

Document downloaded from:

http://hdl.handle.net/10459.1/58426

The final publication is available at:

https://doi.org/10.1016/j.agee.2010.07.015

# Copyright

cc-by-nc-nd, (c) Elsevier, 2010

Està subjecte a una llicència de Reconeixement-NoComercial-SenseObraDerivada 4.0 de Creative Commons

1	Soil CO <sub>2</sub> fluxes during tillage operations and rainfall events in a Mediterranean
2	agroecosystem: effects of tillage systems and nitrogen fertilization
3	
4	
5	F.J. Morell <sup>a, *</sup> , J. Álvaro-Fuentes <sup>b</sup> , J. Lampurlanés <sup>c</sup> C. Cantero-Martínez <sup>a</sup>
6	
7	
8	<sup>a</sup> Departamento de Producción Vegetal y Ciencia Forestal, Universitat de Lleida, Rovira
9	Roure 191, 25198 Lleida, Spain
10	<sup>b</sup> Natural Resource Ecology Laboratory, Colorado State University, 1231 East Drive, Fort
11	Collins, CO 80523, USA
12	<sup>c</sup> Departamento de Ingeniería Agroforestal, Universitat de Lleida, Rovira Roure 191, 25198
13	Lleida, Spain
14	
15	
16	
17	
18	* Corresponding author (pacomorell@yahoo.com)
19	
20	
21	
22	

#### ABSTRACT

1

2 Management practices can influence soil CO<sub>2</sub> efflux in croplands. Under Mediterranean 3 conditions, soil CO<sub>2</sub> flux responds to tillage and precipitation events with different extent 4 and duration. CO<sub>2</sub> fluxes during these events may constitute a significant part of the annual 5 soil CO<sub>2</sub> emission. Further estimation of these events is required for estimation of the soil C 6 balance and modeling of C dynamics. The long-term effects of tillage practices (NT, no-7 tillage, MT, minimum tillage and CT, conventional tillage) and N fertilization level (zero, medium, 60 kg N ha<sup>-1</sup>, and high, 120 kg N ha<sup>-1</sup>) on soil CO<sub>2</sub> fluxes were measured during 8 9 tillage operations in four consecutive years (2005-2008 period) and during four rainfall 10 events. In all the four years studied, tillage implementation led to a pulse of soil CO<sub>2</sub> flux. 11 The extent of this response was linearly related with soil CO<sub>2</sub> flux on the day before tillage operations under MT (slope=4.22;  $R^2$ =0.69; P<0.01) and CT (slope=16.7;  $R^2$ =0.87; 12 13 P<0.01) and indicating the extent of soil disturbances. Cumulative soil CO<sub>2</sub> fluxes 48 hours 14 after tillage were similar for different soil tillage and N fertilization treatments. 15 Precipitation events led to sharp increase of soil CO<sub>2</sub> fluxes that decreased as the soil dried. 16 The response of soil CO<sub>2</sub> fluxes to rainfall events was higher in order NT>MT>CT. Under 17 NT, the soil CO<sub>2</sub> flux after rainfall was linearly related to soil temperatures (slope=0.15; 18 R<sup>2</sup>=0.96, P<0.01). N fertilization affected CO<sub>2</sub> flux occasionally (in 4 out of 35 samplings), 19 with lower fluxes with no N fertilization, and significant interaction with tillage system, 20 where differences among N fertilizer levels were significant under NT and MT, but not 21 under CT. 22 **Abbreviations:** TIL, tillage system factor; NIT, N fertilizer level factor; NT, no tillage; 23 MT, minimum tillage; CT, conventional tillage; SOC, soil organic carbon; SOM, soil 24 organic matter; SWC, soil water content; ZN, no (zero) N fertilizer application; MN,

- 1 medium N fertilizer level (60 kg N/ha); HN, high N fertilizer level (120 kg N/ha); Fb, soil
- $CO_2$  flux on the day before tillage operations; Fa, soil  $CO_2$  flux immediately after tillage
- 3 operations; rainfall events: D06, December 2006; S07, September 2007; O07, October
- 4 2007; J09, July 2009.

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

5 **Key words**: semiarid, precipitation, tillage, temperature

6 INTRODUCTION

Soil organic carbon (SOC) content determines soil quality and fertility. Moreover SOC is the greatest terrestrial reservoir of carbon. Upon conversion to agriculture, most soils lose one-third to one-half of their SOC content (Lal and Bruce, 1999). Part of this SOC can be restored with adequate crop management practices (e.g. cropping systems, tillage practices and cover crops) and agricultural inputs (fertilizers and irrigation). The use of adequate management practices can help to offset the increase of atmospheric CO<sub>2</sub> concentration while improving soil quality and productivity (Lal, 2004; Johnson et al., 2007). The SOC content is determined by the balance between the rate of C inputs from crop production and the rate of C outputs as CO<sub>2</sub> efflux from decomposition of crop residues and soil organic matter (Paustian et al., 1997). Soil microbial activity transforms plant-assimilated C (input) to SOC or CO<sub>2</sub> that returns to the atmosphere (output). This process leads to a flux of CO<sub>2</sub> from the soil to the atmosphere. The process of soil CO2 flux is altered by management practices (e.g. soil tillage operations) and by meteorology (e.g. rainfall events). Additionally, long-term management practices (e.g. tillage systems, N fertilization) will affect soil characteristics and hence soil CO<sub>2</sub> production and flux. It is well sound that soil microbial activity, and hence CO<sub>2</sub> production and subsequent soil CO<sub>2</sub> flux, is commonly limited by C availability and strongly modulated by soil environmental conditions.

Tillage operations lead to different responses that may be related with the cultivation type and history and with the soil type (Calderón et al., 2000), as well as soil conditions when tillage is implemented (Prior et al., 1997; Kessavalou et al., 1998a). Tillage of previously untilled soil under some agroecosystems led to an increase of CO<sub>2</sub> efflux from the soil that starts with residue incorporation and extends for a period of time due to aggregate disruption and exposition of once protected organic matter to decomposition (La Scala et al., 2008). Tillage systems, applied over the medium- to long-term, modify soil environmental conditions and on SOC content and stratification and consequently soil CO<sub>2</sub> flux (Franzluebbers et al., 1995; Bono et al., 2008). In cultivated soils, short-term effects of soil tillage on soil CO<sub>2</sub> fluxes occurred as an initial flush of CO<sub>2</sub> emission followed by a stabilization of CO<sub>2</sub> flux within hours that results from the release of gases entrapped in the soil pores (Reicosky et al., 1997). Thus, tillage implementation produces an associated CO<sub>2</sub>-C loss from the soil (Alvarez et al., 2001). Also, rainfall would have a great effect on soil CO<sub>2</sub> flux that may lead abrupt changes (Rochette et al., 1991). In semiarid agroecosystems and during drought periods, soil CO<sub>2</sub> flux is mainly driven by soil water content (Akinremi et al., 1999). In these ecosystems, precipitation events lead to increased CO<sub>2</sub> emissions from activation of heterotrophic respiration (Inglima et al., 2009), due to a recovery of microbial activity (Borken and Matzner, 2009). In Mediterranean agroecosystems, soil CO<sub>2</sub> flux follows a seasonal trend in relation with soil environmental conditions and crop phenology (Álvaro-Fuentes et al., 2008) and temperature limits the response of soil CO<sub>2</sub> emission to rainfall events (Almagro et al., 2009). However soil tillage operations and precipitations modify the seasonal trends of soil CO<sub>2</sub> emissions (Sanchez et al., 2003). CO<sub>2</sub> fluxes that occur after tillage and precipitation events under these agroecosystems are fairly understood. In Mediterranean agroecosystems, previous work on

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

- these short-term events has focused on the short-term effects of tillage (Álvaro-Fuentes et
- 2 al., 2007) as affected by tillage systems.
- 3 N fertilization affects C inputs to the soil and consequently the CO<sub>2</sub> emission from soil to
- 4 the atmosphere (Sainju et al., 2008; Ding et al., 2007). In Mediterranean conditions, to our
- 5 knowledge, no previous work has explored the effects of N fertilization on soil CO<sub>2</sub> flux.
- 6 As a part of a wider study of the long-term effects of N fertilization on SOC dynamics
- 7 under different tillage systems in a semiarid Mediterranean agroecosystem, in this work we
- 8 are presenting and discussing data on soil CO<sub>2</sub> fluxes induced by tillage implementation
- 9 and rainfall events after 10 years of different treatments of tillage and N fertilization.

### MATERIALS AND METHODS

### Site, tillage and N fertilization

- 12 A long-term tillage and N fertilization experiment in winter-barley was established in 1996
- in Agramunt (41° 48'N, 1° 07'E; Lleida, Spain) (Cantero-Martínez et al., 2003). Three
- levels of N fertilization (zero (ZN), medium (MN) -60 kg N ha<sup>-1</sup>- and high (HN) -120 kg N
- 15 ha<sup>-1</sup>-) were compared in a factorial design with three tillage systems (no-tillage, NT and
- 16 minimum tillage, MT -conservation tillage systems-; and conventional tillage, CT -
- intensive tillage system-) in a three repetition randomized block design with a plot size 50
- 18 m x 6 m.

10

- 19 The soil is Xerofluvent typic with a mean annual precipitation of 430 mm. In 1996, before
- 20 the initiation of the experiment, the soil in the Ap horizon (0-28cm) contained 465 g kg<sup>-1</sup>
- sand, 417 g kg<sup>-1</sup> silt and 118 g kg<sup>-1</sup> clay, an organic carbon concentration ranging form 6 to
- 22 9 g kg<sup>-1</sup> and pH ranging from 7.8 to 8.1.
- 23 In this long-term experiment, winter-barley was cropped every year under rainfed
- 24 conditions. By the end of June, after the grain harvest, the straw residue was spread over

the plot in all the treatments. Since this moment, the field was kept free from vegetation for three-four months fallow. Tillage operations were annually conducted by the end of October or beginning of November. The CT treatment consisted on an intensive tillage with moldboard ploughing to a depth of 25-30 cm soil surface with almost 100% of the residue incorporated in the soil. The moldboard plow consisted of three bottoms of 0.50 m width. Intensive tillage operations in 2007 and 2008 were replaced with disk ploughing to a depth of 25-30cm instead of moldboard ploughing, due to the soil water conditions at the moment of tillage implementation, too dry in 2007 and too wet in 2008. Disk ploughing consisted in full inversion tillage similar to moldboard ploughing in terms of depth of disturbance, soil loosening and residue incorporation. The MT treatment consisted in a cultivator pass to a depth of 10-15 cm with an incorporation of approximately 50% of the crop residue. The plough consisted of 5 rigid shanks spaced 20 cm apart and with a shank width of 5 cm. No soil disturbances were produced in the NT plots. Barley was annually seeded in mid November two-three weeks after tillage operations. N fertilizer was split in two applications: one-third of the dose previous to tillage as ammonium sulfate (21% N) and two-thirds of the dose at the beginning of tillering as ammonium nitrate (33.5% N).

17 Measurements

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

18

19

20

21

22

23

24

Soil CO<sub>2</sub> fluxes were measured during autumn tillage operations in four consecutive years (2005-2008 period). An open chamber system (model CFX-1, PPSystems) connected to an infrared gas analyzer (model EGM-4, PPSystems) were used. The chamber has a cylindrical diameter of 21 cm, covering a soil surface of 346 cm<sup>2</sup>. Two regions of 6 m<sup>2</sup> were defined on each plot, and one measurement within each region was taken on each sampling day. The chamber was directly inserted about 1-2 cm deep in the soil. Flow rate of the chamber was adjusted to 900 mL min<sup>-1</sup>. Flux readings were taken 3 to 4 min after the

chamber had been inserted into the soil, when readings of CO2 flux were stable. All 1 2 measurements were conducted between 09:00 and 13:00 hours. From other experiments on 3 the area, diurnal variations in summer and autumn have been found to be reduced, and measurements at the given time were assumed to provide estimations of daily soil CO2 4 5 emissions for the calculations of cumulated fluxes. 6 The effects of tillage operations on soil CO<sub>2</sub> fluxes were evaluated during tillage operations 7 in four consecutive years (2005-2008). For each tillage system, soil CO<sub>2</sub> flux was measured 8 five times each year (Table 1); 24 hours prior to tillage (-1d), immediately after tillage 9 implementation (0h), two hours after second measurement (2h), 24 hours after tillage (1d) 10 and 48 hours after tillage (2d). For NT plots, the 2h sampling was suppressed since the 11 difference in gas fluxes between 0h and 2h was minimal. 12 The effects of rainfall events on soil CO<sub>2</sub> flux were evaluated in December 2006, in 13 September and October 2007 and in July 2009. Soil CO<sub>2</sub> flux was measured on the day 14 before and after rainfall events. In September and October 2007, we extended the 15 experimental period after each rainfall event for the observation of the evolution of soil 16 CO<sub>2</sub> flux with subsequent soil drying (Table 2). In 2006, when studying the effects of 17 tillage operations, CO<sub>2</sub> flux was also affected by additional 8 mm rainfall, which is 18 considered for discussion on the soil CO<sub>2</sub> fluxes after rainfalls. 19 The measurements during tillage and during rainfalls were conducted during fallow period. 20 During this time, the soil was free from living plants and there was not any live plant root. 21 In December 2006, measurements were conducted at crop emergence, when the 22 contribution of root respiration can be supposed minimal (Rochette et al., 1999a). Emerging 23 green area was cut without altering the soil surface before inserting the soil chamber. For

- these reasons, the CO<sub>2</sub> efflux during these events can be entirely attributed to microbial
- 2 respiration and so it can be accounted as net C losses from the soil.
- 3 Environmental conditions in the soil surface were determined at each sampling point. Soil
- 4 temperature at 5 cm depth was determined with a had-held probe (TM65, Crison).
- 5 Gravimetric soil water content (SWC) in the soil surface (0-5 cm depth) was determined by
- 6 oven drying a soil sample from each sampling point at 105°C. Daily air temperature and
- 7 precipitation were recorded at the experimental site in an automated weather station.

8 Data analysis

9 Statistical analyses of data were performed with SAS (1990). On each sampling date,

analyses of variance (ANOVA) using the general linear model procedure was used was

used to detect significance of the main factor effects (Tillage system (TIL) and N fertilizer

dose (FNT)) and the interaction factor effect (TILxFNT) on each year). Where effects

revealed statistically significant (P<0.05), separation of means was determined by multiple

comparisons of least-squares means (P<0.05).

Rough estimates of cumulative soil C losses after the rainfall event or tillage were

calculated by linearly interpolating CO<sub>2</sub> flux measurements on consecutive days after

tillage operations and after rainfall events (trapezoid rule). The accumulated flux of CO<sub>2</sub>

was divided by the length of the experimental measurement, 2 days for tillage periods, or 1,

3 or 6 days for rainfall events, and the soil C loss is reported in kg CO<sub>2</sub>-C ha<sup>-1</sup>d<sup>-1</sup> for

qualitative comparison among measurements periods.

13

16

17

18

19

# 1 RESULTS

2	1. Tillage effects on short-term soil CO <sub>2</sub> fluxes
3	Soil CO <sub>2</sub> flux on the day before tillage operations (-1 in Fig. 1) was significantly affected
4	by the effect of tillage systems (TIL) (Table 3). This response was related to differences in
5	soil environmental conditions (SWC and soil temperature) and organic C availability from
6	changes on SOC concentration and crop residue production.
7	On day 0, under NT, soil CO <sub>2</sub> flux on day 0 was similar to those on the day before (-1) in
8	three out of the four periods considered indicating steady conditions (Fig 1). Considering
9	the years 2005, 2007 and 2008, soil CO <sub>2</sub> fluxes on these two successive days were similar
10	(slope= 1.03; R <sup>2</sup> =0.87; P<0.05) (Fig 2), further indicating steady fluxes in that period
11	during these years. However, fluxes in 2006 were not steady due to the effects of a rainfall
12	event and soil drying.
13	On day 0, under MT and CT, tillage operations induced an immediate burst of CO <sub>2</sub> efflux
14	ranging from 1 to 8 μmol m <sup>-2</sup> s <sup>-1</sup> (Fig. 1), with major differences among years, in agreement
15	the previous studies in the region (Álvaro-Fuentes et al., 2007). Rochette and Angers
16	(1999b) in a prairie under cultivation observed pulses between 7 and 20 $\mu mol\ m^{-2}\ s^{-1}$
17	Reicosky et al. (1997) observed pulses between 1.6 and 3.2 µmol m <sup>-2</sup> s <sup>-1</sup> in a cultivated
18	land. As it has been already described, tillage operations lead to an immediate burst in soil
19	CO <sub>2</sub> emission rate because of an increase on the transport coefficient from soil loosening
20	(Reicosky and Lindstrom, 1993; Reicosky et al., 1997). The CO <sub>2</sub> flux after tillage
21	operations was similar under MT and CT in all years, with no significant differences
22	between these two systems at this moment.
23	N fertilization led to occasional significant effect on soil CO <sub>2</sub> flux at 2h and 0h observation
24	times respectively in 2006 and 2007 (Table 3). Fluxes under ZN were half those under MN

- and HN. Interaction between tillage and N fertilizer was significant two days after tillage
- 2 operations in 2006, with significant effect of N fertilizer level under MT, where soil CO<sub>2</sub>
- 3 fluxes were 0.49, 0.84 and 1.01 μmol m<sup>-2</sup> s<sup>-1</sup> under ZN, MN and HN respectively.
- 4 The observed burst of CO<sub>2</sub> emission after tillage operation was related to the flux of CO<sub>2</sub> on
- 5 the previous day. CO<sub>2</sub> flux at this moment is closely related to amount of CO<sub>2</sub> entrapped in
- 6 the soil atmosphere and that will be released after tillage operations. From this relation
- 7 between fluxes on the previous day (Fb) and fluxes immediately after tillage operations
- 8 (Fa) the slope of the linear adjustment were 4.22 under MT and 16.7 under CT indicating
- 9 the deeper and consequently higher soil disturbance of this system. Previous work on the
- 10 region reports an increase immediately after tillage operations from 3 to 15 times greater
- than the fluxes observed on the day before (Álvaro-Fuentes et al., 2007). In other studies in
- other agroecosystems, it has been reported an increase of soil CO<sub>2</sub> efflux of 3.8, 6.7, 8.2,
- and 10.3 times larger than the no-tilled soil for 10, 15, 20 and 25 cm plowing depths
- 14 (Reicosky and Archer, 2007). Under these conditions we report a similar relation between
- the  $CO_2$  released immediately after tillage and the  $CO_2$  flux before tillage.
- In all the years, soil CO<sub>2</sub> fluxes under MT and CT sharply decreased 2 hours after tillage
- operations in comparison to the flux immediately after tillage operations (Fig. 1). In 2007,
- 18 under driest conditions (Fig. 3), TIL effect was no longer significant at this moment (Table
- 19 3). The short-term response will depend on the soil environmental conditions when the soil
- 20 is tilled (Prior et al., 1997; Kessavalou et al., 1998a) and cultivation history and soil type
- 21 (Calderón et al., 2000).
- 22 SWC was higher under NT followed by MT and drier under CT during most samplings
- 23 (Figs 3). In 2006 and 2007, at 0h and at 2h measurements, SWC significantly also differed
- 24 among N fertilizer levels. SWC in ZN was on average 0.01-0.02 g·g<sup>-1</sup> (g H<sub>2</sub>O g<sup>-1</sup> dry soil)

- drier than the fertilized treatments (MN and HN). Soil temperatures during the
- 2 experimental periods ranged between 10 and 20 °C, with similar values under different
- 3 tillage systems. Average soil temperatures for each sampling are presented in Figure 4.
- 4 Warmer temperatures were partly responsible for the soil drying, especially in 2006 after an
- 5 8 mm precipitation event on the day before -1 (Figs. 3 and 4).

### 2. Rainfall effects on CO<sub>2</sub> fluxes

- 7 Soil CO<sub>2</sub> flux on the day before rainfall, was close to 0.5 μmol m<sup>-2</sup> s<sup>-1</sup>. The CO<sub>2</sub> flux, in
- 8 µmol m<sup>-2</sup> s<sup>-1</sup>, after rainfalls were 0.33 after 6 mm in December 2006 (D06), 1.68 after 4
- 9 mm in September 2007 (S07), 1.42 after 14 mm in October 2007 (O07) and 2.77 after 29
- 10 mm in July 2009 (J09) (Fig. 5).

- Soil rewetting led to high increases in soil CO<sub>2</sub> flux in three out of the four periods studied,
- with significant effect of tillage systems (TIL) (Table 4). Differences among tillage systems
- were more evident after rainfall events (Fig. 5). In D06, the response depended on the
- 14 tillage systems and was positive under conservation tillage systems (NT and MT) and
- negative under CT. In S07 and O07, soil CO<sub>2</sub> flux after rainfall was high and differences
- among tillage systems were high. Further rewetting in O07 further increased CO<sub>2</sub> flux. In
- J09, after 23 mm rainfall, soil CO<sub>2</sub> flux increased 8 times those on the day before under
- 18 conservation tillage systems.
- 19 In S07 and O07, soil CO<sub>2</sub> fluxes on the following days decreased as the soil dried (Figs. 5
- and 6). A similar trend was observed under NT after precipitation and the following days
- 21 concomitant with soil drying (Figs. 1 and 3) that led to 44% decrease between day -1 and
- day 0 under NT.
- 23 The response of soil CO<sub>2</sub> to N application (NIT) was occasional, as occurred during tillage
- operations. In September 3 days after precipitation, and in October on the previous to

1 rainfall event, average flux was 0.55  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> under ZN and 0.66  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> on

2 fertilized soil.

8

9

10

11

12

13

14

15

16

17

21

22

23

24

3 As found during the tillage implementation periods, the SWC after the rainfall events

4 differed among tillage systems with higher SWC under NT, medium under MT and lowest

5 under CT (Fig. 6). Soil temperature was between 1 and 4 °C higher under CT than under

6 NT, and medium under MT system (Fig 7).

7 Soil CO<sub>2</sub> flux after the observed rainfall events were related to the soil temperatures. Under

NT the soil CO<sub>2</sub> flux after the rainfall event showed a significant linear relationship with

soil temperatures (slope=0.15, R<sup>2</sup>=0.96, P<0.01) (Fig. 8), for temperatures between 10.3

and 24.2° C. This relationship is similar as that obtained by Rochette et al., 1991 under a

maize crop and considering data with volumetric soil water content between 10 and 35 %

(Rsoil= 0.2 Tsoil -0.74; for Tsoil between 13 and 33°C). Under MT and under CT there was

no clear relation with the soil temperature, indicating that other factors may have been of

greater concern during the observed events. Even the increase of soil CO<sub>2</sub> flux after the

observed rainfall events has been related with soil temperature under NT, the extent of soil

rewetting influences the activation, as observed after additional 11 mm rainfall in October

2007, with additional rainfall and rewetting (Fig. 6) and further increase of soil CO<sub>2</sub> flux

18 (Fig. 5).

19 **DISCUSSION** 

20 Soil CO<sub>2</sub> flux during the rainfall events as well as on the day before tillage operations were

higher under conservation tillage systems (NT and MT) than under CT (Fig. 1 and 5). Soil

water status limited soil CO<sub>2</sub> emissions. As observed after rainfall events, once rewetting

occurred, temperature determined the extent of the activation of soil CO<sub>2</sub> flux, and soil

drying the duration of the activation. Hence during summer and autumn, water limited-

seasons in Mediterranean conditions, the effect of rainfalls on soil CO2 flux is persisting for 1 2 a period of time depending on the length of time that the soil remains moist (Fierer et al., 3 2003), and it is not a pulse response, but a period of time during which water-limitation is 4 overcome. Responses of CO<sub>2</sub> fluxes under undisturbed soil in contrast to disturbed soil 5 have been observed in other field experiments with higher response under no-tillage 6 systems or on undisturbed soils in comparison with tilled soils (Kessavalou et al., 1998a; 7 Jackson et al., 2003). 8 The effects of TIL, NIT and the interaction term on soil CO<sub>2</sub> flux (Tables 3 and 4) can be 9 related to differences organic C availability and crop residue production. Annual biomass 10 production differed among tillage systems. Crop residues production was on average 30 11 and 23 % higher under NT and MT than under CT, due to improved water use efficiency 12 under conservation tillage systems in this region (data not shown). Because of tillage 13 operations and residue incorporation in deeper layers, SOC significantly responded to tillage systems and in 2006 the SOC concentration was 12.1, 17.6 and 20.3 g kg<sup>-1</sup>, in the 14 soil surface (0-5cm soil depth) under CT, MT and NT respectively, but no significant 15 16 differences at deeper layers, and this pattern on SOC stratification led to a similar pattern 17 on soluble carbon availability, microbial biomass and biochemical activity (Madejón et al., 18 2009). SOC accumulation and crop residue production enhanced substrate availability. 19 Substrate availability is one major control of the response of soil CO<sub>2</sub> flux to rainfall events 20 (Casals et al., 2009) from the effects on the soil microbial activity. Hence under semiarid 21 Mediterranean conditions, soil CO<sub>2</sub> fluxes after rainfall were more responsive to 22 precipitation events under NT and MT than under CT. Moreover, the increases in SOC 23 concentration is leading to an increase of the capacity of the soil to retain water, as 24 observed on the SWC after rainfall events (Fig. 6).

From all the observations in this work, N fertilization effect was only significant in two samplings during tillage operations and two samplings during rainfall events (Table 3 and 4). In these two samplings during rainfall the interaction term was also significant (Table 4), besides of another sampling during tillage operations in 2006 (Table 3). The interaction indicated N fertilizer application occasionally increased soil CO<sub>2</sub> flux under conservation tillage systems (NT and MT), but were not significant under CT. In a field experiment in Montana, N fertilization increased soil CO<sub>2</sub> emissions by 14% in an annual cropping system, though this effect could be attributed to differences on either root respiration or microbial respiration (Sainju et al., 2008). However during the experimental periods considered in this paper, carried out during fallow periods, there was not any contribution of root respiration to soil CO<sub>2</sub> flux and differences among treatments were ascribed to differences on microbial respiration. The occasional response of soil CO<sub>2</sub> flux to N application in our experiment, and the interaction with tillage systems (Tables 3 and 4) can be related with similar responses on residue production (data not shown). N fertilization led to 10 to 20% increase that led to higher soil cover and slightly wetter conditions and may eventually lead to increased substrate availability and increased soil microbial activity that would hence increase soil CO<sub>2</sub> flux. This may have affected microbial activity and soil CO<sub>2</sub> flux during these moments. In a subhumid environment, N fertilizer applications reduced soil CO<sub>2</sub> flux (Brye, 2006), however the N fertilizer regimes had failed to increase wheat residues levels and other factors may have been involved in this response. The implication of short-term emissions of CO<sub>2</sub> on soil C dynamics was considered in the seven measurement periods studied. Rough estimates of the CO<sub>2</sub>-C emissions were calculated for the three rainfall events and four autumn tillage events. CO<sub>2</sub>-C emission, on a per day basis, is calculated for different tillage systems and N fertilizer levels (Table 5).

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

1 The daily CO<sub>2</sub> -C loss after tillage is within the range of values obtained by Ellert and Janzen, 1999, that reported 2.7 kg C ha<sup>-1</sup> d<sup>-1</sup>, during two days, Prior, 1997, that reported 5.0 2 kg C ha<sup>-1</sup> d<sup>-1</sup>, during 8 days after tillage implementation and McGinn et al., 1998, that 3 reported 8.4 kg C ha<sup>-1</sup> d<sup>-1</sup> during 11 days after tillage. Short-term losses of CO<sub>2</sub>-C after 4 5 tillage are small in terms of C balance (Roberts and Chan, 1990; Álvaro-Fuentes et al., 6 2008) even after initial cultivation of no-tilled soil (Quincke et al., 2007). Despite the initial 7 flush, tillage operations produced a pulse of CO2 emission of short duration that leads to 8 little effect on cumulative CO<sub>2</sub>-C losses (Table 5). 9 From long-term observations (unpublished data), mean annual soil CO<sub>2</sub> flux rates in this experimental area are around 10 kg CO<sub>2</sub>-C ha<sup>-1</sup> d<sup>-1</sup>, and those during summer and autumn 10 are 4 and 7 kg CO<sub>2</sub>-C ha<sup>-1</sup> d<sup>-1</sup>, in agreement with similar agroecosystems in semiarid Spain 11 12 (Sanchez et al., 2003). The calculated rates after rainfall were up to 6 times those mean 13 rates (Table 5). The accumulated CO<sub>2</sub>-C losses after rainfalls during fallow period appeared 14 to be of greater importance than those after tillage operations, due to a more persisting 15 effect (Kessavalou et al., 1998b; Ball et al., 1999). Tillage system had substantial effects on CO<sub>2</sub> emissions after rainfalls (Table 5) that must be taken into account when comparing 16 17 emissions under different tillage systems. Effects of N fertilization on the cumulative fluxes 18 were slight, in accordance with the lack of changes on the SOC content due to long-term N 19 fertilization. Hence, CO<sub>2</sub> emission after rainfalls during the fallow period, especially under 20 semiarid conditions, must be taken into account to accurately estimate annual soil CO<sub>2</sub> 21 emissions and for the evaluation of management practices.

#### 22 CONCLUSIONS

23

24

This experiment highlights the extent of changes on the dynamics of soil CO<sub>2</sub> emission with conservation tillage systems and the contributions of tillage operations and

- 1 precipitation events on the soil CO<sub>2</sub> emissions in semiarid Mediterranean conditions.
- 2 Tillage operations led to pulse emissions that were occasionally affected by N fertilization,
- 3 however these pulse had little effect, and cumulated CO<sub>2</sub> emissions after two days were
- 4 similar. Increases and decreases of soil CO<sub>2</sub> flux occurred with soil rewetting and soil
- 5 drying. Under NT, soil CO<sub>2</sub> flux after rainfalls could be related with soil temperature.
- 6 Emissions after rainfall events are high and must be taken into account when estimating the
- 7 soil CO<sub>2</sub>-C loss in semiarid Mediterranean agroecosystems. During these events,
- 8 conservation tillage systems (NT and MT) increased the soil CO<sub>2</sub> emission.

### 9 ACKNOWLEDGMENTS

- 10 This work was supported with by the Comision Interministerial de Ciencia y Tecnologia of
- 11 Spain (Grants AGL 2004-07763-C02-02 and AGL2007-66320-CO2-02/AGR). Thanks to
- 12 Carlos Cortés and Silvia Martí for technical assistance and to Jaume Prenafeta for his help
- after precipitation events. We also acknowledge the Ministry of Science and Innovation for
- 14 funding the doctorate studies of F.J. Morell.

18

22

#### 15 REFERENCES

- Akinremi, O.O., McGinn, S.M., McLean, H.D.J., 1999. Effects of soil temperature and
- moisture on soil respiration in barley and fallow plots. Can. J. Soil Sci., 79: 5-13.
- 19 Almagro, M., López, J., Querejeta, J.I., Martínez-Mena, M., 2009. Temperature
- 20 dependence of soil CO<sub>2</sub> efflux is strongly modulated by seasonal patterns of moisture
- 21 availability in a mediterranean ecosystem. Soil Biol.Biochem., 41: 594-605.
- 23 Alvarez, R., Alvarez, C.R., Lorenzo, G., 2001. Carbon dioxide fluxes following tillage
- from a mollisol in the argentine rolling pampa. Eur. J. Soil Biol., 37: 161-166.

- 2 Álvaro-Fuentes, J., López, M.V., Arrúe, J.L., Cantero-Martínez, C., 2008. Management
- 3 effects on soil carbon dioxide fluxes under semiarid Mediterranean conditions. Soil Sci.
- 4 Soc. Am. J., 72: 194-200.

5

- 6 Álvaro-Fuentes, J., Cantero-Martínez, C., López, M.V., Arrúe, J.L., 2007. Soil carbon
- 7 dioxide fluxes following tillage in semiarid Mediterranean agroecosystems. Soil Tillage
- 8 Res., 96: 331-341.

9

- 10 Ball, B.C., Scott, A., Parker, J.P., 1999. Field N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> fluxes in relation to
- tillage, compaction and soil quality in scotland. Soil Tillage Res., 53: 29-39.

12

- Bono, A., Alvarez, R., Buschiazzo, D.E., Cantet, R.J.C., 2008. Tillage effects on soil
- carbon balance in a semiarid agroecosystem. Soil Sci. Soc. Am. J., 72: 1140-1149.

15

- Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N
- mineralization and fluxes in soils. Global Change Biol., 15: 808-824.

18

- 19 Brye, K.R., Longer, D.E., Gbur, E.E., 2006. Impact of Tillage and Residue Burning on
- 20 Carbon Dioxide Flux in a Wheat–Soybean Production System. Soil Sci. Soc. Am. J., 70:
- 21 1145-1154.

- 23 Calderón, F.J., Jackson, L.E., Scow, K.M., Rolston, D.E., 2000. Microbial responses to
- simulated tillage in cultivated and uncultivated soils. Soil Biol. Biochem., 32: 1547-1559.

- 2 Cantero-Martínez, C., Angás, P., Lampurlanés, J., 2003. Growth, yield and water
- 3 productivity of barley (hordeum vulgare L.) affected by tillage and N fertilization in
- 4 Mediterranean semiarid, rainfed conditions of Spain. Field Crops Res., 84: 341-357.

5

- 6 Casals, P., Gimeno, C., Carrara, A., Lopez-Sangil, L., Sanz, M.J., 2009. Soil CO<sub>2</sub> efflux
- 7 and extractable organic carbon fractions under simulated precipitation events in a
- 8 Mediterranean Dehesa. Soil Biol. Biochem., 41: 1915-1922.

9

- Ding, W., Cai, Y., Cai, Z., Yagi, K., Zheng, X., 2007. Soil respiration under maize crops:
- Effects of water, temperature, and nitrogen fertilization. Soil Sci. Soc. Am. J., 71: 944-951.

12

- 13 Ellert, B.H., Janzen, H.H., 1999. Short-term influence of tillage on CO<sub>2</sub> fluxes from a semi-
- arid soil on the canadian prairies. Soil Tillage Res., 50: 21-32.

15

- 16 Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide
- production commonly observed following the rapid rewetting of a dry soil. Soil Sci. Soc.
- 18 Am. J., 67: 798-805.

19

- Franzluebbers, A.J., Hons, F.M., Zuberer, D.A., 1995. Tillage and crop effects on seasonal
- 21 dynamics of soil CO<sub>2</sub> evolution, water-content, temperature and bulk-density. Appl. Soil
- 22 Ecol. 2: 95-109.

- 1 Inglima, I., Alberti, G., Bertolini, T., Vaccaris, F.P., Giolis, B., Migliettas, F., Cotrufo,
- 2 M.F., Peressotti, A., 2009. Precipitation pulses enhance respiration of mediterranean
- 3 ecosystems: The balance between organic and inorganic components of increased soil CO<sub>2</sub>
- 4 efflux. Global Change Biol., 15: 1289-1301.

- 6 Jackson, L.E., Calderon, F.J., Steenwerth, K.L., Scow, K.M., Rolston, D.E., 2003.
- 7 Responses of soil microbial processes and community structure to tillage events and
- 8 implications for soil quality. Geoderma, 114: 305-317.

9

- 10 Johnson, J.M., Franzluebbers, A.J., Weyers, S.L., Reicosky, D.C., 2007. Agricultural
- opportunities to mitigate greenhouse gas emissions. Environ. Pollution, 150: 107-124.

12

- 13 Kessavalou, A., Doran, J.W., Mosier, A.R., Drijber, R.A., 1998a. Greenhouse gas fluxes
- 14 following tillage and wetting in a wheat-fallow cropping system. J. Environ. Qual., 27:
- 15 1105-1116.

16

- 17 Kessavalou, A., Mosier, A.R., Doran, J.W., Drijber, R.A., Lyon, D.J., Heinemeyer, O.,
- 18 1998b. Fluxes of carbon dioxide, nitrous oxide, and methane in grass sod and winter wheat-
- 19 fallow tillage management. J. Environ. Qual., 27: 1094-1104.

20

- 21 Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food
- 22 security. Science, 304: 1623-1627.

- 1 Lal, R., Bruce, J.P., 1999. The potential of world cropland soils to sequester C and mitigate
- 2 the greenhouse effect. Environ. Sci. Policy, 2: 177-185.

- 4 La Scala N. Jr., Lopes, A., Spokas, K., Bolonhezi, D., Archer, D.W., Reicosky, D.C., 2008.
- 5 Short-term temporal changes of soil carbon losses after tillage described by a first-order
- 6 decay model. Soil Tillage Res., 99: 108-118.

7

- 8 Madejón, E., Murillo, J.M., Moreno, F., López, M.V., Arrue, J.L., Álvaro-Fuentes, J.,
- 9 Cantero-Martínez, C., 2009. Effect of long-term conservation tillage on soil biochemical
- properties in Mediterranean Spanish areas. Soil Tillage Res., 105: 55-62.

11

- 12 McGinn, S.M., Akinremi, O.O., McLean, H.D.J., Ellert, B., 1998. An automated chamber
- system for measuring soil respiration. Can. J. Soil Sci., 78: 573-579.

14

- Paustian, K., Andrén, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Van
- Noordwijk, M., Woomer, P.L., 1997. Agricultural soils as a sink to mitigate CO<sub>2</sub> emissions.
- 17 Soil use and Manag., 13: 230-244.

18

- 19 Prior, S.A., Rogers, H.H., Runion, G.B., Torbert, H.A., Reicosky, D.C., 1997. Carbon
- 20 dioxide-enriched agroecosystems: Influence of tillage on short-term soil carbon dioxide
- 21 efflux. J. Environ. Qual., 26: 244-252.

- 1 Quincke, J.A., Wortmann, C.S., Mamo, M., Franti, T., Drijber, R.A., 2007. Occasional
- 2 tillage of no-till systems: Carbon dioxide flux and changes in total and labile soil organic
- 3 carbon. Agron. J., 99: 1158-1168.

- 5 Reicosky, D.C., Archer, D.W., 2007. Moldboard plow tillage depth and short-term carbon
- 6 dioxide release. Soil Tillage Res., 94: 109-121.

7

- 8 Reicosky, D.C., Lindstrom, M.J., 1993. Fall tillage method: Effect on short-term carbon
- 9 dioxide flux from soil. Agron. J., 85: 1237-1243.

10

- 11 Reicosky, D.C., Dugas, W.A., Torbert, H.A., 1997. Tillage-induced soil carbon dioxide
- loss from different cropping systems. Soil Tillage Res., 41: 105-118.

13

- Roberts, W.P., Chan, K.Y., 1990. Tillage-induced increases in carbon dioxide loss from
- 15 soil. Soil Tillage Res., 17: 143-151.

16

- 17 Rochette, P., Desjardins, R.L., Pattey, E., 1991. Spatial and temporal variability of soil
- respiration in agricultural fields. Can. J. Soil Sci., 71: 189-196.

19

- 20 Rochette, P., Angers, D.A., 1999b. Soil surface carbon dioxide fluxes induced by spring,
- summer, and fall moldboard plowing in a sandy loam. Soil Sci. Soc. Am. J., 63: 621-628.

- 1 Rochette, P., Flanagan, L.B., Gregorich, E.G., 1999a. Separating soil respiration into plant
- 2 and soil components using analyses of the natural abundance of carbon-13. Soil Sci. Soc.
- 3 Am. J., 63: 1207-1213.

- 5 Sainju UM, Jabro JD, Stevens WB., 2008. Soil carbon dioxide emission and carbon content
- 6 as affected by irrigation, tillage, cropping system, and nitrogen fertilization. J. Environ.
- 7 Qual., 37(1): 98-106.

8

- 9 Sanchez, M.L., Ozores, M.I., Lopez, M.J., Colle, R., De Torre, B., Garcia, M.A., Perez, I.,
- 10 2003. Soil CO<sub>2</sub> fluxes beneath barley on the central Spanish plateau. Agric. For. Meteorol.,
- 11 118: 85-95.

12

- 13 SAS Institute, 1990. SAS User's Guide: Statistics, sixth ed., vol. 2. SAS Institute, Cary,
- 14 NC.

## **TABLES**

- ${\bf 2} \qquad {\bf Table \ 1}. \ {\bf Calendar \ of \ soil \ CO_2 \ measurements \ during \ tillage \ operations \ for \ each \ experimental}$
- 3 period.

Year	Tillage date	Time since tillage		
		implementation in hours		
2005	3 <sup>rd</sup> November	-24, 0, 2, 24, 48		
2006	25 <sup>th</sup> October	-24, 0, 2, 24, 48		
2007	6 <sup>th</sup> November	-24, 0, 2, 24, 48		
2008	6 <sup>th</sup> November	-24, 0, 2, 24, 48		

- **Table 2**. Calendar of soil CO<sub>2</sub> measurements during rainfall events for each experimental period. -
- 6 1, previous day to precipitation event.

Precipitation event (Rainfall)	Precipitation date	Time since precipitation (days)
December 2006 (6mm)	5 <sup>th</sup> December 06	-1, 1
September 2007 (4mm)	22 <sup>nd</sup> September 07	-1, 1, 2, 3
October 2007 (14+10mm)	3 <sup>rd</sup> October 07	-1, 1, 2, 3, 4, 5, 6
July 2009 (29mm)	6 <sup>th</sup> July 09	-1, 1

- 1 Table 3. Significance of main factors (TIL, tillage system, and NIT, N fertilizer levels) and
- 2 the interaction factor (TIL\*NIT) on the analysis of variance for each sampling and year before (-
- 3 1 day), and after tillage operations (After, 2 hours, 1 day and 2 days), (C.V., coefficient of variation;
- 4 NS, non-significant at 0.05 probability level). The number of samples on each sampling was 54
- 5 (two samples per plot) but on the 0 hour sampling when only 27 samples were taken (one per plot).

S	Event	C.v.	TIL	NIT	TIL*NIT	
	2005	31.7	0.0144	NS	NS	
y	2006	30.6	<.0001	NS	NS	
Day	2007	27.4	0.0005	NS	NS	
	2008	47.2	<.0001	NS	NS	
	2005	63.4	0.0005	NS	NS	
ur	2006	55.4	0.0014	NS	NS	
0 hour	2007	64.6	0.0153	0.32	NS	
	2008	56.5	0.0006	NS	NS	
	2005	51.1	0.0107	NS	NS	
2 hours	2006	25.5	0.0022	0.00	NS	
ho	2007	34.6	NS	NS	NS	
(1	2008	14.7	0.001	NS	NS	
er	2005	46.4	0.0123	NS	NS	
1 day after	2006	35.7	NS	NS	NS	
day	2007	29.1	<.001	NS	NS	
1	2008	39.7	0.0063	NS	NS	
	2005	45.2	NS	NS	NS	
ays	2006	34.4	0.0060	NS	0.0057	
2 days	2007	33.5	0.0402	NS	NS	
	2008	32.5	<.0001	NS	NS	

- 1 Table 4. Significance of main factors (TIL, tillage system, and NIT, N fertilizer levels) and
- 2 the interaction factor (TIL\*NIT) on the analysis of variance for each sampling and year before (-
- 3 1 day), and after rainfall events (After, 2 hours, 1 day and 2 days), (C.V., coefficient of variation;
- 4 NS, non-significant at 0.05 probability level). S. Sampling; d, days. The number of samples on each
- 5 sampling was 54 (two samples per plot).

S	Event	C.V.	TIL	NIT	TIL*NIT	
e.	D06	29.5	0.008	NS	NS	
Day before	S07	45.4	<.0001	NS	NS	
ay b	O07	33.3	0.02	0.02	0.04	
D	J09	25.7	NS	NS	NS	
L	D06	29.5	<.0001	NS	NS	
Day after	S07	34.7	<.0001	NS	NS	
)ay	O07	39.6	<.0001	NS	NS	
I	J09	19.7	0.02	NS	NS	
d	S07	39.9	<.0001	NS	NS	
2 d afte	O07	38.8	<.0001	NS	NS	
2.4	S07	41.6	<.0001	0.05	0.02	
3 d	O07	35.4	<.0001	NS	NS	
4 d	O07	34.6	<.0001	NS	NS	
5 d	O07	37.9	<.0001	NS	NS	
6 d	O07	43.9	<.0001	NS	NS	

- 1 Table 5. Estimations of daily soil CO<sub>2</sub>-C loss from tillage-implementation periods (Tillage) and
- 2 rainfall events (Rainfall), in kg of C per hectare and per day. The number in brackets on the Period
- 3 column indicates the number of observation days after tillage or rainfall events.

	Period	Cumulative CO <sub>2</sub>			Cumulative CO <sub>2</sub>			
		NT	MT	CT	ZN	MN	HN	
	2005 (2)	4.7	8.8	6.6	6.7	7.6	7.1	
age	2006 (2)	6.4	9.3	7.2	6.2	8.2	9.2	
Tillage	2007 (2)	3.3	5.3	4.1	4.2	4.5	4.3	
	2008 (2)	10.7	14.4	10.0	12.6	13.0	11.0	
	Dec 06 (1)	5.5	4.2	0.7	3.6	3.5	3.3	
ıfall	Sep 07 (3)	14.7	12.4	6.6	10.0	12.9	10.9	
Rainfall	Oct 07 (6)	19.3	17.0	6.3	14.4	14.9	13.7	
	Jul 09 (1)	28.1	33.4	21.8	25.9	27.0	30.7	

#### FIGURE CAPTIONS

- 2 Fig 1. Soil CO<sub>2</sub> fluxes during tillage operations (CT, conventional tillage; MT, minimum tillage;
- 3 NT, no-tillage) in four consecutive years. Vertical bars indicate standard deviations of the means for
- 4 each tillage system.

5

1

- 6 Fig 2. Relation of soil CO<sub>2</sub> efflux the day before tillage (-24h) and immediately after tillage
- 7 implementation (0h), for each tillage system (CT, conventional tillage; MT, minimum tillage; NT,
- 8 no-tillage). Nine points per treatment corresponding to observations in three years (2005, 2007 and
- 9 2008) and three N fertilization levels for each year and tillage system. Lines were obtained from
- 10 linear adjustments for each tillage system. Fb and Fa are fluxes before and after tillage operations.

11

- 12 Fig 3. Gravimetric soil water content (SWC) in the top 5 cm during the tillage periods studied
- under different tillage treatments (CT, conventional tillage; MT, minimum tillage; NT, no-tillage),
- 14 line plot. Different letters indicate mean separation among tillage systems (P<0.05). Mean
- separation on the following days was the same as indicated for day 1.

16

- 17 **Fig 4.** Mean Trends of soil temperatures at 5 cm soil depth on each experimental period in different
- 18 years, 2005-2008, for the different tillage systems: CT, conventional tillage; MT, minimum tillage;
- 19 and NT, no-tillage.

20

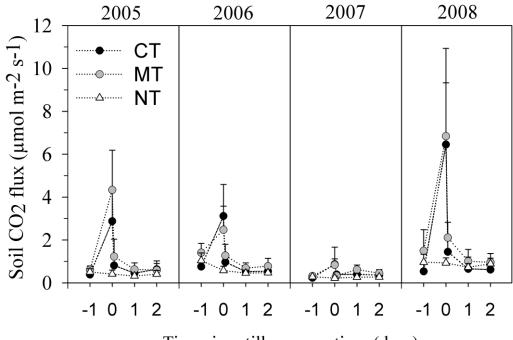
- 21 Fig 5. Soil CO<sub>2</sub> fluxes before and after rainfall events (D06, December 2006; S07, September
- 22 2007; O08, October 2007; O9, July 2009) under different tillage systems (CT, conventional tillage;
- MT, minimum tillage; NT, no-tillage). Vertical bars indicate standard deviations of the means for
- each tillage system.

- 1 Fig 6. Gravimetric soil water content (SWC) in the top 5 cm previous and after rainfall events
- 2 under CT, conventional tillage; MT, minimum tillage; and NT, no-tillage. In -1, values on the
- 3 previous day are indicated. The periods are D06, December 2006; S07, September 2007; O08,
- 4 October 2007; 09, July 2009. Different letters indicate mean separation among tillage systems
- 5 (P<0.05). In S07 and O07 mean separation on the following days was the same as indicated for day
- 6 1.

- 8 Fig 7. Trends of soil temperatures at 5 cm soil depth on each experimental period: D06, December
- 9 2006; S07, September 2007; O08, October 2007; for the different tillage systems: CT, conventional
- tillage; MT, minimum tillage; and NT, no-tillage.

11

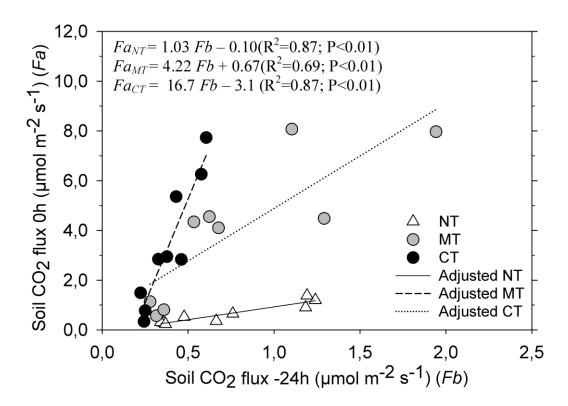
- 12 **Fig 8.** Relation of soil CO<sub>2</sub> flux after rainfall (1) and temperature, for each tillage system (CT,
- conventional tillage; MT, minimum tillage; NT, no-tillage) on the day after rainfall events. Ellipses
- indicate the rainfall events considered: O06, October 2006; D06, December 2006; S07, September
- 15 2007; O07, October 2007; J09, July 2009. The amount precipitated (in mm) is also indicated for
- each event. The adjusted line is drawn for NT system. The adjustments were not significant under
- 17 MT and CT (P<0.05).



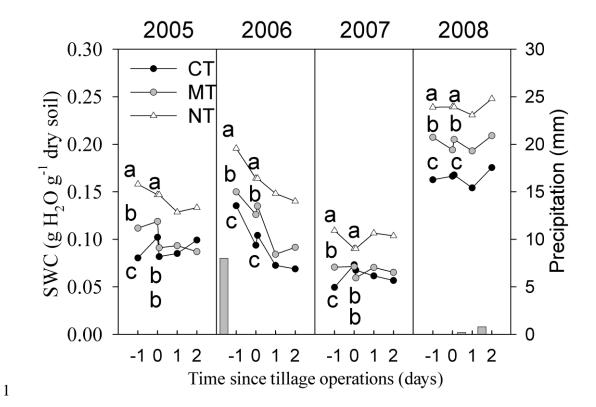
Time since tillage operations (days)

## 2 Figure 1

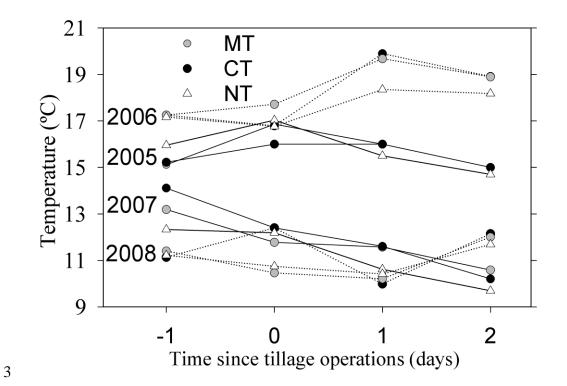
1



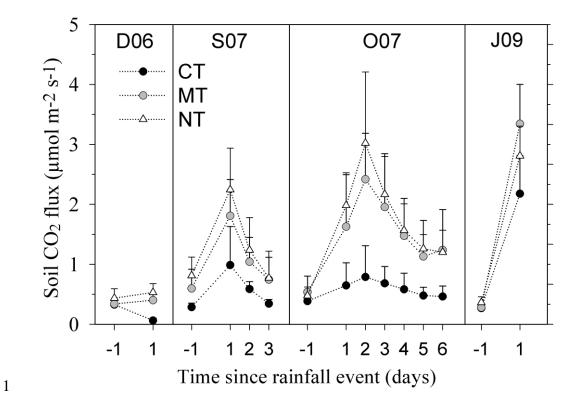
4 Figure 2



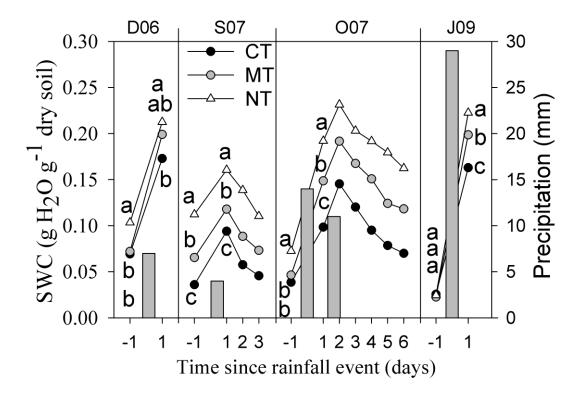
2 Figure 3



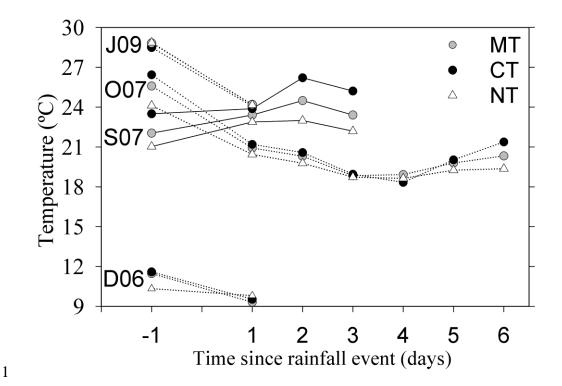
4 Figure 4



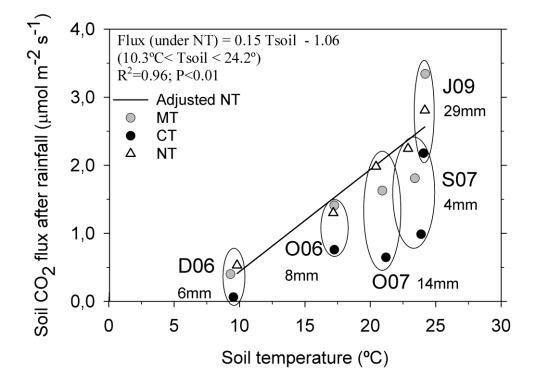
# 2 Figure 5



4 Figure 6



## 2 Figure 7



4 Figure 8