on highly productive summer crops such as corn an increase of SOC sequestration rates occurs 456 reaching a value of 969 kg C ha⁻¹ yr⁻¹ when averaged over all treatments. The application 457 of water through irrigation allows more biomass production and more C-inputs from crop 458 residues. Particulate organic carbon was the main indicator for SOC sequestration due to 459 460 the conversion of the cropping system, which shows a great sensitivity to the new scenario. If NT is used in these newly irrigated systems, SOC sequestration rates can attain 1959 kg 461 C ha⁻¹ yr⁻¹ when applying high N fertilizer rates in comparison to 731 kg C ha⁻¹ yr⁻¹, which 462 463 would be attained when using conventional tillage.

464 Our results indicate that the use of NT practices under rainfed led to an increase of 465 SOC. A second step related to the introduction of irrigation allows the modification of the 466 cropping system to the cultivation of more productive summer crops such as corn,

- enhancing C inputs to the soil and SOC, which is maximized by the use of NT and adjusted
- 468 fertilizer rates.

470 Acknowledgements

We would like to thank the field and laboratory technicians Carlos Cortés, Javier
Bareche and Silvia Martí. This research work was financially supported by the Ministerio
de Economía y Competitividad of Spain (project AGL2013-49062-C4-1-R; PhD
fellowship BES-2014-070039). DPB received a Juan de la Cierva postdoctoral grant from
the Ministerio de Economía y Competitividad of Spain (IJCI-2016-27784) and is Ramón y
Cajal fellow (RYC2018-024536-I) of the Ministerio de Ciencia, Innovacion y
Universidades.

479 **References**

480	Adviento-Borbe, M.A.A., Haddix, M.L., Binder, D.L., Walters, D.T. & Dobermann, A.
481	(2007). Soil greenhouse gas fluxes and global warming potential in four high-yielding
482	maize systems. Global Change Biology. 13, 1972–1988.

- 483 Álvaro-Fuentes, J., Cantero-Martínez, C. & Arrue, J.L. (2008). Tillage Effects on Soil
- 484 Organic Carbon Fraction sin Mediterranean Dryland Agroecosystems. *Soil Science*485 *Society of America Journal*. 72, 541–547.
- 486 Álvaro-Fuentes, J., Cantero-Martínez, C., Lopez, M.V., Paustian, K., Denef, K., Stewart,
- 487 C.E. & Arrué, J.L. (2009). Soil aggregation and soil organic carbon stabilization:
- 488 effects of management in semiarid Mediterranean agroecosystems. *Soil Science Society*
- *d***8**9 *of America Journal*. 73, 1519–1529.
- 490 Álvaro-Fuentes, J., Plaza-Bonilla, D., Arrué, J.L., Lampurlanés, J. & Cantero-Martínez, C.
- 491 (2014). Soil organic carbon storage in a no-tillage chronosequence under Mediterranean
 492 conditions. *Plant Soil*. 376, 31–41.
- Apesteguía, M., Virto, I., Orcaray, L., Enrique, A. & Bescansa, P. (2015). Effect of the
 conversion to irrigation of semiarid Mediterranean dryland agrosecoystems on soil
 carbon dynamics and soil aggregation. *Arid Land Research and Management*. 29, 399414.
- 497 Austin, R.B., Cantero-Martínez, C., Arrúe, J.L., Playán, E. & Cano-Marcellán, P. (1998).
- 498 Yield-rainfall relationships in cereal cropping systems in the Ebro river valley of Spain.
- 499 *European Journal of Agronomy.* 8, 239-248.
- 500 Cambardella, C., & Elliot, E.T. (1992). Particulate soil organic matter changes across a
- 501 grassland cultivation sequence. *Soil Science Society of America Journal*. 56, 777–783.

502	Cantero-Martínez, C., Angás, P., & Lampurlanés, J. (2003). Growth yield and water
503	productivity of barley (hordeum vulgare l.) affected by tillage and N fertilization in
504	Mediterranean semiarid, rainfed condition of Spain. Field Crops Research. 84, 341-357.

505 Cantero-Martínez, C., Angás, P., & Lampurlanés, J. (2007). Long-term yield and water use

- 506 efficiency under various tillage systems in Mediterranean rainfed conditions. *Annals of*
- 507 *Applied Biology*. 150, 293–305.
- 508 Dastane, N.G. (1978). Effective rainfall in irrigated agriculture. Irrigation and Drainage
 509 Paper 25. FAO, Rome.
- 510 DeLuca, T.H., & Zabinski, C.A. (2011). Prairie ecosystems and the carbon problem.
 511 *Frontiers in Ecology and the Environment*. 9, 407–413.
- 512 Denef, K., Stewart, C.E., Brenner, J., & Paustian, K. (2008). Does long-term center-pivot
 513 irrigation increase soil carbon stocks in semi-arid agroecosystems? *Geoderma*. 145,
 514 121–129.
- 515 Dimassi, B., Mary, B., Wylleman, R., Labreuche, J., Couture, D., Piraux, F., & Cohan, J.P.
- 516 (2014). Long-term effect of contrasted tillage and crop management on soil carbon
- 517 dynamics during 41 years. *Agriculture, Ecosystems & Environment.* 188, 134–146.
- Ellert, B.H., & Bettany, J.R. (1995). Calculation of organic matter and nutrients stored in
 soil under contrasting management regimes. *Canadian Journal of Soil Science*. 75, 529–
 538.
- 520 558.
- 521 Elliott, E.T. (1986). Aggregate structure and carbon, nitrogen, and phosphorous in native
- and cultivated soils. *Soil Science Society of America Journal*. 50, 627–633.

- Follett, R.F., Castellanos, J.Z., & Buenger, E.D. (2005). Carbon dynamics and
 sequestration in an irrigated Vertisol in central Mexico. *Soil & Tillage Research*. 83,
 148–158.
- 526 Follett, R.F., Jantalia, C.P., & Halvorson, A.D. (2013). Soil carbon dynamics for irrigated
- 527 corn under two tillage systems. *Soil Science Society of America Journal*. 77, 951–963.
- Gillabel, J., Denef, K., Brenner, J., Merckx, R., & Paustian, K. (2007). Carbon
 sequestration and soil aggregation in center-pivot irrigated and dryland cultivated
 farming systems. *Soil Science Society of America Journal*. 71, 1020–1028.
- 531 Golchin, A., Oades, J., Skjemstad, J., & Clarke, P. (1994). Study of free and occluded
- particulate organic matter in soils by solid state 13C Cp/MAS NMR spectroscopy and
- scanning electron microscopy. *Soil Research*. 32, 285-309.
- Grandy, A.S., & Robertson, G.P. (2006). Initial cultivation of a temperate-region soil
 immediately accelerates aggregate turnover and CO₂ and N₂O fluxes. *Global Change Biology*. 12, 1507–1520.
- Halvorson, A.D., Reule, C.A., & Mosier, A.R. (2004). Nitrogen and crop management
 influence irrigated corn yields and greenhouse gas emissions. *Symposium Proceedings*.
 10, 21-27.
- Hassink, J. (1996). Preservation of plant residues in soils differing in unsaturated protective
 capacity. *Soil Science Society of America Journal*. 60, 487–491.
- Hassink, J. (1997). The capacity of soils to preserve organic C and N by their association
 with clay and silt particles. *Plant Soil*. 191, 77–87.

- Haynes, R.J. (2005). Labile organic matter fractions as central components of the quality
 of agricultural soils: an overview. *Advances in Agronomy*. 85, 221-268.
- Hernanz, J.L., Sánchez-Girón, V., & Navarrete, L. (2009). Soil carbon sequestration and
 stratification in a cereal/leguminous crop rotation with three tillage systems in semiarid
- 548 conditions. *Agriculture, Ecosystems & Environment.* 133, 114–122.
- Krogh, L., Noergaard, A., Hermansen, M., Humlekrog Greve, M., Balstroema, T., &
 Breuning-Madsen, H. (2003). Preliminary estimates of contemporary soil organic
 carbon stocks in Denmark using multiple datasets and four scaling-up methods. *Agriculture, Ecosystems & Environment*. 96, 19–28.
- Li, X.G., Li, Y.K., Li, F.M., Ma, Q., Zhang, P.L., & Yin, P. (2009). Changes in soil organic
 carbon, nutrients and aggregation after conversion of native desert soil into irrigated
 arable land. *Soil & Tillage Research*. 104, 263–269.
- Luo, Z., Wang, E., & Sun, O.J. (2010). Soil carbon change and its response to agricultural
 practices in Australian agro-ecosystems: A review and synthesis. *Geoderma*. 155, 211–
 223.
- 559 Mahal, N.K., Osterholz, W.R., Miguez, F.E., Poffenbarger, H.J, Sawyer, J.E., Olk, D.C.,
- 560 Archontoulis, S.V., & Castellano, M.J. (2009). Nitrogen Fertilizer Suppresses
- Mineralization of Soil Organic Matter in Maize Agroecosystems. *Frontiers in Ecology and Evolution.*7, 59.
- Manzoni, S., Jackson, R.B., Trofymow, J.A., & Porporato, A. (2008). The Global
 Stoichiometry of Litter Nitrogen Mineralization. *Science*, 321, 684-686.

565	Martens, D.A., Emmerich, W., Mclain, J.E.T., & Johnsen, T.N. (2005). Atmospheric
566	carbon mitigation potential of agricultural management in southwestern USA. Soil &
567	Tillage Research. 83, 95–119.

- 568 Morell, F.J., Cantero-Martínez, C., Lampurlanés, J., Plaza-Bonilla, D., & Álvaro-Fuentes,
- 569 J. (2011). Soil carbon dioxide flux and organic carbon content: effects of tillage and
- 570 nitrogen fertilization. *Soil Science Society of America Journal*. 75, 1874–1884.
- 571 Mulvaney, R.L., Khan, S.A., & Ellsworth, T.R. (2009). Synthetic nitrogen fertilizers
- deplete soil nitrogen: A global dilemma for sustainable cereal production. *Journal of Environmental Quality*. 38, 2295–2314.
- 574 Nelson, D.W., & Sommers, L.E. (1982). Total carbon, organic carbon and organic matter.
- In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2. 2nd
- ed. Agronomy, vol. 9. Soil Science Society of America, Madison, WI, USA, pp. 539–
 577 579.
- Nourbakhsh, F. (2006). Fate of carbon and nitrogen from plant residue decomposition in a
 calcareous soil. *Plant, Soil and Environment.* 52, 137–140.
- 580 Pareja-Sánchez, E., Cantero-Martínez, C., Álvaro-Fuentes, J., & Plaza-Bonilla, D. (2020).
- 581 Impact of tillage and N fertilization rate on soil N₂O emissions in irrigated maize in a
- 582 Mediterranean agroecosystem. *Agriculture, Ecosystems & Environment.* 287, 106687.
- 583 Pareja-Sánchez, E., Plaza-Bonilla, D., Ramos, M.C., Lampurlanés, J., Álvaro-Fuentes, J.,
- & Cantero-Martínez, C. (2017). Long-term no-till as a means to maintain soil surface
- structure in an agroecosystem transformed into irrigation. *Soil & Tillage Research*. 174,
- 586 221-230.

- 587 Patrick, M., Tenywa, J.S., Ebanyat, P., Tenywa, M.M., Mubiru, D.N., Basamba, T.A., &
- Leip, A. (2013). Soil organic carbon thresholds and nitrogen management in tropical
- agroecosystems: concepts and prospects. *Journal of Sustainable Development*. 6, 31-43.
- 590 Paustian, K., Collis, H.P., & Paul, E.A. (1997). Management controls on soil carbon. In:
- 591 E.A Paul et al. (Eds.) Soil organic matter in temperate agroecosystems. CRC Press, Boca
- 592 Raton, FL. pp 15-49.
- 593 Plaza-Bonilla, D., Álvaro-Fuentes, J., Arrúe, J.L., & Cantero-Martínez, C. (2014). Tillage
- and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed
 Mediterranean area. *Agriculture, Ecosystems & Environment*. 189, 43–52.
- Plaza-Bonilla, D., Álvaro-Fuentes, J., & Cantero-Martínez, C. (2014). Identifying soil
 organic carbon fractions sensitive to agricultural management practices. *Soil & Tillage Research.* 139, 19–22.
- 599 Plaza-Bonilla, D., Arrúe, J.L., Cantero-Martínez, C., Fanlo, R., Iglesias, A., & Álvaro-
- 600 Fuentes, J. (2015). Carbon management in dryland agricultural systems. A review.
- 601 *Agronomy for Sustainable Development*. 35, 1319-1334.
- Powlson, D.S., Glendining, M.J., Coleman, K., & Whitmore, A.P. (2011). Implications for
 soil properties of removing cereal straw: results from long-term studies. *Agronomy Journal.* 103, 279–287.
- 605 Powlson D.S., Whitmore, A.P., & Goulding, W.T. (2011). Soil carbon sequestration to
- 606 mitigate climate change: a critical reexamination to identify the true and the false.
- 607 *European Journal of Soil Science*. 62, 42–55.

- Puget, P., Chenu, C., & Balesdent, J. (2000). Dynamics of soil organic matter associated
 with particle-size fractions of water-stable aggregates. *European Journal of Soil Science*. 51, 595-605.
- 611 Russell, A.E., Laird, D.A., Parkin, T.B., & Mallarino, A.P. (2005). Impact of Nitrogen
- 612 Fertilization and Cropping System on Carbon Sequestration in Midwestern Mollisols.
- 613 Soil Science Society of America Journal. 69, 413–422.
- 614 SAS Institute Inc, 2018. Using JMP® 13. Cary. SAS Institute Inc., NC.
- Six, J., Bossuyt, H., Degryze, S., & Denef, K. (2004). A history of research on the link
- between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil & Tillage*
- 617 *Research*. 79, 7–31.
- Soil Survey Staff. 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources
 Conservation Service, Washington, DC, pp 306.
- 620 Virto, I., Barré, P., Burlot, A., & Chenu, C. (2012). Carbon input differences as the main
- factor explaining the variability in soil organic C storage in no-tilled compared to
 inversion tilled agrosystems. *Biogeochemistry*. 108, 17–26.
- Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G.,
 Matzner, E., & Marschner, B. (2007). SOM fractionation methods: Relevance to
 functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry*. 39,
 2183–2207.
- Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., & Samson-Liebig, S.E. (2003).
 Estimating active carbon for soil quality assessment: a simplified method for laboratory
 and field use. *American Journal of Alternative Agriculture*. 18, 3–17.
 - 31

- 630 West, T.O., & Post, W.M. (2002). Soil organic carbon sequestration rates by tillage and
- 631 crop rotation: a global data analysis. *Soil Science Society of America Journal*. 66, 1930–
- 632 1946.

634 **Figure captions**

Fig. 1. Monthly precipitation (dark blue columns), irrigation (light blue columns), potential
evapotranspiration (PET, red dash line) and mean air temperature (yellow line) at Agramunt
from 1996 to 2017. For each year total precipitation (above) and irrigation water (below)
are reported.

Fig. 2. Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and nitrogen fertilizer rate (0, Medium, High) effects on SOC sequestration rate (Δ SOC_{rate}) (kg C ha⁻¹ year⁻¹) from 2015 to 2017 (P3-I). Different lower case letters indicate significant differences among treatments at *P*< 0.05. Vertical bars indicate standard deviation.

Fig. 3. Linear regression between soil organic carbon sequestration rate (Δ SOC_{rate}) (kg C ha⁻¹ year⁻¹) and annual carbon inputs (C-input) (kg C ha⁻¹ year⁻¹) from 1996 to 2009 (P1-R), from 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I). For each period, each point represents the average of each treatment (tillage systems: CT, conventional tillage; RT, reduced tillage; NT, no-tillage; N fertilizer rates: 0, Medium, High).

Fig. 4. Permanganate-oxidizable organic C (POxC) (mg C kg⁻¹), particulate organic carbon (POC) and mineral-associated organic carbon (C-Min) as affected by tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) at different soil depths before (2015) (a, c and e) and after (2017) (b, d and f) the conversion of rainfed to irrigated land. Different lower case letters indicate significant differences between tillage systems for a given depth at *P*< 0.05. **Table 1.** Agricultural management practices carried out in the experimental field during the

655 rainfed and irrigated periods.

	Rainfed period (1996 to 2014)	Irrigated period (2015 to 2017)
Tillage	CT: one moldboard plough pass (25-	CT: one rototiller pass (15 cm depth)
	30 cm depth) plus two cultivator	plus one subsoiler pass (35 cm depth)
	passes (15 cm depth) during	and one disk plough pass (20 cm
	September and October.	depth) during March or April.
	RT: one cultivator pass (10 to 15 cm	RT: Strip-tillage (25 cm deep with a
	depth) during September and October.	working width of 25 cm, 12.5 cm on
		each side, reducing the surface tilled
		to 20%).
	NT: No-tillage.	NT: No-tillage.
Growing season	November – June.	April – November.
Sowing/Planting	Barley: cv. Hispanic from 1996 to	Corn: cv. Kopias from 2015 to 2017
	2010 and cv. Cierzo from 2010 to	Planting density of 90,000 seeds ha-
	2014.	in April.
	Planting density of 450 seeds $m^{\!-\!2}$ in	
	November.	
Fertilization	• Three mineral N fertilizer rates 0,	• Three mineral N fertilizer rate
	60 and 120 kg N ha ⁻¹ :	0, 200 and 400 kg N ha ⁻¹ .
	• 1/3 of the rate in one pre-sowing	• 1/3 of the rate in one pre-sowing
	application as ammonium sulphate	application as urea (April before
	(November).	sowing).
	• 2/3 of the rate in one top dressing	• 1/3 of the rate in two top-dressing
	application as ammonium nitrate	applications at V5 (May) and V10
	(between January and February).	(July) as ammonium nitrate.
	• Phosphorous: 40–50 kg P_2O_5 ha ⁻¹	• Phosphorous: 154 kg P_2O_5 ha ⁻
	yr ⁻¹	yr^{-1}
	• Potassium: 90 kg K_2O ha ⁻¹ yr ⁻¹	• Potassium: $322 \text{ kg } \text{K}_2\text{O} \text{ ha}^{-1} \text{ yr}^{-1}$
	(both P and K before sowing in	(both P and K in March or April
	November).	before planting).
Weed control	pre-sowing: 1.5 L ha ⁻¹ of glyphosate	pre-sowing: 1.5 L ha ⁻¹ of glyphosate
	[N-(phosphonomethyl) glycine].	[N-(phosphonomethyl) glycine].
Grain harvest	Commercial combine at the end of	Commercial combine at the
	June or beginning of July.	beginning of November.
Crop residue	Chopped and spread over the soil	Chopped and spread over the soi
management	surface (NT) or incorporated (CT and	surface (NT) or incorporated (CT and
	RT) according to the tillage system.	RT) according to the tillage system.

657	Table 2. Above-ground carbon inputs (C-input) (kg C ha ⁻¹ yr ⁻¹) from 1996 to 2009 (P1-R), from
658	2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I) as affected by tillage (CT; conventional tillage,
659	RT; reduced tillage and NT; no tillage) and N fertilization rate (zero, medium and high). For each
660	variable, different letters indicate significant differences between treatments at P<0.05. Values in
661	brackets indicate standard deviation. Analysis of variance (P-values) of C-inputs of P1-R, P2-R and
662	P3-I as affected by tillage (CT, RT and NT), N fertilization rate (zero, medium and high), and their
663	interaction.

	C-input (kg C ha ⁻¹ yr ⁻¹)			
Treatment	P1-R	P2-R	Р3-І	
СТ-0	1245 (81) a	1066 (17) a	1995 (391)	
RT-0	1263 (105) a	1069 (91) a	2488 (211)	
NT-0	1445 (150) a	1310 (34) a	2651 (332)	
CT-Medium	1490 (84) b	1222 (68) b	2341 (35)	
RT-Medium	1662 (91) b	1391 (151) b	2723 (395)	
NT-Medium	2079 (170) a	1874 (81) a	3191 (267)	
CT-High	1540 (118) b	1249 (162) b	2303 (831)	
RT-High	1697 (85) b	1707 (89) a	2679 (637)	
NT-High	2268 (68) a	2009 (201) a	3921 (96)	
ANOVA				
Tillage (Till)	< 0.001	< 0.001	< 0.001	
N fertilization	(Fert) <0.001	< 0.001	0.02	
Till*Fert	0.01	< 0.001	ns	

675	Table 3. Soil organic carbon sequestration rate (ΔSOC_{rate}) (kg C ha ⁻¹ yr ⁻¹) from 1996 to 2009 (P1-
676	R), from 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I) as affected by tillage (CT; conventional
677	tillage, RT; reduced tillage and NT; no tillage) and N fertilization rate (zero, medium and high.
678	Different lower case letters indicate significant differences among treatments at $P < 0.05$. Values in
679	brackets indicate standard deviation. Analysis of variance (P-values) of soil organic carbon
680	sequestration rate (ΔSOC_{rate}) of P1-R, P2-R and P3-I as affected by tillage (CT, RT and NT), N
681	fertilization rate (zero, medium and high), and their interaction.

Treatment CT	P1-R	P2-R	P3-I
СТ	204 (271) 1		
	394 (271) b	237 (269)	698 (406) b
RT	508 (204) ab	334 (352)	955 (764) b
NT	574 (189) a	94 (245)	1255 (758) a
0	358 (240) b	95 (315)	431 (265) b
Medium	509 (251) ab	321 (272)	1119 (495) a
High	610 (113) a	249 (293)	1357 (813) a
Average	492 (228)	222 (298)	969 (541)
ANOVA			
Tillage (Till)	< 0.001	ns	< 0.001
N fertilization (Fert)	< 0.001	ns	< 0.001
Till*Fert	ns	ns	0.04

697	Table 4. Soil particulate organic carbon (POC) (kg ha ⁻¹) (0-40 cm) and mineral-associated organic
698	carbon (C-Min) (kg ha ⁻¹) (0-40 cm) before (2015) and after (2017) the conversion of rainfed to
699	irrigated land, as affected by tillage (CT; conventional tillage, RT; reduced tillage and NT; no
700	tillage) and N fertilization rate (zero, medium and high). For each variable, different letters indicate
701	significant differences between treatments at P<0.05. Values in brackets indicate standard
702	deviation. Analysis of variance (P-values) of POC and C-Min before (2015) and after (2017) the
703	conversion of rainfed to irrigated land, as affected by tillage (CT, RT and NT), N fertilization rate
704	(zero, medium and high), and their interaction.

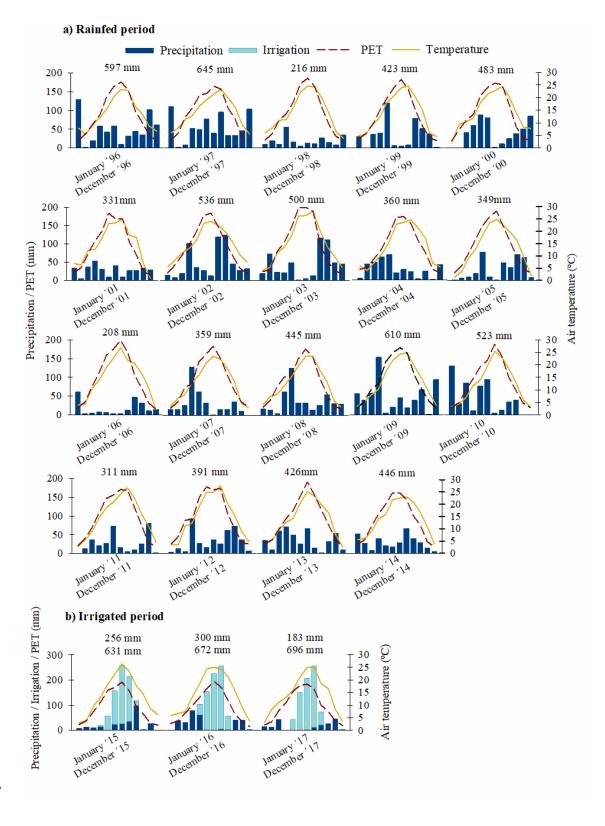
	2	015	2017		
Treatment	POC (kg ha ⁻¹)	C-Min (kg ha ⁻¹)	POC (kg ha ⁻¹)	C-Min (kg ha ⁻¹)	
СТ	21105 (2143)	20170 (4143)	18186 (2749) b	23835 (3777)	
RT	23351(2627)	20372 (2844)	21285 (5339) ab	23893 (3208)	
NT	23570 (3649)	19768 (3099)	24602 (2749) a	22544 (3698)	
0	20483 (1621) b	19974 (3230)	19165 (6923) b	23102 (3395)	
Medium	22377 (1335) b	21270 (3472)	20773 (3248) b	24345 (4063)	
High	25166 (3478) a	19066 (3120)	24136 (5365) a	22825 (3167)	
ANOVA					
Tillage (Till)	ns	ns	0.02	ns	
N fertilization (Fert)	< 0.001	ns	< 0.001	ns	
<u>Till*Fert</u>	ns	ns	ns	ns	

- Table 5. Analysis of variance (*P*-values) of soil permanganate-oxidizable organic C (POxC),
 particulate organic carbon (POC) and mineral-associated organic carbon (C-Min) before (2015)
 and after (2017) the conversion of rainfed to irrigated land, as affected by tillage (CT, conventional
 tillage; RT, reduced tillage; NT, no-tillage), N fertilization rate (zero, medium and high), soil depth
 (0-5, 5-10, 10-20, 20-30, 30-40 cm) and their interaction.
- 713

Year	2015		2017			
Source of variation	POxC	POC	C- Min	POxC	POC	C- Min
Tillage (Till)	ns	< 0.001	ns	ns	ns	ns
N fertilization (Fert)	ns	< 0.001	ns	ns	ns	ns
Till*Fert	ns	ns	ns	ns	< 0.001	ns
Fert*Depth	ns	ns	0.03	ns	ns	0.004
Till*Depth	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Fert*Till*Depth	ns	ns	ns	ns	ns	0.002

ns; non-significant

Fig. 1. Monthly precipitation (dark blue columns), irrigation (light blue columns), potential
evapotranspiration (PET, red dash line) and mean air temperature (yellow line) at Agramunt from
1996 to 2017. For each year total precipitation (above) and irrigation water (below) are reported.



- Fig. 2. Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and nitrogen
 fertilizer rate (0, Medium, High) effects on SOC sequestration rate (ΔSOC_{rate}) (kg C ha⁻¹ year⁻¹)
 from 2015 to 2017 (P3-I). Different lower case letters indicate significant differences among
- 721 treatments at P < 0.05. Vertical bars indicate standard deviation.

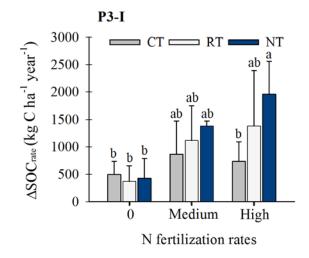


Fig. 3. Linear regression between soil organic carbon sequestration rate (ΔSOC_{rate}) (kg C ha⁻¹ year⁻¹) and annual carbon inputs (C-input) (kg C ha⁻¹ year⁻¹) from 1996 to 2009 (P1-R), from 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I). For each period, each point represents the average of each treatment (tillage systems: CT, conventional tillage; RT, reduced tillage; NT, no-tillage; N fertilizer rates: 0, Medium, High).

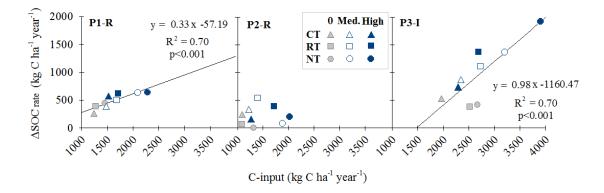


Fig. 4. Permanganate-oxidizable organic C (POxC) (mg C kg⁻¹), particulate organic carbon (POC) (g C 100g⁻¹) and mineral-associated organic carbon (C-Min) (g C 100g⁻¹) as affected by tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) at different soil depths before (2015) (a, c and e) and after (2017) (b, d and f) the conversion of rainfed to irrigated land. Different lower case letters indicate significant differences between tillage systems for a given depth at *P*< 0.05.

