



Universitat de Lleida

## The role of sustainable agricultural practices to mitigate greenhouse gases and to sequester soil carbon under newly irrigated Mediterranean agroecosystems

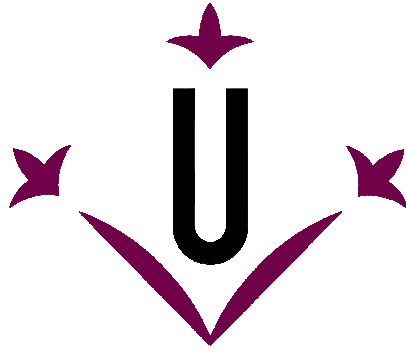
Evangelina Pareja Sánchez

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**DOCTORAL THESIS**

**The role of sustainable agricultural practices to mitigate greenhouse  
gases and to sequester soil carbon under newly irrigated  
Mediterranean agroecosystems**

Evangelina Pareja Sánchez

Dissertation to obtain the degree of Doctor by the University of Lleida. Doctorate Program in  
Agricultural and Food Science and Technology

Doctoral Thesis

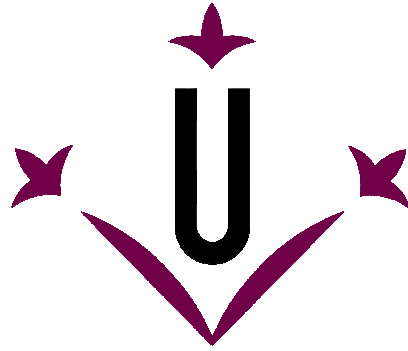
Supervised by:

Dr. Carlos Cantero Martínez

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2019





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# Agradecimientos

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## Summary

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In the Mediterranean rainfed area of the Ebro valley (NE of Spain), farmers have adopted conservation agriculture during the last 35 years. However, a significant fraction of the agricultural area is being converted to irrigation to ensure greater yields, changing winter cereals to new more productive crops like maize. In these newly irrigated areas, farmers are returning to adopt intensive tillage systems, which are the most usual in irrigated maize production and therefore, putting at risk the soil quality benefits achieved with long-term conservation tillage. Moreover, the gradual occupation of irrigation, leads to crops which require more nitrogen use, which if not adapted to the needs of the crop, can lead to a greater environmental impact, such as increasing greenhouse gas (GHG) emissions. In these new conditions, there is a lack of knowledge about the best combination of tillage and N fertilization rates to reduce soil GHG emissions while maintaining maize productivity as well as keeping soil quality. Therefore, the main objective of this study was the identification of the effect of different tillage systems and N fertilizer rates on GHG emissions (methane, CH<sub>4</sub>; carbon dioxide, CO<sub>2</sub>; nitrous oxide, N<sub>2</sub>O) to the atmosphere, as well as, soil C sequestration, soil surface structure and crop productivity when converting rainfed lands to irrigated. In order to achieve that objective a study was carried out in NE Spain in a long-term (LTE) tillage and N rate field experiment established in 1996 under rainfed barley (*Hordeum vulgare* L.) conditions which was converted to irrigation with maize (*Zea mays* L.) monoculture as cropping system in 2015. This study was conducted during three consecutive maize growing seasons (i.e. years 2015, 2016, and 2017). Three types of tillage (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) and three mineral N fertilization rates (0, 200, 400 kg N ha<sup>-1</sup>) were compared in a randomized block design with three replications. In 2015, an adjacent experiment (short-term experiment, STE) with the same layout as the LTE but with different previous management based on NT was set up. In the LTE, soil CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions were quantified during three years. Also, N<sub>2</sub>O emission factor (EF) and yield-scaled N<sub>2</sub>O emissions were determined. In addition, annual SOC sequestration rate ( $\Delta\text{SOC}_{\text{rate}}$ ) (0-40 cm depth) was calculated for each treatment in three different periods (P1-, P2-, P3-) under rainfed (-R) conditions and irrigated (-I) conditions (P1-R, from 1996 to 2009; P2-R,



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2009 to 2015; P3-I, from 2015 to 2017). Moreover, in LTE and STE soil surface (0-5 cm) dry and water-stable macroaggregates and their C concentration, as well as other soil fractions (total SOC concentration and labile C concentration) were measured. Also soil surface penetration resistance (PR), and water infiltration were analyzed during the second maize growing season (i.e. year 2016). In addition, in both experimental fields, aboveground biomass, maize grain yield, yield components and water and nitrogen use efficiency (WUE and NUE, respectively) were measured annually. In the LTE, under CT, a surface crusting and deterioration of soil structure caused low water infiltration (1.70, 2.40 and 3.14 mm h<sup>-1</sup> for CT, RT and NT, respectively) reduced soil water availability for the crop. Crop establishment was also affected by soil surface degradation, showing a 22% and 19% lower density of plants in CT compared to NT and RT, respectively. The main process cause behind soil crusting was low SOC concentration, making the soil more susceptible to breakdown of aggregates. The lack of available water under CT caused lower maize aboveground biomass, yield, and yield components. However, under NT and RT combined with the application of rates 400 kg N ha<sup>-1</sup>, led to a greater grain yield (7306, 11291 and 13797 kg grain ha<sup>-1</sup> for CT, RT, and NT with rate 400 kg N ha<sup>-1</sup>, respectively). In the STE, greater resilience to soil degradation and crust formation due to the former soil management under NT, enhanced water infiltration and water-stable macroaggregates in the soil surface, favoring an early development of the crop, regardless of the tillage system. In general, tillage systems and nitrogen fertilizer rates affected the emissions of soil GHG to the atmosphere in LTE. In this experiment, NT with high N application showed greater N<sub>2</sub>O and CO<sub>2</sub> emissions compared to CT. Meanwhile greater CH<sub>4</sub> oxidation was observed in RT and NT compared to CT. Cumulative CO<sub>2</sub> emissions were 37% greater (3856 vs. 2854 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>) in NT compared to CT while cumulative N<sub>2</sub>O emissions when 400 kg N ha<sup>-1</sup> was applied were 76 % greater (0.83 vs. 0.20 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) compared to rate of 200 kg N ha<sup>-1</sup>. Contrarily, cumulative CH<sub>4</sub> emissions were not significantly affected by any effect, although a trend of greater uptake of CH<sub>4</sub> was observed under RT compared with NT and CT (-0.77, -0.52 and -0.36 kg CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). In all treatments, the N<sub>2</sub>O EF was much lower than the 1% factor proposed by the IPCC. N fertilizer treatments significantly affected the yield-scaled N<sub>2</sub>O emissions, increasing when increasing N fertilizer rate in the first year of study. In LTE, SOC sequestration was 492, 222 and 969 kg C ha<sup>-1</sup> year<sup>-1</sup> for P1-R, P2-R and P3-I, respectively, as an average of treatments. In P1-R, NT presented the highest  $\Delta\text{SOC}_{\text{rate}}$  compared to CT with intermediate values in RT, while the high N rate showed greater  $\Delta\text{SOC}_{\text{rate}}$  compared to the control with intermediate values in the medium N rate. Contrarily, in P2-R  $\Delta\text{SOC}_{\text{rate}}$  did not show differences between treatments. The conversion

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from rainfed to irrigated conditions led to a greater amount of crop residues returned to the soil resulting in a SOC increase, mainly through an accumulation of POC. In the irrigated period (P3-I) NT showed greater  $\Delta\text{SOC}_{\text{rate}}$  than CT at the highest N rate, with intermediate values in RT (1959, 731, and 1380 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). In Mediterranean agroecosystems recently transformed to irrigated land, a reduction in N fertilization rate together with a reduction in tillage are optimum strategies in terms of maintenance of crop productivity. In addition, reductions of tillage improve the structural state of the soil, in order to provide the soil enough resilience and ensure an optimum development of crops. Although the reduction of tillage generates higher GHG emissions from the soil to the atmosphere, this is compensated by a greater maize yield and SOC sequestration.



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## Resumen

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En la zona de secano Mediterránea del valle del Ebro (NE de España), los agricultores han adoptado la agricultura de conservación durante los últimos 35 años. Sin embargo, una fracción significativa del área agrícola se está convirtiendo en regadío para asegurar mayores rendimientos, cambiando los cereales de invierno a nuevos cultivos más productivos como el maíz. En estas áreas recién irrigadas, los agricultores están volviendo a adoptar sistemas de laboreo intensivos, que son los más habituales en la producción de maíz en regadío y, por lo tanto, ponen en riesgo los beneficios de calidad del suelo logrados con el laboreo de conservación a largo plazo. Además, la ocupación gradual del riego lleva a cultivos que requieren un mayor uso de nitrógeno, que si no se adapta a las necesidades del cultivo, puede generar un mayor impacto ambiental, como el aumento de las emisiones de gases de efecto invernadero (GEI). En estas nuevas condiciones, existe una falta de conocimiento sobre la mejor combinación de prácticas de laboreo y fertilización N para reducir las emisiones de GEI del suelo, al mismo tiempo que se mantiene la productividad del maíz y la calidad del suelo. Por lo tanto, el objetivo principal de este estudio fue la evaluar los efectos de los diferentes sistemas de laboreo y las dosis de fertilizantes de N en las emisiones de GEI (metano, CH<sub>4</sub>; dióxido de carbono, CO<sub>2</sub>, óxido nitroso, N<sub>2</sub>O) a la atmósfera, así como, el secuestro de C del suelo, la estructura de la superficie del suelo y la productividad del cultivo en un área recientemente transformada a regadío. Para lograr ese objetivo, se llevó a cabo un estudio en NE España en un experimento de larga duración (LTE) de laboreo y dosis de fertilización N establecido en 1996 bajo la producción de cebada (*Hordeum vulgare* L.) en secano, posteriormente, se transformó en monocultivo maíz (*Zea mays* L.) con riego por aspersión en 2015. Este estudio se realizó durante tres campañas consecutivas de cultivo de maíz (es decir, los años 2015, 2016 y 2017). Se compararon tres tipos de laboreo (laboreo convencional, CT, laboreo reducido, RT, No laboreo, NT) y tres dosis de fertilización mineral N (0, 200, 400 kg N ha<sup>-1</sup>) en un diseño de bloques al azar con tres repeticiones. En 2015, se creó un experimento adyacente (experimento a corto plazo, STE) con el mismo diseño que el LTE pero con una gestión anterior diferente basada en NT. En el LTE, las emisiones de CO<sub>2</sub>, CH<sub>4</sub> y N<sub>2</sub>O del suelo se cuantificaron durante tres años. Además, se calculó el

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factor de emisión de N<sub>2</sub>O (EF) y las emisiones de N<sub>2</sub>O a escala de rendimiento. También, se calculó la tasa anual de secuestro de SOC ( $\Delta\text{SOC}_{\text{rate}}$ ) (0-40 cm de profundidad) para cada tratamiento en tres periodos diferentes (P1-, P2-, P3-) en condiciones de secano (-R) y condiciones de riego (-I) (P1-R, de 1996 a 2009; P2-R, de 2009 a 2015; P3-I, de 2015 a 2017). Además, en LTE y STE superficie del suelo (0-5 cm) se midieron macroagregados secos y estables en agua y su concentración de C, así como otras fracciones del suelo (concentración total de SOC y concentración de C lábil). Asimismo, se analizaron la resistencia a la penetración en la superficie del suelo (PR) y la infiltración de agua durante la segunda temporada de cultivo de maíz (es decir, el año 2016). Inicialmente, en ambos campos experimentales, se midió anualmente la biomasa aérea, el rendimiento de grano, los componentes de rendimiento y la eficiencia de uso de agua y nitrógeno (WUE y NUE, respectivamente). En la LTE, bajo CT, una menor estabilidad estructural provocó encostramiento superficial del suelo, que produjo una menor infiltración de agua (1.70, 2.40 y 3.14 mm h<sup>-1</sup> para CT, RT y NT, respectivamente) reduciendo la disponibilidad de agua en el suelo para el cultivo. El establecimiento de cultivos también se vio afectado por la degradación de la superficie del suelo, observándose una densidad de plantas 22% y 19% menor en CT en comparación con NT y RT, respectivamente. El proceso principal detrás de la formación de costra superficial en el suelo se debió a una menor concentración de SOC, haciendo que el suelo sea más susceptible a la descomposición de los agregados. La falta de agua disponible en el CT causó un descenso de la biomasa, el rendimiento y los componentes del rendimiento del maíz. Sin embargo, bajo NT y RT cuando se aplicó una dosis de 400 kg N ha<sup>-1</sup>, se obtuvo un mayor rendimiento de grano (7306, 11291 y 13797 kg de grano ha<sup>-1</sup> para CT, RT y NT, respectivamente). En el caso de STE, una mayor resistencia a la degradación del suelo y la formación de costra superficial debido al manejo anterior del suelo en el NT, dio lugar a una mayor infiltración de agua y macroagregados estables al agua en la superficie del suelo, favoreciendo un desarrollo temprano del cultivo, independientemente del sistema de laboreo. En general, los sistemas de laboreo y las dosis de fertilización N afectaron las emisiones de GEI del suelo a la atmósfera en LTE. En este experimento, el NT con una aplicación alta de N mostró mayores emisiones de N<sub>2</sub>O y CO<sub>2</sub> en comparación con CT. Mientras tanto, se observó una mayor oxidación de CH<sub>4</sub> en RT y NT en comparación con CT. Las emisiones acumuladas de CO<sub>2</sub> fueron 37% mayores (3856 vs. 2854 kg CO<sub>2</sub>-C ha<sup>-1</sup> año<sup>-1</sup>) en NT en comparación con CT, mientras que las emisiones acumuladas de N<sub>2</sub>O cuando se aplicaron 400 kg N ha<sup>-1</sup> fueron de 76% mayores (0.83 vs. 0.20 kg N<sub>2</sub>O-N ha<sup>-1</sup> año<sup>-1</sup>) en comparación con la dosis de 200 kg N ha<sup>-1</sup>. Por el contrario, las emisiones acumuladas de CH<sub>4</sub> no se vieron afectadas

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significativamente por ningún efecto, aunque se observó una tendencia de mayor captación de CH<sub>4</sub> en RT en comparación con NT y CT (-0.77, -0.52 y -0.36 kg CH<sub>4</sub>-C ha<sup>-1</sup> año<sup>-1</sup>, respectivamente). En todos los tratamientos, el N<sub>2</sub>O EF fue mucho más bajo que el factor del 1% propuesto por el IPCC. Los tratamientos con fertilización N afectaron significativamente las emisiones de N<sub>2</sub>O a escala de rendimiento, aumentando con el aumento de la dosis de aplicación de fertilizantes en el primer año de estudio. En LTE, el secuestro de SOC fue de 492, 222 y 969 kg C ha<sup>-1</sup> año<sup>-1</sup> para P1-R, P2-R y P3-I, respectivamente como promedio de los tratamientos. En P1-R, el NT presentó el  $\Delta\text{SOC}_{\text{rate}}$  más alto en comparación con CT y con valores intermedios en RT. Mientras que el uso de la dosis de N alta mostró un  $\Delta\text{SOC}_{\text{rate}}$  mayor en comparación con el control y con valores intermedios en la dosis de N media. Por el contrario, en P2-R el  $\Delta\text{SOC}_{\text{rate}}$  no se vió afectado significativamente por ningún tratamiento. La conversión de secano a regadío llevó a una mayor cantidad de residuos de cultivos que se devolvieron al suelo, lo que resultó en un aumento de SOC, principalmente a través de una acumulación de POC. En el periodo de regadío (P3-I), el uso de NT generó un mayor  $\Delta\text{SOC}_{\text{rate}}$  que CT con la dosis más alta de N, y con valores intermedios en RT (1959, 731 y 1380 kg C ha<sup>-1</sup> año<sup>-1</sup>, respectivamente). En los agroecosistemas Mediterráneos recientemente transformados a regadío, una reducción de la dosis de fertilización N junto con una reducción en el laboreo, es una estrategia óptima en términos de mantenimiento de la productividad de los cultivos. Además, la disminución del laboreo mejora el estado estructural del suelo, a fin de proporcionar al suelo suficiente resistencia y asegurar un desarrollo óptimo de los cultivos. Si bien la reducción del laboreo genera mayores emisiones de GEI del suelo a la atmósfera, estas se compensan con un mayor rendimiento de maíz y secuestro de SOC.



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## Resum

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A la zona del secà Mediterrània de la vall de l'Ebre (NE d'Espanya), els agricultors han adoptat l'agricultura de conservació durant els últims 35 anys. No obstant això, una fracció significativa de l'àrea agrícola s'està convertint en regadiu per assegurar majors rendiments, canviant els cereals d'hivern a nous cultius més productius com el blat de moro. En aquestes àrees recentment irrigades, els agricultors estan tornant a adoptar sistemes de conreu intensius, que són els més habituals en la producció de blat de moro en regadiu i, per tant, posen en risc els beneficis de la qualitat del sòl assolits amb el treball de conservació a llarg termini. A més, l'ocupació gradual del reg porta a cultius que requereixen un major ús de nitrogen, que si no s'adapta a les necessitats del cultiu, pot generar un major impacte ambiental, com l'augment de les emissions de gasos d'efecte hivernacle (GEH). En aquestes noves condicions, hi ha una manca de coneixement sobre les millors combinacions de pràctiques de conreu i fertilització N per reduir les emissions de GEH del sòl, al mateix temps que es manté la productivitat del blat de moro i la qualitat del sòl. Per tant, l'objectiu principal d'aquest estudi va ser la d'avaluar els efectes dels diferents sistemes de conreu i les dosis de fertilitzants de N en les emissions de GEH (metà, CH<sub>4</sub>; diòxid de carboni, CO<sub>2</sub>, òxid nitrós, N<sub>2</sub>O) a l'atmosfera, així com, el segrest de C del sòl, l'estructura de la superfície del sòl i la productivitat del cultiu en una àrea recentment transformada a regadiu. Per aconseguir aquest objectiu, es va dur a terme un estudi en NE Espanya en un experiment de llarga durada (LTE) de conreu i dosis de fertilització N establert el 1996 sota la producció d'ordi (*Hordeum vulgare* L.) en secà, posteriorment, es transformar en monocultiu blat de moro (*Zea mays* L.) amb reg per aspersió en 2015. Aquest estudi es va realitzar durant tres campanyes consecutives de cultiu de blat de moro (és a dir, els anys 2015, 2016 i 2017). Es van comparar tres tipus de conreu (conreu convencional, CT, conreu reduït, RT, No conreu, NT) i tres dosis de fertilització mineral N (0, 200, 400 kg N ha<sup>-1</sup>) en un disseny de blocs a l'atzar amb tres repeticions. El 2015, es va crear un experiment adjacent (experiment a curt termini, STE) amb el mateix disseny que el LTE però amb una gestió anterior diferent basada en NT. En el LTE, les emissions de CO<sub>2</sub>, CH<sub>4</sub> i N<sub>2</sub>O del sòl es van quantificar durant tres anys. A més, es va calcular el factor d'emissió de N<sub>2</sub>O (EF) i les emissions de N<sub>2</sub>O a escala de rendiment. També, es va calcular la taxa anual del segrest de SOC ( $\Delta\text{SOC}_{\text{rate}}$ ) (0-40 cm de profunditat) per a



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cada tractament en tres períodes diferents (P1-, P2-, P3-) en condicions de secà (-R) i condicions de regadiu (-I) (P1-R, de 1996 a 2009; P2-R, de 2009 al 2015; P3-I, de 2015 a 2017). A més, en LTE i STE superfície del sòl (0-5 cm) es van mesurar macroagregats secs i estables en l'aigua i la seva concentració de C, així com altres fraccions del sòl (concentració total de SOC i concentració de C làbil). Així mateix, es va analitzar la resistència a la penetració en la superfície del sòl (PR) i la infiltració d'aigua durant la segona temporada de cultiu de blat de moro (és a dir, l'any 2016). Ingualement, en els dos camps experimentals, es va mesurar anualment la biomassa aèria, el rendiment de gra, els components de rendiment i l'eficiència d'ús d'aigua i nitrogen (WUE i NUE, respectivament). A la LTE, sota CT, una menor estabilitat estructural provocar encrostament superficial del sòl, que va produir una menor infiltració d'aigua (1.70, 2.40 i 3.14 mm h<sup>-1</sup> per a CT, RT i NT, respectivament) reduint la disponibilitat d'aigua en el sòl per al cultiu. L'establiment de cultius també es va veure afectat per la degradació de la superfície del sòl, observant-se una densitat de plantes 22% i 19% menor en CT en comparació amb NT i RT, respectivament. El procés principal darrere de la formació de crosta superficial a terra es va deure a una menor concentració de SOC, fent que el sòl sigui més susceptible a la descomposició dels agregats. La manca d'aigua disponible al CT va causar un descens de la biomassa, el rendiment i els components del rendiment del blat de moro. No obstant això, sota NT i RT quant es va aplicar una dosi de 400 kg N ha<sup>-1</sup>, es va obtenir un major rendiment de gra (7306, 11291 i 13797 kg de gra ha<sup>-1</sup> per a CT, RT i NT, respectivament). En el cas de STE, una major resistència a la degradació del sòl i la formació de crosta superficial a causa del maneig anterior del sòl en el NT, va donar lloc a una major infiltració d'aigua i macroagregats estables a l'aigua a la superfície del sòl, afavorint un desenvolupament primerenc del cultiu, independentment del sistema de conreu. En general, els sistemes de conreu i les dosis de fertilització N van afectar les emissions de GEH del sòl a l'atmosfera en LTE. En aquest experiment, el NT amb una aplicació alta de N va mostrar majors emissions de N<sub>2</sub>O i CO<sub>2</sub> en comparació amb CT. Mentrestant, es va observar una major oxidació de CH<sub>4</sub> en NT en comparació amb CT. Les emissions acumulades de CO<sub>2</sub> van ser 37% més grans (3856 vs. 2854 kg CO<sub>2</sub>-C ha<sup>-1</sup> any<sup>-1</sup>) en NT en comparació amb CT, mentre que les emissions acumulades de N<sub>2</sub>O quan es van aplicar 400 kg N ha<sup>-1</sup> van ser de 76% majors (0.83 vs. 0.20 kg N<sub>2</sub>O-N ha<sup>-1</sup> any<sup>-1</sup>) en comparació amb la dosi de 200 kg N ha<sup>-1</sup>. Per contra, les emissions acumulades de CH<sub>4</sub> no es van veure afectades significativament per cap efecte, tot i que es va observar una tendència de major captació de CH<sub>4</sub> a RT en comparació amb NT i CT (-0.77, -0.52 i -0.36 kg CH<sub>4</sub>-C ha<sup>-1</sup> any<sup>-1</sup>, respectivament). En tots els tractaments, el N<sub>2</sub>O EF va ser molt més baix que el factor de l'1% proposat per l'IPCC. Els tractaments amb fertilització N van afectar

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significativament les emissions de N<sub>2</sub>O a escala de rendiment, augmentant amb l'increment de la dosi d'aplicació de fertilitzants en el primer any d'estudi. En LTE, el segrest de SOC va ser de 492, 222 i 969 kg C ha<sup>-1</sup> any<sup>-1</sup> per a P1-R, P2-R i P3-I, respectivament com a mitjana dels tractaments. En P1-R, el NT va presentar el  $\Delta SOC_{rate}$  més alt en comparació amb CT i amb valors intermedis en RT. Mentre que l'ús de la dosi de N alta va mostrar un  $\Delta SOC_{rate}$  més gran en comparació amb el control i amb valors intermedis en la dosi de N mitjana. Per contra, en P2-R el  $\Delta SOC_{rate}$  no es va veure afectat significativament per cap tractament. La conversió de secà a regadiu va portar a una major quantitat de residus de cultius que es van retornar a terra, el que va resultar un augment de SOC, principalment a través d'una acumulació de POC. En el període de regadiu (P3-I), l'ús de NT va generar un major  $\Delta SOC_{rate}$  que CT amb la dosi més alta de N, i amb valors intermedis en RT (1959, 731 i 1380 kg C ha<sup>-1</sup> any<sup>-1</sup>, respectivament). En els agroecosistemes Mediterranis recentment transformats a regadiu, una reducció de la dosi de fertilització N juntament amb una reducció en el conreu, és una estratègia òptima en termes de manteniment de la productivitat dels cultius. A més, la reducció del conreu millora l'estat estructural del sòl, per tal de proporcionar al sòl suficient resistència i assegurar un desenvolupament òptim dels cultius. Si bé la reducció del conreu genera més emissions de GEH del sòl a l'atmosfera, aquestes es compensen amb un major rendiment de blat de moro i segrest de SOC.



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## Abbreviations

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|                                  |                                               |
|----------------------------------|-----------------------------------------------|
| BD                               | Bulk density                                  |
| C                                | Carbon                                        |
| C <sub>2</sub> H <sub>2</sub>    | Acetylene                                     |
| CH <sub>4</sub>                  | Methane                                       |
| CO <sub>2</sub>                  | Carbon dioxide                                |
| CT                               | Conventional tillage                          |
| C-Min                            | Mineral-associated organic matter             |
| $\Delta\text{SOC}_{\text{rate}}$ | Soil organic carbon sequestration rate        |
| ECD                              | Electrical conductivity detector              |
| EF                               | Emission factor                               |
| ET <sub>c</sub>                  | Evapotranspiration                            |
| FID                              | Flame ionization detector                     |
| GHG                              | Greenhouse gases                              |
| I                                | Irrigation                                    |
| IPCC                             | The intergovernmental Panel on Climate Change |
| K                                | Potassium                                     |
| K <sub>c</sub>                   | Crop coefficient                              |
| LTE                              | Long-term experiment                          |
| MaP                              | Macroporosity                                 |

---

|                              |                                                   |
|------------------------------|---------------------------------------------------|
| MiP                          | Microporosity                                     |
| N                            | Nitrogen                                          |
| NE                           | Northeast                                         |
| N <sub>2</sub>               | Molecular nitrogen                                |
| N <sub>2</sub> O             | Nitrous oxide                                     |
| NH <sub>4</sub> <sup>+</sup> | Ammonium                                          |
| NO                           | Nitric oxide                                      |
| NO <sub>3</sub> <sup>-</sup> | Nitrate                                           |
| NT                           | No-tillage                                        |
| NUE                          | Nitrogen use efficiency                           |
| NAR                          | Apparent nitrogen recovery efficiency             |
| NHI                          | Nitrogen harvest index                            |
| P                            | Phosphorous                                       |
| P1-R                         | Period 1 (1996-2009) under rainfed conditions     |
| P2-R                         | Period 2 (2009-2015) under rainfed conditions     |
| P3-I                         | Period 3 (2015-2017) under irrigated conditions   |
| POC                          | Particulate organic carbon                        |
| PR                           | Soil penetration resistance                       |
| POxC                         | Soil permanganate-oxidizable carbon concentration |
| RT                           | Reduced tillage                                   |
| SNC                          | Soil nitrate content                              |
| STE                          | Short-term experiment                             |

---

|                  |                                              |
|------------------|----------------------------------------------|
| SOC              | Soil organic carbon                          |
| SR               | Soil respiration                             |
| SWC              | Soil water content                           |
| SWI              | Soil water infiltration                      |
| T                | Temperature                                  |
| TKW              | Thousand kernels weight                      |
| TP               | Total porosity                               |
| WFPS             | Soil water filled pore space                 |
| WU               | Water use                                    |
| WUE              | Water use efficiency                         |
| WUE <sub>B</sub> | Water use efficiency for aboveground biomass |
| WUE <sub>Y</sub> | Water use efficiency for yield               |
| YSNE             | Yield-scaled N <sub>2</sub> O emissions      |



# **General introduction**





# General introduction

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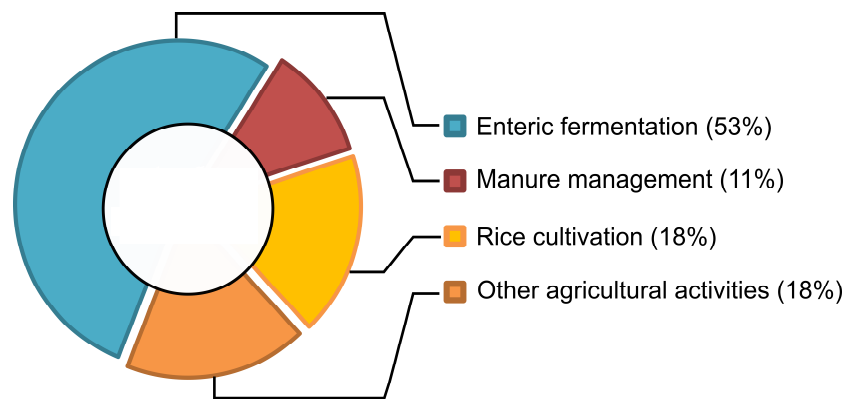
## 1. *Potential of agriculture to mitigate greenhouse gas emissions.*

In recent decades there has been increasing concern about the effects of man on climate. The so-called climate change, which encompasses global warming, has been identified as one of the great environmental threats of our time, modifying our economy, health and communities in different ways. The main factors that cause global warming are greenhouse gases (GHG). These gases include carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), which are released by industry, agriculture and the combustion of fossil fuels. In the last three decades the atmospheric concentrations of GHG have increased considerably due to anthropic activity (IPCC, 2013). Spain, which is located in the Mediterranean basin, is one of the countries most vulnerable to climate change, being the agricultural sector one of the most affected. Agriculture and livestock account for 12% of total GHG emissions in Spain (MAGRAMA, 2019). The greatest emissions from the agricultural sector are produced by the soil and in particular from the application of nitrogen fertilizers, both from mineral and organic sources.

Among GHG, N<sub>2</sub>O plays an important role on climate change, since its global warming potential is 265 times greater than that of CO<sub>2</sub>. Global atmospheric concentration of N<sub>2</sub>O has risen from pre-industrial levels of ~270 ppb to over 330 ppb currently (IPCC, 2014). Agriculture is the main source of global N<sub>2</sub>O emissions, thus contributing up to 14% global warming potential in a 100-year horizon (IPCC, 2014). Nitrous oxide production in agricultural soils is mainly linked to two biological processes. The first is the biological conversion of ammonium (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) and then nitrate (NO<sub>3</sub><sup>-</sup>) by ammonia (NH<sub>3</sub>) oxidizing bacteria or archaea, which is called nitrification. Meanwhile, the second is the denitrification which consists of the reduction of soil NO<sub>3</sub><sup>-</sup> to N oxides (NO, N<sub>2</sub>O) or N<sub>2</sub> by a diverse array of bacteria that use NO<sub>3</sub><sup>-</sup> as a terminal electron acceptor in the absence of oxygen. Nitrification and denitrification are predominantly controlled by N availability, temperature, oxygen content, soil moisture and soil C (only denitrification) (Van Groenigen *et al.*, 2015), which are modified, in turn, by a range of agricultural management practices.

Methane is considered to be the second most important greenhouse gas in the atmosphere (Motzka *et al.*, 2011). Moreover, it has a global warming potential 25 times greater

than that of CO<sub>2</sub> and a residence time in the atmosphere of 9 to 15 years (IPCC, 2013). Global atmospheric concentration of CH<sub>4</sub> has risen from pre-industrial levels of ~800 ppb to over 1800 ppb currently (IPCC, 2014). Methane from agricultural sector proceeds mainly from the enteric fermentation in livestock, manure and waste management, rice cultivation and biomass burning (Fig. 1) (Yusuf *et al.*, 2012). Soils can also be either a weak source or sink for CH<sub>4</sub> (USEPA, 2011) consequence of the balance of two opposite processes, the production and oxidation of CH<sub>4</sub>. The production of CH<sub>4</sub> takes place in anaerobic environments by methanogenic bacteria, while oxidation by methanotrophic bacteria occurs in aerobic environments. Upland soils usually act as net CH<sub>4</sub> oxidizers (Conrad, 1995). Tillage can influence the processes that regulate the uptake and emission of CH<sub>4</sub> through its impact on soil structure, soil water content and microbial diversity (Mitra *et al.*, 2002).



**Fig. 1.** Methane emissions from agriculture. Reproduced from Yusuf *et al.* (2012).

Carbon dioxide has the biggest impact among greenhouse gases in atmosphere, counting for approximately 76% of all greenhouse gases (IPCC, 2014). Global atmospheric concentration of CO<sub>2</sub> has risen from pre-industrial levels of ~260 ppm to over 400 ppm currently (IPCC, 2014). Carbon dioxide primarily increased due to fossil fuel burning and land use change. In the soil, CO<sub>2</sub> is produced mainly through the microbial decomposition of organic compounds. Therefore, agricultural soils can represent an important source of CO<sub>2</sub> (Follett, 2001) during the decomposition of organic matter, when a fraction of carbon is returned to the atmosphere as CO<sub>2</sub>, while another fraction is transformed into simpler compounds.

The reduction of GHG emissions from agriculture is an enormous challenge. Some strategies to mitigate soil GHG emissions may include efficient N fertilizer management, and use of conservation tillage systems to sustain soil quality and crop yields (Johnson *et al.*, 2007). The reduction of N fertilization rate can have a positive effect on reducing N<sub>2</sub>O emissions. Also, N

fertilization can inhibited aerobic conditions that result in CH<sub>4</sub> oxidation (Plaza-Bonilla *et al.*, 2014) therefore a reduction in the rate of N could avoid or reduce the inhibition. Meanwhile, the use of NT can reduce SOC mineralization potentially lowering soil CO<sub>2</sub> emissions (Snyder *et al.*, 2009). Agricultural soils not only act as emitting sources of GHG but also as sinks of these gases. The use of suitable management practices, including better management of fertilization, crop rotation, tillage and the use of more productive crops favours the fixation of atmospheric CO<sub>2</sub> through carbon sequestration (Cole *et al.*, 1996). Soils have a great capacity to store carbon. It is estimated that global croplands could sequester between 0.90 and 1.85 Pg C yr<sup>-1</sup>, i.e. 26–53% of the objective of the 4p1000 Initiative (Zomer *et al.*, 2017). At the same time, this process provides other important benefits like prevention of erosion and desertification. Moreover, an improvement in soil quality through the increase in SOC stocks could lead to an improvement in crop yields. Therefore, the identification of management practices that maintain or even sequester SOC is an important challenge.

## 2. Conversion of rainfed land to irrigation in Mediterranean agroecosystems.

The agroecosystems of the Ebro valley (NE Spain) are representative of agricultural production in the Mediterranean area. The Ebro valley presents a continental Mediterranean climate, where, the scarcity of rainfall, high potential evapotranspiration and strong thermal oscillation are the most characteristic features that define the climate of this area (Vicente-Serrano *et al.*, 2003). The main production system in the rainfed areas of the valley has been the monoculture of winter cereals, mostly barley and wheat, whose yield (ranging from 1 to 5 t ha<sup>-1</sup> as average) is highly dependent on seasonal rainfall and water storage capacity of the soil (Austin *et al.*, 1998). Currently, this rainfed production system is being adapted to more diverse crop rotations. Currently, a significant fraction of the rainfed agroecosystems of the Ebro valley are being converted to irrigation (906,000 ha of irrigated land out of a surface of 3.8 million ha of cropland in the Ebro basin), allowing farmers the cultivation of more productive summer crops such as maize. Maize is one of the most important field crops in the irrigated area of the Ebro valley. Its grain yields range from 12 to 16 t ha<sup>-1</sup> and nitrogen uptake is over 250–300 kg ha<sup>-1</sup> (Berenguer *et al.*, 2008). Therefore, nitrogen fertilisation is one of the most important factors influencing maize production. Farmers do not usually consider the levels of residual soil N before planting that are commonly high in the area (Ballesta and Lloveras, 1996; Vázquez *et al.*, 2006). Therefore, over-fertilization is common, being mineral N rates applied to maize about 300–450 kg ha<sup>-1</sup> (Sisquella *et al.*, 2004). Over-fertilization has led to environmental impacts like reactive N

losses by nitrate leaching (Quemada *et al.*, 2013) and the increase in N<sub>2</sub>O emissions to atmosphere (Meijide *et al.*, 2009).

Conservation agriculture, which involves reduced and no-tillage (RT and NT, respectively), is currently common in the Mediterranean rainfed area of the Ebro valley (80% of the surface for more than 35 years). The introduction of NT in rainfed areas has been encouraged as a way to reduce costs, increase available soil water, water use efficiency and grain yield by 10-20 % (Cantero-Martinez *et al.*, 2003) as well as to preserve soil fertility and increase soil C (Morell *et al.*, 2011). In the Ebro valley, farmers using NT and RT who made the conversion from rainfed to irrigated land are returning to adopt intensive tillage (CT) systems, which are commonly used in irrigated cropping systems. In the traditional irrigated areas, the limited or nil previous experience about the implementation of RT or NT systems constrains their adoption by farmers and puts at risk the soil quality benefits achieved with the long-term use of NT under former rainfed conditions. The use of intensive tillage results in the release of organic matter due to aggregate breakdown (Álvaro-Fuentes *et al.*, 2008). The lower stability of soil aggregates under intensive tillage systems is aggravated by the incorporation of crop residues in the soil leaving the soil surface exposed to water drops. This last process can lead to soil crusting in susceptible soils such the ones of the Ebro valley (Pareja-Sánchez *et al.*, 2017). Soil surface crusts, affect negatively seedling emergence and reduce water infiltration, favoring runoff and soil erosion (Fox *et al.*, 2004). For instance, in irrigated areas of the Ebro valley Amezketa *et al.* (2003), indicated that soil crusting could reduce maize emergence by 20-30% and production by up to 50%. Moreover, conversion from rainfed land to irrigation entails an increase in soil moisture which has strong implications in relation to carbon and nutrient cycles (Apesteguía *et al.*, 2015), by creating more favourable soil conditions for microbial activity (Calderon and Jackson, 2002) which may increase GHG emissions (Aguilera *et al.*, 2013). However, higher productivity and, consequently, availability of crop residues under irrigated conditions can also enhance SOC sequestration, counteracting a potential increase in direct GHG emissions from soils.

### *3. Tillage system and N fertilization rate affect GHG emissions, soil C sequestration and soil quality.*

Management practices like tillage and N fertilization can affect soil biochemical and physical properties, consequently influencing the release of GHG. Conservation tillage systems like NT or RT are practices that can reduce erosion and increase SOC sequestration, which leads to a reduction of CO<sub>2</sub> emissions from soil to the atmosphere (Denef *et al.*, 2004). For instance,

Smith *et al.*, (2012) showed an increase in CO<sub>2</sub> emissions under CT compared to NT in a maize crop in Alabama, USA. This result was due to greater contact of crop residues with the soil in the plow layer, which increased soil temperature and aeration, providing better conditions for microbial respiration. However, the mitigating effect of NT can be neutralized by an increase in soil emissions to the atmosphere of N<sub>2</sub>O (Yonemura *et al.*, 2014). This last is due to the interaction of tillage with several factors, e.g. soil type, climatic conditions (which determine the prevalence of nitrification or denitrification), N fertilization rate, soil moisture content and crop residues (van Kessel *et al.*, 2013). In this line, authors like Venterea *et al.* (2005) have reported greater emissions of N<sub>2</sub>O following broadcast urea application under NT and RT compared to CT as a result of increased soil moisture content and lower soil gas diffusivity. Agricultural activities usually have negative effects on the activity of CH<sub>4</sub> oxidizing bacteria, being tillage and N fertilization the main practices responsible for the reduction of CH<sub>4</sub> oxidation in agricultural soils (Hutsch, 2001). The use of tillage can reduce oxidation of CH<sub>4</sub> due to the alteration of methanotrophic microorganisms in the soil, as well as changes in soil structure. Therefore, structural degradation, which is a common problem in intensively tilled soils, can adversely affect CH<sub>4</sub> consumption by a reduction in gas diffusion through the soil pores (Ball *et al.*, 1999). In this regard, Venterea *et al.* (2005) observed higher CH<sub>4</sub> soil uptake rates and lower emissions of this gas under reduced tillage compared to CT.

Nitrogen fertilization can also play an important role in the release of GHG, being N<sub>2</sub>O the most affected by N fertilization since it can be nitrified or denitrified in soil and released as N<sub>2</sub>O (Eichner, 1990). Therefore, estimating N<sub>2</sub>O emissions and N<sub>2</sub>O emission factors (EF) under different practices, is essential for assessing the impact of agriculture on GHG and identify strategies to mitigate it. Regarding to this, the IPCC uses a default EF of 1% to estimate the percentage of fertilizer N applied that is transformed and emitted on-site as N<sub>2</sub>O (IPCC, 2006). In this line, in a meta-analysis of studies carried out under Mediterranean conditions Cayuela *et al.*, 2017 showed that the most important factors controlling the magnitude of N<sub>2</sub>O EF in irrigated soils were related to water availability through irrigation water management and/or N fertilizer rate and type (organic vs synthetic fertilizers). Also, N fertilization can affect CH<sub>4</sub>, since the application of fertilizers based on ammonium inhibits the oxidation of methane in the soil, an effect that can be maintained over time modifying the microbial community (Hütsch *et al.*, 1994). Carbon dioxide emissions are also affected by N fertilization through an increase in crop biomass production, which can influence soil microbial activity through decomposition of organic matter and root respiration (Hanson *et al.*, 2000). To our knowledge there is a lack of studies

about the impacts of tillage and N fertilization rate on GHG in soils recently converted to irrigation under Mediterranean conditions. Moreover, it is important to estimate local EF in the new irrigation systems management under different practices such as tillage systems and N fertilization rates. Moreover, the quantification of other new metrics that combine cropproductivity and environmental impact are needed. For example, one option is to assess GHG emissions per unit yield, termed yield-scaled emissions (Van Groenigen *et al.*, 2010).

The effects of fertilizer N rate on SOC stocks still remains unclear (Blanco Canqui and Schlegel, 2013). Authors like Jantalia and Halvorson, (2011) or Follett *et al.* (2013) suggested that increasing N fertilization rates may increase SOC stocks depending on soil depth considered and tillage practice. In contrast, other authors showed that fertilizer N accelerate SOC mineralization based (Khan *et al.* 2007, Mulvaney *et al.* 2009). Presently long-term NT and other conservation practices are recommended to maintain or increase SOC stocks in rainfed areas (Plaza-Bonilla *et al.*, 2015) but less is known in irrigated areas. Halvorson *et al.*, (2004) reported that the annual rate of SOC sequestration increased ( $1.4 \text{ Mg SOC ha}^{-1} \text{ yr}^{-1}$ , 0-15 cm depth) with increasing N rate under NT in irrigated continuous maize with respect to CT that showed a rate of  $0.2 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in Colorado (USA).

In Mediterranean agroecosystems it is essential to identify management practices that enhance crop productivity, mitigate soil GHG emissions avoiding soil degradation after converting rainfed lands to irrigations.

## **General objectives**

The main objective of this doctoral thesis was the quantification of the effect of different types of tillage systems and N fertilizer rates on greenhouse gases emissions, soil C sequestration, surface structure and crop productivity in a rainfed semiarid area converted to irrigated land.

To achieve the overall goal, four specific objectives were proposed:

- (i) To determine to what extent soil management practices affect both soil surface characteristics and crop establishment when transforming a rainfed area into irrigation under Mediterranean conditions.
- (ii) To evaluate the combined impact of tillage and mineral N fertilization rates analyzed on irrigated maize grain yield, WUE and NUE according to previous soil management.
- (iii) To quantify the emission of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> under different tillage systems and rates of N fertilization.
- (iv) To evaluate the impacts of the conversion of rainfed land to irrigation on SOC sequestration after 21 years of differential soil tillage systems and N fertilization rates.

## **Structure of the thesis**

This thesis is structured in 5 chapters. The contents of each chapter are briefly described below:

### ***Chapter I: Long-term no-till as a means to maintain soil surface structure in an agroecosystem transformed into irrigation.***

This chapter focuses on determining to what extent previous and current soil management practices affect soil surface characteristics and crop establishment when transforming a rainfed area to irrigation.

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 Res.* 174, 221-230.



***Chapter II: Is it feasible to reduce tillage and N use while improving maize yield in irrigated Mediterranean agroecosystems?***

In this chapter we analyze the combined effect of tillage and mineral N fertilization rates on maize grain yields and water and nitrogen use efficiency, in two field experiments differing on previous soil management which recently have been converted to irrigation under Mediterranean conditions.

Submitted to: *European Journal of Agronomy*.

***Chapter III: Impact of tillage and N fertilization rate on soil N<sub>2</sub>O emissions in irrigated maize in a Mediterranean agroecosystem.***

This chapter focuses on determining the best management system to reduce maize yield-scaled N<sub>2</sub>O emissions among different soil management practices and N fertilization rates.

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

This chapter focuses on the quantification of the interactive effects of tillage and N fertilization rates on soil CO<sub>2</sub> and CH<sub>4</sub> emissions to identify the most environmentally sustainable practices.

Pareja-Sánchez, E., Cantero-Martínez, C., Álvaro-Fuentes, J., Plaza-Bonilla, D., 2019. Tillage and nitrogen fertilization in irrigated maize: key practices to reduce soil CO<sub>2</sub> and CH<sub>4</sub> emissions. *Soil Tillage Res.* 191, 29-36.

***Chapter V: Soil organic carbon sequestration after conversion of a rainfed Mediterranean agroecosystem into irrigated land managed under different long-term tillage systems and N fertilization rates.***

In this chapter we analyze the effect of the the conversion of rainfed land to irrigation on SOC sequestration after 21 years of differential soil tillage systems and N fertilization rates.

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## **Chapter I**

**Long-term no-till as a means to maintain soil surface structure in an agroecosystem transformed into irrigation.**





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# Chapter I

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## Long-term no-till as a means to maintain soil surface structure in an agroecosystem transformed into irrigation.

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### Abstract

The aim of this study was to determine the most appropriate soil management to reduce the structural degradation of soils susceptible to crusting in Mediterranean areas recently transformed into irrigation. A long-term field experiment (LTE) under rainfed conditions was established in 1996 in NE Spain to compare three tillage systems (no-tillage, NT; reduced tillage, RT; conventional tillage, CT). The experiment was transformed to irrigated maize in 2015. In 2015, an adjacent experiment with the same layout was created (short-term experiment, STE) in an area previously managed under long-term NT. The study was carried out during the second maize growing season (i.e. year 2016). Soil samples were collected from 0-5 cm depth at different dates during maize season. Dry and water-stable macroaggregates and their C concentration, soil organic carbon (SOC) and labile C concentration, soil respiration, bulk density, penetration resistance (PR), water infiltration, macroporosity, microporosity, amount of crop residues and ground cover, maize development, aerial biomass, and grain yield were measured. In LTE and STE tillage led to a breakdown of dry sieved aggregates (of 2-4 and 4-8 mm size) in RT and CT, being slowly reconsolidated throughout the maize growing season. However, macroaggregate water-stability did not increase in CT and RT compared to NT due to a lower SOC concentration, making the soil more susceptible to its degradation by the action of water. SOC differences between treatments were more pronounced in LTE than STE given the long-term differential management in the first, which allowed greater accumulation of SOC under NT. In LTE, PR between maize rows was greater under NT than CT and RT and non-significantly different between treatments within the row. In the case of STE, PR increased over time after tillage (CT and RT) to match NT in the last sampling. Crop establishment was slower in CT than NT in LTE highlighting the impact of soil surface degradation on crop development. However, contrarily to the differences in maize yield in

2015, a careful planting in 2016 led to a lack of differences between tillage systems on maize yield. Our results indicate that in areas transformed into irrigation intensive tillage leads to greater susceptibility to soil structural degradation. Thus, in these areas the adoption of conservation agriculture practices such RT and NT enhances soil resilience to degradation processes and ensures an adequate development of the crop.

## **Keywords**

Maiz; soil crust; soil degradation; tillage systems; transformation into irrigation.

## **1. Introduction**

Soil management practices affect both soil surface characteristics and crop productivity. Tillage exposes soil to erosive agents such as wind and water, inducing its degradation. Under severe erodible forces, soils are exposed to the impact of water-drops, either produced by irrigation or by rainfall. This last process results in the release of organic matter and, generally, in soil crusting (Awadhwal and Thierstein, 1985). In bare soils, structural crusts are a major problem facing many agricultural areas worldwide (Mbuvi *et al.*, 2009). Structural crusts, developed on soil surface, negatively affect seedling emergence and reduce infiltration, favoring runoff and soil erosion (Fox *et al.*, 2004). Furthermore, crusting is closely related to soil aggregation. In that sense, Bouaziz *et al.* (1990) found a linear relationship between soil aggregate size and the proportion of non-emerged wheat seedlings due to soil crusting.

In Mediterranean climate regions, an increasing number of rainfed areas are transformed into irrigation to stabilize or increase crop yields (Apesteguía *et al.*, 2015). This conversion generates significant consequences in agroecosystems. Greater biomass production by irrigation leads to an increase in crop residues which can be returned to the soil. The increase of organic C inputs to the soil usually entails an increase in soil organic carbon (SOC) (Franzluebbers, 2005) and, concomitantly, an improvement in soil quality (Wick *et al.*, 1998; Dexter *et al.*, 2008). Moreover, C inputs play an essential role in the formation of soil aggregates, which physically protect SOC from microbial degradation (Beare *et al.*, 1994) boosting SOC sequestration and climate change mitigation (Lal, 2011).

C-enriched aggregates are more stable to alterations such as rainfall, irrigation or tillage. Furthermore, crop residues protect the soil surface, preventing the formation of crusts (Jordán *et al.*, 2010). Besides its importance in the Mediterranean climate regions, the impact of rainfed into irrigation transformation on soil surface characteristics (e.g., soil aggregation, soil organic

carbon, bulk density, infiltration, penetration resistance and soil porosity) has been scarcely studied. Regarding to this, Apesteguía *et al.* (2015) observed an increase of the proportion of large macroaggregates under maize and wheat cropping systems managed under conventional tillage (chisel plow) when transforming a Mediterranean rainfed area into irrigation in north of Spain. Also, in Central Great Plains, Deneff *et al.* (2008) found greater SOC storage in the surface soil layer (0–20 cm) in pivot-irrigated areas compared to dryland areas.

Tillage operations that incorporate crop residues into the soil increase soil susceptibility to degradation. When intensive tillage systems are adopted, soil remains bare until the next planting. Bare soils are more exposed to erosive agents and to drop impact promoting soil surface sealing and crusting and, at the end, water runoff (Pagliai *et al.*, 2004). Tillage generally decreases soil bulk density compared to no-tillage (NT) (Lal, 1999) and it can negatively influence soil water infiltration, depending on soil type and properties (Dexter *et al.*, 2004). For instance, Chan and Heenan (1993) and McGarry *et al.* (2000) reported lower infiltration rates under conventional tillage (CT) compared to NT. The adoption of NT systems has been identified as an optimal practice to reduce soil degradation and to improve soil aggregation in rainfed Mediterranean areas (Álvaro-Fuentes *et al.*, 2009; Plaza-Bonilla *et al.*, 2010). Moreover, it has been proved that long-term use of NT increases soil organic carbon (SOC) sequestration (Plaza-Bonilla *et al.*, 2015). Similarly, Follett *et al.* (2013) showed that CT induced greater losses of old organic matter than NT in irrigated maize systems influencing soil physical properties. Soil organic matter plays a fundamental role in the formation and maintenance of aggregates, positively influencing the soil water retention capacity, water infiltration, and avoiding the formation of superficial crusts which improves seed germination and crop emergence.

In Mediterranean irrigated agroecosystems, typical soil management strategies include intensive tillage with deep subsoilers and mouldboard ploughs. However, unlike in irrigated systems, in Mediterranean rainfed areas an increasing adoption of reduced tillage (RT) or NT techniques has been taking place over the last 30 years (Lampurlanés *et al.*, 2016). In Mediterranean irrigated areas, the limited knowledge associated to the use of RT or NT systems, makes farmer adoption difficult and jeopardizes the soil quality benefits attained with long-term NT. As a consequence, the aim of this study was to determine to what extent soil management practices affect soil surface characteristics and crop establishment when transforming a rainfed area into irrigation in Mediterranean conditions.

## 2. Materials and methods

### 2.1 Experimental design

A field experiment was conducted in Agramunt, NE Spain (41°48' N, 1°07' E, 330 m asl), where the soil was classified as *Typic Xerofluvent* (Soil Survey Staff, 2014). Soil characteristics are presented in Table 1. The climate is semiarid Mediterranean with a mean annual precipitation of 430 mm and a potential evapotranspiration of 855 mm. Mean annual air temperature is 13.8°C.

**Table 1.** Soil characteristics of Ap horizon (0-28 cm) at the beginning of the field experiment (1996).

| Soil characteristic                                  |      |
|------------------------------------------------------|------|
| pH                                                   | 8.5  |
| EC <sub>1:5</sub> (dS m <sup>-1</sup> )              | 0.15 |
| Organic matter (g kg <sup>-1</sup> )                 | 9    |
| P Olsen (ppm)                                        | 12   |
| K (ppm)                                              | 155  |
| Water retention (-33 kPa) (%) (g g <sup>-1</sup> )   | 16   |
| Water retention (-1500 kPa) (%) (g g <sup>-1</sup> ) | 5    |
| Sand (%)                                             | 46.5 |
| Silt (%)                                             | 41.7 |
| Clay (%)                                             | 11.8 |

A rainfed long-term field experiment (LTE) was established in 1996 to compare three tillage systems (no-tillage, NT; reduced tillage, RT; conventional tillage, CT) under barley monocropping (Angás *et al.*, 2006). In 2015 the LTE was transformed into irrigation by installing a fixed sprinkler irrigation system with a 18 x 18 m spacing and a maximum flow rate of 2.07 m<sup>3</sup> h<sup>-1</sup> coming pressurized from the Segarra-Garrigues channel, and maize (*Zea mays* L.) monoculture as cropping system. The experimental design in LTE consisted of randomized blocks with three replications and a plot size of 50x6 m. After the transformation into irrigation, the LTE maintained the same tillage treatments (NT, RT and CT) and the same experimental layout as the previous rainfed experiment. At the same time, in 2015, a new tillage experiment was set up adjacent to the LTE (separated by a 15-m corridor). The layout of this new experiment (so called short-term experiment, STE) was exactly the same as the LTE (same tillage treatments, spatial arrangement and cropping system) but with different historical tillage management. For the last 20 years, the entire surface occupied by the STE consisted of a rainfed NT winter cereal system.

In LTE and STE, the CT treatment was implemented according to the traditional practices of the area for maize cultivation. It consisted of one pass of rototiller to 15 cm depth followed by subsoiler to 35 cm depth and to finish one pass of a disk plough to 20 cm depth with almost

100% of the crop residues incorporated into the soil. The RT treatment consisted of one pass of a strip-till implement on the maize planting row to 30 cm depth reducing the total area tilled to ca. 20 %. Finally, the NT consisted of weed control with a non-selective herbicide (i.e. glyphosate) at 1.5 L ha<sup>-1</sup> without no soil disturbance. Planting was carried out with a pneumatic row direct drilling machine equipped with double disc furrow openers (model Prosem K, Solà, Calaf, Spain) in the three tillage systems (NT, RT, and CT). Rotary residue row cleaners were installed to clear the path for the row unit openers. In this work we evaluated the second year after conversion into irrigation in LTE and STE. Tillage operations were conducted at the end of March and beginning of April 2016 (Table 2). Planting of maize (cv. Kopias) was performed on 22 April, at a rate of 90.000 seeds ha<sup>-1</sup> with the rows 0.73 m apart. Mineral N fertilization was split in one pre-planting application of 50 kg N ha<sup>-1</sup> with urea (46% N), on 12 April, and two top-dressing applications of 75 kg N ha<sup>-1</sup> with calcium ammonium nitrate (27% N), on 31 May and 5 July, respectively. P and K fertilization consisted of 154 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 322 kg ha<sup>-1</sup> K<sub>2</sub>O applied at pre-planting, respectively. Irrigation was supplied to meet the estimated evapotranspiration (ET) of the crop minus the effective precipitation. Reference ET was computed with the FAO Penman–Monteith method from meteorological data obtained from an automated weather station located near the experimental site. Crop coefficients (Kc) were estimated as a function of the thermal time (Martínez-Cob, 2008). Weekly maize evapotranspiration was calculated from the corresponding weekly values of ET and Kc. Irrigation began on 19 April and ended on 14 September with a total of 77 irrigation dates. The amount of water applied by irrigation was 672 mm. Harvesting was done by the end of October with a commercial combine which chopped and spread the crop residues over the soil. The fallow period was maintained free of weeds with an application of glyphosate at 1.5 L ha<sup>-1</sup>.

**Table 2.** Sampling and key crop management practices dates in the field experiments. Dates of first and second irrigation are included given its presumable impact on soil consolidation after tillage.

| <b>Crop management practices</b>                                             |            |
|------------------------------------------------------------------------------|------------|
| Soil sampling                                                                | 03/01/2016 |
| Tillage: Rototiller (CT treatment)                                           | 03/07/2016 |
| Tillage: Subsoiler (CT treatment)                                            | 03/29/2016 |
| Crop residues sampling                                                       | 03/30/2016 |
| Pre-planting N fertilization                                                 | 04/12/2016 |
| Tillage: disk plough and strip-till<br>(CT and RT treatments, respectively). | 04/19/2016 |
| First irrigation                                                             |            |
| Cultipacker-rolling                                                          | 04/26/2016 |
| Planting                                                                     | 04/26/2016 |
| Second irrigation                                                            | 05/15/2016 |
| Soil sampling                                                                | 04/27/2016 |
| Soil sampling                                                                | 05/04/2016 |
| Macroporosity and microporosity                                              |            |
| Soil sampling                                                                | 05/25/2016 |
| Macroporosity and microporosity                                              |            |
| First top-dressing N fertilization                                           | 06/31/2016 |
| Second top-dressing N fertilization                                          | 07/05/2016 |
| Crop residues sampling                                                       | 10/04/2016 |
| Soil sampling                                                                | 10/05/2016 |
| Macroporosity and microporosity                                              |            |
| Gran yield and aboveground biomass sampling                                  | 10/19/2016 |
| Commercial harvest                                                           | 11/11/2016 |

### 2.1.1 Soil and crop samplings and analyses

Soil and crop measurements were carried out during the maize growing season of 2016, at the next key dates: before tillage operations, right after planting, two weeks and one month after planting, and right before maize harvest (Table 2). In the first two measurement dates, all variables except soil porosity were measured. Moreover, a weekly monitoring of crop development was carried out on two sampling areas of 2.5 x 2.5 m<sup>2</sup> per plot.

### 2.1.2 Soil water-stable macroaggregates, bulk density, moisture, organic C fractions, and soil respiration.

From each plot, two composite soil samples, one within the row (WR) and one between the rows (BR), were prepared from two samples taken randomly from 0 to 5 cm soil depth using a flat spade and stored in crush-resistant airtight containers. Additionally, soil cylinders from 0 to 5-cm depth were obtained to quantify soil bulk density (BD) and soil moisture by drying the

samples at 105°C during 48 h until constant weight (Grossman and Reinsch, 2002). Once in the laboratory, unaltered soil samples were gently passed through an 8-mm sieve and air-dried at room temperature. Water-stable macroaggregate size separation was performed using a method adapted from Elliott (1986). Briefly, 100 g of the 8-mm sieved soil was placed on the top of a 2-mm sieve and submerged for 5 min in deionized water at room temperature. The sample was manually sieved 50 times for 2 min to achieve aggregate separation. The slurry was further sieved through a 0.250 mm sieve using the same procedure. Therefore, two aggregate-size fractions were obtained: large water-stable macroaggregates (2–8 mm) and small water-stable macroaggregates (0.250–2 mm). Soil aggregates were oven-dried at 50°C during 48 h in aluminum trays and weighed. Sand correction was performed to each aggregate size according to Elliott *et al.* (1991) since sand was not considered to be part of the aggregates. Sand content was determined by dispersing a 5 g subsample in a sodium hexametaphosphate solution (5 g L<sup>-1</sup>) using a reciprocal shaker, sieved with the corresponding sieve, oven-dried at 50°C during 24 h and weighed. Furthermore, the dry aggregate size distribution was conducted placing 100 g of air-dried sub-sample (8 mm sieved) on an electromagnetic sieve apparatus (Filtrá FTL-0200, Badalona, Spain) with a series of sieves (4, 2, 1, 0.25 and 0.05 mm). A sieving time of 1 min and the lowest power program of the machine were used.

Two fractions of bulk soil organic C were determined: the permanganate-oxidizable organic C (POxC) and the dichromate oxidizable soil organic C (SOC). SOC concentration of each water-stable aggregate-size fraction was also quantified. POxC was quantified according to the method of Weil *et al.* (2003), while SOC was determined using the wet oxidation method of Walkley & Black described by Nelson and Sommers (1996). This last method was modified to increase the oxidation of SOC, heating the sample externally at 150 °C for 30 min.

Soil respiration (SR) was measured in LTE using non-steady state static chambers (Hutchinson and Mosier, 1981). Gas samples were taken at 0, 20 and 40 min after the closure of the chamber and stored in vials, being subsequently analyzed by a gas chromatography system equipped with a flame ionization detector coupled to a methanizer. Gas fluxes were calculated taking into account the linear increase of CO<sub>2</sub> within the chamber with time (40 min) and correcting the values for air temperature (Holland *et al.*, 1999).

### 2.1.3 Soil penetration resistance and water infiltration.

Soil penetration resistance (PR) was measured using a pocket penetrometer (Facchini srl. mod. FT 327, Alfonsine, Italy). The apparatus consists of a short rod finishing in a tip which



penetrates at a constant rate up to a depth of 5 cm. Four randomly selected points were measured in each sampling area and position (WR, BR).

The rate of soil water infiltration (SWI) was quantified near saturation with a disc permeameter (CSIRO Permeameter, A.L. Franklin Precision Engineers) similar to the design of Perroux and White (1988), used to carry out ponded measurements (positive water potentials). One measurement per plot was performed on each sampling date. The level of water infiltrated was measured every ten seconds for the first two minutes and every one minute until a steady state was reached.

#### *2.1.4 Soil macroporosity, microporosity and total porosity.*

Undisturbed soil samples (0-5 cm depth) were taken in each plot using stainless steel cylinders 6 cm i.d. and 5 cm in height (141.4 cm<sup>3</sup>). In the laboratory, the samples were placed in porous ceramic plates to saturation. After saturation, the samples were weighed (saturated weight) and left in the same ceramic plates at -10 kPa for 24 h to drain all water not hold against the gravitational force. They were weighed again and the difference with the saturated weight divided by the sample volume was the fraction of soil volume corresponding to macropores or macropore porosity (MaP). Later, the samples were dried at 105 °C. The difference between the saturated and dried weight divided by the sample volume, was the fraction of soil volume corresponding to pores or total porosity (TP). The difference between TP and MaP was the fraction of soil volume corresponding to the micropores or micropore porosity (MiP).

#### *2.1.5 Crop residues and maiz early development, biomass and grain yield.*

The proportion of soil surface covered by crop residues was estimated (i) before planting (for NT and RT, since crop residues were already incorporated in the soil in CT) and (ii) right after harvest. The measurements were done at four positions per plot using metal frames of 0.5 m x 0.5 m with a 0.25 m x 0.25 m grid to help visually estimate the percent of soil surface covered by residues (CIAT, 1982). Afterwards, the residues within the grids were oven-dried at 60 °C during 48 h and weighed.

Monitoring of the maiz emergence and early development was carried out once a week by counting the number of plants per row (2 m) and recording their phenological stage from planting to V6 stage (Ritchie *et al.*, 1997).

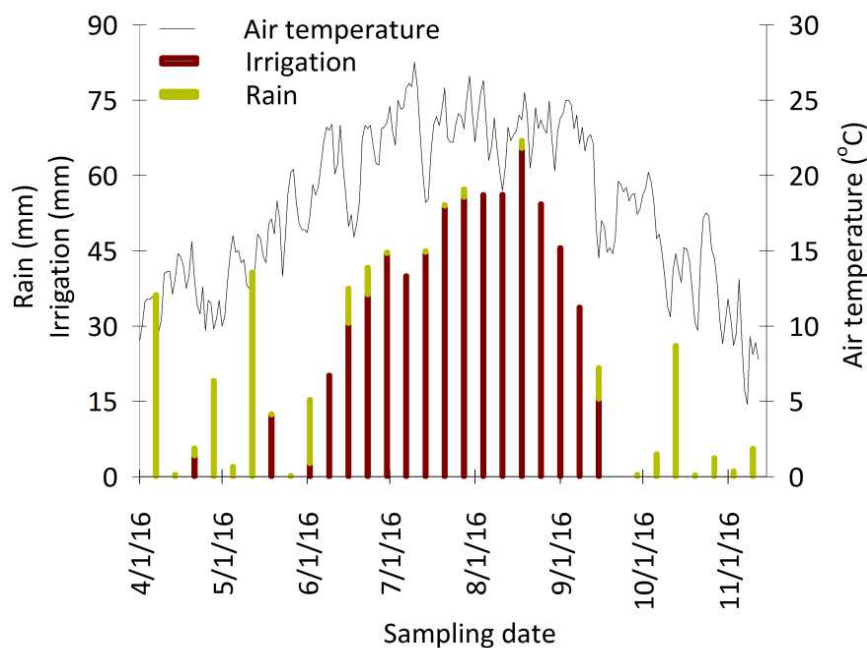
Maiz aboveground biomass and grain yield were determined in mid-October by cutting the plants at the soil level along 2 m of two central rows of each plot. The number of plants and ears was counted and registered. Afterwards, a sub-sample of two entire plants and five ears were taken, oven-dried at 60°C for 48 h and weighed. Next, the grain was threshed and weighed. Grain yield was adjusted to 14% moisture.

### 2.3 *Statistical analyses*

For each experiment (LTE and STE), analysis of variance (ANOVA) was performed with tillage, sampling position, sampling date and their interaction as effects. For variables measured under different soil moisture levels (SWI, PR and BD), soil moisture was added to the ANOVA as co-variable. When significant, differences among treatments were identified at 0.05 probability level of significance with a protected t-Student test. A Sqrt-transformation was carried out to normalize BD, SWI (LTE and STE), sand-free large water stable macroaggregate-POxC (2-8 mm) (STE) and dry aggregate size distribution (4-8 mm) (LTE) data. All the statistical analysis were performed with the statistical package JMP 12 (SAS Institute Inc, 2016).

## 3. Results

Rainfall, irrigation events and air temperature during the entire experimental period are shown in Fig. 2. Air temperature increased from the beginning of the experimental period, reaching a maximum in summer months (July-August), to decrease later during autumn months. Total rainfall during the crop cycle was 140 mm with the greatest rainfall recorded in May (43 mm), which was far from the evapotranspiration needs. The amount of water applied by irrigation was 672 mm, 80% of this considered effective irrigation, i.e. 538 mm.



**Fig. 2** Daily air temperature (continuous line), and weekly rainfall and irrigation (green and red columns, respectively) during the experimental period.

### 3.1 Soil management effect on water infiltration, soil penetration resistance (PR), macroporosity (MaP), microporosity (MiP), total porosity (TP) and bulk density (BD) dynamics.

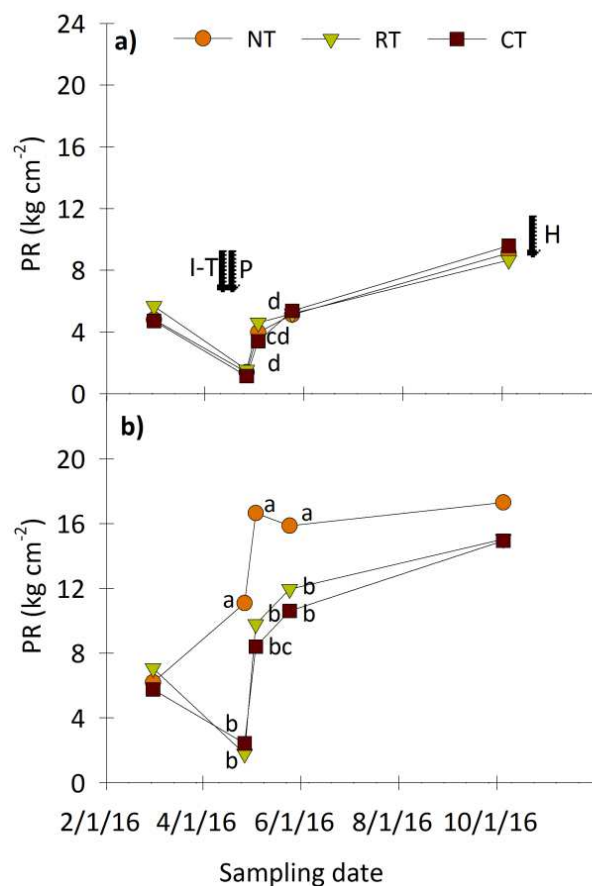
In the STE, the interaction between tillage, sampling date and position significantly affected PR (Table 3). In the case of the LTE, PR was significantly affected by the interaction between tillage and position and between sampling date and position. Soil water infiltration, PR, MaP, MiP, TP, BD, and SR showed significant differences between sampling dates in LTE (Table 3). However, in STE differences between sampling dates were only observed on TP, PR, and BD. Bulk density was also significantly affected by tillage, and the interaction between sampling date and position in LTE and by tillage in STE (Table 3).

**Table 3.** Analysis of variance (*P*-values) of soil water infiltration (SWI), penetration resistance (PR), macroporosity (MaP), microporosity (MiP), total porosity (TP), bulk density (BD) and soil respiration (SR) as affected by tillage, sampling date, sampling position and their interactions in a long-term (LTE) and a short-term (STE) field experiment. Soil moisture was included as a co-variable.

| Experiment | Source of variation | SWI  | PR    | MaP  | MiP  | TP    | BD    | SR    |
|------------|---------------------|------|-------|------|------|-------|-------|-------|
| LTE        | Tillage (Till)      | ns   | ns    | 0.06 | 0.05 | ns    | <0.01 | 0.01  |
|            | Date                | 0.04 | <0.01 | 0.04 | 0.04 | 0.02  | <0.01 | <0.01 |
|            | Position            | -    | <0.01 | -    | -    | -     | ns    | -     |
|            | Till*Date           | ns   | ns    | ns   | ns   | ns    | ns    | 0.01  |
|            | Till*Position       | -    | 0.01  | -    | -    | -     | ns    | -     |
|            | Date*Position       | -    | <0.01 | -    | -    | -     | 0.04  | -     |
|            | Till*Date*Position  | -    | ns    | -    | -    | -     | ns    | -     |
|            | Soil moisture       | ns   | 0.07  | 0.05 | 0.05 | ns    | ns    | -     |
| STE        | Tillage (Till)      | 0.09 | <0.01 | ns   | ns   | ns    | <0.01 | -     |
|            | Date                | 0.09 | <0.01 | ns   | ns   | <0.01 | <0.01 | -     |
|            | Position            | -    | <0.01 | -    | -    | -     | ns    | -     |
|            | Till*Date           | ns   | <0.01 | ns   | ns   | ns    | ns    | -     |
|            | Till*Position       | -    | <0.01 | -    | -    | -     | ns    | -     |
|            | Date*Position       | -    | <0.01 | -    | -    | -     | ns    | -     |
|            | Till*Date*Position  | -    | 0.03  | -    | -    | -     | ns    | -     |
|            | Soil moisture       | ns   | ns    | ns   | ns   | ns    | ns    | -     |

ns, non-significant

In the STE and for the three sampling dates right after tillage, NT showed greater PR in the BR position than the other two treatments (Fig. 3b). The lowest PR values were found in the WR position in the second sampling date, just after tillage (1.38, 1.54 and 1.13 kg cm<sup>-2</sup> for NT, RT and CT, respectively). However, the highest PR values were found in the BR position in the last sampling date, seven months after the first measurement (17.31, 15.04 and 14.94 kg cm<sup>-2</sup> for NT, RT and CT, respectively) (Fig. 3 a and b). In LTE, PR showed significant differences between tillage treatments in the BR position, being greater under NT (13.2 kg cm<sup>-2</sup>) than under RT (10.6 kg cm<sup>-2</sup>) and CT (11.1 kg cm<sup>-2</sup>), as an average of all five sampling dates. Contrarily, tillage treatments showed similar PR in the WR position. Significant differences in PR between sampling dates and sampling positions were also observed in LTE, being greatest in the fifth sampling date in both positions with values of 19.0 and 10.2 kg cm<sup>-2</sup> for BR and WR, respectively.



**Fig. 3** Soil penetration resistance (PR) dynamics under three tillage treatments (NT, no-tillage; RT, reduced tillage; CT, conventional tillage) within (WR) (a) and between (BR) maize rows (b), in a short-term field experiment (STE). For a given date, different lowercase letters indicate significant differences between tillage treatments at  $P < 0.05$ . Arrows represent key dates (H, harvest; I, first irrigation; T, tillage; P, planting).

As an average of sampling dates and positions, BD was greater under NT (1.47 and 1.49 g cm<sup>-3</sup> for LTE and STE, respectively) than RT (1.36 and 1.40 g cm<sup>-3</sup> for LTE and STE, respectively) and CT (1.43 and 1.33 g cm<sup>-3</sup> for LTE and STE, respectively). No significant differences were found between tillage treatments on SWI neither in LTE nor STE (Table 3). Mean SWI values were 3.14, 2.40 and 1.70 mm h<sup>-1</sup> for NT, RT and CT, respectively, in the LTE, and 3.80, 3.60 and 2.60 mm h<sup>-1</sup> for the same tillage treatments in the STE.

### 3.2 Soil management effect on water-stable macroaggregate and dry aggregate distribution dynamics.

In the LTE, the interaction between tillage and sampling date significantly affected sand-free water-stable macroaggregates (0.250-2 and 2-8 mm sizes). However, in the STE, small water-stable macroaggregates were only affected by tillage (Table 4). In LTE the proportion of

large sand-free water-stable macroaggregates decreased from the second sampling date (just after tillage) up to the fourth sampling date (Table 5). However, in the last sampling (just before harvest), the proportion of large sand-free water-stable macroaggregates was not different to the first sampling value. In this same experiment, the proportion of small sand-free water-stable macroaggregates only showed significant differences between tillage treatments in the first and fourth sampling dates. In these two sampling dates, small sand-free water-stable macroaggregates were greater in CT compared to RT and NT (Table 5). In the STE, a greater proportion of small sand-free water-stable macroaggregates was observed under NT and RT (0.42 and 0.41 g g<sup>-1</sup>, respectively) compared to CT (0.35 g g<sup>-1</sup>) as an average of sampling dates and sampling positions.

**Table 4.** Analysis of variance (*P*-values) of sand-free water-stable aggregate classes (2-8 and 0.250-2 mm), dry aggregate size distribution (4-8, 2-4, 1-2, 0.250-1, 0.05-0.250, and <0.05 mm), bulk soil organic C (SOC) and permanganate-oxidizable organic C (POxC) concentration, and aggregate-C as affected by tillage, sampling date and sampling position and their interactions in a long-term (LTE) and a short-term (STE) field experiment.

| Experiment | Source of variation | Aggregate (mm) |         | Dry aggregate size distribution (mm) |       |       |         |            |       | Bulk soil |       | Aggregate-C (mm) |
|------------|---------------------|----------------|---------|--------------------------------------|-------|-------|---------|------------|-------|-----------|-------|------------------|
|            |                     | 2-8            | 0.250-2 | 4-8                                  | 2-4   | 1-2   | 0.250-1 | 0.05-0.250 | <0.05 | SOC       | POxC  | 0.250-2          |
| LTE        | Tillage (Till)      | <0.01          | <0.01   | <0.01                                | <0.01 | <0.01 | <0.01   | <0.01      | ns    | <0.01     | <0.01 | ns               |
|            | Date                | <0.01          | ns      | ns                                   | <0.01 | ns    | ns      | <0.01      | ns    | <0.01     | <0.01 | <0.01            |
|            | Position (Ps)       | ns             | 0.09    | ns                                   | ns    | ns    | ns      | ns         | ns    | ns        | ns    | ns               |
|            | Till*Date           | 0.01           | 0.03    | 0.04                                 | <0.01 | 0.01  | 0.02    | 0.03       | ns    | ns        | ns    | <0.01            |
|            | Till*Ps             | ns             | ns      | ns                                   | ns    | ns    | ns      | ns         | ns    | ns        | ns    | ns               |
|            | Date*Ps             | ns             | ns      | ns                                   | ns    | ns    | ns      | ns         | ns    | ns        | ns    | ns               |
|            | Till*Date*Ps        | ns             | ns      | ns                                   | ns    | ns    | ns      | ns         | ns    | ns        | ns    | ns               |
| STE        | Till                | ns             | <0.01   | <0.01                                | <0.01 | ns    | <0.01   | <0.01      | ns    | <0.01     | <0.01 | ns               |
|            | Date                | <0.01          | <0.01   | ns                                   | <0.01 | <0.01 | <0.01   | <0.01      | <0.01 | <0.01     | <0.01 | <0.01            |
|            | Position (Ps)       | ns             | ns      | ns                                   | 0.01  | ns    | 0.08    | ns         | ns    | 0.09      | ns    | ns               |
|            | Till*Date           | ns             | ns      | <0.01                                | <0.01 | ns    | <0.01   | <0.01      | ns    | 0.03      | ns    | ns               |
|            | Till*Ps             | ns             | ns      | ns                                   | ns    | 0.06  | ns      | ns         | ns    | ns        | ns    | 0.08             |
|            | Date*Ps             | ns             | ns      | ns                                   | ns    | ns    | ns      | ns         | ns    | ns        | ns    | ns               |
|            | Till*Date*Ps        | ns             | ns      | ns                                   | ns    | ns    | ns      | ns         | ns    | ns        | 0.04  | ns               |

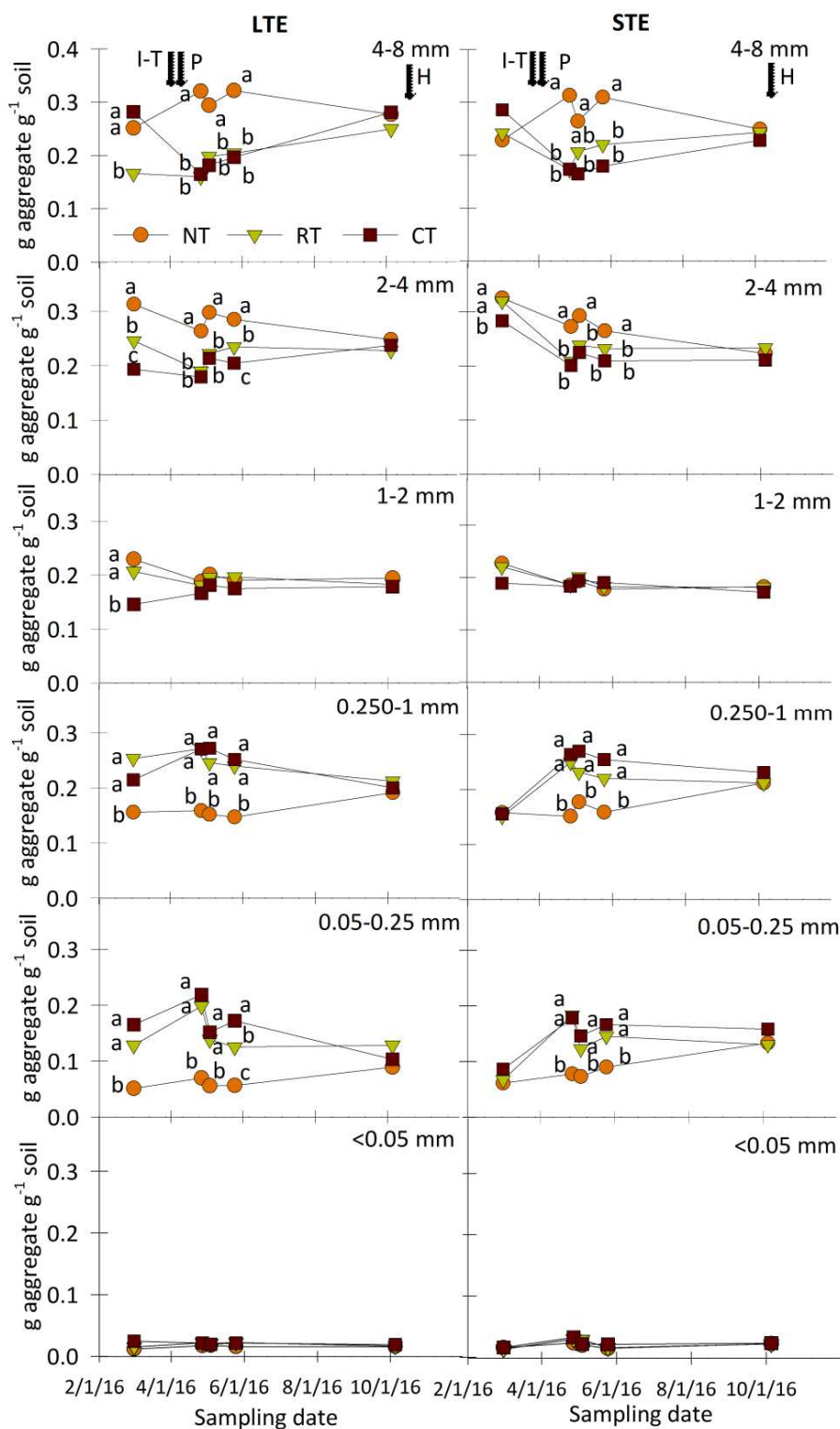
ns, non significant

In the LTE and STE, most dry aggregate sizes were significantly affected by the tillage x sampling date interaction (Table 4). In both experiments, dry aggregate classes of 4-8 and 2-4 mm size showed significant differences between tillage systems in all the sampling dates except for the last one, and the first one for the 4-8 mm fraction in the STE (Fig. 4). Compared to NT, a decrease in the proportion of dry-sieved aggregates of 4-8 and of 2-4 mm was observed under

RT and CT in the second sampling date, after tillage. Average dry-sieved 4-8 mm aggregate values in the second, third and fourth sampling dates were 0.18 and 0.17 g g<sup>-1</sup> under CT, 0.18 and 0.20 g g<sup>-1</sup> under RT and 0.31 and 0.20 g g<sup>-1</sup> under NT in LTE and STE, respectively (Fig. 4). After the fourth sampling date, the proportion of aggregates in RT and CT gradually increased until the last sampling (133 days after tillage) when no differences between tillage systems were observed (Fig. 4). Unlike, the smaller dry-sieved aggregate sizes (i.e., 0.250-1 mm and 0.05-0.250 mm) showed opposite results with greater values under CT and RT than under NT in the first four sampling dates in the LTE and in the second to fourth sampling dates in the STE. Regarding to this, the proportion of this aggregate sizes increased right after the implementation of tillage (second sampling date) in RT and CT in both field experiments.

**Table 5.** Sand-free water-stable large (2-8 mm) and small (0.250-2 mm) macroaggregates at 0–5 cm soil depth as affected by tillage system (NT, no-tillage; RT, reduced tillage; CT, conventional tillage) and sampling date in a long-term (LTE) field experiment. For a given date and aggregate class, different lowercase letters indicate significant differences between tillage treatments at  $P < 0.05$ . Different uppercase indicate significant differences between sampling dates at  $P < 0.05$ .

| Sampling date | Tillage treatment | Sand-free water-stable aggregate classes (g g <sup>-1</sup> ) |            |
|---------------|-------------------|---------------------------------------------------------------|------------|
|               |                   | 2-8 mm                                                        | 0.250-2 mm |
| 03/01/2016    | NT                | 0.23 a                                                        | 0.22 b     |
|               | RT                | 0.12 b                                                        | 0.24 b     |
|               | CT                | 0.15 b                                                        | 0.46 a     |
|               | Average           | 0.17 A                                                        | 0.29       |
| 04/27/2016    | NT                | 0.18 a                                                        | 0.30 a     |
|               | RT                | 0.08 b                                                        | 0.25 a     |
|               | CT                | 0.09 b                                                        | 0.30 a     |
|               | Average           | 0.11B                                                         | 0.26       |
| 05/04/2016    | NT                | 0.14 a                                                        | 0.27 a     |
|               | RT                | 0.06 b                                                        | 0.25 a     |
|               | CT                | 0.08 ab                                                       | 0.33 a     |
|               | Average           | 0.09 B                                                        | 0.28       |
| 05/25/2016    | NT                | 0.15 a                                                        | 0.26 b     |
|               | RT                | 0.05 b                                                        | 0.24 b     |
|               | CT                | 0.10 a                                                        | 0.37 a     |
|               | Average           | 0.09 B                                                        | 0.29       |
| 10/05/2016    | NT                | 0.31 a                                                        | 0.27a      |
|               | RT                | 0.15 b                                                        | 0.24 a     |
|               | CT                | 0.07 c                                                        | 0.29 a     |
|               | Average           | 0.18 A                                                        | 0.27       |

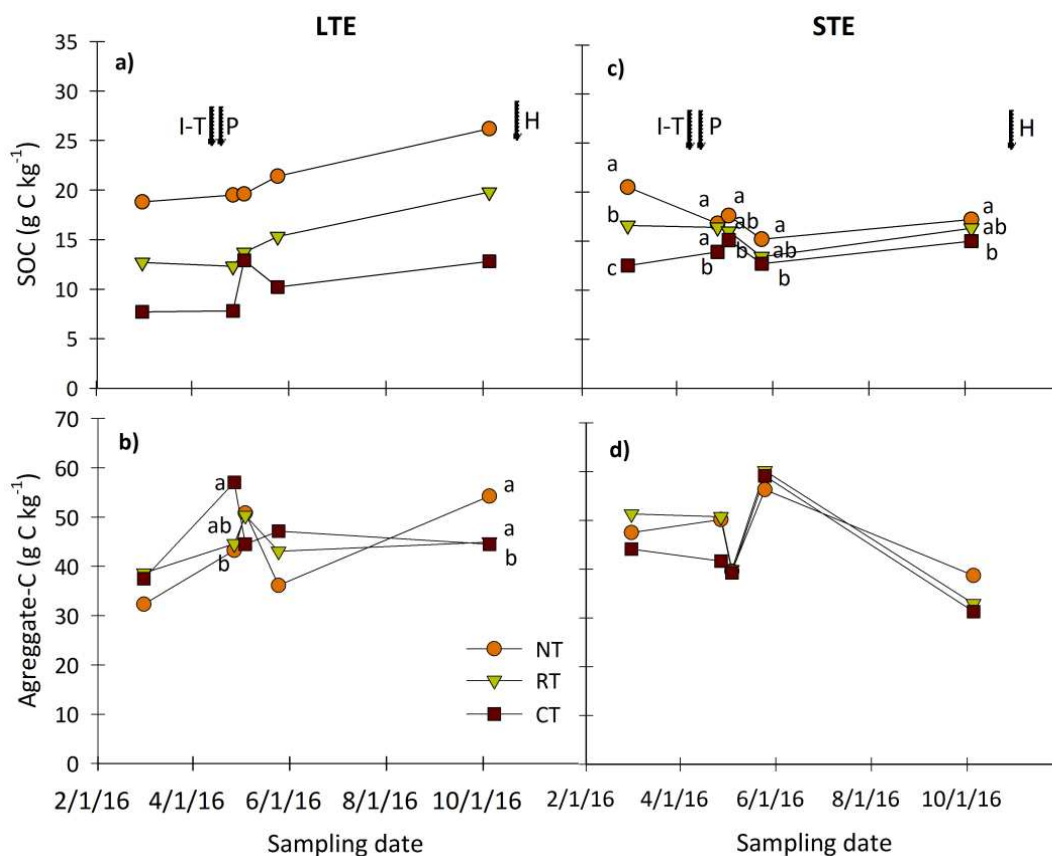


**Fig. 4** Dry-sieved aggregate size distribution (4-8, 2-4, 1-2, 0.250-1, 0.05-0.25 and < 0.05 mm) at 0–5cm soil depth as affected by tillage system (NT, no-tillage; RT, reduced tillage; CT, conventional tillage) and sampling date in a long-term (LTE) and a short-term (STE) field experiment. For each experiment, aggregate fraction, and sampling date, different lowercase letters indicate significant differences between tillage treatments at  $P < 0.05$ . Arrows represent key dates (H, harvest; I, first irrigation; T, tillage; P, planting).



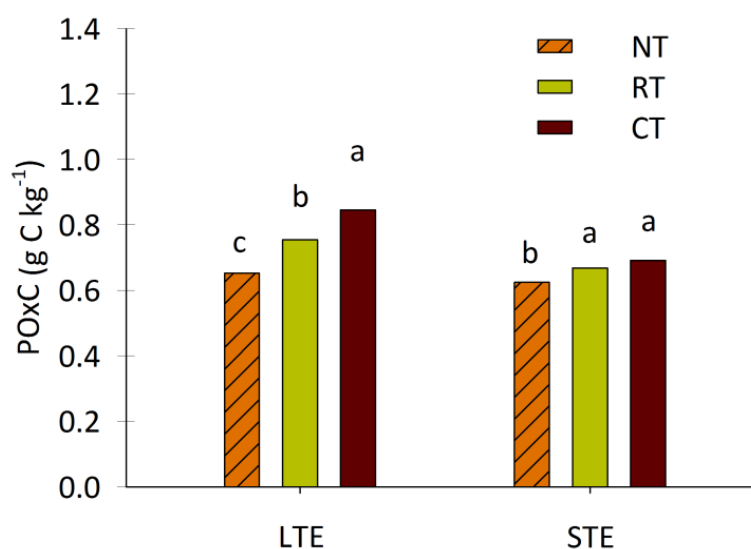
### 3.3 Soil management effect on bulk soil organic C, water-stable macroaggregate C and soil respiration.

Bulk SOC concentration was significantly affected by tillage systems and sampling date effects in LTE and also by their interaction in STE (Table 4). In the LTE, the SOC concentration (0-5 cm depth) was 21.1, 14.8 and 10.3 g C kg<sup>-1</sup> soil for NT, RT and CT, respectively, as an average of sampling dates. In the STE, SOC differences between tillage systems were found in all sampling dates (Fig. 5c). SOC concentration followed the order NT>RT>CT in the first sampling date, and showed greater values under NT than CT and intermediate values in RT in the last fourth sampling dates (Fig. 5c). SOC values in CT were about 36% higher in STE compared with LTE, as an average of all sampling dates (Fig. 5a and c).



**Fig. 5** Bulk soil organic carbon (SOC) and sand-free water-stable small macroaggregate (0.250-2 mm) organic carbon (aggregate-C) concentration at 0-5 cm depth as affected by tillage system (NT, no-tillage; RT, reduced tillage; CT, conventional tillage) in a long-term (LTE) and a short-term (STE) field experiment. For each experiment and sampling date, different lowercase letters indicate significant differences between tillage treatments at  $P < 0.05$ . Arrows represent key dates (H, harvest; I, first irrigation; T, tillage; P, planting).

Bulk soil POxC concentration showed significant differences between tillage systems and sampling dates in both field experiments (Table 4). In LTE, POxC concentration decreased in the following order: CT>RT>NT, while in STE NT presented lower bulk soil POxC concentration than CT and RT (Fig. 6).



**Fig. 6** Bulk soil permanganate-oxidizable organic carbon (POxC) concentration at 0-5 cm depth in a long-term (LTE) and a short-term (STE) field experiment. For a given experiment, different lower case letters indicate significant differences between tillage treatments at  $P<0.05$ .

Not enough water-stable large macroaggregates (2-8 mm) were obtained in the wet sieving procedure for aggregate-C determination. The C concentration of the small size water-stable macroaggregates (aggregate-C) showed significant interaction between tillage and sampling date in the LTE (Table 4). Significant differences between tillage systems were observed in the second sampling date (just after tillage) with greater aggregate-C in CT than NT and intermediate values under RT. Differences also occurred in the last sampling date (right before harvest) with greater values in NT and RT than CT (Fig. 5b). Differently, in the STE, aggregate-C was only affected by the sampling date (Table 4).

The interaction between sampling date and tillage system significantly affected soil respiration (Table 3). Soil respiration increased from the second sampling date (coinciding just after tillage, Table 6) with values of 504, 615, and 276 mg CO<sub>2</sub>-C m<sup>-2</sup> day<sup>-1</sup> for NT, RT and CT, respectively, until the last sampling with values of 1203, 940 and 921, mg C-CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> for the

same tillage treatments. Significant differences between tillage treatments were found in the second, third and fourth sampling dates with CT showing the lowest values (Table 6).

**Table 6.** Soil respiration as affected by tillage system (NT, no-tillage; RT, reduced tillage; CT, conventional tillage) and sampling date in a long-term (LTE) field experiment. For a given date, different lowercase letters indicate significant differences between tillage treatments at  $P < 0.05$ .

| Sampling date | Tillage treatment | Soil respiration<br>(mg CO <sub>2</sub> -C m <sup>-2</sup> day <sup>-1</sup> ) |
|---------------|-------------------|--------------------------------------------------------------------------------|
| 03/01/2016    | NT                | 630 a                                                                          |
|               | RT                | 540a                                                                           |
|               | CT                | 378 a                                                                          |
| 04/27/2016    | NT                | 504 ab                                                                         |
|               | RT                | 615 a                                                                          |
|               | CT                | 276 b                                                                          |
| 05/04/2016    | NT                | 796 a                                                                          |
|               | RT                | 820 a                                                                          |
|               | CT                | 442 b                                                                          |
| 05/25/2016    | NT                | 846 a                                                                          |
|               | RT                | 722 ab                                                                         |
|               | CT                | 56 b                                                                           |
| 10/05/2016    | NT                | 1203 a                                                                         |
|               | RT                | 940 ab                                                                         |
|               | CT                | 921 a                                                                          |

### 3.4 Soil management effect on maize emergence and early development, crop residues, crop biomass and grain yield.

In the LTE, maize emergence was slower under CT than under NT and RT. In this experiment, 21 days after planting 64,957 emerged plants ha<sup>-1</sup> were observed under NT, while under RT and CT the plants observed dropped to about 97% and 59% of the NT value, respectively. However, 44 days after planting the number of plants emerged was similar between tillage systems. In contrast, in the STE, maize emergence was similar in the three tillage treatments, with final density of 70,513, 76,068 and 69,231 plants ha<sup>-1</sup> in NT, RT, and CT, respectively.

In both experimental fields, the proportion of the soil surface covered by crop residues was significantly affected by tillage systems (Table 2). Before planting, the surface covered by crop residues was 86% and 70% for NT and RT, respectively, in LTE, and 88% and 73% for the same tillage treatments in STE. Seven months later, right before harvest, the proportion of

surface covered by residues was 77%, 45% and 10% for NT, RT and CT, respectively, in LTE, and 81%, 53% and 28% for the same tillage systems in STE, respectively.

Maiz yield and aboveground biomass were not significantly affected by tillage treatments in any of the field experiments (Table 2). However, in the LTE, a non-significant trend of greater grain yield was observed under NT than CT (11,680 vs. 9,864 kg ha<sup>-1</sup>).

## 4. Discussion

### 4.1 Effect of long-term and short-term management practices on soil surface and maiz development.

The different historical management of the two experiments tested had a great impact on the results obtained. In the LTE, soil inversion with moldboard plough for the last 20 years (CT treatment) led to soil crusting (Fig. 7b). However, NT for 20 years provided greater resilience to soil degradation and crust formation, enhancing water infiltration, with almost two-fold greater water infiltration in NT compared to CT.

In the STE, tillage treatments significantly affected PR between maiz rows, being the greatest values in NT. However, within maiz rows, similar PR values were observed among tillage treatments. Also, greater BD was observed in NT when compared to the rest of treatments in both experiments. Bulk density, penetration resistance and water content in the soil are closely related (Ferrerias *et al.*, 2000). Soil PR tends to increase when there is an increase in bulk density (Lampurlanés *et al.*, 2003). However, in our study, PR and BD would not be limiting crop growth, since the highest yields were observed under NT. According to Neave and Rayburg (2007) soil PR is usually related to the presence of soil crusts. Our results suggest that PR was not a good indicator to relate the presence of soil crust with maiz development failures since the highest values of PR were measured in NT (Fig. 7a). Similarly, Martínez *et al.* (2008) observed a significantly higher PR under NT than CT but only for the 0-5 cm soil depth when comparing tillage systems for 4 years under wheat cultivation. Therefore, it is necessary to test the performance of other variables as indicators of soil crusting. Soil crusting in CT could be the consequence of a lower cover of soil surface by crop residues than NT, resulting in the degradation of soil aggregates by the impact of water drops of irrigation or rain (Ruan *et al.*, 2001).



**Fig. 7** (a) Development of maize in conventional tillage (CT) (plot on left) and no-tillage (NT) (plot on right) 50 days after planting, and (b) soil crusting and sediment movement due to irrigation in CT in the long-term tillage experiment (LTE).

In both experiments, tillage operations led to a breakdown of dry-sieved aggregates of greater size (4-8 mm and 2-4 mm). However, during crop growth, the CT and RT treatments showed an increase in the proportion of these 4-8- and 2-4-mm sized macroaggregates resulting in no differences with NT by the end of the experiment. This increase in the proportion of soil macroaggregates in RT and CT may be explained by the contribution of organic matter when crop residues are incorporated with tillage. Fresh organic matter from crop residues activates the aggregation cycle being firstly incorporated into macroaggregates (Six *et al.*, 2000). Although

the greater fractions of dry-sieved aggregates in CT and RT increased over time, the proportion of water-stable macroaggregates of size 2-8 mm did not, which demonstrates that the stability of soil structure in those treatments was still lower compared to NT. When using NT, aggregate turnover is decreased, promoting the formation of macroaggregates with higher stability (Álvaro-Fuentes *et al.*, 2009; Panettieri *et al.*, 2013). Consequently, soil aggregates in CT are less resistant to the action of water, either received as irrigation or rainfall. In our experiment, greater SOC concentration was observed in NT compared to RT and CT at the soil surface. The contribution of crop residues and the stimulation of biological activity leads to the formation of stable macroaggregates in NT (Martens *et al.*, 2004; Tisdall and Oades, 1982). Álvaro-Fuentes *et al.* (2013) and Martínez *et al.* (2013) observed higher SOC and microbial activity in surface NT soils compared with CT soils. Also, in the same study area, Cantero-Martínez *et al.*, (2004) observed greater activity of earthworms in the first 30 centimeters of soil when using NT. Similarly, Baker *et al.*, (1993) working under Mediterranean conditions of southern Australia, observed greater activity in the first 10 centimeters of soil in NT, since this soil management does not alter earthworm activity, favoring their development. In NT, the accumulation of SOC is promoted (Balesdent *et al.*, 1990; Plaza-Bonilla *et al.*, 2013), mainly in the first centimeters of the soil profile (Franzluebbers, 2001; Reyes *et al.*, 2002). It is expected that the increase in SOC and the concomitant improvement of soil biological activity in NT result in higher soil CO<sub>2</sub> emissions to the atmosphere (Reicosky, 2007). In our experiment, the NT treatment presented the highest soil respiration throughout the study period (only in the first sampling date, CO<sub>2</sub> emission values were similar among tillage treatments). As a difference to SOC, which is less responsive to changes in management, POxC is a highly active fraction, sensitive to management changes in the very short term. Regarding POxC, higher contents were observed in CT followed by RT and NT being greater in LTE than STE. Therefore, an increase in POxC was observed as tillage intensity increased. This result is contrary to what was expected, according to other authors (Hurisso *et al.*, 2016; Panettieri *et al.*, 2013) and previous results obtained in the LTE under dryland conditions (Plaza-Bonilla *et al.*, 2014). Consequently, further research is needed to explain these differences.

In 2015 (the first year of irrigation and the previous maize season), maize yield was greatly affected by soil degradation. In 2015, significantly lower yield values were found in CT (5,876 kg grain ha<sup>-1</sup> at 14% moisture) compared to RT and NT (10,649 and 12,747 kg grain ha<sup>-1</sup> at 14% moisture, respectively). The strong impact of soil crusting on maize yield observed in 2015

motivated us to implement in 2016 an adequate cultipacker-rolling pass just after sowing to break soil crust (in CT and RT) and also to change irrigation management based on short and more frequent irrigation events. These two management changes intended to prevent soil crusting and, consequently, yield losses. According to the results presented in this study, these strategies successfully avoided yield losses in 2016. Despite maize emergence was earlier under NT and RT than CT in both experiments, 44 days after planting the number of maize plants were similar among tillage systems. Interestingly, despite the impact of tillage treatments on soil surface structure, the implementation of a successful planting led to the same yield between treatments. Similar to our results, after two years of study, Alletto *et al.* (2011) observed a delay in maize development in CT compared to conservation tillage early in the growing season when assessing the impact of soil management and cover crops in maize production in an irrigated area in SW France. The last authors related the delay in maize development to greater soil drying under CT which negatively affected the final maize grain yield.

#### *4.2 Effect of soil management change during the transformation into irrigation on soil surface properties.*

In the LTE, two decades of contrasting tillage under rainfed conditions led to different initial soil conditions between tillage treatments when transforming the area into irrigation. The continuous use of CT in the LTE resulted in a decrease in SOC compared with NT and RT as a result of the lower amount of crop residues returned to the soil as C inputs (Morell *et al.*, 2011). In rainfed Mediterranean conditions, the use of NT enhances the amount of water stored in the soil (Lampurlanés *et al.*, 2016), increasing crop biomass and, consequently, C inputs as crop residues. Regarding to this, Virto *et al.*, (2012) demonstrated that SOC storage is mainly explained by the amount of C inputs returned to the soil. In the LTE, the lower SOC levels found in the CT treatment made the soil more susceptible to degradation. However, in the STE, the 20 years of NT management prior to the transformation from rainfed into irrigation and to the setup of the different tillage treatments favoured those differences between tillage systems on SOC concentration were minimal (3.7% difference between NT and CT). Consequently, soil surface in the CT treatment was more resilient to the impact of water on soil crusting. The resilience to soil crusting found in the CT plots of the STE contrasted with the susceptibility to crusting and soil surface degradation found in the CT plots of the LTE. Thus, initial SOC concentration and soil surface structural condition played a major role on the response of soil to the transformation from rainfed conditions to irrigation. In general, high SOC levels tend to

increase the rate of water infiltration into the soil (Martinez *et al.*, 2008). In our study, although water infiltration did not differ significantly between tillage treatments, a marked trend existed in the rates found between tillage systems, in the order NT > RT > CT. Furthermore, infiltration rates were higher in STE than in LTE, coinciding with the greater SOC concentration found in STE compared to LTE. However, water infiltration was quantified by performing ponded measurements, where water movement on the soil surface is impeded. The presence of a soil crust in CT could have increased water runoff, mainly through the surface between rows, which is the preferential route of irrigation water in row crops. This last process would be aggravated by the lack of crop residues on soil surface during the most part of the crop growing period when CT is used. This hypothesis would be supported by our field observations in which soil crusting and the presence of soil sediment prevailed in between rows in the CT treatment of the LTE (Fig. 6b). Regarding to this, Osunbitan *et al.* (2005) compared tillage systems in the short term, concluding the presence of higher BD in NT compared to CT, but with higher hydraulic conductivity in NT because of its pore continuity. Moret and Arrúe, (2007) compared different tillage systems (NT, RT and CT) in a fallow-wheat rotation in the Ebro valley. The authors observed a more compacted topsoil layer under NT compared with CT and RT. Contrarily to Osunbitan *et al.* (2005) they observed lower soil hydraulic conductivity near saturation in NT than CT and RT.

## 5. Conclusions

Our study shows that the long-term use of intensive tillage in areas recently transformed into irrigation leads to a greater susceptibility to soil crust formation, and structural degradation. The results of this study have shown that the main process behind soil crusting was the breakdown of dry-sieved aggregates.

Although the proportion of dry-sieved aggregates increased after tillage (even reaching similar values than NT at the end of maize growing season) their water stability was lower. Differences in the stability of aggregates between tillage treatments were explained by different SOC levels as a result of long-term (20 years) of contrasted tillage during the previous rainfed conditions in the LTE. By contrast, in the STE, soil structural degradation was minor, given its previous management based on NT which provided higher resilience to soil crusting, although higher penetration resistance was observed between rows under NT.



The previous NT management during 20 years stimulated the biological activity and the formation of water-stable macroaggregates in the soil, favoring the early development of the crop. Our data highlights the need to maintain NT over time in rainfed areas transformed into irrigation prone to soil structural degradation, in order to provide the soil enough resilience and ensure an optimum development of crops.

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## **Chapter II**

**Is it feasible to reduce tillage and N use while improving maize yield in irrigated Mediterranean agroecosystems?**

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## Chapter II

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### Is it feasible to reduce tillage and N use while improving maize yield in irrigated Mediterranean agroecosystems?

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#### Abstract

Mediterranean rainfed areas are transformed into irrigation to stabilize or increase crop yields. The gradual occupation of irrigation leads to an increase in nitrogen use and intensity of tillage. The aim of this work was to evaluate the combined impact of tillage systems and mineral N fertilization rates on maize grain yield, water and nitrogen use efficiencies (WUE and NUE) under Mediterranean irrigated conditions. The study was carried out in NE Spain during three maize growing seasons (i.e. years 2015, 2016 and 2017). A long-term (LTE) tillage and N rate field experiment established in 1996 under rainfed conditions was transformed into irrigation with maize (*Zea mays* L.) monoculture as cropping system in 2015. Three types of tillage (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) and three mineral N fertilization rates (0, 200, 400 kg N ha<sup>-1</sup>) were compared. In 2015, an adjacent experiment (short-term experiment, STE) with the same layout was set up in an area previously managed under long-term rainfed NT for the last 21 years. In the long-term tillage and N fertilization combination (LTE), the reduction of tillage (NT and RT) led to greater grain yield when applying 200 and 400 kg N ha<sup>-1</sup> compared to the use of the same rates under CT. Differently, in the short-term experiment with preceding NT (STE), tillage systems did not influence grain yields, while N application led to greater yields than the control (0 kg N ha<sup>-1</sup>). In both situations (LTE and STE), NT and RT enhanced soil water content before planting leading to greater crop growth compared to CT. The lack of available water under CT caused lower maize aboveground biomass, yield, and yield components in LTE and, therefore, lower WUEB (for biomass) and WUEY (for yield). In LTE, the use of long-term CT led to a significant accumulation of nitrate compared to NT. Differently, in the STE, soil nitrate content did not show differences between tillage systems. In the LTE, water and N were used more efficiently to produce aboveground

biomass and grain yield in RT and NT. Our study shows that in Mediterranean agroecosystems transformed into irrigation the use of NT and RT with medium rates of N leads to greater maize yield, WUE and NUE than the traditional management based on CT with high rates of mineral N. In rainfed areas with long-term history of no-till, this soil management system can be successfully maintained if transformed into irrigation.

## **Keywords**

Grain yield; maize; N fertilization; N use efficiency; tillage systems; water use.

## **1. Introduction**

In the Mediterranean rainfed area of the Ebro valley (NE Spain), an increasing adoption of reduced tillage (RT) and no-tillage (NT) techniques has taken place over the last 35 years (Lampurlanés *et al.*, 2016). Currently, a significant fraction of the area is being transformed into irrigation to ensure greater yields of winter and spring crops and to develop summer crops. However, in these newly irrigated areas, farmers have been induced to return to intensive tillage systems to overcome the difficulties to handle the increased level of crop residues from irrigated production (Pareja-Sánchez *et al.*, 2017). In these irrigated areas, the limited knowledge about the correct use of RT or NT systems makes difficult their adoption by farmers and puts at risk the soil quality benefits attained with the long-term use of NT in rainfed conditions. In general, in Mediterranean irrigated areas traditional soil management has been based on conventional tillage (CT) with deep subsoilers, mouldboard ploughs and rototillers. Therefore, the preservation of adequate management practices, such as NT or the implementation of new strategies of RT adapted to irrigated row crops like strip-tillage are important to improve water capture and retention (Unger *et al.*, 1991), avoid soil degradation (Pareja-Sánchez *et al.*, 2017), increase soil fertility (Alvarez, 2005) as well as enhance crop productivity (Lamm *et al.*, 2009).

The gradual increasing surface transformed into irrigation leads to an increase in nitrogen use, concomitant with increasing crop yield potential. Nitrogen is a key factor determining crop yield, being one main input in maize production (Parry *et al.*, 2005). However, the use of N fertilizer is generally inefficient with farmers applying it in important quantities to achieve high crop yields and without taking into account soil N availability, which leads to an over-fertilization. This strategy does not increase grain yield but, instead, wastes fertilizer, increases costs, and cause potential nitrate pollution to groundwater (Bowman *et al.*, 2008).

Surveys conducted in the Ebro valley by Sisquella *et al.* (2004) showed that traditional mineral N rates applied to maize by farmers were about 300–350 kg ha<sup>-1</sup>, with grain yields in the area ranging from 12,000 to 16,000 kg ha<sup>-1</sup>. Therefore, the most effective means to ensure high yields while reducing N loss and thus environmental damage is to improve the nitrogen use efficiency (NUE) of crops (Davidson *et al.*, 2015). In a long-term irrigated experiment with maize managed under CT and comparing different mineral N rates (0, 100, 150, 200, 250, 300 and 400 kg N ha<sup>-1</sup>) also in the Ebro valley, Martínez *et al.* (2017) observed the highest NUE and grain yield when applying 200 kg N ha<sup>-1</sup>. Therefore, in the study area, it is feasible the reduction in maize N fertilization while maximizing maize yields.

Crop nitrogen uptake is strongly influenced by water supply (Martin *et al.*, 1982), thereby farmers should optimize water use to enhance NUE and reduce economic losses and environmental pollution. In pressurized irrigated systems, a proper water management is important, since irrigation accounts for a great proportion of the production costs that farmers face, due to electric energy needs and high costs of infrastructure establishment. This fact forces to redesign current cropping systems and, more specifically, the management practices to increase WUE in irrigated areas. The simultaneously combination of an efficient management of water, N fertilization and tillage is crucial for closing the yield gap of main cereal crops as well as to prevent water and soil pollution. In this line, authors like Cullum, (2012) have shown that NT systems achieved higher maize grain yields than CT based on chisel/disk under the same N rate in northern Mississippi. In turn, Fabrizzi *et al.* (2005) evaluated the effects of RT and NT and two N fertilization rates (0 and 150 kg N ha<sup>-1</sup>) on maize yield in Buenos Aires (Argentina) and observed lower grain yields under NT than under RT in the control treatment and no differences between tillage systems when applying 150 kg N ha<sup>-1</sup>. Soil tillage and N fertilization influence soil N dynamics due to their impact on crop residues production and decomposition, soil organic nitrogen mineralization and water dynamics in the soil profile. Under NT crop residues are maintained on the soil surface which reduces their decomposition rate (Salinas-García *et al.*, 2002). In that context, N fertilizer could be immobilized if applied on soil surface (Kitur *et al.*, 1984). Moreover, the lack of soil alteration under NT maintains organic nitrogen protected, reducing its mineralization by soil microbes (Doran, 1980). Consequently, different authors pointed out the need to increase N fertilizer rates during the first years of NT implementation (Sims *et al.*, 1998; McConkey *et al.*, 2002). Indeed, other authors suggest to maintain this strategy until the increase in soil organic matter levels is sufficient to assure enough N from

mineralization and available to crops. Therefore, optimum N fertilization level is dependent on the type of tillage implemented (Baker *et al.*, 1996).

Besides a tillage system change, the gradual transformation into irrigated areas could represent an intensification in the use of N. Currently, knowledge about the combined effect of tillage and N fertilization on crop production and WUE and NUE in the Mediterranean areas is limited to rainfed conditions (Cantero-Martínez *et al.*, 2007; Morell *et al.*, 2011; Plaza-Bonilla *et al.*, 2017). Therefore, the aim of this study was to evaluate the combined impact of tillage and mineral N fertilization rates on irrigated maize grain yield, WUE and NUE in two field experiments differing on previous soil management under Mediterranean conditions.

## **2. Materials and methods**

### *2.1 Experimental design and management practices.*

The study was performed in Agramunt, NE Spain (41°48' N, 1°07' E, 330 m asl). The climate is semiarid Mediterranean with a continental trend. The mean annual precipitation in the last 30 years is 401 mm, the mean annual of temperature is 14.1°C, and the annual potential evapotranspiration is 855 mm.

A rainfed long-term field experiment (LTE) was established in 1996 to compare 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<sup>-1</sup>) under barley monocropping (Angás *et al.*, 2006). In 2015 the LTE was transformed to irrigation with solid set sprinklers and 3 years of maize (*Zea mays* L.) monoculture as the main cropping system in the area. After the transformation to irrigation, the same tillage intensity treatments (CT, RT and NT) and three mineral N fertilization rates adapted to maize cultivation (0, 200, 400 kg N ha<sup>-1</sup>) were compared in LTE maintaining the same experimental layout as the previous rainfed experiment. At the same time, in 2015, a new experiment was created adjacent to the LTE (separated by a 15 m corridor). The layout of this new experiment (so called short-term experiment, STE) was exactly the same as the LTE (same tillage and N fertilization treatments, spatial arrangement and cropping system) but with different historical tillage management. For the previous 21 years, the entire surface occupied by the STE consisted of a rainfed NT winter cereal-based cropping system and the new experiment was implemented directly in no-tillage soil. The experimental design in LTE and STE consisted of a randomized block design with three replications and plot size of 50x6 m. Soil was classified as Typic Xerofluvent (Soil Survey Staff, 2014) with a silt loam texture (sand, 30.8%; silt,

57.3%; clay, 11.9%) in the upper (0-28 cm) horizon. The main physico-chemical properties at the beginning of the experiment (2015) were as follows: pH (H<sub>2</sub>O, 1:2.5) 8.5; electrical conductivity (1:5) 0.15 dS m<sup>-1</sup>; soil organic carbon (SOC) concentration (0-30 cm) 7, 9 and 9 g kg<sup>-1</sup> under CT, RT and NT, respectively in LTE and 10, 9 and 10 g kg<sup>-1</sup> under CT, RT and NT, respectively in STE; P Olsen, 35 mg kg<sup>-1</sup>; K (neutral ammonium acetate), 194 mg kg<sup>-1</sup>; water retention (-33 kPa), 16 kg kg<sup>-1</sup>; water retention (-1500 kPa), 5 kg kg<sup>-1</sup>.

The experiment was implemented in three successive maize growing seasons (2015, 2016 and 2017). In LTE and STE, tillage operations were carried out at the end of March to the beginning of April in the three growing seasons. The CT treatment consisted of one pass of rototiller (15 cm depth) followed by subsoiler (35 cm depth), finished by one pass of a disk plough (20 cm depth) with almost 100% of the crop residues incorporated into the soil before planting. The RT treatment consisted of a pass of strip-till to a depth of 20-25 cm, implemented on the maize planting row reducing the surface tilled to ca. 20%. In the RT treatment no chemical weeding was used. Finally, the NT treatment consisted of weed control with a non-selective herbicide (i.e. glyphosate) at 1.5 L ha<sup>-1</sup>. Planting was carried out with a pneumatic row direct drilling machine equipped with double disc furrow openers (model Prosem K, Solà, Calaf, Spain) in the three tillage systems (CT, RT, and NT). Rotary residue row cleaners were installed to clear the path for the row unit openers (both in RT and NT treatments). The planting depth was adapted to each tillage treatment to reach a constant value (ca. 4 cm). Mineral P and K fertilization was applied prior to maize planting based on soil analysis at rates of 154 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 322 kg K<sub>2</sub>O ha<sup>-1</sup>, respectively, in the first two years. In the third year the levels of available P and K in the soil were appropriate for the crop, therefore P and K were not applied in 2017. The N fertilizer rates compared were split in one pre-planting application with urea (46% N) on April, and two top-dressing applications on May and July (V5 and V10 stages, respectively) with calcium ammonium nitrate (27% N) with 50, 75 and 75 kg N ha<sup>-1</sup> applied, respectively, in the three splits in the 200 kg N ha<sup>-1</sup> rate, being doubled in the 400 kg N ha<sup>-1</sup> rate. For the three years, maize (cv. Kopias) was planted in late April at a rate of 90,000 seeds ha<sup>-1</sup> with a 73-cm width between rows. Irrigation was supplied to meet the estimated evapotranspiration (ET) of the crop minus the effective precipitation, which was estimated as 75% of precipitation when precipitation > 5 mm. (Dastane, 1978). Weekly maize evapotranspiration (ET<sub>c</sub>) was calculated from the corresponding weekly values of ET and K<sub>c</sub>. Reference ET was computed with the FAO Penman–Monteith method from meteorological data obtained from an automated weather station located near the experimental site. Crop coefficients (K<sub>c</sub>) were estimated in function of

crop development. The experiment received a total of 631, 672 and 696 mm of irrigation water in 2015, 2016 and 2017, respectively, during the maize growing period. Harvesting was carried out at the beginning of November with a commercial combine. The crop residues were chopped and spread over the soil. During the winter periods between crops the soil was maintained free of weeds with an application of 1.5 L ha<sup>-1</sup> of commercial product which contains a 36% glyphosate [(N-(phosphonomethyl)-glycine)].

### *2.2 Soil and crop samplings and measurements.*

Within each plot, two sampling areas were defined. Soil samples from each area were collected prior to maize planting (mid-March) and after harvesting (mid-November). Soil water, ammonium and nitrate contents were quantified at three depth intervals (0–30, 30–60 and 60–90 cm depth). The soil nitrate (NO<sub>3</sub><sup>-</sup>) contents were quantified by extracting 50 g of fresh soil with 100 mL of 1 M KCl. The extracts were analyzed with a continuous flow autoanalyzer (Seal Autoanalyzer 3, Seal Analytical, Norderstedt, Germany). Gravimetric soil water content was determined for every depth interval by oven drying a soil sample at 105°C until constant weight. Concentrations were transformed to volume-based values using soil bulk density determined by the soil core method (Grossman and Reinsch, 2002).

One week before the commercial harvest, aboveground biomass was quantified by sampling two central rows of 2–5 m long, depending on plant density, in three sampling areas per plot. For the aboveground biomass, ears were not included. The first year of sampling 5 m length was taken in all treatments due to the low plant density in CT. The rest of the period (second and third year) the length of the sampling row was 2 m. The number of plants and ears were counted and registered. Afterwards, a sub-sample of two entire plants and five ears was taken, oven-dried at 60°C for 48 h and weighed. Afterwards, the grain was threshed and weighed. Grain moisture was adjusted to 14% moisture content. These determinations allowed calculating above-ground biomass (excluding the grain) as well as maize yield components: plants per square meter, number of ears per plant and thousand kernels weight (TKW). Nitrogen concentration of maize grain and aboveground biomass were determined by dry combustion (model Truspec CN, LECO, St Joseph, MI, USA). Afterwards, grain N and aboveground biomass N excluding the grain were calculated by multiplying the biomass of each fraction by its N concentration. Aboveground N uptake was calculated by the sum of N content in both fractions. Grain protein was calculated by multiplying the grain N concentration by 6.25 (Jones, 1941).

### 2.3 Water and nitrogen related indicators.

Water use (WU) was calculated as the difference in soil water content (SWC) between planting and harvest plus the rainfall received and the irrigation water applied between both dates. Water use efficiency for aboveground biomass ( $WUE_B$ ) and yield ( $WUE_Y$ ) were calculated as follows:

$$WUE_B (\text{kg biomass ha}^{-1}) = \frac{\text{Aboveground biomass}}{\text{WU}}$$

$$WUE_Y (\text{kg grain ha}^{-1}) = \frac{\text{Grain yield}}{\text{WU}}$$

The following N-related parameters were calculated for each treatment:

N use efficiency (NUE;  $\text{kg kg}^{-1}$ ):

$$\text{NUE} = \frac{\text{Grain yield}}{\text{N supply}}$$

Where N supply is the sum of soil nitrate–N at planting and N fertilizer applied.

N harvest index (NHI):

$$\text{NHI} = \frac{\text{N grain}}{\text{Aboveground N uptake}}$$

Where N grain is grain N concentration.

N apparent recovery efficiency for each fertilizer treatment:

$$\text{NAR} = \frac{\text{Aboveground N uptake} - \text{N uptake}_{0\text{N}}}{\text{N fertilizer}}$$

Where N uptake is the aboveground biomass N of the crop for a given fertilizer treatment and  $\text{N uptake}_{0\text{N}}$  is the aboveground biomass N of the control.

### 2.4 Statistical analyses.

Statistical analyses were performed using the JMP 12 statistical package (SAS Institute Inc, 2015). Data were checked for normality with the Shapiro-Wilk Test. All data complied with normality. Therefore, data were not transformed before analysis. For each experiment (LTE and STE), analysis of variance (ANOVA) was performed with tillage system, N fertilization rate,

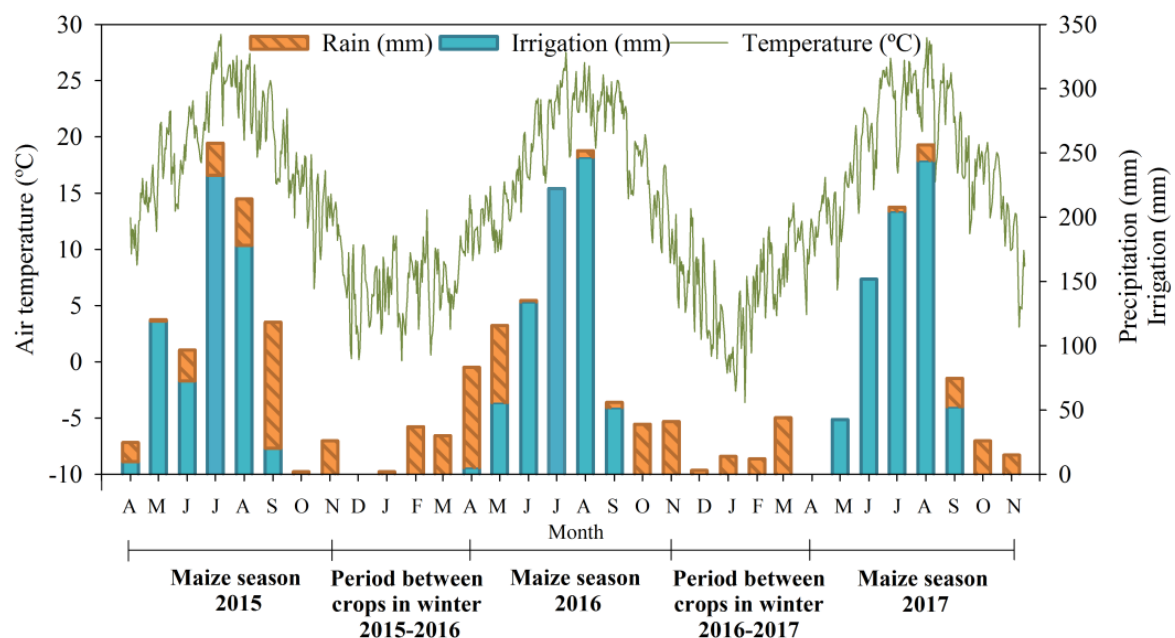


year/sampling date and the interactions among them as fixed effects. In the analysis year was used to the grain yield, aboveground biomass, plants  $m^{-2}$ , TKW, grain protein, grain N content, WU,  $WUE_B$ ,  $WUE_V$ , N uptake, NUE, NHI and NAR variables, while sampling date was used to the SWC and SNC variables. When significant effects were found, means separation was carried out using a Tukey HSD test at the 0.05 probability level.

### 3. Results

#### 3.1 Weather conditions during the experimental period.

Air temperature, precipitation and irrigation applied during the three maize growing seasons are shown in Fig. 8. Air temperature increased from the beginning of each maize season, reaching a maximum in summer months (June to August), to decrease later during autumn and winter months, being the minimum in December and January. The maximum temperature was reached in 2017, being the average of June, July and August 22.6, 24.0 and 24.6 °C, respectively. Precipitation varied considerably between maize seasons being 226, 151 and 78 mm for 2015, 2016 and 2017, respectively (Fig. 8). During the two periods between crops in winter (2015-2016 and 2016-2017) rainfall was 108 mm and 106 mm, respectively. The amount of water applied by irrigation was 631, 672 and 696 mm in 2015, 2016 and 2017, respectively (Fig. 2).



**Fig. 8** Monthly precipitation and irrigation (orange and turquoise columns, respectively) and daily air temperature (continuous line), during the experimental period (April 2015 to November 2017). Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and periods between crops in winter (2015-2016 and 2016-2017).

### 3.2 Maize grain yield, aboveground biomass, yield components and grain protein.

The interaction between tillage and N fertilization, tillage and year and N fertilization and year had a significant effect on maize grain yields in LTE (Table 7). In 2016 and 2017, the application of 200 and 400 kg N ha<sup>-1</sup> led to greater yields than the control treatment (Fig. 9). In 2015 and 2017, grain yields in LTE were higher under NT and RT than under CT, without differences between tillage treatments in 2016 (Fig. 10). In the STE, the interaction between N fertilization and year had a significant effect on maize grain yields. Nitrogen application led to greater maize grain yields compared to the control without N in 2016 and 2017 (Fig. 9).

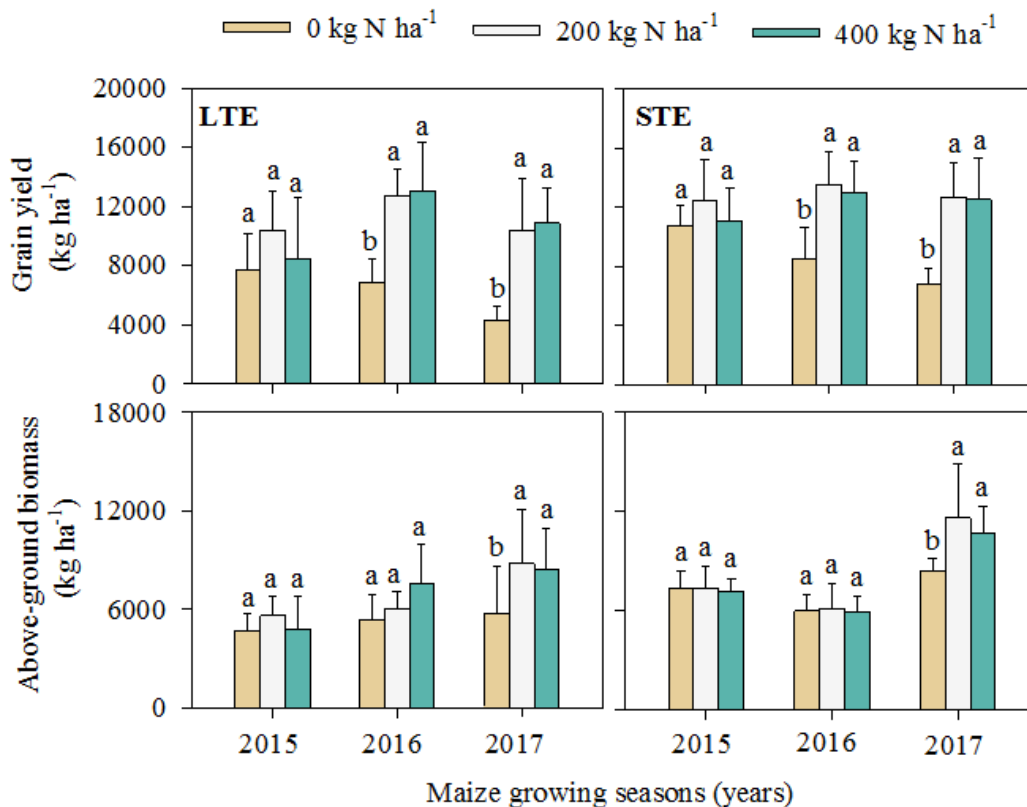
**Table 7.** Analysis of variance (P-values) of maize grain yield, aboveground biomass, plant population (plants m<sup>-2</sup>), thousand kernels weight (TKW) and grain protein as affected by tillage, N fertilization, year and their interactions in a long-term (LTE) and a short-term (STE) field experiment.

| Experiment | Source of variation    | Grain yield | Aboveground biomass | Plants m <sup>-2</sup> | TKW    | Grain protein |
|------------|------------------------|-------------|---------------------|------------------------|--------|---------------|
| LTE        | Tillage (Till)         | <0.001      | <0.001              | <0.001                 | ns     | <0.001        |
|            | N fertilization (Fert) | <0.001      | 0.001               | ns                     | <0.001 | <0.001        |
|            | Year                   | <0.001      | <0.001              | <0.001                 | 0.005  | ns            |
|            | Till*Fert              | <0.001      | ns                  | ns                     | 0.01   | 0.03          |
|            | Till*Year              | 0.01        | ns                  | <0.001                 | 0.003  | ns            |
|            | Fert*Year              | <0.001      | 0.03                | ns                     | ns     | ns            |
|            | Till*Year*Fert         | ns          | ns                  | ns                     | ns     | ns            |
| STE        | Tillage (Till)         | ns          | ns                  | ns                     | ns     | 0.01          |
|            | N fertilization (Fert) | <0.001      | 0.003               | ns                     | <0.001 | <0.001        |
|            | Year                   | ns          | <0.001              | <0.001                 | ns     | 0.003         |
|            | Till*Fert              | ns          | ns                  | ns                     | 0.03   | ns            |
|            | Till*Year              | ns          | 0.003               | ns                     | ns     | ns            |
|            | Fert*Year              | 0.004       | 0.002               | ns                     | ns     | ns            |
|            | Till*Year*Fert         | ns          | ns                  | ns                     | ns     | ns            |

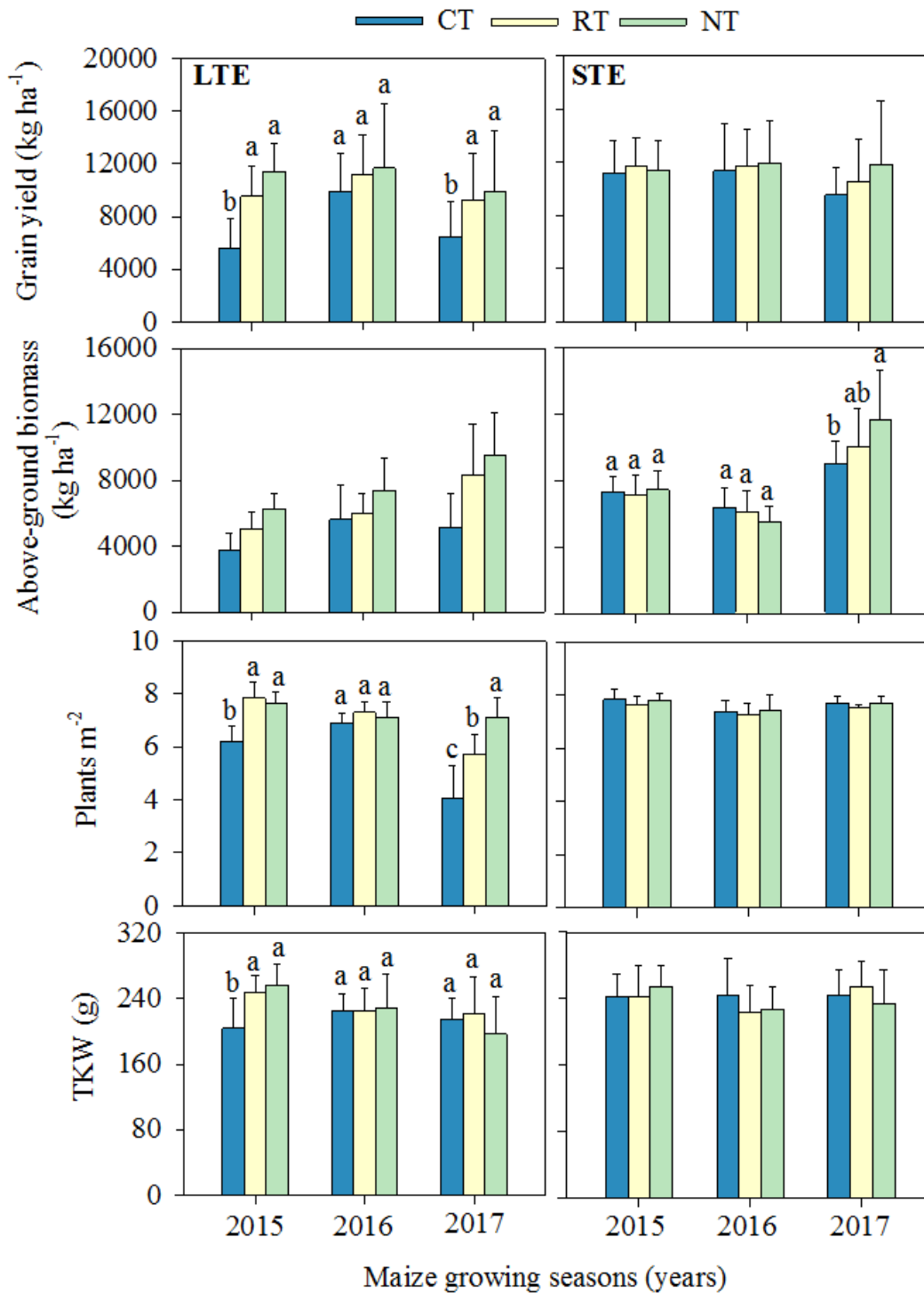
ns, non-significant

Maize aboveground biomass in the LTE was significantly affected by tillage and the interaction between N fertilization and year (Table 7). In the LTE, when comparing among tillage treatments and as an average of the three years, greater aboveground biomass was found under NT than under RT or CT, being NT values 60% greater than CT (7728 vs 4839 kg ha<sup>-1</sup>, respectively). Greater aboveground biomass was observed when applying 200 and 400 kg N ha<sup>-1</sup> in 2017 compared to the control treatment (Fig. 9). In the STE, maize aboveground biomass was significantly affected by the interaction between tillage and year and N fertilization and year

(Table 7). Regarding to this, in 2017 the application of 200 and 400 kg N ha<sup>-1</sup> led to greater aboveground biomass production than the control (Fig. 9). Moreover, greater aboveground biomass was observed under NT than CT, with intermediate values in RT in 2017 (Fig. 10).



**Fig. 9** Maize grain yield and aboveground biomass as affected by N fertilization treatments (0, 200 and 400 kg N ha<sup>-1</sup>) in three consecutive maize growing seasons (2015, 2016 and 2017) in a long-term (LTE) and a short-term (STE) field experiment. For each experiment, different lowercase letters indicate significant differences between N fertilization treatments for a given year at  $P < 0.05$ . Vertical bars indicate standard deviation.



**Fig. 10** Maize grain yield, aboveground biomass, yield components (thousand kernels weight, TKW and plants populations) as affected by tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage). Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) in a long-term (LTE) and a short-term (STE) field experiment. Different lowercase letters indicate significant differences between tillage treatments for a given year at  $P < 0.05$ . Vertical bars indicate standard deviation.

Plant population was only affected by the interaction between tillage and year in the LTE (Table 7). In 2015, NT and RT showed greater number of plants per square meter than CT. In 2016, similar values were observed in the different tillage treatments. In 2017 plant population followed the order NT>RT>CT (Fig. 10). Differently, in the STE, plant population was only affected by year. In the LTE, TKW was affected significantly by the interaction between tillage and N fertilization and between tillage and year (Table 7). When using NT, the 400 kg N ha<sup>-1</sup> treatment showed greater TKW than the control, with 257 g and 190 g, respectively, as an average of the three cropping seasons studied. Thousand kernel weight was higher under NT and RT than under CT in 2015, without differences between tillage treatments in 2016 and 2017 (Fig. 10). In the STE, TKW was significantly affected by the interaction between tillage and N fertilization (Table 7). The rates of 200 and 400 kg N ha<sup>-1</sup> led to greater TKW than the control in NT and CT, without differences between rates in RT, as an average of the three cropping seasons studied.

In the LTE, grain protein was significantly affected by the interaction between tillage and N fertilization (Table 7). In this regard, greater grain protein concentration was found under CT when applying 400 kg N ha<sup>-1</sup> (100 g kg<sup>-1</sup>) compared with RT and NT without N application (67 and 62 g kg<sup>-1</sup>, respectively), as an average of the three cropping seasons studied. In the STE, grain protein was significantly affected by tillage, N fertilization and year simple effects (Table 7). Greater grain protein was found under CT and RT compared with NT (89, 89 and 79 g kg<sup>-1</sup>, respectively). Moreover, the 400 and 200 kg N ha<sup>-1</sup> rates showed greater grain protein compared to the control (95, 88 and 74 g kg<sup>-1</sup>, respectively). Furthermore, in 2017 greater grain protein was observed compared with 2015 and 2016 (93, 82 and 83 g kg<sup>-1</sup>, respectively).

### *3.3 Soil water content dynamics and maize water-use efficiency.*

In the LTE, SWC was significantly affected by the interaction between tillage and N fertilization and by the interaction of these last with the sampling date. In the STE, SWC was significantly affected by the sampling date and the interaction between tillage and N fertilization (Table 8). In the LTE, SWC dynamics followed a similar pattern during the 2015 and 2016 cropping seasons, with greater SWC in NT and RT than in CT and a trend of increasing SWC from planting to harvest in the three years studied. However, SWC did not show differences between treatments after harvesting in 2017 (Fig. 11). In contrast, SWC showed similar behavior in the different N fertilization rates. In the STE, the control in NT showed greater SWC compared to CT

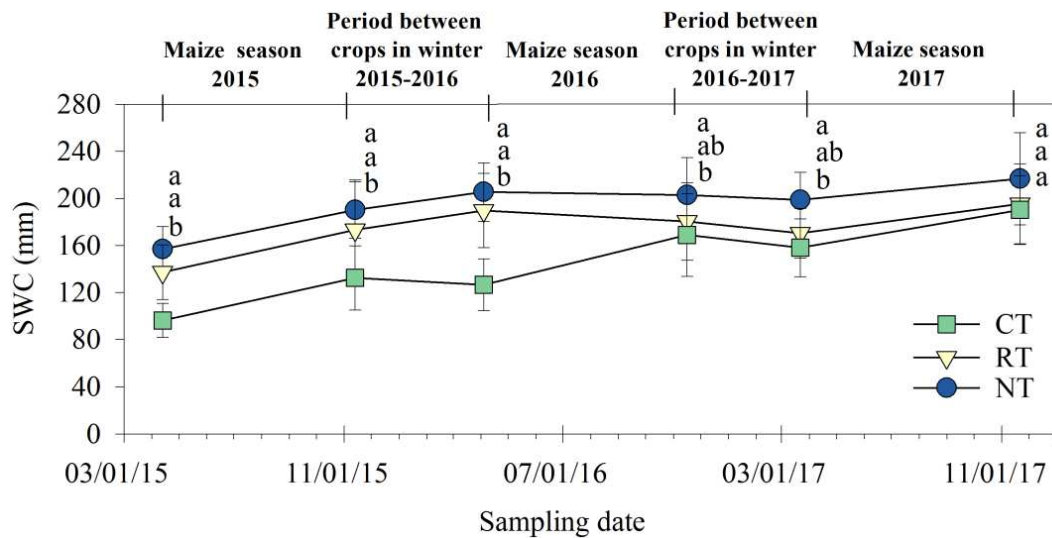
when applying 400 kg N ha<sup>-1</sup> which were observed the lowest values, as an average of the three cropping seasons studied.

In the LTE, water use (WU) was significantly affected by the interaction between tillage and N fertilization and the interaction between tillage and year (Table 8). Greater WU was observed under RT and NT when applying 400 kg N ha<sup>-1</sup> (820 and 806 mm, respectively) than CT under the same N rate (780 mm), as an average of three years. Furthermore, the rate of 200 kg N ha<sup>-1</sup> showed greater WU in NT (815 mm) than CT (789 mm). In the STE, the interaction between tillage and year significantly affected WU (Table 8). In 2015, 2016 and 2017, WU was similar between tillage systems (data not shown).

**Table 8.** Analysis of variance (P-values) of soil water content (SWC), soil nitrate content (SNC) (0-90 cm depth), maize water use (WU), water-use efficiency for aboveground biomass (WUE<sub>B</sub>), water-use efficiency for yield (WUE<sub>Y</sub>), aboveground N uptake (N uptake), grain N content, N use efficiency (NUE), N harvest index (NHI), N apparent recovery fraction (NAR) as affected by tillage, N fertilization, year or date (year/date) and their interactions in a long-term (LTE) and a short-term (STE) field experiment.

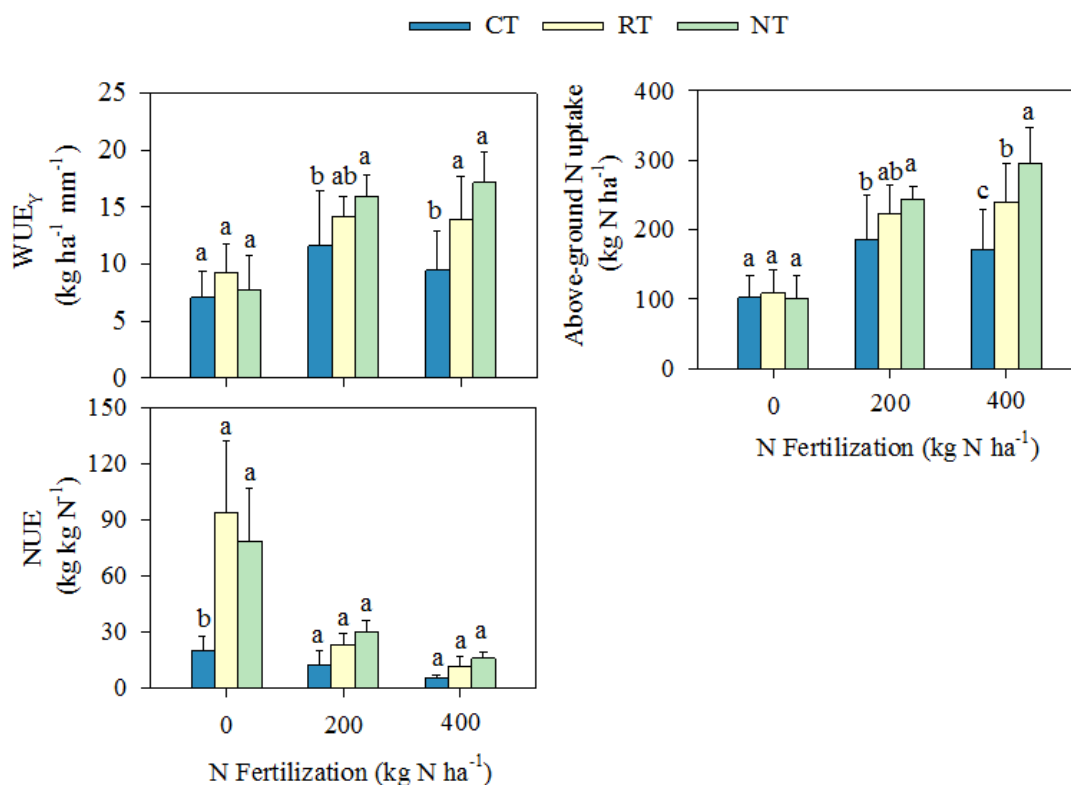
| Experiment | Source of variation | SWC    | SNC    | WU     | WUE <sub>B</sub> | WUE <sub>Y</sub> | N uptake | Grain N content | NUE    | NHI    | NAR    |
|------------|---------------------|--------|--------|--------|------------------|------------------|----------|-----------------|--------|--------|--------|
| LTE        | Tillage (Till)      | <0.001 | <0.001 | <0.001 | <0.001           | <0.001           | <0.001   | <0.001          | <0.001 | 0.004  | <0.001 |
|            | N fertilization     | ns     | <0.001 | <0.001 | <0.001           | <0.001           | <0.001   | <0.001          | <0.001 | 0.01   | <0.001 |
|            | Year/Date           | <0.001 | <0.001 | <0.001 | <0.001           | <0.001           | <0.001   | <0.001          | 0.001  | <0.001 | <0.001 |
|            | Till*Fert           | <0.001 | 0.005  | 0.002  | ns               | 0.001            | <0.001   | <0.001          | <0.001 | <0.001 | ns     |
|            | Till*Year/Date      | <0.001 | 0.001  | <0.001 | 0.006            | 0.006            | 0.005    | 0.004           | ns     | ns     | 0.004  |
|            | Fert* Year/Date     | 0.007  | 0.004  | ns     | 0.002            | <0.001           | <0.001   | <0.001          | 0.01   | 0.003  | ns     |
|            | Till* Year/Date     | ns     | ns     | ns     | ns               | ns               | ns       | 0.01            | ns     | 0.003  | 0.03   |
| STE        | Tillage (Till)      | 0.002  | ns     | ns     | ns               | ns               | ns       | ns              | ns     | ns     | ns     |
|            | N fertilization     | 0.008  | <0.001 | ns     | <0.001           | <0.001           | <0.001   | <0.001          | <0.001 | ns     | 0.007  |
|            | Year/Date           | <0.001 | <0.001 | <0.001 | <0.001           | ns               | 0.01     | ns              | <0.001 | <0.001 | <0.001 |
|            | Till*Fert           | 0.03   | ns     | ns     | ns               | ns               | ns       | ns              | ns     | ns     | ns     |
|            | Till* Year/Date     | ns     | ns     | 0.01   | ns               | ns               | ns       | ns              | ns     | ns     | ns     |
|            | Fert* Year/Date     | ns     | <0.001 | ns     | 0.003            | 0.002            | 0.04     | 0.008           | <0.001 | ns     | ns     |
|            | Till* Year/Date     | ns     | ns     | ns     | ns               | ns               | ns       | ns              | ns     | ns     | ns     |

ns, non-significant



**Fig. 11** Soil water content (SWC) (0–90 cm depth) dynamics as affected by tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) in a long-term field experiment (LTE) during three consecutive maize growing seasons (2015, 2016 and 2017). For a given date, different lowercase letters indicate significant differences between treatments at  $P < 0.05$ . Vertical bars indicate standard deviation.

The analysis of variance revealed significant effects of the interaction between tillage and year and between N fertilization and year on  $WUE_B$  and  $WUE_Y$  in the LTE. Differently, the interaction between tillage and N fertilization only affected significantly  $WUE_Y$  (Table 8). In this experiment, NT and RT showed larger  $WUE_Y$  compared to CT when applying  $400 \text{ kg N ha}^{-1}$  as an average of the three years studied (Fig. 12), while NT showed greater  $WUE_Y$  than CT when applying  $200 \text{ kg N ha}^{-1}$ . In the same experiment and in 2016 and 2017, the rates of 200 and  $400 \text{ kg N ha}^{-1}$  led to greater  $WUE_B$  and  $WUE_Y$  than the control (Fig. 13). In the STE, the  $WUE_B$  and  $WUE_Y$  were significantly affected by the interaction between N fertilization and year. Regarding to this, the application of N fertilizer led to greater  $WUE_B$  than the control in 2017 and greater  $WUE_Y$  than the control in 2016 and 2017 (Fig. 13).



**Fig. 12** Maize water-use efficiency for yield ( $WUE_y$ ), aboveground N uptake (N uptake) and N use efficiency (NUE) as affected by tillage treatments (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and N fertilization rates (0, 200 and 400 kg N ha<sup>-1</sup>) during three consecutive maize growing seasons (2015, 2016 and 2017) in a long-term experiment (LTE). Different lowercase letters indicate significant differences between tillage for a given N fertilization treatments at  $P < 0.05$ . Vertical bars indicate standard deviation.

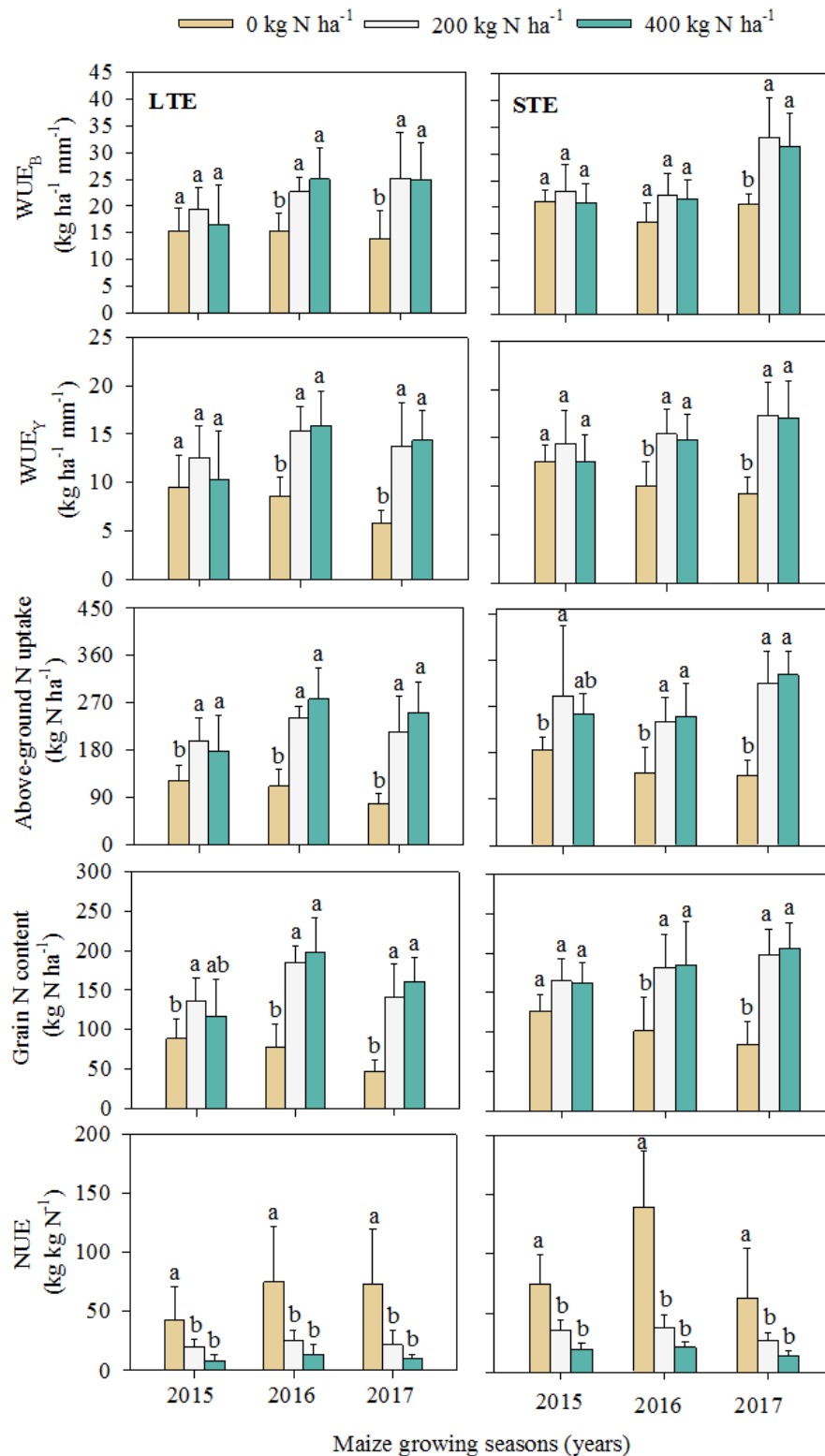
### 3.4 Soil nitrate nitrogen content, nitrogen use efficiency and grain N content.

In the LTE, soil nitrate nitrogen content (SNC) was significantly affected by the interaction between tillage and N fertilization and by the interaction of these last with the sampling date (Table 8). The use of increasing rates of N fertilizer under CT led to greater SNC as an average of the different sampling dates covered by the experiment compared to NT. In the STE, SNC was significantly affected by the interaction between N fertilization and sampling date (Table 8). In this experiment, the rate of 400 kg N ha<sup>-1</sup> showed greater values than the rate of 200 kg N ha<sup>-1</sup> and the control in all sampling dates, except before planting in 2015.

In the LTE, aboveground N uptake was significantly affected by the interaction between tillage and N fertilization and by their interaction with the year. Grain N content was affected by



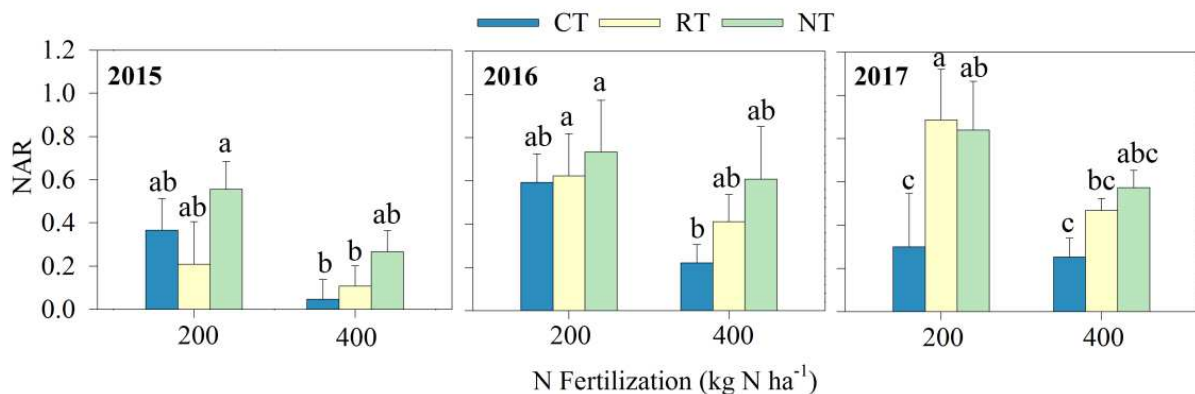
the interaction between tillage, N fertilization and year (Table 8). In this regard, as an average of the three years, the application of 200 kg N ha<sup>-1</sup> under NT showed greater aboveground N uptake than CT with intermediate values under RT (Fig. 6). Differently, when applying 400 kg N ha<sup>-1</sup> N uptake followed the order NT > RT > CT (294, 239 and 171 kg N ha<sup>-1</sup>, respectively). When comparing between N rates greater aboveground N uptake was observed under the application of 400 kg N ha<sup>-1</sup> compared to the control in 2015, 2016 and 2017, in the LTE (Fig. 13). In the three years studied, NT led to greater grain N content compared to CT with intermediate values in RT when applying 400 kg N ha<sup>-1</sup>. The rate of 200 kg N ha<sup>-1</sup> only showed differences in 2017 with greater values in NT and RT compared to CT. In the STE, aboveground N uptake and grain N content were significantly affected by the interaction between N fertilization and year (Table 8). Regarding to this, greater aboveground N uptake was found under the rate of 200 kg N ha<sup>-1</sup> compared to the control in 2015, while in 2016 and 2017 greater aboveground N uptake was observed when applying N fertilizer compared to the control (Fig. 13). In turn, in STE greater grain N content was observed under the application of 200 and 400 kg N ha<sup>-1</sup> compared with the control in 2016 and 2017, whereas in 2015 no differences were observed between N fertilization rates (Fig. 13).



**Fig. 13** Water-use efficiency for biomass ( $WUE_B$ ) and yield ( $WUE_Y$ ), aboveground N uptake, grain N content and N use efficiency (NUE) as affected N fertilization treatments ( $0, 200$  and  $400 \text{ kg N ha}^{-1}$ ). Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) in a long-term (LTE) and a short-term (STE) field experiment. For each experiment, different lowercase letters indicate significant differences between N fertilization treatments for a given years at  $P < 0.05$ . Vertical bars indicate standard deviation.

In the LTE, NUE was affected by the interaction between tillage and N fertilization and between N fertilization and year while NHI was affected by the interaction between tillage, N fertilization and year (Table 8). When N fertilizer was not applied greater NUE was observed under NT and RT compared to CT (78, 93 and 20 kg kg N<sup>-1</sup>, respectively) as an average of the three years covered by the experiment (Fig. 12). In 2015, NHI did not show significant differences between treatments, whereas in 2016 and 2017 the lowest NHI was found under NT in the 0 kg N ha<sup>-1</sup> treatment (data not shown). In the STE, NUE was affected by the interaction between N fertilization and year, with greater NUE in the control treatment compared with the application of N fertilizer in the three years of study (Fig. 13).

Finally, NAR was significantly affected by the interaction between tillage, N fertilization and year in the LTE (Table 8). In 2015 and 2016, NAR did not show differences between treatments, although a trend of greater values was observed when reducing tillage intensity. However, in 2017 NT and RT led to greater NAR compared to CT when applying 200 kg N ha<sup>-1</sup> (Fig. 14). In the STE, NAR was affected by N fertilization, with a 47% increase on NAR when applying 200 kg N ha<sup>-1</sup> compared to 400 kg N ha<sup>-1</sup>.



**Fig. 14** Nitrogen apparent recovery efficiency (NAR) as affected by tillage treatments (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and N fertilization rates (0, 200 and 400 kg N ha<sup>-1</sup>) during three consecutive maize growing seasons (2015, 2016 and 2017) in a long-term experiment (LTE). For a given year, different lowercase letters indicate significant differences between tillage and N fertilization treatments at  $P < 0.05$ . Vertical bars indicate standard deviation.

#### 4. Discussion

This study, carried out during three campaigns, has demonstrated that soil tillage exerts a significant impact on maize performance in Mediterranean irrigated conditions. On this point, lower yield was observed in CT compared to NT and RT in long-term experiment. The impact of

tillage systems on soil structure played a major role on crop productivity. In this regard, in a study recently published on this experimental field, it was reported that long-term CT leads to a deterioration of the soil physical properties. This degradation was due to a lower structural stability, causing soil surface crusting, which resulted in lower water infiltration (Pareja-Sánchez *et al.*, 2017). Therefore, although the contribution of irrigation water was the same for all tillage systems (666 mm of water as an average of the three years), the worse structural conditions under CT reduced soil water availability for the crop, causing lower maize yields. In addition, crop establishment was also affected by soil surface degradation, showing a density of plants 22% and 19% lower in CT compared to NT and RT, respectively, which could have been another key cause behind the greater maize yield in NT and RT. Regarding to this, it is well known that grain yield of modern hybrid maize varieties is highly sensitive plant density (Tokatlidis and Koutroubas, 2004; Grassini *et al.*, 2011). Other studies have compared the impact of different tillage systems on maize production in soils previously managed under conventional tillage, with opposite or similar results. For instance, Alletto *et al.* (2011) compared the impact of CT, consisting of one pass of moldboard plow followed by one pass of cultivator and one of roller, with RT, consisting of one pass of harrow and another of roller, on maize production in an irrigated area in SW France. The last authors observed lower soil moisture under CT compared to RT during the growing period of maize, which led to similar or greater grain yields under RT. Differently, Salem *et al.* (2015) determined the short-term impact (1 year) of four tillage treatments on soil physical properties and maize productivity in a central Spain area transformed into irrigation with previous management based on continuous CT under rainfed barley. Salem *et al.* (2015) observed a decrease in maize grain yields and yield components when using NT compared to CT and RT. They pointed out that the higher soil compaction under NT would be the cause for maize yield decrease. Unlike the previous author, in our study we obtained more production under NT because in STE the soil presents a long history (21 years, in our case) of continued management under NT before the transformation from rainfed to irrigation, while they only did it for a year. Therefore, maize yield as well as the use of water and N resources differs depending on the years in which the tillage system was used. This last aspect indicates a soil structure maintenance effect (i.e. soil structure resilience), since the maintenance of NT in the long-term results in the formation of soil macroaggregates with greater stability (Álvaro-Fuentes *et al.*, 2009, Panettieri *et al.*, 2013) and greater SOC levels (Plaza-Bonilla *et al.*, 2013), specially in the soil surface (0-10 cm). Therefore, the greater SOC concentration and better structural condition of the soil surface played an important role in the response of the soil to the

transformation from rainfed to irrigated conditions (Pareja-Sánchez *et al.*, 2017). Due to this aspect, maize grain yields as well as yield components were not negatively influenced by the use of CT over the three years of the experiment in STE. In the opposite case, where CT has been transformed to NT in dry climates, Van Kessel *et al.* (2013) observed a greater tendency for yield decline for NT and RT being implemented in short-term (<10 years) while in long-term (>10 years) did not show differences between tillage system which could be attributed to the improvement soil aggregation (Six *et al.*, 2004). These studies show that it takes more than 10 years to begin to notice changes in grain yield after a tillage transformation. The authors also showed that physical properties are much related to the yield since it affects establishment of the crop. However, our study is not long enough to determine how many years of intensive tillage would be needed to find significant differences in soil structural condition and crop yield between tillage systems. Moreover, these differences between tillage systems and between both scenarios of historical soil management influenced the use of resources. In relation to this last, although WU was similar among tillage systems in the short-term experiment, we hypothesized that the degradation of soil structure would have reduced water infiltration. Therefore, the CT treatment would have led to less water available to the crop, resulting in a lower WUE.

Over-fertilization with N does not provide any extra grain yield, but simply wastes fertilizer, reduces crop profitability and is a potential source of reactive N contamination (Cox *et al.*, 1993). Therefore, a reduction in fertilizer application could lead to a better balance between crop demand and soil N supply (Cassman *et al.*, 2002) when over-fertilized. In Mediterranean conditions where water is a limiting factor, yield varies according to the amount of water available for the crop and its use efficiency. Soil management techniques and N fertilization rates affect both factors. For instance, conservation tillage reduces the evaporation of the water stored in the soil due to the presence of crop residues on the surface, therefore promoting greater soil water availability than intensive tillage (Lafond, 1994). The water used by the crop through transpiration is strongly affected by N fertilization, with a positive relationship between foliar area and water transpired in N fertilized crops (Samuelson *et al.*, 2007). In our study, in the LTE the application of mineral nitrogen under long-term NT and RT produced an increase in maize  $WUE_y$  compared to long-term CT at the same N rates. Lower water infiltration in CT compared to NT and RT (1.70, 2.40 and 3.14 mm h<sup>-1</sup> for CT, RT and NT, respectively) reduced available water (Pareja-Sánchez *et al.*, 2017), partially explaining the lack of response of CT in  $WUE_y$  to the application of N fertilization in LTE. Similarly, Lamm *et al.* (2009) observed a greater

water use efficiency when using strip-till and NT in comparison to CT, in a field of irrigated maize in Kansas. Another factor that is influenced by tillage and N fertilization is the residual N content in the soil and, therefore, it is important to adjust these two cultivation techniques in combination. In the maize-based cropping systems under Mediterranean irrigated conditions, farmers normally over-fertilize (Berenguer *et al.*, 2009). In this line, our results showed that in the scenario of long-term conventional tillage prior to the transformation into irrigation (LTE), the application of high rates of nitrogen under CT leads to a greater content of mineral N in the soil than under NT. This result would be explained by several causes. First, the long-term use of CT during the previous rainfed experimental period (from 1996 to 2014) led to an accumulation of soil nitrate due to the limited soil water available for barley N uptake (Angás *et al.*, 2006; Morell *et al.*, 2011). Secondly, the lower production of maize biomass in CT mostly attributed to the lower N uptake, due to lower available soil water caused by deterioration of the soil physical properties under long-term CT. On the other hand, in the control plots without nitrogen fertilization, the soil mineral N content (0-90 cm depth) was found to be greater in the long-term experiment (272, 97 and 104 kg N ha<sup>-1</sup> for CT, RT and NT, respectively) than in the short-term one (101 kg N ha<sup>-1</sup>) very likely due to the tillage performed during the previous years (from 1996 to 2014) as commented previously. Therefore, the higher levels of soil mineral nitrogen in the CT control pots led to the aboveground N uptake decreasing in a greater extent in LTE compared to STE. Accordingly, the optimal rate of N fertilization for maize should consider the amount of N available in the soil to avoid over-fertilization and long-term N accumulation in the soil profile. In a study with sprinkler-irrigated maize carried out in Colorado (USA), Halvorson *et al.* (2006) observed that the residual soil nitrate tended to be slightly higher in a CT system fertilized with 202 kg N ha<sup>-1</sup> than under NT at the same N rate, indicating excess of applied N. The high residual soil nitrate content in CT had a great influence on the NUE measured. In the unfertilized treatment (0 kg N ha<sup>-1</sup>), NUE was higher in the conservation tillage systems compared to CT but without differences in the rates of 200 and 400 kg N ha<sup>-1</sup>. The cause was because in RT and NT the residual nitrogen is lower than in CT, since in CT the mineralization rate is higher. Similarly, in a NT maize production system in Argentina, Barbieri *et al.* (2008) reported a greater NUE when nitrogen was not applied. In our study, the high availability of soil mineral N before planting also caused a lack of response to the application of N of the grain N content and the NAR. In this line, lower NAR values were observed at the beginning of the experiment as a result of the high initial soil nitrate content which led to a lower recovery of N applied. In contrast, in the third year of the experiment, the NAR increased, showing higher values when using NT and RT with a rate of

200 kg N ha<sup>-1</sup> compared with CT at the same rate. These data suggest that under NT and RT the crop makes a better use of the nitrogen fertilizer applied during the crop cycle leading to better yields. In this study, similar grain yields were obtained when 200 and 400 kg N ha<sup>-1</sup> were applied in NT and RT, with CT showing the lowest yields in both N rates. Therefore, the application of a high rate when NT or RT is used does not lead to a higher yield of maize grain than the medium rate. This could be due to the high initial soil nitrogen content in the plots fertilized with 200 kg N ha<sup>-1</sup>, which would be enough N to cover the needs of the plant. Consequently, a reduction in N fertilization with a reduction of tillage could help to increase the productivity and profitability of maize crops and reduce the risk of N losses by leaching (Quemada *et al.*, 2013) and the increase in greenhouse gas emissions to atmosphere (Meijide *et al.*, 2009; Sanz-Cobena *et al.*, 2012).

Differently in STE, when the soil was continuously managed under NT during the 21 years previous to the transformation from rainfed to irrigated conditions, the first year of the study (2015) already showed differences in the mineral nitrogen content in post-harvest, showing greater soil nitrogen content in the rate of 400 kg N ha<sup>-1</sup> compared to the application of 0 and 200 kg N ha<sup>-1</sup>. This trend was maintained over the three years of study. This fact indicates the importance of considering the residual levels of nitrogen in this area, due to the high rates of N fertilization that are handled and the long-term accumulation of nitrate in the soil profile, susceptible of being lost by leaching. In addition, the rate of 200 kg N ha<sup>-1</sup> increased grain yield, aboveground biomass, TKW and grain protein, with no increases when applying beyond 200 kg N ha<sup>-1</sup>. These data suggest that the N rate could be reduced by half without compromising grain yield or yield components. In this line, Al-Kaisi and Yin (2003) suggested that the traditional application rate used by farmers in north eastern Colorado in maize production (250 kg N ha<sup>-1</sup>) could be reduced to 140 kg N ha<sup>-1</sup> without losses in grain yield, since high N rates led to a decrease in nitrogen use efficiency as soil water content decreased. Similarly, in a maize experiment in the NE Spain comparing different mineral N application rates, Martínez *et al.*, (2017) reported that the lowest N fertilization rate sufficient to achieve optimal yields was 200 kg N ha<sup>-1</sup>. In our experiment, in all the three years studied, the NUE and the NAR decreased when increasing the rate of N from 200 to 400 kg N ha<sup>-1</sup>, obtaining an improvement of 47% in NAR when 200 kg N ha<sup>-1</sup> were applied compared with 400 kg N ha<sup>-1</sup>. This would prove that the rates of fertilization adapted to the needs of the crop are used more efficiently.

## 5. Conclusions

In the Mediterranean region, large rainfed areas managed under long-term conservation tillage practices are being transformed into irrigation. In this context, the limited knowledge existing on the performance of conservation tillage under irrigation systems, move farmers to return to intensive tillage and high N fertilization rates. The results of this study have shown that conservation tillage must be maintained after the transformation into irrigation. In this context, the use of NT and RT in combination to medium N rates (i.e. 200 kg N ha<sup>-1</sup>) led to greater WUE, which was sufficient to produce optimal grain yield while also achieving relatively high NUE. Adverse soil structural conditions under long-term CT led to lower available soil water, leading to crop water stress, causing lower maize yields and therefore reducing water and nitrogen use efficiency. Moreover, the application of N fertilizer under CT led to the accumulation of residual nitrate in the soil over time. The traditional application of high N fertilizer rates did not bring improvements in grain yield, WUE and NUE compared to medium rates. The use of less aggressive tillage practices, such as no-tillage and strip-tillage, as well as the reduction of N fertilization, could be viable options to stabilize or, even, increase crop yields and optimize NUE and water use simultaneously, saving production costs in comparison with the traditional management based on conventional tillage with high rates of mineral N.

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## **Chapter III**

**Impact of tillage and N fertilization rate on soil N<sub>2</sub>O emissions in irrigated maize in a Mediterranean agroecosystem.**

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## Chapter III

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### Impact of tillage and N fertilization rate on soil N<sub>2</sub>O emissions in irrigated maize in a Mediterranean agroecosystem.

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#### Abstract

In irrigated Mediterranean conditions there is a lack of knowledge about the best combination of tillage and N fertilization practices to reduce soil nitrous oxide (N<sub>2</sub>O) emissions while maintaining maize productivity. The aim of this work was to investigate the effects of different soil management practices and synthetic N fertilization rates on soil N<sub>2</sub>O emissions and their relationship with maize grain yield to determine the best management system to reduce yield-scaled N<sub>2</sub>O emissions (YSNE) in a semiarid area recently converted to irrigation under Mediterranean conditions. A long-term tillage and N rate field experiment established in 1996 under barley rainfed conditions, was converted to irrigated maize (*Zea mays* L.) in 2015. Three types of tillage (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) and three mineral N fertilization rates (0, 200, 400 kg N ha<sup>-1</sup>) were compared during three years (2015, 2016 and 2017) in a randomized block design with three replications. Soil N<sub>2</sub>O emissions, water-filled pore space, soil temperature, mineral N content (as NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), denitrification potential and maize grain yield and aboveground N uptake were quantified. Moreover, the emission factor (EF) and YSNE were calculated. The results showed that the combination of NT and the highest rate of N fertilization led to greater N<sub>2</sub>O emissions. Furthermore, the lowest N<sub>2</sub>O fluxes were observed in CT when WFPS was below 40% and the highest N<sub>2</sub>O fluxes were seen in NT when WFPS was above 60% coinciding with the greatest denitrification potential. Cumulative N<sub>2</sub>O emissions in 2017 and 2015 followed the order 400>200>0 kg N ha<sup>-1</sup>, while in 2016, rate of 400 and 200 kg N ha<sup>-1</sup> showed greater cumulative N<sub>2</sub>O emission compared to the rate control. Differently, only RT showed differences between growing seasons on cumulative N<sub>2</sub>O emissions, with greater values in 2017 compared to 2015, and intermediate values in 2016. In all treatments, the N<sub>2</sub>O EF was much lower than the default IPCC emission factor (1%). NT and RT increased the grain production compared to CT which was affected by severe soil crusting



causing water deficit. Likewise, N fertilizer treatments significantly affected the YSNE, increasing with increasing fertilizer N application rate in the first year of study. Our data show that the use of NT or RT does not lead to more yield-scaled N<sub>2</sub>O emissions than CT in Mediterranean agroecosystems recently converted to irrigation.

### **Keywords**

Soil N<sub>2</sub>O emissions; Irrigated maize; N fertilization; Yield-scaled N<sub>2</sub>O emissions; Tillage systems; Emission Factor.

### **1. Introduction**

Mediterranean climate is characterized by high evapotranspiration, relatively mild temperatures in winter and summer drought. Precipitation is highly variable, becoming deficient in some areas of the Mediterranean, leading to yield constraints. Consequently, rainfed areas are increasingly being converted to irrigation to stabilise or increase yields of traditional crops such as wheat or barley or to allow the establishment of more water demanding crops such as maize, alfalfa or fruit trees. Apart from an increase in crop yield, this conversion to irrigated land also generates an increase in nitrogen fertilizer use which, if not adapted to the needs of the crop, can lead to adverse environmental impacts such N<sub>2</sub>O emitted to the atmosphere (Smith *et al.*, 2008), soil nitrate leached (Quemada *et al.*, 2013), or ammonia gas volatilized (Erisman *et al.*, 2007). Irrigation increases soil water availability, which in combination with elevated temperatures, induces better conditions for biological activity, favouring denitrification. It is assumed that denitrification becomes the dominant mechanism when soil water-filled pore space is above 60%; due to low oxygen availability, rapidly increasing the rate of emission of N<sub>2</sub>O (Skiba and Ball, 2002).

In Mediterranean irrigated conditions, summer crops such as maize can have high productivity, which leads to significant requirements for N. The application of high rates of irrigation water combined with high rates of N offers an elevated potential for the formation of N<sub>2</sub>O (Ellert and Janzen, 2008). Mineral N availability is a key process controlling soil N<sub>2</sub>O emissions. An excess of mineral N accompanied by high N fertilizer rates increases soil mineral N losses as N<sub>2</sub>O through higher nitrification and denitrification rates (Chantigny *et al.*, 1998), increasing the EF. Authors such as Ma *et al.* (2010) and Hoben *et al.* (2011) have reported that an increase in N fertilization rates leads to higher N<sub>2</sub>O emissions in maize. In the Mediterranean area, different studies have provided similar EF for maize production under sprinkler irrigation to

the current IPCC default of 1% (Aguilera *et al.*, 2013; Cayuela *et al.*, 2017). However, in these previous works the impact of other management practices such as tillage on soil N<sub>2</sub>O emissions was not elucidated.

Different to N fertilization, the impact of tillage on soil N<sub>2</sub>O emissions is highly variable (Gregorich *et al.*, 2008). The effects of conservation tillage on N<sub>2</sub>O emissions depend on soil properties, climate conditions, and the number years since conservation tillage was implemented (van Kessel *et al.*, 2013). Six *et al.* (2004) suggested that the emissions of N<sub>2</sub>O could be reduced when maintaining NT over time, as a result of an improvement in soil structure and porosity, thus reducing the formation of anaerobic microsites. For instance, use of NT is a means to conserve water and reduce soil organic matter losses compared with CT, and usually increases bulk density (Lampurlanés and Cantero-Martínez, 2003). This increase in bulk density reduces gas diffusivity, which combined with an increase in surface soil moisture, stimulates the probability of anaerobic conditions, favouring denitrification and N<sub>2</sub>O fluxes (Mosier *et al.*, 2002). On the other hand, long-term use of NT can improve soil structure (Pareja-Sánchez *et al.*, 2017) and lower soil temperature, which in turn can reduce N<sub>2</sub>O emissions relative to CT (Grandy *et al.*, 2006). In past studies reporting tillage effects on N<sub>2</sub>O emissions, several authors found greater fluxes under NT compared with CT (Baggs *et al.*, 2003; Ball *et al.*, 2008). However, others reported higher fluxes under CT (Elder and Lal, 2008). These differences between studies may be attributed to soil properties, climate conditions or the number years under each treatment.

There is a need to identify practices that minimize net greenhouse gas emissions (Follett *et al.*, 2005) while meeting agricultural production. Therefore, a good indicator of the performance of a cropping system in terms of productivity and environmental impact is the yield-scaled N<sub>2</sub>O emissions (YSNE). This indicator is proposed as a metric of the important global challenge of ensuring food security whilst reducing N<sub>2</sub>O emissions (Van Groenigen *et al.*, 2010).

Over the last three decades, in the Mediterranean rainfed area of the Ebro Valley, NE Spain, RT and NT systems have been introduced with the purpose of mitigating soil erosion as well as for reducing production costs (Moreno *et al.*, 2010). However, as in many arid and semiarid regions, rainfed areas are being converted to irrigation and changing to new more productive crops such as maize, which require more nitrogen input than the traditional winter cereal production. Nevertheless, in these newly irrigated areas, farmers are returning to adopt intensive tillage systems, which are common in irrigation production. The limited knowledge about the correct use of RT or NT systems in irrigated land, including their interactive effects

with N fertilization, makes their adoption by farmers difficult and compromises the soil quality benefits attained with long-term NT use.

Different studies have focused on N fertilization strategies in irrigated maize under Mediterranean conditions (e.g. Martínez *et al.*, 2017; Berenguer *et al.*, 2009). However, to our knowledge none of them have been tested the performance of conservation tillage and its interaction with N fertilization on irrigated maize productivity. Moreover, as far as we know, there are no studies that have investigated the interactions of fertilizer N rates and tillage practices on yield-scaled N<sub>2</sub>O emissions in maize production in irrigated Mediterranean conditions. Our main hypotheses were that i) reducing N fertilizer rate in combination with a decrease of tillage intensity, would reduce N<sub>2</sub>O emissions, while ii) the greater N<sub>2</sub>O emissions under NT would be compensated by greater grain yield. Therefore, according to that hypothesis, the objectives of the present study were to investigate the effects of different tillage systems and N application rates on maize grain yields and N<sub>2</sub>O emissions and to determine the best combination to reduce YSNE.

## **2. Materials and methods**

### *2.1. Site description and experimental design.*

The study was carried out in Agramunt, NE Spain (41°48' N, 1°07' E, 330 m asl). The climate is semiarid Mediterranean with a mean annual precipitation of 401 mm and potential evapotranspiration (PET) of 855 mm, (1984–2014). Mean annual air temperature is 14.1°C.

A long-term field experiment was established in 1996 to compare three tillage systems (CT, RT and NT) and three increasing rates of mineral N fertilizer (0, 60 and 120 kg N ha<sup>-1</sup>) under rainfed barley monoculture (Angás *et al.*, 2006). In 2015 the experimental field was converted to irrigation with solid set sprinklers of 18 x 18 m spacing. Three successive maize growing seasons (2015, 2016 and 2017) were studied, corresponding to the typical irrigated cropping system in the area. After the conversion to irrigation, the field experiment maintained the same tillage treatments (CT, RT and NT) while N fertilization rates were adapted to maize (0, 200, 400 kg N ha<sup>-1</sup>). Traditionally, farmer of the área apply N fertilizer rates ranging between 300 and 450 kg ha<sup>-1</sup> (Sisquella *et al.*, 2004). Therefore, in our study the rate of 400 kg N ha<sup>-1</sup> reflects the worst case scenarios used by some farmers and the medium rate (200 kg N ha<sup>-1</sup>) aims to demonstrate that N fertilizer application can be reduced to a half to achieve optimal yields reducing the environmental impact. The experiments were laid out in a randomized block design with three

replications and plot size of 50x6 m. Site characteristics and soil properties are detailed in Table 9. The CT treatment consisted of one pass of rototiller (15 cm depth) followed by one pass of subsoiler (35 cm depth) and one pass of a disk plough (20 cm depth) before planting during March or April with almost 100% of the crop residues incorporated into the soil before planting. This tillage system represents the traditional practice for maize production in the area. The RT treatment consisted of one pass of a strip-till implement on the maize planting row to 25 cm depth reducing the surface tilled to 20%. Finally, NT consisted of a total herbicide application ( $1.5 \text{ L ha}^{-1}$ , 36% glyphosate) without soil disturbance. Planting was carried out with a pneumatic row direct drilling machine equipped with double disc furrow openers (model Prosem K, Solà, Calaf, Spain). The planting depth was adapted to each tillage system. Rotary residue row cleaners were installed to clear the path for the row unit openers. The N fertilizer rates were split in one pre-planting application with urea (46% N) in April, which was surface broadcasted and incorporated with tillage in CT and RT, with  $50 \text{ kg N ha}^{-1}$  applied in the one splits in the  $200 \text{ kg N ha}^{-1}$  rate being doubled in the  $400 \text{ kg N ha}^{-1}$  rate. Afterwards, two top-dressing applications were carried out by broadcasting calcium ammonium nitrate (27% N), in May and July (V5 and V10 stages, respectively) with  $75 \text{ kg N ha}^{-1}$  applied, respectively, in the two splits in the  $200 \text{ kg N ha}^{-1}$  rate, being doubled in the  $400 \text{ kg N ha}^{-1}$  rate. Mineral P and K fertilization was applied prior to maize planting based on soil analysis at rates of  $154 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$  and  $322 \text{ kg K}_2\text{O ha}^{-1}$ , respectively, in the first two years. In the third year the levels of available P and K in the soil were appropriate for the crop, making unnecessary further P and K applications. In the three years maize (cv. Kopias) was planted late April at a rate of  $90,000 \text{ seeds ha}^{-1}$  with a 73 cm width between rows. Irrigation began in April and ended in September being supplied to meet the estimated evapotranspiration of the crop (ETc) minus the effective precipitation, which was estimated as 75% of precipitation (for any precipitation > 5 mm) (Dastane, 1978). Weekly ETc was calculated from the corresponding values of PET and the crop coefficient (Kc). Potential evapotranspiration was computed with the FAO Penman–Monteith method from meteorological data obtained from an automated weather station located near the experimental site. Crop coefficients (Kc) were estimated based on crop development, ranging between 0.3 and 1.2. Irrigation was carried out each 3 to 4 days when crop evapotranspiration was lower (April, May, June and September) and daily in July and August, when the crop water needs are higher. Harvesting was done at the beginning of November with a commercial combine. Afterwards, crop residues were chopped and spread over the soil. During the periods between crops in winter the soil was maintained free of weeds with an application of glyphosate at  $1.5 \text{ L ha}^{-1}$ .

**Table 9.** Soil characteristics of Ap horizon (0-28 cm depth) in 1996. Initial soil organic carbon content (SOC<sub>i</sub>) (1996) and soil organic carbon content (SOC) (0-30 cm) in three tillage systems (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) in 2015.

| Soil Characteristic                              |                   |
|--------------------------------------------------|-------------------|
| Soil classification*                             | Typic Xerofluvent |
| pH (H <sub>2</sub> O, 1:2.5)                     | 8.5               |
| EC <sub>1:5</sub> (dS m <sup>-1</sup> )          | 0.15              |
| P Olsen (ppm)                                    | 35                |
| K Amm. Ac. (ppm)                                 | 194               |
| Water retention (-33 kPa) (g g <sup>-1</sup> )   | 16                |
| Water retention (-1500 kPa) (g g <sup>-1</sup> ) | 5                 |
| SOC <sub>i</sub> (g kg <sup>-1</sup> )           | 7.6               |
| Sand (%)                                         | 30.8              |
| Silt (%)                                         | 57.3              |
| Clay (%)                                         | 11.9              |
| SOC (g kg <sup>-1</sup> )                        |                   |
| CT                                               | 7                 |
| RT                                               | 9                 |
| NT                                               | 9                 |

\*According to the USDA classification (Soil Survey Staff, 2014)

## 2.2 Soil N<sub>2</sub>O emissions and denitrification potential.

During the three years studied, the emission of N<sub>2</sub>O from soil was measured with the non-steady-state chamber method (Hutchinson and Mosier, 1981), using the same chambers described by Plaza-Bonilla *et al.* (2014). Two polyvinylchloride rings (31.5 cm internal diameter) were inserted into the soil to a depth of 5 cm. Chambers of 20-cm height were constructed with same material. A metal fitting was attached in the center of the top of the chamber and was lined with two silicon-Teflon septa as sampling port. To reduce internal temperature fluctuations the chambers were covered with a reflective insulation layer (model Aislatermic, Arelux, Zaragoza, Spain). Soil N<sub>2</sub>O fluxes were measured in two observations per plot, with weekly measurements during the growing season (April to November), greater measurement intensity during fertilizer applications (i.e. 24 h. prior and 3 h., 24 h. and 48 h. after) and measured every 21 days in the periods between crops in winter (November to March). Gas samples were taken at 0, 20 and 40 min after the closure of the chamber and stored into 15 mL Exetainer® borosilicate vials (model 038 W, Labco, High Wycombe, UK). Samples were subsequently analyzed by a gas chromatography system (7890A, Agilent, Santa Clara, CA, United States) equipped with an electrical conductivity detector (ECD) and an HP-Plot Q column (30 m long,

0.32 mm of section and 20 $\mu$ m) with a pre-column 15 m long of the same characteristics. The injector and oven temperatures were set to 50°C. The temperature of the detector was set to 300°C, using a 5% methane in Argon gas mixture as a make-up gas at a flow of 30 mL min<sup>-1</sup>. The system was calibrated using analytical grade standards (Carbueros Metálicos, Barcelona, Spain). Gas fluxes were calculated taking into account the linear increase in the N<sub>2</sub>O concentration inside the chamber with time (40 min) and correcting the values for air temperature.

Soil denitrification potential was determined 5 days after the three fertilizer applications of the second maize season (2016) by quantifying the activity of denitrifying enzyme as described by Groffman *et al.* (1999). 25 g of fresh soil and 25 mL of a solution containing 1M glucose, 1 nM KNO<sub>3</sub> and 1 g L<sup>-1</sup> chloramphenicol were added into 125 mL hermetic glass jars. The jars were sealed and repeatedly flushed with N<sub>2</sub> for 2 min in order to create anaerobic conditions. Afterwards, acetylene 5% was added to the jars to determine denitrification potential (Estavillo *et al.*, 2002). The jars were incubated in an orbital shaker at room temperature. After incubation at 30 and 90 minutes, 15 ml gas samples were removed from the jar headspace using a syringe and then stored in vials. Sample N<sub>2</sub>O concentration was analyzed by gas chromatography as described above.

### 2.3 Soil sampling and plant analysis.

At the same sampling dates as soil N<sub>2</sub>O emissions measurements, soil samples (0-5 cm depth) were obtained for mineral N (as ammonium, NH<sub>4</sub><sup>+</sup>, and nitrate, NO<sub>3</sub><sup>-</sup>) and gravimetric moisture determination in two observations per plot. Soil temperature (10 cm depth) was measured using a handheld probe (TM65, Crison). Soil gravimetric moisture was transformed into water-filled pore space (WFPS) using soil bulk density, which was measured monthly at two positions per plot, and assuming a theoretical particle density of 2.65 g cm<sup>-3</sup>. Soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> contents were quantified by extracting 50 g of fresh soil with 100 mL of 1M KCl. The extracts were analyzed with a continuous flow autoanalyzer (Seal Autoanalyzer 3, Seal Analytical, Norderstedt, Germany).

At harvest, maize aboveground biomass and grain yield were measured by collecting plant samples of two central rows 2-5 m long, depending on plant density, in three sampling areas per plot. The number of plants and ears was counted and registered. Afterwards, a sub-sample of two entire plants and five ears were taken to determine the yield components and moisture. The sub-sample was oven-dried at 60°C for 48 h and weighed. Next, the grain was

threshed and weighed. Grain moisture was adjusted to 14% moisture content. These determinations allowed calculating the total aboveground biomass as well as maize yield components: number of plants per square meter, number of ears per plant and thousand kernels weight (TKW). Grain and aboveground biomass N concentration were determined by dry combustion (Dumas method) (Truspec CN, LECO, St Joseph, MI, USA). Afterwards, N content of the grain and the rest of aboveground biomass were calculated by multiplying the biomass of each fraction by its N concentration. Aboveground N uptake was calculated by the sum of N content in both fractions.

#### *2.4 Cumulative N<sub>2</sub>O emissions, emission factor and yield-scaled N<sub>2</sub>O emissions.*

Cumulative N<sub>2</sub>O emissions were quantified with the trapezoid rule, differentiating three maize growing seasons from April to November in 2015, 2016, and 2017, and two periods between maize crops from November 2015 to March 2016 and from November 2016 to March 2017. Yield-scaled N<sub>2</sub>O emissions were calculated dividing the cumulative N<sub>2</sub>O emission in CO<sub>2</sub> equivalents (assuming a global warming potential of 298 as suggested by IPCC, 2013) by maize grain yield (dry matter), for each maize growing season.

The EF was calculated for each year using the following equation:

$$EF (\%) = (E_i - E_0) / (N Rate_i) \times 100$$

where  $E_i$  are the cumulative N<sub>2</sub>O emissions from the  $i$  treatment (kg N<sub>2</sub>O-N ha<sup>-1</sup>),  $E_0$  are the cumulative N<sub>2</sub>O emissions (kg N<sub>2</sub>O-N ha<sup>-1</sup>) from the control treatment without N fertilizer, and  $N Rate_i$  is the N fertilization rate in the  $i$  treatment (kg N ha<sup>-1</sup>). Note that to complete the cumulative N<sub>2</sub>O emissions for 2017; we assumed that the emissions of the period between crops in winter are equal to those measured in the season 2016-2017.

#### *2.5 Statistical analysis.*

Statistical analyses were performed with the statistical package JMP 13 (SAS Institute Inc, 2018). Data were checked for normality by plotting a normal quartile plot. All data complied with normality. A repeated measures analysis of variance (ANOVA) was performed with tillage, N fertilization, sampling date or year or period and their interactions as effects. Sampling date was used as an effect to analyse WFPS, soil ammonium and nitrate contents, N<sub>2</sub>O emissions, and denitrification potential. Period (i.e. growing seasons and winter periods between crops) was used as an effect to analyse cumulative N<sub>2</sub>O emissions. Finally year was used as an effect to

analyse aboveground biomass, grain yield, N-uptake, and YSNE. When significant, differences among treatments were identified at 0.05 probability level of significance with a Tukey HSD test.

### **3. Results**

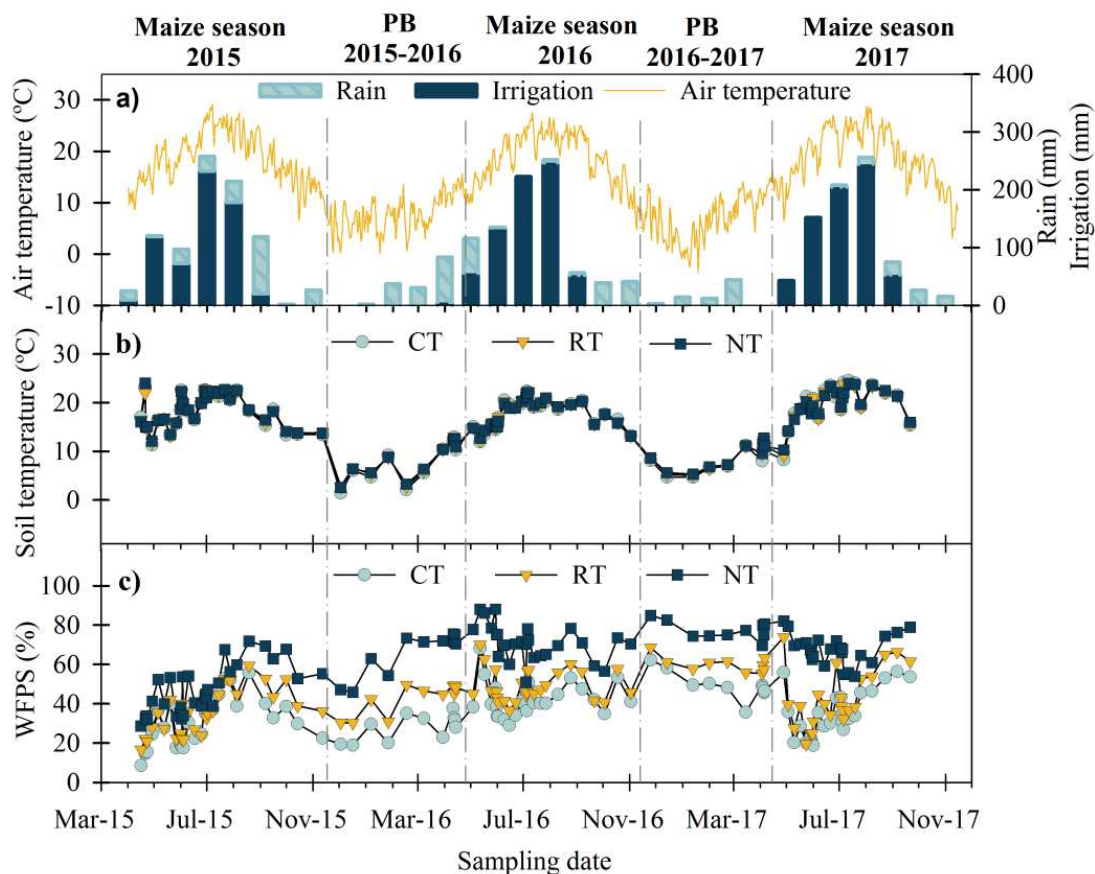
#### *3.1 Weather conditions during the experimental period.*

Mean air temperatures were 19.3, 18.8 and 18.8 °C for the maize season in 2015, 2016 and 2017 respectively. Meanwhile in periods between crops in winter 2015-2016 and 2016-2017 mean air temperatures were 7.7 and 7.1 °C, respectively (Fig. 15a). Cumulative rainfall was 226, 151 and 78 mm for 2015, 2016 and 2017, respectively, during the maize growing season. In the same growing seasons the amount of water applied by irrigation was 631, 672 and 696 mm, respectively (Fig. 15a). During the periods between crops, rainfall was 108 mm and 106 mm in 2015-2016 and 2016-2017, respectively.

#### *3.2. Soil temperature, WFPS, soil ammonium and soil nitrate content.*

Mean soil temperatures at the 10-cm soil depth were 18.6, 17.1 and 19.8 °C for in the 2015, 2016 and 2017 maize seasons, respectively. Meanwhile in periods between crops in 2015-16 and 2016-17, mean soil temperatures were 6.9 and 8.7 °C, respectively (Fig. 15b). Mean WFPS (0–5-cm soil depth) for CT, RT and NT were 36, 44 and 63 %, respectively, as average of the three years of sampling (Fig. 15c).





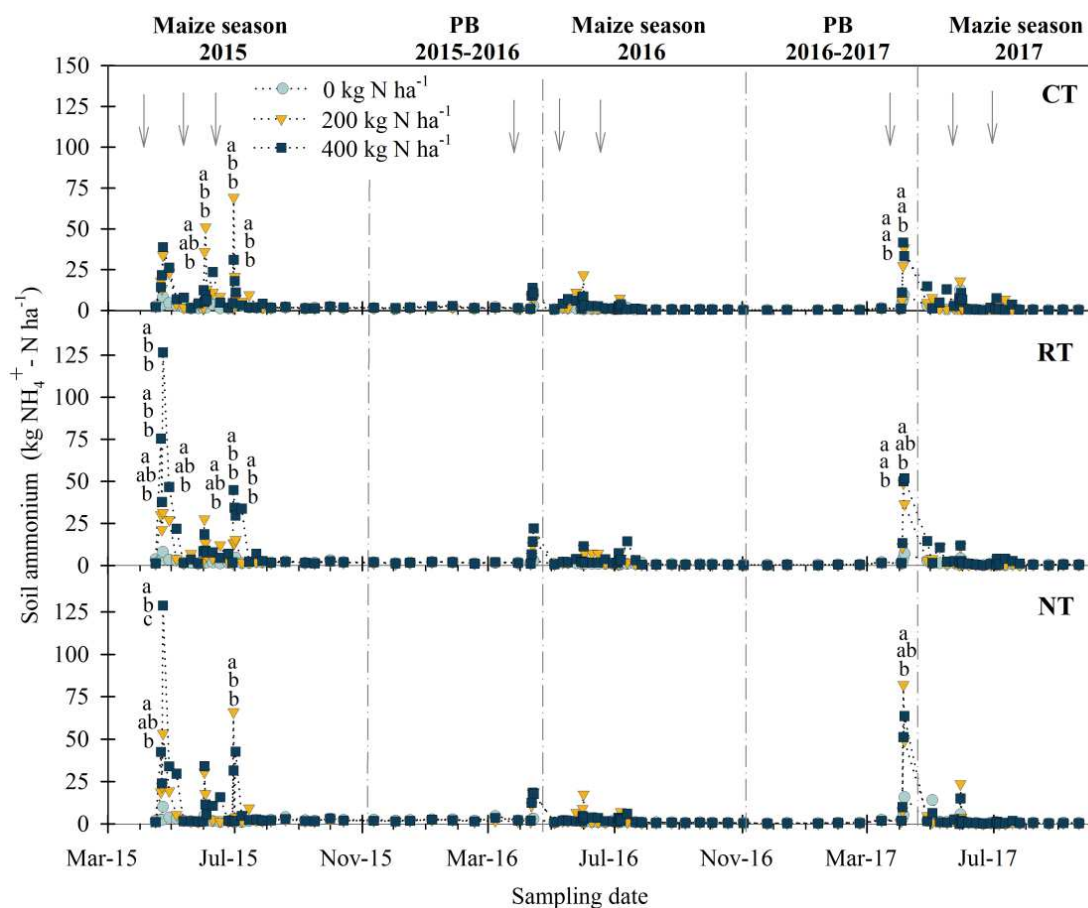
**Fig. 15** Monthly precipitation and irrigation (light blue and dark blue columns, respectively) and daily air temperature (continuous line) (a), soil temperature (10 cm depth) (b), and soil water-filled pore space (WFPS) (0-5 cm depth) (c) in plots managed under conventional tillage (CT), reduced tillage (RT) and no-tillage (NT) during the 2015, 2016 and 2017 maize growing seasons and periods between crops in winter (PB 2015-2016 and PB 2016-2017).

Soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents (0–5-cm soil depth) were significantly affected by the interaction between tillage, N fertilization and sampling date (Table 10). Mean soil  $\text{NH}_4^+$  values remained low ( $< 5 \text{ kg NH}_4^+\text{-N ha}^{-1}$ ) during most of the period studied and increased rapidly after N fertilizer applications (Fig. 16). Soil  $\text{NO}_3^-$  content peaked after fertilization events (Fig. 17). The application of increasing N rates were accompanied by increasing amounts of  $\text{NO}_3^-$  in the soil surface (0–5 cm) during the subsequent month, and this trend was of a greater magnitude under CT (Fig. 17).

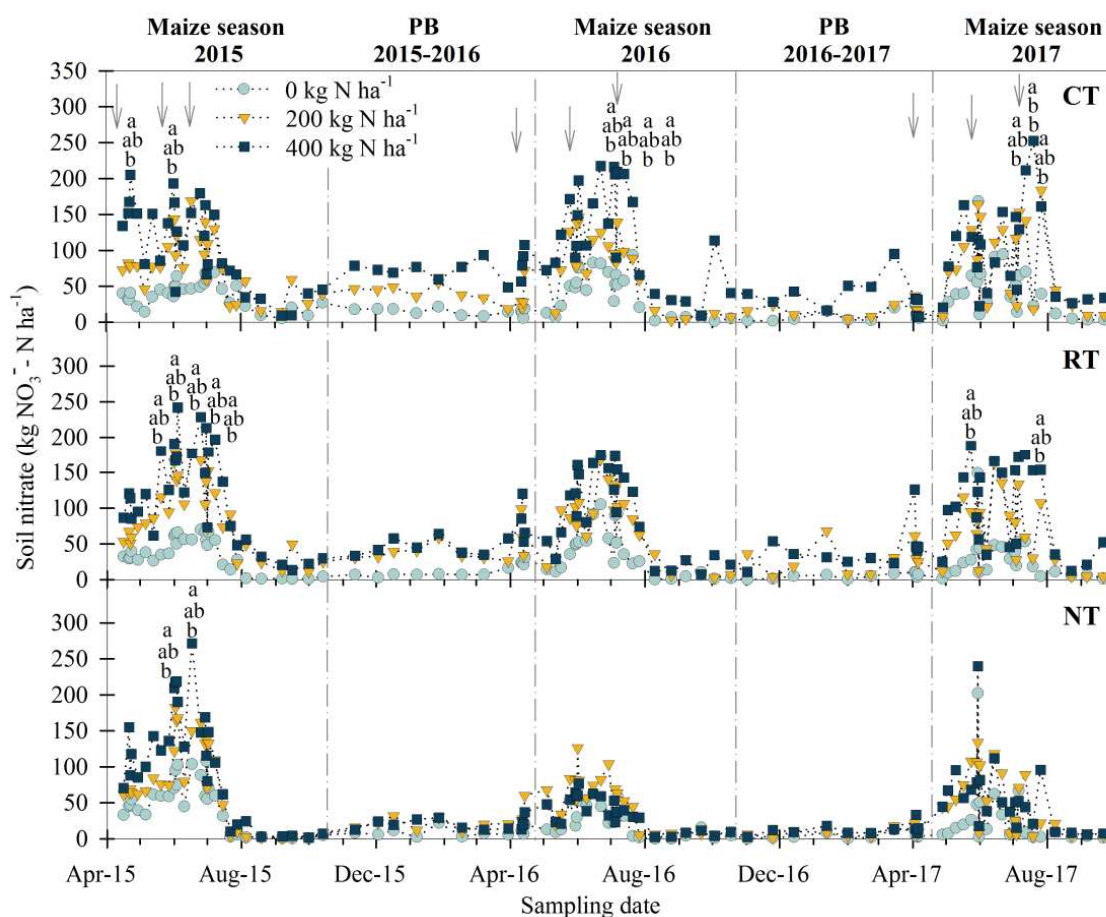
**Table 10.** Analysis of variance (*P*-values) of soil water-filled pore space (WFPS), soil ammonium and nitrate contents (0-5 cm depth), soil N<sub>2</sub>O emissions, denitrification potential, cumulative N<sub>2</sub>O emissions for each maize growing season (2015, 2016 and 2017) and period between crops in winter (2015-2016 and 2016-2017), grain yield, aboveground N uptake and yield-scaled N<sub>2</sub>O emissions (YSNE), as affected by tillage, N fertilization rate, date/year/period and their interactions.

| Source of variation               | Soil ammonium | Soil nitrate | N <sub>2</sub> O | Denitrification | Cumulative | Aboveground N              |             |        |        |
|-----------------------------------|---------------|--------------|------------------|-----------------|------------|----------------------------|-------------|--------|--------|
|                                   | WFPS          | (0–5 cm)     | (0–5 cm)         | emissions       | potential  | N <sub>2</sub> O emissions | Grain yield | uptake | YSNE   |
| <b>Tillage (Till)</b>             | <0.001        | ns           | <0.001           | <0.001          | <0.001     | ns                         | <0.001      | <0.001 | ns     |
| <b>N fertilization (Fert)</b>     | <0.001        | <0.001       | <0.001           | <0.001          | 0.01       | <0.001                     | <0.001      | <0.001 | <0.001 |
| <b>Date/Year/Period</b>           | <0.001        | <0.001       | <0.001           | <0.001          | <0.001     | <0.001                     | <0.001      | <0.001 | <0.001 |
| <b>Till*Fert</b>                  | <0.001        | <0.001       | <0.001           | ns              | ns         | ns                         | <0.001      | <0.001 | ns     |
| <b>Till*Date/Year/Period</b>      | <0.001        | <0.001       | <0.001           | <0.001          | 0.008      | 0.01                       | 0.01        | 0.006  | ns     |
| <b>Fert*Date/Year/Period</b>      | 0.02          | <0.001       | <0.001           | <0.001          | ns         | <0.001                     | <0.001      | <0.001 | 0.04   |
| <b>Till*Date/Year/Period*Fert</b> | ns            | <0.001       | 0.003            | 0.001           | ns         | ns                         | ns          | ns     | ns     |

ns, non-significant



**Fig. 16** Tillage system (CT, conventional tillage; RT, reduced tillage; NT no-tillage) and N fertilizer rate (0, 200, 400 kg N ha<sup>-1</sup>) effects on soil ammonium (NH<sub>4</sub><sup>+</sup>-N) (0-5 cm depth) during the 2015, 2016 and 2017 maize growing seasons and periods between crops in winter (PB 2015-2016 and PB 2016-2017). Arrows indicate dates of N fertilizer application. For a given date and tillage treatment, different lower case letters indicate significant differences between N fertilization rates at  $P < 0.05$ .

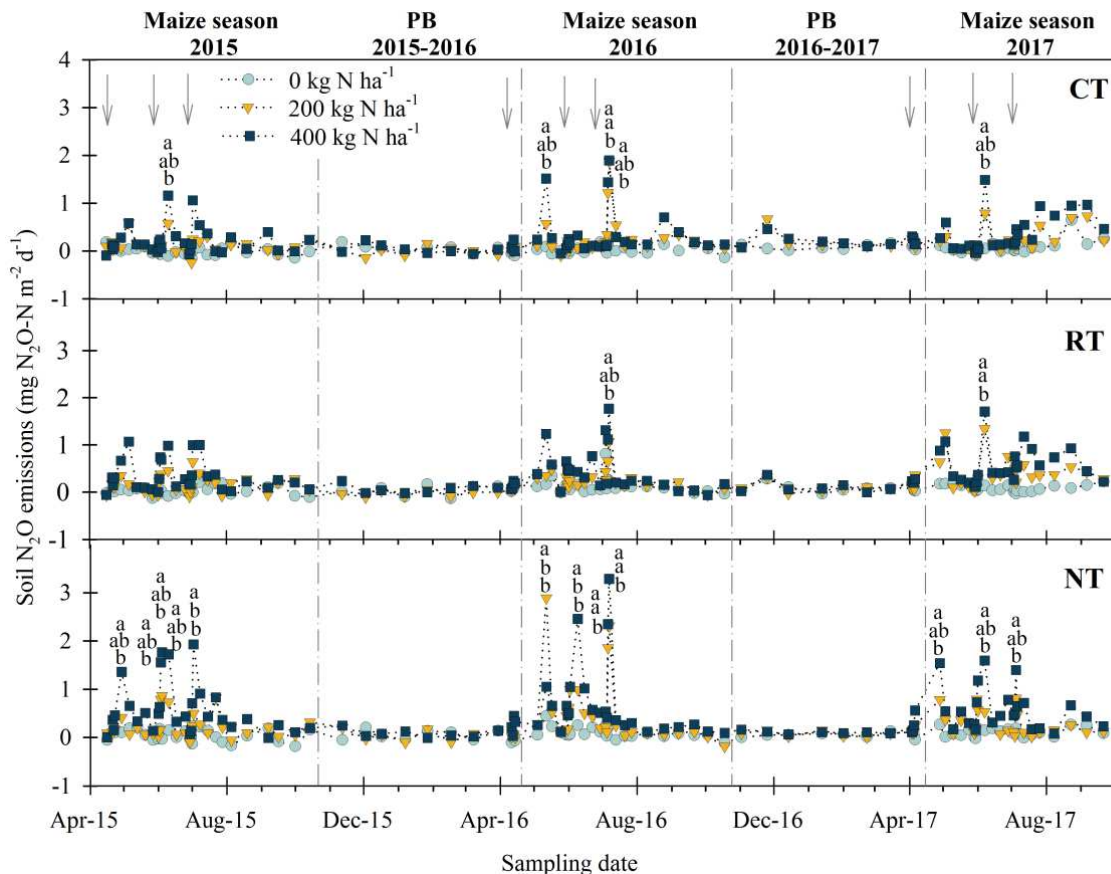


**Fig. 17** Tillage system (CT, conventional tillage; RT, reduced tillage; NT no-tillage) and N fertilizer rate (0, 200, 400 kg N ha<sup>-1</sup>) effects on soil nitrate (NO<sub>3</sub><sup>-</sup>-N) (0-5 cm depth) during the 2015, 2016 and 2017 maize growing seasons and periods between crops in winter (PB 2015-2016 and PB 2016-2017). Arrows indicate dates of N fertilizer application. For a given date and tillage treatment, different lower case letters indicate significant differences between N fertilization rates at  $P < 0.05$ .

### 3.3 Soil N<sub>2</sub>O emissions and denitrification potential.

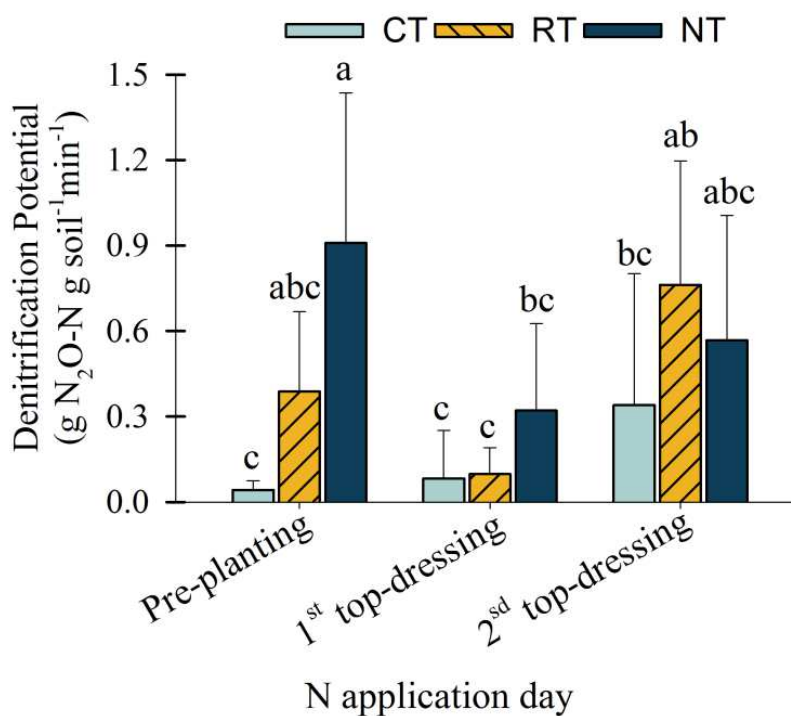
Soil N<sub>2</sub>O fluxes ranged from -0.24 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> (CT-200 on 1<sup>st</sup> July 2015) to 3.29 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> (NT-400 on 7<sup>th</sup> July 2016) (Fig. 18). The interaction between tillage, N fertilization and sampling date had a significant effect on soil N<sub>2</sub>O emissions (Table 10). Several N<sub>2</sub>O emission peaks occurred during the maize growing period, which were observed within a few days after N fertilizer application (Fig. 18). In the three maize seasons, NT presented the highest N<sub>2</sub>O emission values in most sampling dates compared with RT and CT, showing the rate of 400 kg N ha<sup>-1</sup> greater soil N<sub>2</sub>O emissions compared to the control and 200 kg N ha<sup>-1</sup> rates (Fig. 18). For instance, for the NT tillage system, the average soil N<sub>2</sub>O emission for the 0, 200 and 400 kg N ha<sup>-1</sup> rates (considering the three maize seasons) were 0.08, 0.29 and 0.52 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup>, respectively. In the case of the CT system, the average emission values dropped to 0.04, 0.18 and 0.27 mg N<sub>2</sub>O-N

$\text{m}^{-2} \text{d}^{-1}$  for the 0, 200 and 400  $\text{kg N ha}^{-1}$  rates, respectively (Fig. 18). Increases in soil  $\text{N}_2\text{O}$  fluxes also occurred after pre-planting fertilizer application in maize season 2015 only under NT (Fig. 18). Conversely, in the two periods between crops in winter, all  $\text{N}_2\text{O}$  fluxes observed were lower than  $0.3 \text{ mg N}_2\text{O-N m}^{-2} \text{d}^{-1}$  without significant differences between treatments.



**Fig. 18** Tillage system (CT, conventional tillage; RT, reduced tillage; NT no-tillage) and N fertilizer rate (0, 200, 400  $\text{kg N ha}^{-1}$ ) effects on soil  $\text{N}_2\text{O}$  emissions during the 2015, 2016 and 2017 maize growing seasons and periods between crops in winter (PB 2015-2016 and PB 2016-2017). Arrows indicate dates of N fertilizer application. For a given date and tillage treatment, different lower case letters indicate significant differences between N fertilization rates at  $P < 0.05$ .

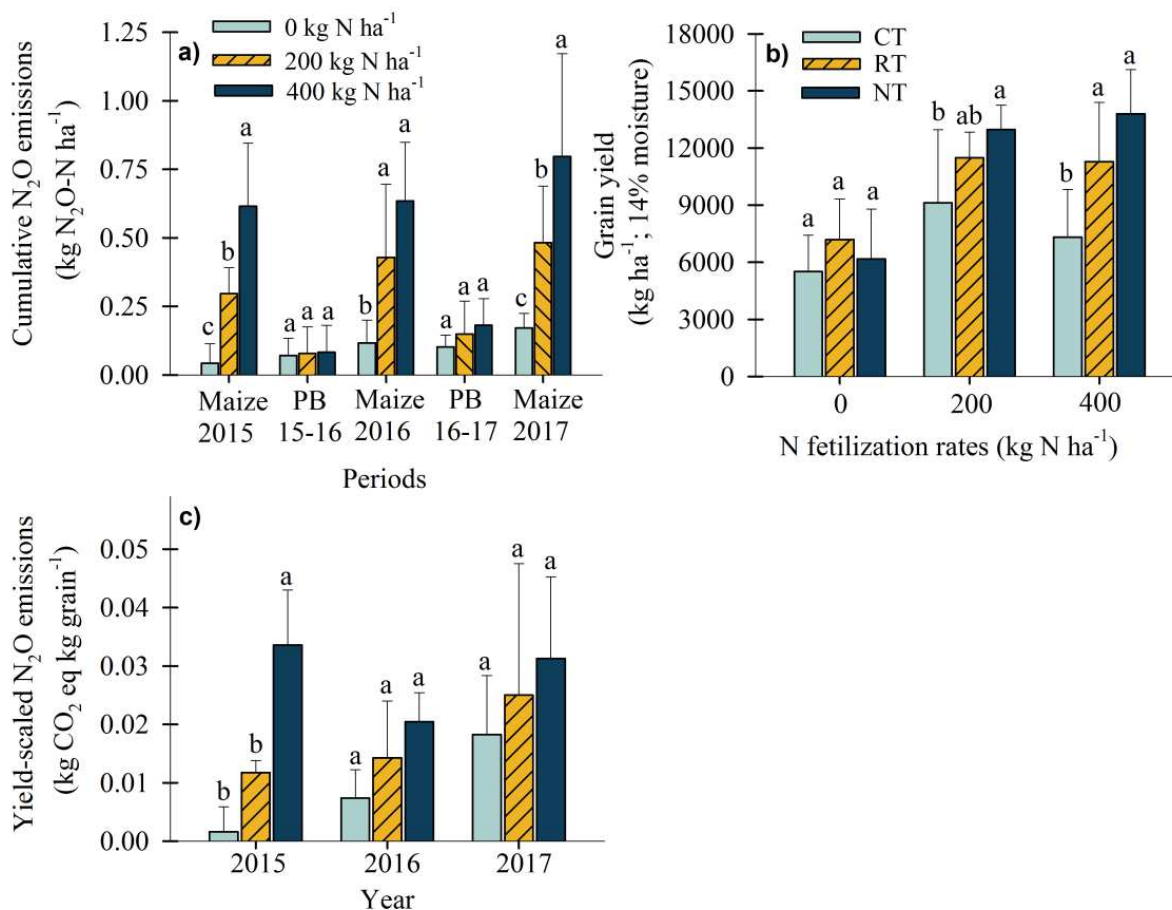
Soil denitrification potential was significantly affected by the interaction between tillage and N application date and N fertilization single effect (Table 10). Soil denitrification potential just after pre-planting fertilizer application was higher under NT compared to CT with intermediate values under RT, while no differences between tillage systems were found after top-dressing N applications (Fig. 19). In turn, of the application of 200 and 400  $\text{kg N ha}^{-1}$  led to greater soil denitrification potentials compared to the control, with mean values of 0.46, 0.48 and 0.22  $\text{g N}_2\text{O-N g soil}^{-1} \text{min}^{-1}$ , respectively.



**Fig. 19** Tillage system (CT, conventional tillage; RT, reduced tillage; NT no-tillage) effects on soil potential denitrification 5 days after pre-planting N fertilizer application, 1<sup>st</sup> top-dressing application and 2<sup>nd</sup> top-dressing application during the 2016 maize growing season. Different lower case letters indicate significant differences between tillage systems at  $P < 0.05$ . Vertical bars indicate standard deviation.

### 3.4 Cumulative soil N<sub>2</sub>O emissions and emission factor.

The interaction between N fertilization rates and maize growing season and between tillage system and maize growing season had a significant effect on cumulative N<sub>2</sub>O emissions (Table 10). In the 2015 and 2017 growing seasons, cumulative N<sub>2</sub>O emissions followed the order  $0 < 200 < 400$  kg N ha<sup>-1</sup>. In 2016, the N rates of 200 and 400 kg N ha<sup>-1</sup> showed greater values compared to the control (Fig. 20a). No-tillage and CT did not show differences between growing seasons on cumulative N<sub>2</sub>O emissions. Differently, under RT differences between maize seasons were found, with greater cumulative N<sub>2</sub>O emission in 2017 compared to 2015 and intermediate values in 2016 (0.57, 0.30 and 0.35 kg N<sub>2</sub>O-N ha<sup>-1</sup>, respectively). However, no differences between N rates or between tillage systems were found in the periods between crops in winter 2015-2016 and 2016-2017 (Fig 20a).



**Fig. 20** Nitrogen fertilizer rate (0, 200, 400 kg N ha<sup>-1</sup>) effects on cumulative N<sub>2</sub>O emissions (a) and yield-scaled N<sub>2</sub>O emissions (c), and tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) effects on grain yield (b). Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and two periods between crops in winter (PB 2015-2016 and PB 2016-2017). Different lowercase letters indicate significant differences between N fertilization rates for a given period (a and b) and significant differences between tillage systems for a given N fertilization rate (c) at  $P < 0.05$ . Vertical bars indicate standard deviation.

The EF showed the greatest value when applying 200 kg N ha<sup>-1</sup> (0.20%) compared to the application of 400 kg N ha<sup>-1</sup> (0.18%) as an average of the three years studied (Table 11). Meanwhile, the EF ranged between 0.16 and 0.23% and between 0.10 and 0.22%, under NT and CT respectively, when applying 400 kg N ha<sup>-1</sup>.

**Table 11.** Soil N<sub>2</sub>O emission factor (EF) (%) in 2015, 2016 and 2017 as affected by N fertilization rate (200 and 400 kg N ha<sup>-1</sup>) and tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage). Average of the three years studied in rate of 200 and 400 kg N ha<sup>-1</sup>.

| Year | Tillage system | EF (%)                    |                           |
|------|----------------|---------------------------|---------------------------|
|      |                | 200 kg N ha <sup>-1</sup> | 400 kg N ha <sup>-1</sup> |
| 2015 | CT             | 0.09                      | 0.10                      |
|      | RT             | 0.07                      | 0.15                      |
|      | NT             | 0.17                      | 0.22                      |
| 2016 | CT             | 0.33                      | 0.20                      |
|      | RT             | 0.12                      | 0.12                      |
|      | NT             | 0.27                      | 0.23                      |
| 2017 | CT             | 0.31                      | 0.22                      |
|      | RT             | 0.29                      | 0.22                      |
|      | NT             | 0.13                      | 0.16                      |
|      | <b>Average</b> | 0.20                      | 0.18                      |

### 3.5 Maize grain yield, aboveground N uptake and yield-scaled N<sub>2</sub>O emissions.

The interaction between tillage and N fertilization and their interaction with year had a significant effect on maize grain yields (Table 10). In 2016 and 2017, the application of 200 (12,760 and 10,425 kg ha<sup>-1</sup>, respectively) and 400 kg N ha<sup>-1</sup> (13,067 and 10,879 kg ha<sup>-1</sup>, respectively) led to greater yields than the control treatment (6,870 and 4,297 kg ha<sup>-1</sup>, respectively). In 2015 and 2017, grain yields were higher under NT (11,406 and 9,844 kg ha<sup>-1</sup>, respectively) and RT (9,548 and 9,278 kg ha<sup>-1</sup>, respectively) than under CT (5,594 and 6,478 kg ha<sup>-1</sup>, respectively), without differences between tillage treatments in 2016. No differences between tillage systems on grain yield were observed in the control treatment, as an average of the three years studied (Fig. 20b). In contrast, greater grain yield was observed under NT compared to CT with intermediate values in RT when applying 200 kg N ha<sup>-1</sup>. Moreover, as an average of years, greater grain yield was observed under NT and RT when 400 kg N ha<sup>-1</sup> were applied, in comparison with CT at the same rate (Fig. 20b).

Maize aboveground N uptake was significantly affected by the interaction between tillage and N fertilization and by the interaction between N fertilization and year (Table 10). In this regard, greater aboveground N uptake was observed under NT than CT, with intermediate values in RT when applying 200 kg N ha<sup>-1</sup>, (243, 186 and 223 kg ha<sup>-1</sup>, respectively). Moreover, greater aboveground N uptake was found under NT when applying 400 kg N ha<sup>-1</sup> followed by RT and finally by CT at the same rate (295, 240 and 172 kg ha<sup>-1</sup>, respectively) as an average of the



different years covered by the experiment. In 2015, 2016 and 2017 greater aboveground N uptake was observed under the application of 200 (197, 241 and 214 kg N ha<sup>-1</sup>, respectively) and 400 kg N ha<sup>-1</sup> (178, 277 and 252 kg N ha<sup>-1</sup>, respectively) compared to the control (123, 111 and 79 kg N ha<sup>-1</sup>, respectively).

Yield-scaled N<sub>2</sub>O emissions were significantly affected by the interaction between N fertilization and year (Table 10). In 2015, YSNE showed greater values when applying 400 kg N ha<sup>-1</sup>, compared to the control and the rate of 200 kg N ha<sup>-1</sup>. Differently, no significant differences between treatments were found in 2016 and 2017, although a trend of greater YSNE at higher N rates was observed (Fig. 20c).

## 4. Discussion

### 4.1 Impacts of tillage and N fertilization rates on soil N<sub>2</sub>O emission.

When converting rainfed Mediterranean agroecosystems to irrigation, conservation tillage systems like no-tillage and strip-tillage should be maintained for a sustainable crop production as well as to avoid environmental impacts like N<sub>2</sub>O emissions. This study, carried out during three maize seasons, has demonstrated that soil tillage combined with mineral N fertilization rate exerts a significant impact on soil N<sub>2</sub>O emissions in Mediterranean irrigated conditions. In this regard, different studies in irrigated Mediterranean conditions have shown that high rates of N fertilizer, lead to greater soil N<sub>2</sub>O fluxes (Meijide *et al.*, 2007; López-Fernández *et al.*, 2007; Álvaro-Fuentes *et al.*, 2016; Guardia *et al.*, 2017). However, the present study demonstrates that the effect of N fertilizer on N<sub>2</sub>O emissions in Mediterranean irrigated areas is determined by soil tillage. The different tillage systems studied influenced N<sub>2</sub>O emissions through variations in the WFPS and mineral nitrogen content, which play a substantial role in N<sub>2</sub>O emissions, by influencing microbial activity and the behavior of water in the soil matrix (Rees *et al.*, 2013). The results clearly show that the highest rates of N fertilization had major impacts on N<sub>2</sub>O emission under NT (Fig. 18). In all three tillage systems, the highest N<sub>2</sub>O fluxes occurred within a few days after N fertilization, contributing about 60% of the total emissions in the three years studied. Exceptionally, in the first year of study, NT was the only tillage system that showed a N<sub>2</sub>O peak associated with the pre-planting fertilizer application. This could be due to the incorporation of the fertilizer by tillage (CT and RT) in very dry soil conditions, since irrigation began a week after the N application.

During the three years of study, the highest N<sub>2</sub>O fluxes were observed when WFPS was above 60 % under NT with 400 kg N ha<sup>-1</sup>. In contrast, CT showed WFPS values lower than 40% resulting in lower emissions, while RT showed values lower than 50% resulting in intermediate N<sub>2</sub>O emissions between NT and CT. N fertilizer and soil moisture are the two main factors influencing soil N<sub>2</sub>O emissions (Gao *et al.*, 2014). In this study, soil water content and soil bulk density were higher under NT than under CT, which resulted in generally higher levels of WFPS. Under NT, higher levels of WFPS could also lead to greater denitrification rates stimulated by the greater levels of SOC in NT compared to the CT systems (Plaza-Bonilla *et al.*, 2013; Álvaro-Fuentes *et al.*, 2014). It is well known that denitrifying bacteria require available C as energy source before the reduction of added nitrogen can occur (Saggar *et al.*, 2013). In our conditions, it is likely that a fast nitrification of the ammonium to nitrate could have been the main N<sub>2</sub>O production process which is justified by the low levels of soil NH<sub>4</sub><sup>+</sup> (4.8 kg NH<sub>4</sub><sup>+</sup> - N ha<sup>-1</sup> as an average of three years of study) and the low WFPS, especially in CT and RT treatments (<40 and 50%, respectively, as an average of three years of study). Differently, under NT, in some periods, denitrification could have also produced N<sub>2</sub>O emissions due to the higher WFPS (>60% as an average of three years of study) as observed by other authors (Venterea *et al.*, 2005). This last assumption would be supported by the greater denitrification potential of NT treatment compared to CT and RT observed in the study. Additionally, in our study established plant density was 19% and 22% lower in CT compared to RT and NT, respectively (Pareja-Sánchez *et al.*, 2017). The higher plant density in NT and RT could have reduced soil N<sub>2</sub>O emissions through increased N uptake (Jiang *et al.*, 2016). During the periods between crops (winter months) N<sub>2</sub>O emissions were low, and did not show significant differences between treatments. The low N<sub>2</sub>O emissions during these periods could be explained by the soil temperatures, which were lower than 8° C leading to low activity levels of nitrifying bacteria in the soil (Smith *et al.*, 2010). However, WFPS during the winter was similar to crop season. Therefore, it could not influence the low N<sub>2</sub>O emissions, being the decrease of the temperatures the main factor. As N<sub>2</sub>O emissions are mainly driven by soil moisture and soil mineral N levels, careful management of agricultural practices involving fertilization, tillage and irrigation are very important when it comes to minimizing gaseous losses. Management can be key through porosity or by delaying the timing of application may have helped to reduce N<sub>2</sub>O emissions (Venterea *et al.*, 2012). Moreover, N fertilizer rate adapted to the needs of the crop could lead to a decrease of N<sub>2</sub>O. Also proper irrigation management can reduce N<sub>2</sub>O emission, since not applying irrigation water immediately after fertilization could decrease N<sub>2</sub>O emissions.

#### 4.2 Cumulative N<sub>2</sub>O emissions and emission factor.

Previously, in the same experimental field, under rainfed CT barley cumulative N<sub>2</sub>O emissions were lower compared to the values found in our study in irrigated conditions (0.43 vs. 0.52 kg N<sub>2</sub>O-N ha<sup>-1</sup>, respectively), and in NT this difference were even lower compared to irrigated conditions (0.33 vs. 0.63 kg N<sub>2</sub>O-N ha<sup>-1</sup>, respectively) (Plaza-Bonilla *et al.*, 2014) due to the increased soil moisture and N fertilization rate in the irrigation experiment. The lower increase of N<sub>2</sub>O emissions in CT (only 17%) could be due to the low WFPS which was caused by surface crusting that reduced the infiltration of water into the soil. Moreover, in CT the reduced availability of water in the soil influenced negatively crop N uptake and led to an accumulation of soil nitrate. Consequently, although a higher soil NO<sub>3</sub><sup>-</sup> content was observed under CT N<sub>2</sub>O emissions remained low since WFPS values were generally below 40% under this tillage system.

In all three maize growing seasons, the greatest cumulative soil N<sub>2</sub>O emissions were obtained with the highest N rates (400 kg ha<sup>-1</sup>) and declined as the rate of N decreased. The high cumulative N<sub>2</sub>O emissions found in the treatments with the greatest N fertilization rate could be related to the high NO<sub>3</sub><sup>-</sup> concentration in the soil when applying high rates of N, favoring denitrification. The addition of N fertilizer increases soil mineral N losses as N<sub>2</sub>O through higher nitrification and denitrification rates (Bouwman *et al.*, 2002).

In our study the EF (the percentage of fertilizer N applied that is emitted on-site as N<sub>2</sub>O) of irrigated maize was lower than the default 1% factor currently proposed by the IPCC (IPCC, 2006). The highest EF estimated in our experiment was 0.24% for CT when applying 200 kg N ha<sup>-1</sup> and 0.20% in NT when applying 400 kg N ha<sup>-1</sup>, as an average of the three years of studied. In a meta-analysis of N<sub>2</sub>O emissions in Mediterranean cropping systems, Cayuela *et al.* (2017) showed that irrigated maize production presents an average EF of 0.83%, a value higher than the ones obtained in our study. This disagreement could be explained by different causes. One hypothesis could be soil texture, which was fine-textured in our study. Soil texture affects soil N<sub>2</sub>O production through its influence on soil aeration which, in turn, modulates nitrification and denitrification processes. Cayuela *et al.* 2017 suggested that larger EFs could be expected from coarse (EF: 0.58%) and medium-textured soils (EF: 0.48%) compared to fine textured soils (EF: 0.27%). This last value agrees with the one found in our study and would confirm that fine-textured Mediterranean soils usually present low N<sub>2</sub>O EF. This could be due to the fact that in fine-textured soils, aeration is lower and therefore less oxygen is available for the microorganisms in microsites, even in rather low WFPS levels. Under these conditions

microorganism would further reduce  $\text{N}_2\text{O}$  decreasing the amount of this gas emitted to the atmosphere. Another hypothesis that could explain the low  $\text{N}_2\text{O}$  EF found in our study would be related to management of irrigation. In order to reduce the emission of  $\text{N}_2\text{O}$  as much as possible, we did not apply irrigation water immediately after N fertilization, maintaining soil WFPS at low levels, avoiding the rapid burst of  $\text{N}_2\text{O}$  emission usually found in other experiments (e.g. *Álvaro-Fuentes et al.*, 2016). Irrigation water was applied 3 days after application at low and frequent rates equivalent to crop needs. In this aspect, when the concentration of nitrate in the soil is high and the WFPS is low the emission of  $\text{N}_2\text{O}$  could be reduced. *Venterea et al.* (2011) in rainfed maize in Minnesota, with a mean annual precipitation of 879 mm, obtained EF in the range of 0.14 to 0.42% of the applied N ( $146 \text{ kg N ha}^{-1}$ ) as averaged across all treatments. They concluded that the timing of fertilizer application could reduce  $\text{N}_2\text{O}$  emissions leading to lower EF values.

#### *4.3 Impacts of tillage and N fertilization rates on maize productivity and yield-scaled $\text{N}_2\text{O}$ emissions.*

In this study, averaged over 3 years, grain yields in NT and RT were similar when applying 200 and  $400 \text{ kg N ha}^{-1}$ , while CT showed the lowest yields at both N rates (Fig. 20b). The lack of yield difference between 200 and  $400 \text{ kg N ha}^{-1}$  could be attributed to the high initial N availability for crop growth in the plots fertilized with  $400 \text{ kg N ha}^{-1}$ . Therefore, these data suggest that the use of less aggressive tillage practices, such as no-tillage and strip-tillage, as well as the reduction of N fertilization, could be viable options to stabilize or, even, increase crop yields. Moreover, it could lead to a decrease of  $\text{N}_2\text{O}$  emissions to the atmosphere simultaneously, saving production costs in comparison with the traditional management based on conventional tillage with high rates of mineral N. Hence, it is interesting to analyze  $\text{N}_2\text{O}$  emissions in relation to the yield obtained, since it provides a good indicator of the environmental impacts of intensive agricultural production systems. In our study, an increase in the N rate led to an increase in the yield-scaled  $\text{N}_2\text{O}$  emissions only in the first out three years, although a similar trend was observed in the subsequent two years (Fig. 20c). But, as explained before, similar yields were obtained with both  $400 \text{ kg N ha}^{-1}$  and  $200 \text{ kg N ha}^{-1}$ . These results suggest that optimal N rates can produce maximum yields while reducing annual yield-scaled emissions by 40%. Moreover, although the yield-scaled  $\text{N}_2\text{O}$  emissions did not differ significantly between tillage treatments, a marked trend existed in the rates found between tillage systems, in the order  $\text{CT} > \text{RT} > \text{NT}$ . Conventional tillage was greatly affected by soil degradation and led to lower plant density inducing lower grain yield, compared to NT that showed greater grain yield.

These results suggest that although cumulative N<sub>2</sub>O emissions under CT are lower, the reduction in crop yield in CT led to an increase in yield-scaled emission compared to NT. Contrary, Venterea *et al.* (2011), in a maize-soybean rotation in SE Minnesota (USA), observed that the yield-scaled N<sub>2</sub>O emission for CT was 40.7 % lower than in NT with a urea fertilizer N input of 146 kg N ha<sup>-1</sup>. In their case, averaged over 3 years study, the grain yield were 14.2% lower in NT than in CT. Lower yield NT was attributed to cooler soil temperatures in the spring, which may inhibit early-season plant development (Venterea *et al.* 2011). Our results demonstrated that in order to keep low yield-scaled N<sub>2</sub>O emissions, it is necessary to obtain adequate crop productivity. Reducing yield-scaled emissions is consistent with aim of ensure the sustainability of production and minimizing environmental impacts (Powlson *et al.*, 2011).

## 5. Conclusions

In this cropping system and climate regime, the mean N<sub>2</sub>O EF measured was 0.19%, much lower than the 1% factor currently default by the IPCC. The increase of WFPS under NT had a major effect on N<sub>2</sub>O emissions especially when combined with the high rate of N fertilization that increased soil mineral N. The yield-scaled N<sub>2</sub>O emissions did not differ significantly between tillage treatments since greater grain yield under NT offset the N<sub>2</sub>O emissions. However, the use of a high N rate led to an increase in the yield-scaled N<sub>2</sub>O emissions in the first out of three years of study.

When converting rainfed Mediterranean systems to irrigation, conservation tillage should be maintained for sustainable maize production. No-tillage is an adequate technological opportunity for the transition from rainfed to irrigated land. If some problems arise under NT during this period, such as those related to crop residue management and/or soil compactation in the planting row, the implementation of new strategies of strip-tillage would be a key alternative. Moreover, the use of an appropriate N fertilizer rate according to crop needs may achieve a yield advantage while decreasing soil N<sub>2</sub>O emissions, independently of the tillage treatment. This combination of strategies is important to reduce N<sub>2</sub>O emissions as well as enhance crop productivity.

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## **Chapter IV**

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



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## Chapter IV

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

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#### Abstract

In newly irrigated Mediterranean agroecosystems, the combined effect of tillage and N fertilization on soil carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) fluxes is at present poorly understood. The goal of this study was to quantify both soil CO<sub>2</sub> and CH<sub>4</sub> emissions as well as crop performance under different tillage systems and N fertilization rates during three maize (*Zea mays* L.) growing seasons (2015-2017) in a semiarid area converted to irrigated. Three types of tillage (conventional tillage, CT, reduced tillage, RT, and no-tillage, NT) and three mineral N fertilization rates (0, 200, and 400 kg N ha<sup>-1</sup>) were compared in a randomized block design with three replications. Weekly soil CO<sub>2</sub> and CH<sub>4</sub> emissions, soil temperature and gravimetric moisture were measured. Moreover, maize aboveground biomass, grain yield, and aboveground C-inputs were quantified. Carbon dioxide emissions ranged from 173 to 4378 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>. No-tillage showed a greater mean soil CO<sub>2</sub> flux than CT when applying the highest rate of N (400 kg N ha<sup>-1</sup>). Although some emissions of CH<sub>4</sub> were observed, all treatments acted as net CH<sub>4</sub> sinks during most of the experimental period. A linear multiple relationships between soil CO<sub>2</sub> fluxes and soil gravimetric moisture (0-5 cm depth) and temperature (10 cm depth) were found. In the 2015 growing season, greater cumulative CO<sub>2</sub> emissions were found under NT and RT compared with CT, while in 2016 NT showed the highest values compared to CT with intermediate values in RT. Differently, in 2017 no differences between tillage systems were found. When applying N fertilizer, NT and RT increased maize grain production and aboveground C-inputs compared to CT, since a severe soil crusting occurred in this last, which caused crop water deficit. The results suggest that tillage intensity and N fertilization rate reduction can increase maize biomass production and yield which leads to greater C-input that returns to the soil.

#### Keywords

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

## 1. Introduction

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

Tillage practices can affect soil biochemical and physical properties, consequently influencing the release of CO<sub>2</sub>. In the last decades carbon loss from soils to the atmosphere as CO<sub>2</sub> has increased due to inappropriate tillage practices (Rakotovao *et al.*, 2017). During intensive tillage operations, like moldboard plowing, soil structure is greatly disturbed and the CO<sub>2</sub> contained in the soil pore system is lost while releasing organic C protected within aggregates making it more accessible to decomposers. In addition, tillage practices can affect soil temperature and soil moisture conditions which are strongly related to soil CO<sub>2</sub> fluxes (Ren *et al.*, 2007). Meanwhile, the use of conservation tillage systems, such as reduced tillage (RT) and no-tillage (NT), has been promoted as a practice to enhance soil carbon levels. The increase in soil organic carbon (SOC) pool under conservation tillage can be attributed to either an increase in C inputs from crop residues (Stewart *et al.*, 2016) or a decrease in CO<sub>2</sub> efflux from the soil. Therefore, the effect of soil C sequestration is particularly crucial for predicting the future trend of CO<sub>2</sub> concentration in the atmosphere (DeLuca and Zabinski, 2011). This importance is reflected in recent international initiatives such as the 4 per 1000 which was launched during the COP21 in Paris and promotes the adoption of recommended management practices aimed to increase SOC sequestration.

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

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intensively tilled soils, can adversely affect CH<sub>4</sub> consumption (Ball *et al.*, 1999).

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

In different rainfed Mediterranean areas, RT and NT techniques have been introduced during the last two to three decades aimed at reducing costs, saving time and increase soil water conservation (Lampurlanés *et al.*, 2016). Currently, a significant fraction of the Mediterranean rainfed areas are being transformed into irrigation, allowing farmers the cultivation of more productive summer crops such as maize, which require greater nitrogen fertilizer rates to maximize yields. However, in these newly transformed irrigated cropping systems, farmers have been induced to return to intensive tillage systems, which are the most common in irrigated Mediterranean cropping systems. In these areas, the limited previous experience about the implementation of RT or NT systems constrains the adoption by farmers and puts at risk the soil quality benefits achieved with the long-term use of NT under former rainfed conditions. Moreover, conversion from rainfed to irrigation may lead to increases of GHG emissions (Aguilera *et al.*, 2013) by creating more favorable soil conditions for microbial activity (Calderon and Jackson, 2002) which may counterbalance the increase in C inputs returned to the soil negatively affecting the overall C budget.

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



## 2. Materials and methods

### 2.1 Experimental site and treatments.

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

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

The CT treatment was implemented according to the traditional practices of maize cultivation in the area. Tillage was performed before planting during March or April, consisting of one pass of a rototiller to 15 cm depth, followed by one pass of a subsoiler to 35 cm depth and finally one pass of a disk plough to 20 cm depth with almost 100% of the crop residues incorporated into the soil. The RT consisted of one pass of a strip-till implement during April. Finally, in the NT treatment no tillage practices were carried out. Weeds were controlled by applying a non-selective herbicide (i.e. glyphosate) at 1.5 L ha<sup>-1</sup> before planting. Planting was performed in April with the use of a pneumatic row direct drilling machine equipped with double disc furrow openers (model Prosem K, Solà, Calaf, Spain) in the three tillage systems. Planting depth was adapted to each tillage treatment. Rotary residue row cleaners were installed to clear the path for the row unit openers. Maize (cv. Kopias) was planted during the last week of April,

in rows 73 cm apart at a planting density of 90,000 plants ha<sup>-1</sup> in the three years. Nitrogen fertilization treatments were split in one pre-planting application with urea (46% N) (April) and two top-dressing applications at the V5 to V10 stages (May and July, respectively) with calcium ammonium nitrate (27% N) with 50, 75 and 75 kg N ha<sup>-1</sup> applied in each split in the 200 kg N ha<sup>-1</sup> rate, being doubled in the 400 kg N ha<sup>-1</sup> rate. Mineral P and K applications were oriented to satisfy crop needs at the same rate for all tillage treatments: ca. 154 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> yr<sup>-1</sup> and 322 kg K<sub>2</sub>O ha<sup>-1</sup> yr<sup>-1</sup>, in the first two years. In the third year the levels of available P and K in the soil were appropriate for the crop, making unnecessary further P and K applications. Irrigation was supplied to meet the estimated evapotranspiration (ET) of the crop minus the effective precipitation, which was estimated as 75% of precipitation (when precipitation > 5 mm) (Dastane 1978). Maize evapotranspiration (ET<sub>c</sub>) was calculated with the corresponding weekly ET values multiplied by the crop coefficient. Crop coefficients were estimated in accordance with crop development (ranging between 0.3-1.2). The ET was calculated using the Penman-Monteith equation. Meteorological data were obtained from an automated weather station located near the experimental site. The amount of water applied by irrigation was 631, 672 and 696 mm in 2015, 2016 and 2017, respectively, during the maize growing period. Maize grain was harvested at the beginning of November using a commercial combine. Afterwards, crop residues were chopped and spread over the soil. Weed and pest control were carried out according to the standard practices in the area.

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

Soil CO<sub>2</sub> and CH<sub>4</sub> emissions were simultaneously measured with the non-steady-state chamber method (Hutchinson and Mosier, 1981), following the procedure of Plaza-Bonilla *et al.* (2014). In each plot, two polyvinylchloride rings with a 315-mm-diameter were inserted 5 cm into the soil. The rings were only removed during tillage, planting and harvesting operations, allowing a minimum lapse of 24 h following ring rearrangement at the initial location before any gas sampling to avoid the concomitant effects of soil disturbance on gas emissions. For the two top-dressing applications, the rings were not removed since the fertilizer was not incorporated. When the measurements were performed, polyvinylchloride chambers 20-cm high were fitted into the rings. The chambers were covered with a reflective insulation layer (model Aislatermic, Arelux, Zaragoza, Spain). As a sampling port, a metal fitting was attached in the center of the top of the chamber and was lined with two silicon-Teflon septa. Soil CO<sub>2</sub> and CH<sub>4</sub> fluxes were measured weekly during the growing season (April to November), with more intensive samplings

during N fertilizer applications (i.e. 24 h. prior and 3 h. and 72 h. after) and every 21 days in the period between crops in winter (November to March) in order to quantify the emissions for the entire year. This process was repeated for 3 consecutive years (from April 2015 to November 2017). Gas samples were collected at regular intervals of 0, 20 and 40 minutes after closure of the chamber, using 20 mL polypropylene syringes and stored into 15 mL borosilicate vials (Exetainer, model 038 W, Labco, High Wycombe, UK). A total of 162 gas samples were collected on every sampling date (27 plots x 2 observations per plot x 3 sampling times per chamber). The concentration of CO<sub>2</sub> and CH<sub>4</sub> in the samples was determined using an Agilent 7890A gas chromatography system equipped with a flame ionization detector (FID) coupled to a methanizer and three valves in order to obtain the gases of interest for each gas injection. A HP-Plot Q column (30 m long, 0.32 mm in section and 20 µm thick) was used, with a 15-m long pre-column of the same characteristics. The injector and the oven temperatures were set to 50°C. The FID and the methanizer were set to 250 and 375°C, respectively. For the detector, H<sub>2</sub> was used as a carrier gas and N<sub>2</sub> as a make-up gas at a flow of 35 and 25 mL min<sup>-1</sup>, respectively. Soil fluxes of CO<sub>2</sub> and CH<sub>4</sub> were calculated taking into account the linear accumulation of the gases in the chamber headspace over the closure period (40 min) and correcting the values for air temperature.

### *2.3 Soil and crop measurements.*

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

At harvest, maize plants were cut at the soil level along 2-5 m (depending on plant density) from the three central rows of each plot. The number of plants and ears was counted and registered. Afterwards, a sub-sample of two entire plants and five ears was dried at 65°C, weighed and ground. Grain moisture was adjusted to 14% moisture content. The C content of maize grain and aboveground biomass was determined by dry combustion (model Truspec CN, LECO, St Joseph, MI, USA).

### *2.4 Data analysis.*

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

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

### 3. Results

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

Air temperature, precipitation and irrigation water during the three years are presented in Table 12. During the maize growing seasons studied, daily mean air temperature ranged from 6.5 to 29.1 °C (average of 19.3 °C), from 8.7 to 27.5 °C (average of 18.8 °C) and 3.1 to 28.8 °C (average of 18.8 °C) in 2015, 2016 and 2017, respectively. Meanwhile, in the 2015-2016 and 2016-2017 periods between crops in winter, air temperature ranged from 0.1 to 14.8 °C (average of 7.7 °C) and -3.6 to 16.4 °C (average of 7.1 °C), respectively. Cumulative rainfall was 226, 151 and 78 mm for the 2015, 2016 and 2017 growing seasons, respectively (Table 12). During the periods between crops in winter in 2015-2016 and 2016-2017 rainfall was 108 mm and 106 mm, respectively. Irrigation water applied was 631, 672 and 696 mm for 2015, 2016 and 2017, respectively (Table 12).

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

**Table 12** Mean monthly air temperature (T) (°C) monthly precipitation (P) (mm) and monthly irrigation (I) (mm) in the field experiment during three consecutive maize growing seasons (2015, 2016 and 2017) and periods between crops in winter (PC 2015-2016 and PC 2016-2017).

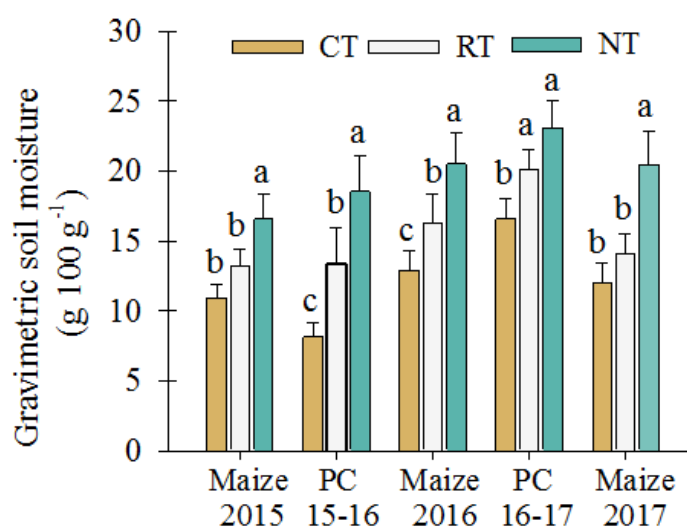
### 3.2 Soil environment during the experimental period.

Over the experimental period, soil temperature ranged from 1.1 to 27.3°C. Mean soil temperature during the maize growing seasons 2015, 2016 and 2017 were 18.6, 17.1 and 19.8 °C, respectively. Meanwhile, in the periods between crops in winter 2015-16 and 2016-17 mean soil temperature were 6.9 and 8.7 °C, respectively. During the study, gravimetric soil moisture was significantly influenced by the interaction between tillage system and period (Table 13). In the 2015 and 2017 maize growing seasons, mean of gravimetric soil moisture showed greater values in NT compared to RT and CT, while in 2016 and in the period between crops in winter 2015-2016 gravimetric soil moisture followed the order NT>RT>CT. In addition, in the period between crops in winter 2016-2017 NT and RT showed greater gravimetric soil moisture than CT (Fig 21).

**Table 13** Analysis of variance ( $P$ -values) of gravimetric soil moisture, soil CO<sub>2</sub> flux, soil CH<sub>4</sub> flux and cumulative CO<sub>2</sub> and CH<sub>4</sub> emissions, aboveground biomass, grain yield and aboveground biomass C inputs (C-inputs) for each maize growing season (2015, 2016 and 2017) and periods between crops in winter (2015-2016 and 2016-2017), as affected by tillage, N fertilization, date/year/period and their interaction.

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

ns, non-significant

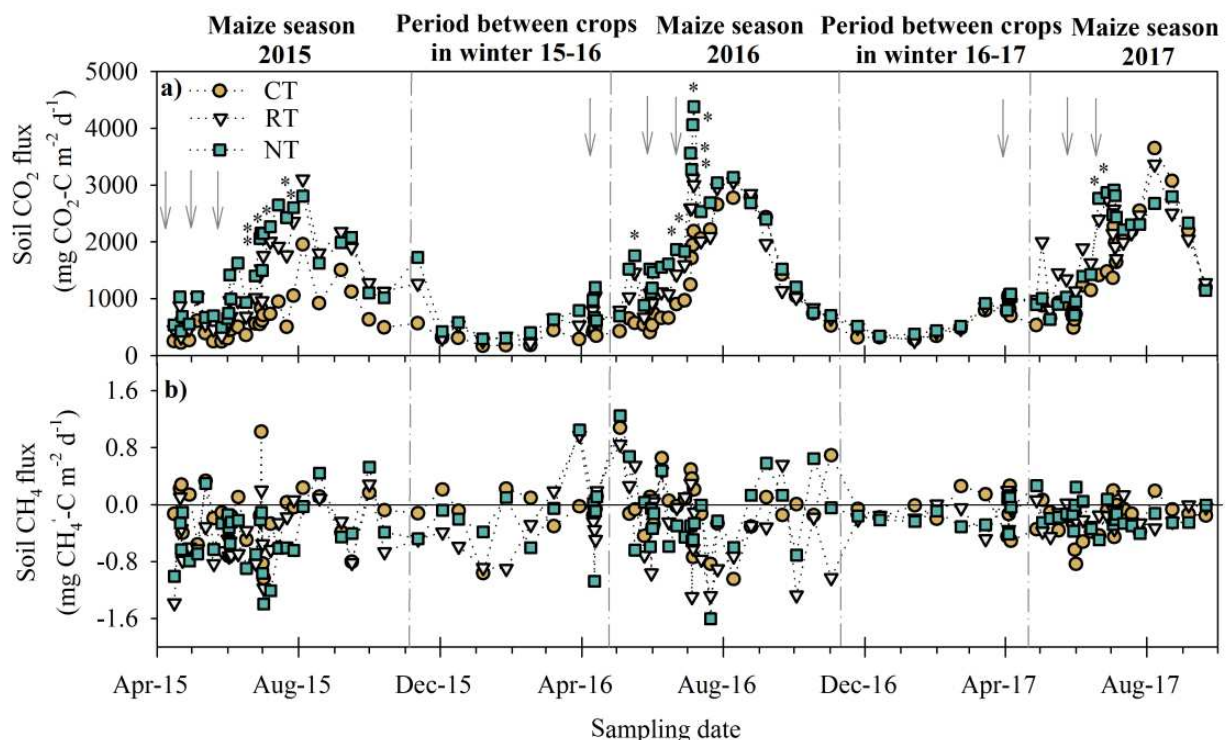


**Fig. 21** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) effects on gravimetric soil moisture at 0-5 cm depth. Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and periods between crops in winter (PC 2015-2016 and PC 2016-2017). Different lowercase letters indicate significant differences between tillage treatments for a given period at  $P < 0.05$ . Vertical bars indicate standard deviation.

### 3.3 Soil carbon dioxide and methane emissions.

The interaction between tillage and sampling date had a significant effect on soil CO<sub>2</sub> emissions (Table 13). Greater CO<sub>2</sub> fluxes were observed under NT and RT than under CT on most

sampling dates during maize growing seasons. There was an increase in CO<sub>2</sub> emissions in the months of July and August, coinciding with the period of maximum maize growth (Fig. 22a). In the three years of study, the largest differences between tillage systems occurred just after the second top-dressing N application. Conversely, in the two periods between crops in winter (2015-2016 and 2016-2017), no differences between tillage treatments on CO<sub>2</sub> fluxes were found, with an average emission of 420 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup> (Fig. 22a). In the control treatment, greater soil CO<sub>2</sub> emissions were observed under NT (1557 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) compared to CT (855 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) with intermediate values in RT (1372 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) as an average of the three years covered by the experiment. When applying 200 kg N ha<sup>-1</sup> greater soil CO<sub>2</sub> emissions were observed in NT and RT (1401 and 1287 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>, respectively) than in CT (1041 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>). Finally, greater soil CO<sub>2</sub> emissions were observed under NT when applying 400 kg N ha<sup>-1</sup> (1559 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) compared to CT under the same rate application (1007 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) with intermediate values in RT (1328 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) as an average of the three years covered by the experiment.



**Fig. 22** Soil CO<sub>2</sub> (a) and CH<sub>4</sub> (b) emissions as affected by tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) during the 2015, 2016 and 2017 maize growing seasons and two periods between crops in winter (2015-2016 and 2016-2017). Arrows indicate dates of N fertilizer application. For a given date, asterisks indicate significant differences between tillage systems at  $P < 0.05$ .

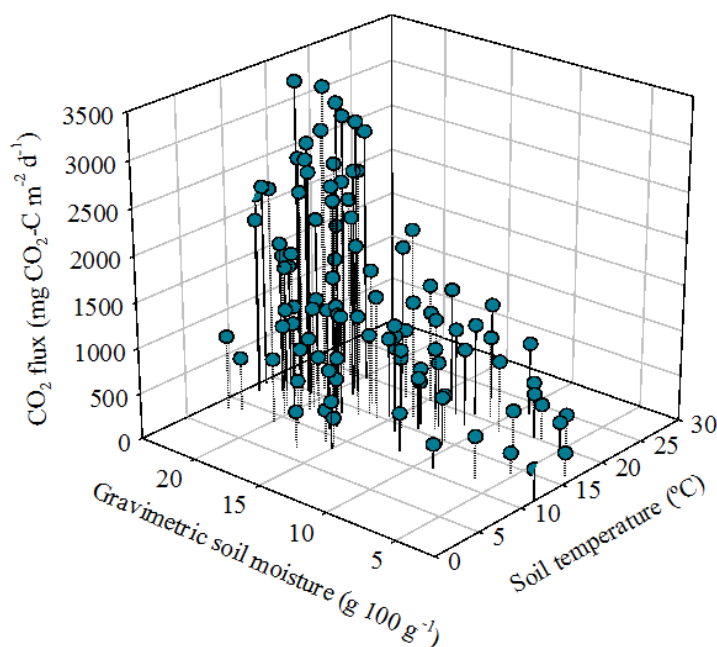
Methane fluxes ranged from -1.60 to 1.24 mg CH<sub>4</sub>-C m<sup>-2</sup> d<sup>-1</sup> (Fig. 22b). Although some emissions to the atmosphere were observed, all treatments acted as net CH<sub>4</sub> sinks during most of the experimental period. Only tillage and sampling date single effects significantly influenced CH<sub>4</sub> fluxes (Table 13). Regarding to this, greater uptake of CH<sub>4</sub> was observed in the three tillage systems compared following the order RT>NT>CT (-0.29, -0.23 and -0.14 mg CH<sub>4</sub>-C m<sup>-2</sup> d<sup>-1</sup>, respectively). Soil uptake of CH<sub>4</sub> tended to decrease with increasing fertilizer N rates.

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

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

Log (soil CO<sub>2</sub> emissions) = Exp (4.30 + 0.10 \* Soil temperature + 0.06 \* Gravimetric soil moisture).

High soil CO<sub>2</sub> fluxes (> 1500 mg CO<sub>2</sub>-C m<sup>-2</sup> d<sup>-1</sup>) were observed when soil temperature at 10 cm depth exceeded 15 °C and gravimetric soil moisture (0-5 cm) was greater than 20 g 100<sup>-1</sup> (Fig. 23).

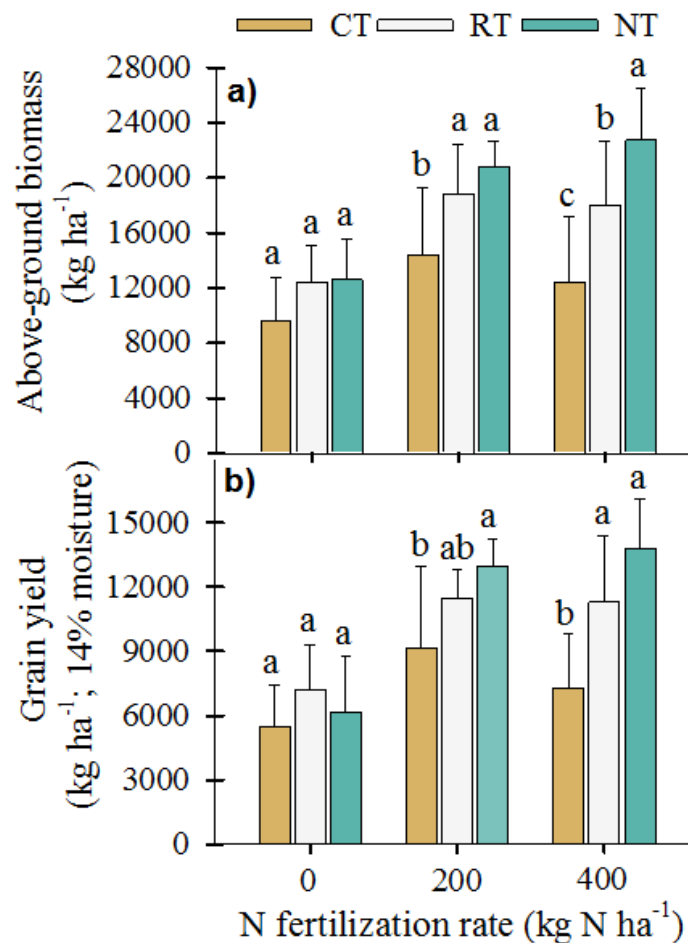


**Fig. 23** Effects of soil temperature at 10 cm depth and gravimetric soil moisture (0-5 cm depth) on CO<sub>2</sub> fluxes. Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and two periods between crops in winter (2015-2016 and 2016-2017). Each point corresponds to the average of the different treatments for a given sampling date (n = 102).



### 3.5 Aboveground biomass and maize grain yield.

Aboveground biomass was significantly affected by the interaction between tillage system and N fertilization and the interaction between N fertilization and year (Table 13). In this regard, NT and RT led to greater aboveground biomass when applying 200 kg N ha<sup>-1</sup> compared to the use of the same rate under CT. Meanwhile, the use of 400 kg N ha<sup>-1</sup> led to greater values in NT followed by RT and CT (Fig. 24a). Moreover, in 2016 and 2017, the rate of 200 (18785 and 19230 kg ha<sup>-1</sup>, respectively) and 400 kg N ha<sup>-1</sup> (20632 and 19348 kg ha<sup>-1</sup>, respectively) showed greater aboveground biomass compared to the control (12256 and 10005 kg ha<sup>-1</sup>, respectively) without significant differences between N rates in 2015.



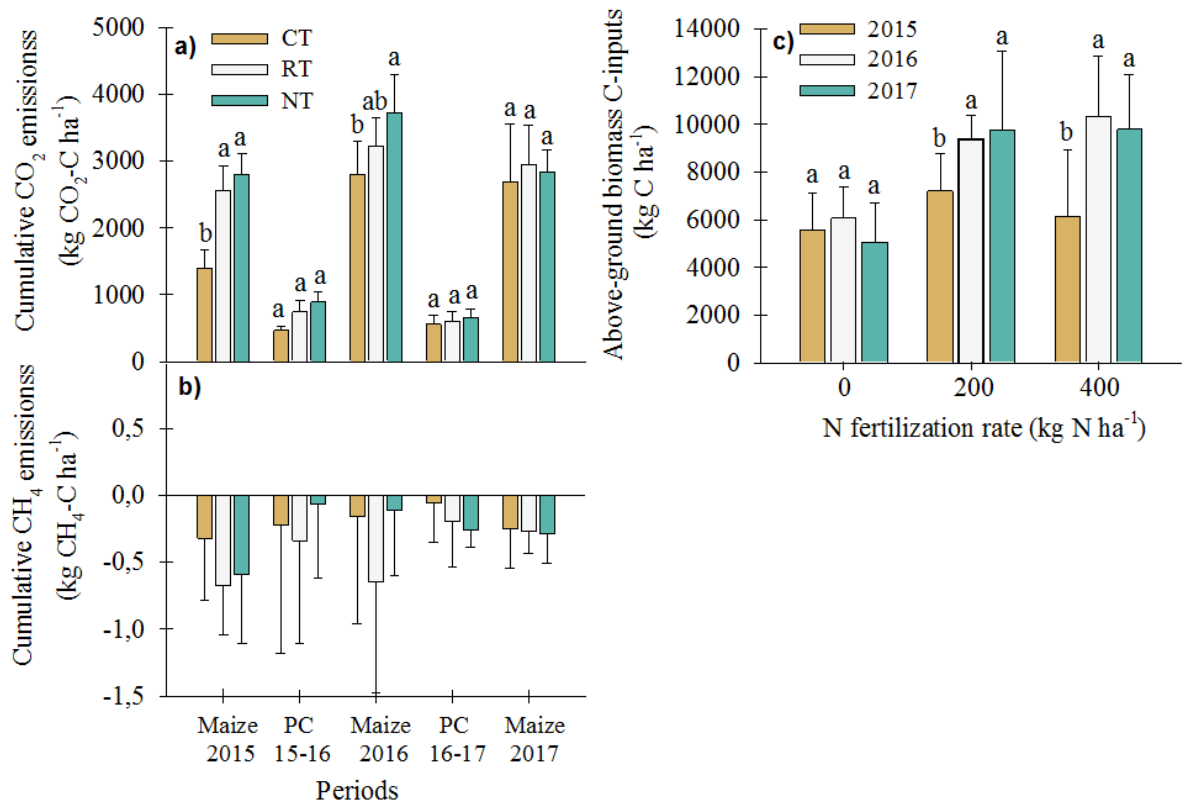
**Fig. 24** Aboveground maize biomass (a) and grain yield (b) as affected by tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and N fertilization rate (0, 200 and 400 kg N ha<sup>-1</sup>). Values correspond to the means of three consecutive maize growing seasons (2015, 2016 and 2017). Different lowercase letters indicate significant differences between tillage systems for a given N fertilization rate at  $P < 0.05$ . Vertical bars indicate standard deviation.

The interaction between tillage and N fertilization and their interaction with the year had a significant effect on maize grain yields (Table 13). In 2015 and 2017, grain yields were higher under NT (11406 and 9844 kg ha<sup>-1</sup>, respectively) and RT (9548 and 9278 kg ha<sup>-1</sup>, respectively) than under CT (5594 and 6478 kg ha<sup>-1</sup>, respectively), without differences between tillage treatments in 2016. As an average of the three seasons, the rate of 200 kg N ha<sup>-1</sup> showed greater grain yield under NT compared to CT with intermediate values in RT. Meanwhile, when applying 400 kg N ha<sup>-1</sup>, NT and RT led to greater grain yield compared to CT (Fig. 24b).

### *3.6 Cumulative soil CO<sub>2</sub> and CH<sub>4</sub> emissions and aboveground biomass C inputs*

Cumulative CO<sub>2</sub> emissions were significantly affected by the interaction between tillage and period (Table 13). In the 2015 growing season, greater cumulative CO<sub>2</sub> emissions were found under NT and RT compared with CT. In 2016 NT showed the highest values compared to CT with intermediate values in RT. Differently, in 2017 no differences between tillage systems were observed (Fig 25a). Contrarily to CO<sub>2</sub>, cumulative CH<sub>4</sub> emissions were not significantly affected by any effect (Table 13), although a trend of greater uptake of CH<sub>4</sub> was observed under RT compared with CT and NT (Fig 25b).

The interaction between tillage and N fertilization and between N fertilization and year had a significant effect on aboveground biomass C-input (Table 13). In 2016 and 2017 the fertilizer rates of 200 and 400 kg N ha<sup>-1</sup> showed greater values compared to the control, while in 2015 no significant differences were observed between N rates (Fig 25c). Moreover, the rates of 200 and 400 kg N ha<sup>-1</sup> led to greater aboveground biomass C-input in NT (10159 and 11124 kg C ha<sup>-1</sup>, respectively) and RT (9210 and 9003 kg C ha<sup>-1</sup>, respectively) than in CT (6978 and 6131 kg C ha<sup>-1</sup>, respectively), as an average of the three years covered by the experiment. Contrarily, no significant differences between tillage systems on aboveground biomass C-inputs were found in the control treatment.



**Fig. 25** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) effects on cumulative soil CO<sub>2</sub> (a), and CH<sub>4</sub> emissions (b) and year (2015, 2016 and 2017) effects on aboveground biomass C-inputs (c). Values correspond to three consecutive maize growing seasons (2015, 2016 and 2017) and two periods between crops in winter (PC 2015-2016 and PC 2016-2017). Different lowercase letters indicate significant differences between tillage treatments for a given period (a and b) and significant differences between years for a given N fertilization rate (c) at  $P < 0.05$ . Vertical bars indicate standard deviation.

## 4. Discussion

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

The combined effects of tillage and N fertilization on soil CO<sub>2</sub> emissions observed in this study would be related to the impact of these practices on soil environmental conditions such as soil temperature and soil moisture and on the availability of organic C due to changes in crop residue production. No-tillage showed higher CO<sub>2</sub> emissions when applying high rates of N (400 kg N ha<sup>-1</sup>) compared to CT. Although irrigation water supply was the same for all treatments, NT led to greater soil moisture (being 60% higher NT than CT). The presence of crop residues on the soil surface under NT enhances soil water availability compared to CT by reducing soil water evaporation and increasing infiltration (Lampurlanés *et al.*, 2016). The increase in soil moisture observed in NT was accompanied by higher CO<sub>2</sub> emissions denoting greater enzymatic activity

stimulated by the greater levels of SOC concentration in NT (being 58% greater in NT compared to CT (42.2 vs. 17.8 g kg<sup>-1</sup>, respectively in the 0–10 cm layer)) and root respiration in the soil in this treatment compared to CT. These data show that in spite of the higher CO<sub>2</sub> emissions in NT, these are compensated by its high capacity for C sequestration. Authors like Manzoni *et al.* (2012) showed that as soil water availability increases, the metabolic activity of soil fauna is enhanced, resulting in higher soil heterotrophic respiration, impacting soil carbon content. The low emissions in CT, even when using high rates of mineral N, could be due to long-term CT leads to a deterioration of the soil physical properties. This degradation was due to a lower structural stability, causing soil surface crusting, which resulted in lower water infiltration (1.70, 2.40 and 3.14 mm h<sup>-1</sup> for CT, RT and NT, respectively) into the soil profile, compromising plantlet establishment (with a density of plants 19% and 22% lower in CT compared to RT and NT, respectively) and leading to plant water stress (Pareja-Sánchez *et al.*, 2017). Under these circumstances, the impact of tillage systems on soil structure played a major role on crop productivity. Therefore, soil respiration and the amount of C returned to the soil as crop residues were reduced under CT.

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

Previously, in the same experimental field, in rainfed NT barley conditions cumulative CO<sub>2</sub> emissions were 37% lower compared to the values found in our study in irrigated conditions and in CT this difference dropped to 46% (Plaza-Bonilla *et al.*, 2014). The difference between these studies could probably be attributed to the increase in heterotrophic and autotrophic respiration taking place under irrigated conditions. In particular, the N fertilization applied in our experiment led to higher aboveground biomass and therefore higher heterotrophic respiration rate under NT and RT than in CT. On the other hand, the increase in autotrophic respiration under NT and

RT was caused by an increase in root respiration which led to significant increase in crop production (Kou *et al.*, 2008) as well as more organic C available to decomposers. This fact would explain the higher cumulative CO<sub>2</sub> emissions observed under NT and RT in comparison with CT (38% and 28% higher, respectively). Contrarily, the application of N did not enhance soil CO<sub>2</sub> emissions under CT. There is no consensus about the impact of N availability on soil C mineralization with studies showing either a stimulatory or a suppressive effect (Al-Kaisi and Guzman, 2013). In this line, although the application of mineral nitrogen generally stimulates CO<sub>2</sub> emissions (Iqbal *et al.*, 2009) throughout the rise in C inputs, some authors have pointed out that CO<sub>2</sub> fluxes can decrease when using high N rates, as a consequence of a lower activity of enzymes (DeForest *et al.*, 2004). A higher soil mineral N availability under CT could have decreased the need for soil microorganisms to mineralize soil organic matter to obtain the necessary N for growth and reproduction (Smith *et al.*, 2012) and, as a consequence, reduce soil respiration.

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

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

The highest cumulative emissions in 2016 coincided with an increase in aboveground biomass C-inputs to the soil from the previous campaign. Therefore, cumulative values would be closely related to the amount of C available for microbial decomposition. Moreover, an increase in soil CO<sub>2</sub> emissions was observed during July and August in the three maize growing seasons studied (2015, 2016 and 2017), when the maximum maize growth rate occurs. During this period (July and August) around 50% of soil respiration is the result of root respiration plus the decomposition of root exudates (Rochette *et al.*, 1999). Additionally, these results during July and August could also be attributed to an enhancement in soil organic matter mineralization as a result of greater soil surface temperature and moisture as the multiple linear relationship obtained between these variables corroborates. The highest CO<sub>2</sub> fluxes occurred when soil temperatures were greater than 15 °C and gravimetric soil moisture was greater than 20%. This

demonstrates that soil temperature and gravimetric soil moisture regulated CO<sub>2</sub> emissions interactively (Almagro *et al.*, 2009).

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

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

Soil properties such as temperature, water content, bulk density, and SOC have an important role in the activity of methanogens and methanotrophs, affecting the sign of CH<sub>4</sub> emissions (Mitra *et al.*, 2002). In the present study, the soil acted as a net sink of CH<sub>4</sub> in all treatments, indicating a greater proportion of methanotrophic activity. Other authors have also reported that CH<sub>4</sub> is primarily consumed by soils under upland field crops cultivation (Kessavalou *et al.*, 1998; Venterea *et al.*, 2005). About 42% and 49% higher uptake of CH<sub>4</sub> occurred during the 2015 maize growing season compared to the 2016 and 2017 seasons, respectively. These results could be explained by the lower soil water contents during the previous rainfed management, closer in time in 2015 compared to 2016 and 2017, which could have favored the methanotrophic communities (von Fischer and Hedin, 2007). This hypothesis would be supported by the greater amount of methane uptake by soil found during a barley rainfed season in the same experiment, which reached average values -0.78 and -1.72 kg CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> under CT and NT, respectively (Plaza-Bonilla *et al.*, 2014) while in irrigated maize were -0.55 and -0.65 kg CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> under CT and NT, respectively.

## 5. Conclusion

This experiment highlights the extent of changes on the dynamics of soil CO<sub>2</sub> and CH<sub>4</sub> emission in Mediterranean agroecosystems recently transformed into irrigation. In this study, soil CO<sub>2</sub> emissions were greater when increasing the rate of N in combination with a tillage reduction as result of enhanced maize growth promoting root respiration and greater soil organic C availability to decomposers. Differently, under CT an increase in N fertilization reduced

soil respiration. The higher soil mineral N availability under CT could have decreased the need for soil microorganisms to mineralize soil organic matter. In general the soil acted as a CH<sub>4</sub> sink in all treatments. However, CH<sub>4</sub> oxidation was lower in CT compared with RT and NT, which could be the result of the effect of tillage on gas diffusivity. Compared to a previous period under rainfed barley production, annual soil CO<sub>2</sub> emissions were 37 to 46% greater, depending on the type of tillage. Contrarily, CH<sub>4</sub> uptake decreased after the transformation into irrigation, as a result of the wetter soil conditions which could have affected the methanotrophic communities by reducing soil CH<sub>4</sub> oxidation. Our study suggests that, in maize-based Mediterranean agroecosystems recently transformed to irrigation, the reduction in tillage intensity and N fertilization rates increases soil CO<sub>2</sub> emissions and CH<sub>4</sub> uptake. The higher maize growth and organic C production under these management practices could lead to soil C storage, reducing the C footprint of the cropping system.

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## **Chapter V**

**Soil organic carbon sequestration after conversion from rainfed to irrigated in a Mediterranean agroecosystem subjected to different tillage systems and N fertilization rates**

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## Chapter V

### Soil organic carbon sequestration after conversion from rainfed to irrigated in a Mediterranean agroecosystem subjected to different tillage systems and N fertilization rates

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#### Abstract

The increase in soil organic carbon (SOC) can help to mitigate global warming through the sequestration of atmospheric carbon. The aim of this work was to evaluate the impact on SOC sequestration of the transformation from rainfed to irrigation under different long-term tillage systems and N fertilization rates. The study was carried out in NE Spain in a long-term tillage and N rate field experiment established in 1996 under barley rainfed conditions which in 2015 was transformed into irrigation with maize monoculture as cropping system. Three types of tillage (conventional tillage, CT; reduced tillage, RT; no-tillage, NT) and three mineral N fertilization rates (0, medium, and high) were compared in a randomized block design with three replications. Soil organic carbon changes were quantified for 21 years. Annual SOC sequestration rate ( $\Delta\text{SOC}_{\text{rate}}$ ) (0–40 cm soil depth) was calculated for each treatment in three different periods (P1-, P2-, P3-) under rainfed (-R) and irrigated (-I) conditions (P1-R, from 1996 to 2009; P2-R, from 2009 to 2015; P3-I, from 2015 to 2017). Moreover, at the 0–5, 5–10, 10–20, 20–30 and 30–40 cm soil depths, particulate organic carbon (POC), mineral-associated organic carbon (C-Min) and permanganate-oxidizable organic C (POxC) were measured at the beginning and at the end of P3-I to elucidate their role on SOC sequestration due to conversion to irrigated land. Annual C-inputs as aboveground crop residues were also quantified. As an average of treatments,  $\Delta\text{SOC}_{\text{rate}}$  was 492, 222 and 969 kg C ha<sup>-1</sup> year<sup>-1</sup> for P1-R, P2-R and P3-I, respectively. In P1-R and P3-I, C-input explained 70% of the variability of  $\Delta\text{SOC}_{\text{rate}}$ . Differently, in P2-R  $\Delta\text{SOC}_{\text{rate}}$  was not correlated with C-input. In P1-R, NT presented the highest  $\Delta\text{SOC}_{\text{rate}}$  compared to CT with intermediate values in RT, while high N rate showed greater  $\Delta\text{SOC}_{\text{rate}}$  compared to the control with intermediate values in the medium N rate. Contrarily, in P2-R  $\Delta\text{SOC}_{\text{rate}}$  did not show differences between treatments.

In P3-I, NT showed greater  $\Delta\text{SOC}_{\text{rate}}$  than CT at the highest N rate, with intermediate values in RT (1959, 731 and 1380 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). SOC increase after the transformation to irrigation was mainly explained by POC, which increased by 75% compared to the previous rainfed period. Our results indicate that the introduction of irrigation and crop management practice based in no-tillage and medium N fertilizer rate can contribute to the sequestration of large amounts of atmospheric CO<sub>2</sub>.

## Keywords

Climate-smart agriculture; Conservation tillage; conversion of rainfed to irrigated land; long-term field experimentation; soil carbon sequestration.

## 1. Introduction

Carbon sequestration using long-term adequate soil and crop management practices is needed not only to mitigate greenhouse gas emissions (Smith *et al.*, 2008), but also to improve soil quality and increase crop production. Changes in SOC levels are detectable certain time after a modification in agricultural management occurs. For this reason, long-term experiments are essential to determine the impact of agricultural management on SOC changes. Indeed, the identification and 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-Bonilla *et al.*, 2014). An adequate use of different agronomic practices (e.g. soil tillage, N fertilization, irrigation, etc.) may boost C sequestration in the soil (West and Post, 2002). In semiarid Mediterranean rainfed conditions, several studies have shown significant increases of SOC stocks after the adoption of conservation tillage. For example, authors like Álvaro-Fuentes *et al.*, (2009) and Hernanz *et al.*, (2009) demonstrated that continuous NT leads to SOC sequestration with rates ranging from 0.40 to 0.50 t C ha<sup>-1</sup> yr<sup>-1</sup>. Similarly, in a global meta-analysis West and Post (2002) examined 67 long-term agricultural experiments and showed that the conversion from conventional tillage (CT) to NT can sequester  $0.57 \pm 0.14$  t C ha<sup>-1</sup>yr<sup>-1</sup>. In addition, N fertilization may also increase SOC stocks, since an increase in crop productivity can lead to greater C-inputs returned to the soil (Luo *et al.*, 2010). However, N fertilization may lead to a decrease in the soil carbon/nitrogen ratio, enhancing microbial activity and decomposability of soil organic matter (Li *et al.*, 2009) and, consequently, increasing C losses to the environment (Patrick *et al.*, 2013).

Another important management practice which can enhance SOC sequestration is the provision of irrigation water in arid and semiarid regions where crop productivity is severely limited by water availability. In this aspect there is little information on the effect of the conversion of rainfed to irrigated on SOC levels. In these conditions SOC can increase from 11 % to 35 % (Trost *et al.*, 2013). Greater crop biomass production due to irrigation leads to an increase in crop residues which can be returned to the soil. The increase of organic C-inputs to the soil usually entails an increase in SOC (Dimassi *et al.*, 2014) and, concomitantly, an improvement in soil quality (Wick *et al.*, 1998; Dexter *et al.*, 2008). However, irrigation can also reduce SOC accumulation by stimulating microbial activity which accelerates soil organic matter decomposition (De Bona *et al.*, 2006). For instance, under semiarid conditions in N Spain, Apesteguía *et al.* (2015) did not find changes in SOC stocks under irrigated maize in relation to the non-irrigated treatment after two years since the conversion to irrigated land. The authors suggested that the lack of differences between rainfed and irrigated treatments on SOC levels could be explained by the slight difference in C inputs and the relatively short period considered (2 years).

The conversion from rainfed to irrigated land can be a potential strategy to further sequester soil C if combined with adequate agricultural practices that reduce SOC decomposition and/or enhance C-inputs. Regarding to this, the combination of irrigation and the use of NT or RT is assumed to have a larger potential to increase SOC contents than irrigation in combination with CT (Martens *et al.*, 2005). For instance, Halvorson *et al.*, (2004) observed an increase in SOC when using NT in irrigated continuous maize systems compared to CT although both tillage treatments (NT and CT) produced similar levels of crop residues. Greater SOC decomposition under CT would explain that result. Soil tillage disrupts soil aggregates, reducing physical protection of C, and increasing mineralization of SOC (Grandy and Robertson, 2006). Therefore, there is a need to identify the most adequate management practices to enhance SOC sequestration when converting rainfed land into irrigation.

This study is developed in a long-term tillage and N fertilization experiment established in 1996 in rainfed land which was transformed in 2015 into irrigated land. In a first rainfed period, Morell *et al.*, 2011 already observed a positive effect of NT in SOC. This study is a step forward in which we evaluate (i) 19 years of impact of tillage and N fertilization and (ii) the conversion of rainfed into irrigated land on SOC.



### 3. Materials and methods

#### 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, NE Spain (41°48' N, 1°07' E, 330 m asl). The climate is semiarid Mediterranean with a continental trend. Mean annual precipitation, potential evapotranspiration, and temperature are 401 mm, 855 mm, and 14.1°C, respectively (data from 1985 to 2015).

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<sup>-1</sup>) under barley (*Hordeum vulgare* L.) monoculture. In 2015, the rainfed experiment was converted to irrigation with solid set sprinklers and maize (*Zea mays* L.) monoculture as cropping system. After the shift from rainfed to irrigation, the field experiment maintained the same tillage intensities (CT, RT, and NT) while N fertilization rates were adapted to maize needs (0, 200, and 400 kg N ha<sup>-1</sup>) (Table 14). A total of 27 plots (50x6 m) were arranged in a randomized complete block design with three replications. The soil was classified as Typic Xerofluvent (Soil Survey Staff, 2014) and presented a silt clay loam texture (sand, 30.8%; silt, 57.3%; clay, 11.9%) in the upper (0-28 cm) horizon. Other main physico-chemical properties (0-28 cm soil depth) at the beginning of the experiment (1996) were as follows: pH (H<sub>2</sub>O, 1:2.5): 8.5; electrical conductivity (1:5): 0.15 dS m<sup>-1</sup>; soil organic carbon concentration 9 g kg<sup>-1</sup>; P Olsen: 35 mg kg<sup>-1</sup>; K (Amm. Ac.): 194 mg kg<sup>-1</sup>; water retention (-33 kPa): 16 kg kg<sup>-1</sup>; water retention (-1500 kPa): 5 kg kg<sup>-1</sup>. Crop management practices were conducted following the local traditional practices (Table 14). After 2015, irrigation was supplied to meet the estimated evapotranspiration (ET) of maize minus the effective precipitation, which was estimated as 75% of precipitation (when precipitation > 5 mm) (Dastane, 1978). Maize evapotranspiration (ET<sub>c</sub>) 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 0.3-1.2). The ET was calculated using the Penman-Monteith equation. Meteorological data were obtained from an automated weather station located near the experimental site.

**Table 14.** Agricultural management practices carried out in the experimental field during the rainfed and irrigated periods.

|                                | <b>Rainfed period (1996 to 2014)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <b>Irrigated period (2015 to 2017)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tillage</b>                 | <p><b>CT:</b> one moldboard plough pass (25-30 cm depth) plus two cultivator passes (15 cm depth) during September and October.</p> <p><b>RT:</b> one cultivator pass (10 to 15 cm depth) during September and October.</p> <p><b>NT:</b> No-tillage.</p>                                                                                                                                                                                                                                                                                                                                                         | <p><b>CT:</b> one rototiller pass (15 cm depth) plus one subsoiler pass (35 cm depth) and one disk plough pass (20 cm depth) during March or April.</p> <p><b>RT:</b> Strip-tillage (25 cm depth reducing the surface tilled to 20%).</p> <p><b>NT:</b> No-tillage.</p>                                                                                                                                                                                                                                                                                                                                               |
| <b>Growing season</b>          | November – June.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | April – November.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Sowing/<br/>Planting</b>    | <p><b>Barley:</b> cv. Hispanic from 1996 to 2010 and cv. Cierzo from 2010 to 2014.</p> <p>Planting density of 450 seeds m<sup>-2</sup> in November.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p><b>Maize:</b> cv. Kopias from 2015 to 2017.</p> <p>Planting density of 90,000 seeds ha<sup>-1</sup> in April.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Fertilization</b>           | <ul style="list-style-type: none"> <li>• <b>Three mineral N fertilizer rates</b> 0, 60 and 120 kg N ha<sup>-1</sup>: <ul style="list-style-type: none"> <li>▪ 1/3 of the rate in one pre-sowing application as ammonium sulphate (November).</li> <li>▪ 2/3 of the rate in one top dressing application as ammonium nitrate (between January and February).</li> </ul> </li> <li>• <b>Phosphorous:</b> 40–50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> yr<sup>-1</sup></li> <li>• <b>Potassium:</b> 90 kg K<sub>2</sub>O ha<sup>-1</sup> yr<sup>-1</sup> (both P and K before sowing in November).</li> </ul> | <ul style="list-style-type: none"> <li>• <b>Three mineral N fertilizer rates</b> 0, 200 and 400 kg N ha<sup>-1</sup>. <ul style="list-style-type: none"> <li>▪ 1/3 of the rate in one pre-sowing application as urea (April before sowing).</li> <li>▪ 1/3 of the rate in two top-dressing applications at V5 (May) and V10 (July) as ammonium nitrate.</li> </ul> </li> <li>• <b>Phosphorous:</b> 154 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> yr<sup>-1</sup></li> <li>• <b>Potassium:</b> 322 kg K<sub>2</sub>O ha<sup>-1</sup> yr<sup>-1</sup> (both P and K in March or April, before planting).</li> </ul> |
| <b>Weed control</b>            | Pre-sowing: 1.5 L ha <sup>-1</sup> of glyphosate [N-(phosphonomethyl) glycine].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Pre-sowing: 1.5 L ha <sup>-1</sup> of glyphosate [N-(phosphonomethyl) glycine].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Grain harvest</b>           | Commercial combine at the end of June or beginning of July.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Commercial combine at the beginning of November.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Crop residue management</b> | Chopped and spread over the soil surface (NT) or incorporated (CT and RT) according to the tillage system.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Chopped and spread over the soil surface (NT) or incorporated (CT and RT) according to the tillage system.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

## 2.2 Soil sampling and analysis.

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 land and maize planting in March 2015. The last sampling was carried out in November 2017 after maize harvest.

Soil samples were collected at 0–5, 5–10, 10–20, 20–30, and 30–40 cm soil depth in two samples per plot. In each plot and depth, soil bulk density was quantified 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 of Walkley and Black (Nelson and Sommers, 1982) with a 1 g subsample. SOC contents were calculated on a mass per unit area basis by multiplying the C concentration values obtained from the oxidation method by the corresponding soil bulk density values. Moreover, the SOC stock ( $\text{kg C ha}^{-1}$ ) was corrected in terms of equivalent soil mass following the procedure of Ellert and Bettany (1995) for the 0 to 40 cm soil depth interval. The annual SOC sequestration rate ( $\Delta\text{SOC}_{\text{rate}}$ ) ( $\text{kg C ha}^{-1} \text{ year}^{-1}$ ) (0-40 cm soil depth) was calculated for each treatment in three different periods: from 1996 to 2009 (P1-R), from 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I). The present work builds on the publication of Morell *et al.*, (2011) focused on the period 1996-2009 (P1-R).

Furthermore, at the beginning and at the end of the period under irrigation (2015 and 2017), 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, and 30–40 cm depth). These C fractions were chosen since they were identified as highly sensitive to changes in management practices in our semiarid areas (Plaza-Bonilla *et al.*, 2014). C-Min and POC were isolated using a physical fractionation method adapted from Cambardella and Elliot (1992). Briefly, twenty-gram subsamples of soil from each depth and plot were dispersed in 100 mL of 5 g  $\text{L}^{-1}$  sodium hexametaphosphate for 15 h on a reciprocal shaker. Then the samples were passed through a 50- $\mu\text{m}$  sieve to separate the POC and C-Min. The material passing through the sieve (C-Min) was collected in aluminum pans and oven dried at 50°C. The wet oxidation method of Walkley and Black was then used to measure the C concentration in the C-Min fraction. The POC content was determined as the difference between total SOC content and C-Min content. The POxC was quantified according to the method of Weil *et al.* (2003).

### 2.3 Carbon inputs.

In the rainfed period, barley aboveground biomass was determined just before 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 seeding line. In the irrigated period, maize aboveground 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 sampling areas per plot. In 2015 a 5 m sampling length was taken in all treatments due to the low plant density in CT.

In 2016 and 2017 the length of the sampling row was 2 m. Barley and maize aboveground 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 and maize 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 biomass by their C concentration.

#### *2.4 Statistical analysis.*

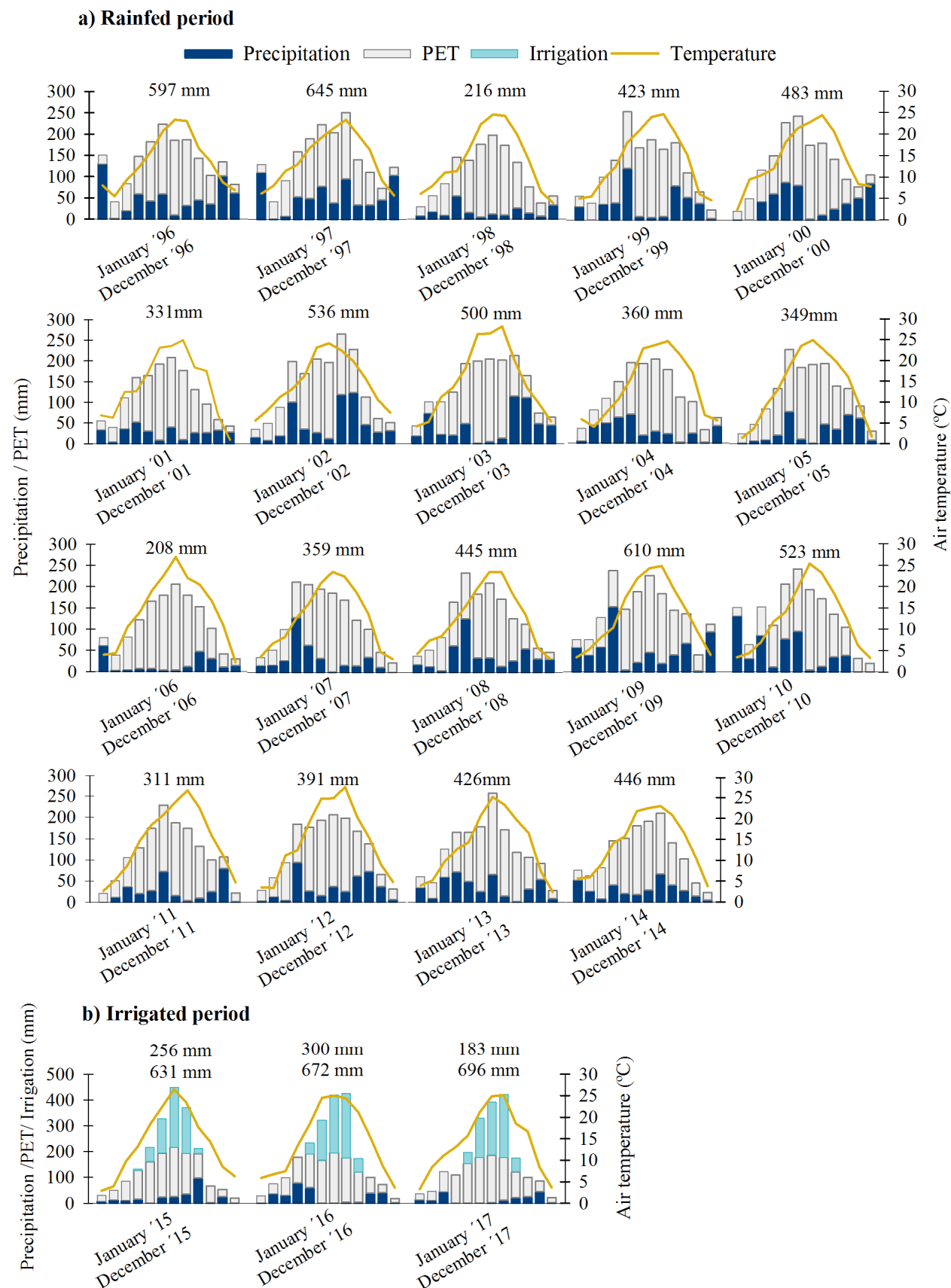
Statistical analyses were performed with the statistical package JMP 13 (SAS Institute Inc, 2018). Data were checked for normality with the Shapiro-Wilk Test. All data complied with normality. Measured  $\Delta\text{SOC}_{\text{rate}}$  and C-input were statistically tested with analysis of variance (ANOVA), which was performed for each period (P1-R, P2-R and P3-I) with tillage, N fertilization and their interaction as fixed effects. Meanwhile, an ANOVA of POxC, POC and C-Min was performed with tillage, N fertilization, soil depth, year, and their interaction as fixed effects. Since the interaction of year with the rest of effects was non-significant, a separate ANOVA was finally varied out for each year (2015 and 2017). When significant, differences 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 (SAS Institute Inc, 2018) to test the presence of relationships between  $\Delta\text{SOC}_{\text{rate}}$  and C-input for each period (P1-R, P2-R and P3-I).

### **3. Results**

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

Monthly rainfall, potential evapotranspiration, air temperature and irrigation for each year are presented in Fig. 26. The precipitation from January to December was highly variable during the 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 which affected the entire autumn soil water recharge period (September-December). Contrarily, 1996, 1997, 2002, 2003, 2009 and 2010 were characterized by 25 to 60% more rainfall than the 30-yr average (i.e. 401 mm). Differently, during the irrigated period, the water deficit was replaced by irrigation, with 631, 672 and 696 mm in 2015, 2016 and 2017, respectively, during the maize growing season (April to September).

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



**Fig. 26.** Monthly precipitation (dark blue columns), potential evapotranspiration (PET, white columns), irrigation (light blue columns) and mean air temperature (yellow line) at Agramunt from 1996 to 2017. For each year total precipitation (above) and irrigation water (below) are reported.

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

Crop residue C-inputs were significantly affected by the interaction between tillage system and N fertilization under rainfed conditions (P1-R and P2-R). However, in P3-I only tillage and N fertilization single effects significantly influenced C-inputs (Table 15). Regarding to this, in P1-R and P2-R, when no N fertilizer was applied, C-inputs did not show differences between tillage systems while greater values were observed in NT compared to RT and CT when applying medium N fertilization rates. Moreover, in P1-R, NT showed greater C-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 fertilization. In P3-I, greater C-inputs were observed under NT compared to RT and CT (3255, 2630 and 2213 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). Furthermore, in P3-I the high rate of N fertilization showed greater C-inputs than the control rate with intermediate values in the medium rate of N fertilization (2968, 2378, and 2752 C ha<sup>-1</sup> yr<sup>-1</sup>, respectively).

**Table 15.** Aboveground carbon inputs (C-input) (kg C ha<sup>-1</sup> year<sup>-1</sup>) from 1996 to 2009 (P1-R), from 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I) as affected by tillage (CT; conventional tillage, RT; reduced tillage and NT; no tillage) and N fertilization rate (zero, medium and high). For a given N fertilization rate and period different lowercase letters indicate significant differences between tillage treatments at  $P < 0.05$ . Values between brackets indicate standard deviation. Analysis of variance ( $P$ -values) of C-inputs of P1-R, P2-R and P3-I as affected by tillage (CT, RT and NT), N fertilization rate (zero, medium and high), and their interaction.

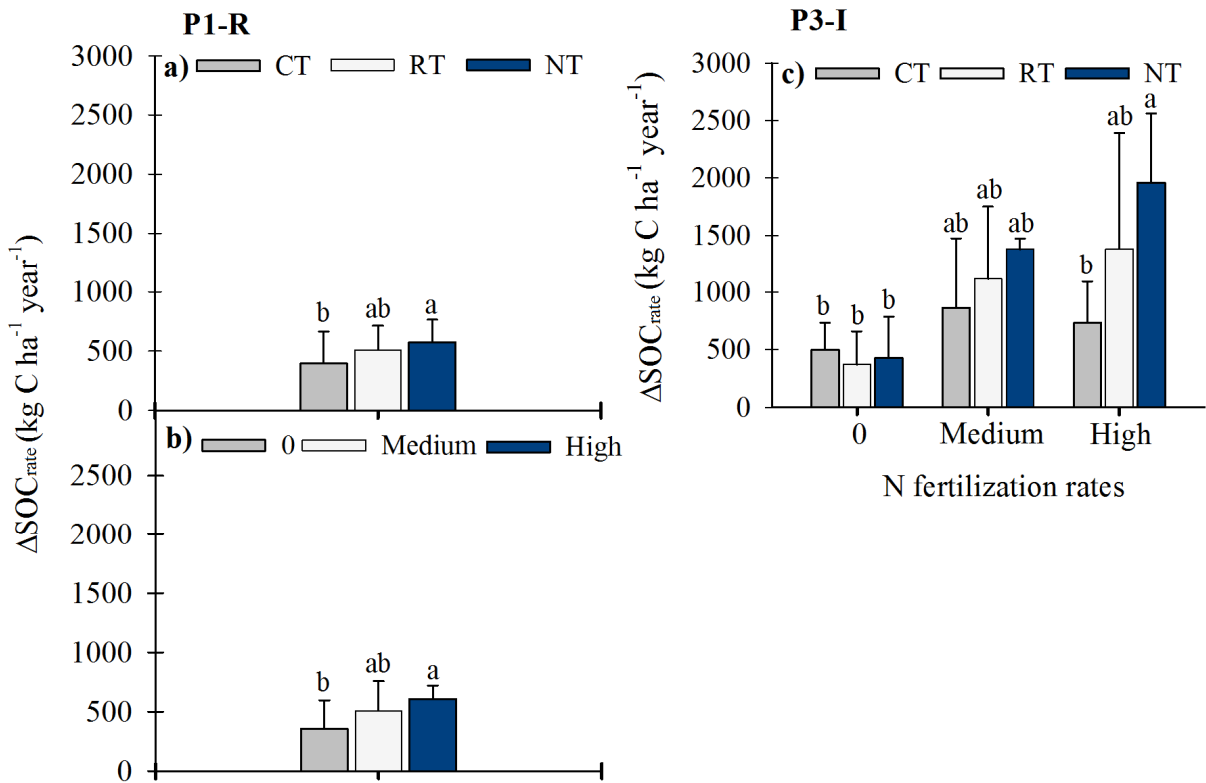
|                               |           | C-input (kg C ha <sup>-1</sup> year <sup>-1</sup> ) |              |            |
|-------------------------------|-----------|-----------------------------------------------------|--------------|------------|
| N fertilization rate          | Tillage   | P1-R                                                | P2-R         | P3-I       |
| <b>0</b>                      | <b>CT</b> | 1245 (81) a                                         | 1066 (17) a  | 1995 (391) |
|                               | <b>RT</b> | 1263 (105) a                                        | 1069 (91) a  | 2488 (211) |
|                               | <b>NT</b> | 1445 (150) a                                        | 1310 (34) a  | 2651 (332) |
| <b>Medium</b>                 | <b>CT</b> | 1490 (84) b                                         | 1222 (68) b  | 2341 (35)  |
|                               | <b>RT</b> | 1662 (91) b                                         | 1391 (151) b | 2723 (395) |
|                               | <b>NT</b> | 2079 (170) a                                        | 1874 (81) a  | 3191 (267) |
| <b>High</b>                   | <b>CT</b> | 1540 (118) b                                        | 1249 (162) b | 2303 (831) |
|                               | <b>RT</b> | 1697 (85) b                                         | 1707 (89) a  | 2679 (637) |
|                               | <b>NT</b> | 2268 (68) a                                         | 2009 (201) a | 3921 (96)  |
| <b>ANOVA</b>                  |           |                                                     |              |            |
| <b>Tillage (Till)</b>         |           | <0.001                                              | <0.001       | <0.001     |
| <b>N fertilization (Fert)</b> |           | <0.001                                              | <0.001       | 0.02       |
| <b>Till*Fert</b>              |           | 0.01                                                | <0.001       | ns         |
| ns; non-significant           |           |                                                     |              |            |

Soil organic carbon sequestration rate ( $\Delta\text{SOC}_{\text{rate}}$ ) (0–40 cm) varied significantly with respect to the period, with mean values of 492, 222 and 969 kg C ha<sup>-1</sup> yr<sup>-1</sup>, in P1-R, P2-R and P3-I, respectively. In P1-R (1996 to 2009),  $\Delta\text{SOC}_{\text{rate}}$  was significantly affected by tillage and N fertilization single effects while in P3-I (2015 to 2017)  $\Delta\text{SOC}_{\text{rate}}$  was affected by the interaction between tillage and N fertilization. Differently, in P2-R (2009 to 2015)  $\Delta\text{SOC}_{\text{rate}}$  did not show differences between treatments (Table 16). In P1-R, NT presented the highest  $\Delta\text{SOC}_{\text{rate}}$  compared to CT with intermediate values in RT (Fig. 27a), while high N rate showed greater  $\Delta\text{SOC}_{\text{rate}}$  compared to the control with intermediate values in the medium N rate (Fig 27b). In P3-I, the high rate of N fertilizer in NT led to greater  $\Delta\text{SOC}_{\text{rate}}$  compared to CT with intermediate values in RT (1959, 731, and 1380, kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively), while under the control and medium N rate  $\Delta\text{SOC}_{\text{rate}}$  did not show differences between tillage systems (Fig. 27c).

**Table 16.** Analysis of variance (*P*-values) of soil organic carbon sequestration rate ( $\Delta\text{SOC}_{\text{rate}}$ ) from 1996 to 2009 (P1-R), from 2009 to 2015 (P2-R), and from 2015 to 2017 (P3-I) as affected by tillage (CT; conventional tillage, RT; reduced tillage and NT; no-tillage), N fertilization rate (zero, medium and high), and their interaction.

| Source of variation           | $\Delta\text{SOC}_{\text{rate}}$ |      |        |
|-------------------------------|----------------------------------|------|--------|
|                               | P1-R                             | P2-R | P3-I   |
| <b>Tillage (Till)</b>         | <0.001                           | ns   | <0.001 |
| <b>N fertilization (Fert)</b> | <0.001                           | ns   | <0.001 |
| <b>Till*Fert</b>              | ns                               | ns   | 0.04   |

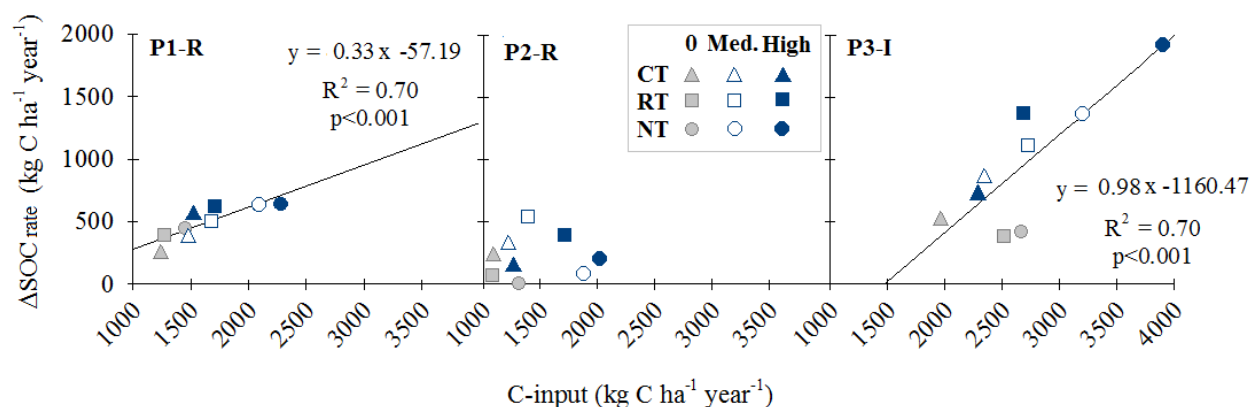
ns; non-significant



**Fig. 27.** Tillage system (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) (a) and nitrogen fertilizer rate (0, Medium, High) (b) effects on soil organic carbon (SOC) sequestration rate ( $\Delta SOC_{rate}$ ) (kg C ha<sup>-1</sup> year<sup>-1</sup>) from 1996 to 2009 (P1-R) and tillage system and nitrogen fertilizer rate (c) effects on SOC sequestration rate ( $\Delta SOC_{rate}$ ) (kg C ha<sup>-1</sup> year<sup>-1</sup>) from 2015 to 2017 (P3-I). Different lower case letters indicate significant differences between treatments (c) at  $P < 0.05$ . Vertical bars indicate standard deviation.

In P1-R and P3-I, a significant positive linear relationship was found between  $\Delta SOC_{rate}$  and annual C-inputs, explaining 70% of the variance of  $\Delta SOC_{rate}$  ( $r^2 = 0.70$ ;  $p < 0.001$ ) (Fig. 28). Contrarily, in P2-R  $\Delta SOC_{rate}$  was not correlated with C-inputs.





**Fig. 28.** Linear regression between soil organic carbon sequestration rate ( $\Delta\text{SOC}_{\text{rate}}$ ) ( $\text{kg C ha}^{-1} \text{ year}^{-1}$ ) and annual carbon inputs (C-input) ( $\text{kg C ha}^{-1} \text{ year}^{-1}$ ) 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).

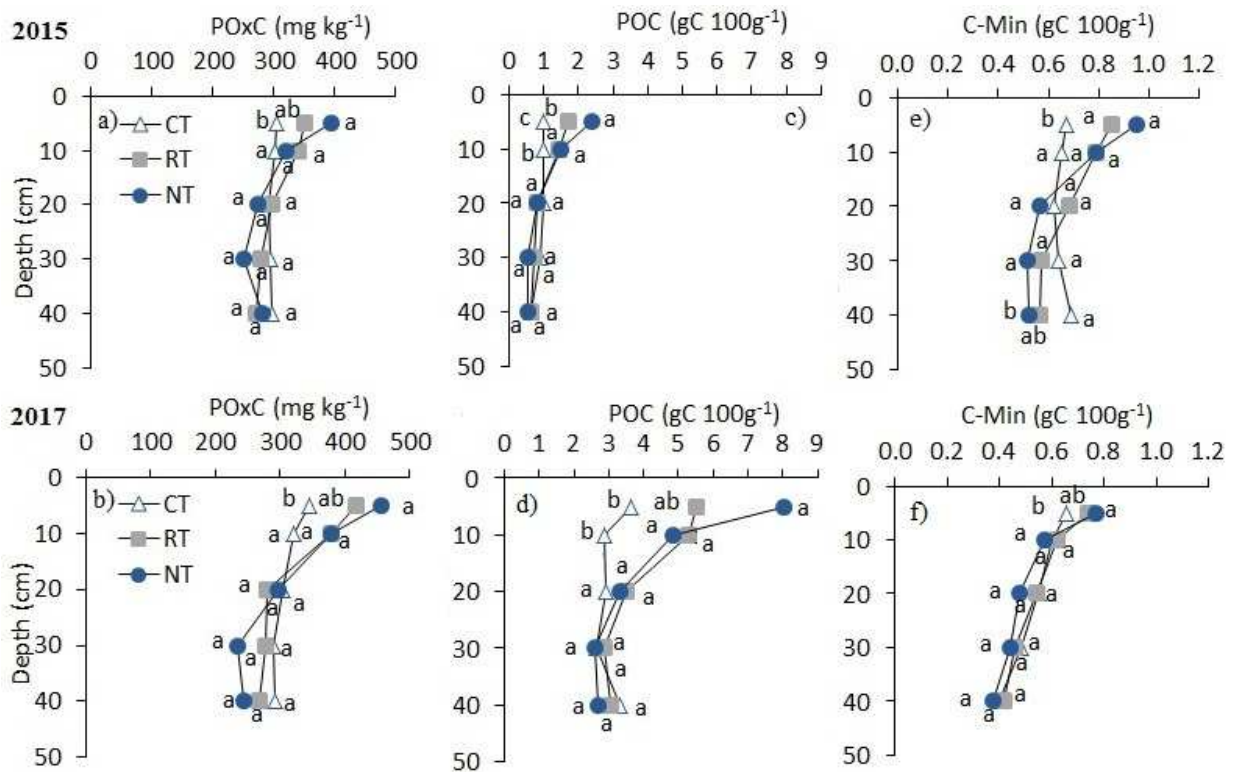
### 3.3 Soil C fractions influenced by the conversion of rainfed to irrigated land.

At the beginning and at the end of P3-I (2015 and 2017), POxC, POC and C-Min were significantly affected by the interaction between tillage system and soil depth. Also, in 2015 POC was significantly affected by N fertilization and tillage system as single effects, while POxC was affected by the interaction between tillage system and N fertilization. In 2017, POxC and C-Min were significantly affected by N fertilization, while POC was affected by the interaction between tillage system and N fertilization (Table 17). Soil POxC and POC increased from 2015 to 2017 in all depths. Differently, C-Min did not show differences between years. In 2015 and 2017 POxC was significantly affected by tillage systems at the soil surface (0–5 cm depth), being the values under NT greater than under CT with intermediate values in RT (Fig. 29a and 29b). Differently, POC was significantly affected by tillage systems at 0-5 and 5-10 cm depth in both years with a significant trend of decreasing POC when increasing tillage intensity (NT>RT>CT) (Fig. 29c and 29d). In 2015, C-Min was higher in NT and RT compared to CT at the soil surface (0-5 cm), while the contrary occurred at the deepest soil layer (30-40 cm) (Fig. 29e). Interestingly, in 2017 C-Min concentration only showed differences between tillage systems in the surface layer (0-5 cm), with greater C-Min under NT than CT and intermediate values in RT (Fig. 29f).

**Table 17.** 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.

| Year                          | 2015   |        |        | 2017   |        |        |
|-------------------------------|--------|--------|--------|--------|--------|--------|
| Source of variation           | POxC   | POC    | C- Min | POxC   | POC    | C- Min |
| <b>Tillage (Till)</b>         | ns     | <0.001 | ns     | ns     | ns     | ns     |
| <b>N fertilization (Fert)</b> | 0.01   | <0.001 | ns     | 0.04   | ns     | <0.001 |
| <b>Till*Fert</b>              | <0.001 | ns     | ns     | ns     | <0.001 | ns     |
| <b>Depth</b>                  | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| <b>Fert*Depth</b>             | ns     | ns     | ns     | ns     | ns     | ns     |
| <b>Till*Depth</b>             | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.01   |
| <b>Fert*Till*Depth</b>        | ns     | ns     | ns     | ns     | ns     | ns     |

ns; non-significant



**Fig. 29** Permanganate-oxidizable organic C (POxC) ( $\text{mg C kg}^{-1}$ ), 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  $P < 0.05$ .

## 4 Discussion

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 *et al.*, 2012), since SOC mainly results from the decomposition of biotic residues. Our results are fully consistent with this assumption, since C-inputs explained 70% of the variation in SOC sequestration rates both in P1-R and in P3-I. According to the C saturation concept, the response of SOC in relation to C-inputs depends on the amount of C that can be associated with clay and silt particles (Hassink, 1996; Hassink, 1997). Consequently, while the finer particles are not saturated with C a soil will have a further capacity to hold more C, protecting it in aggregates, provided that appropriate agricultural practices are carried out. In our study, SOC content at the beginning of the experiment (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 incorporation in RT and CT once the experiment began, would have favoured the increase of SOC in all three tillage systems. In our study, in P1-R  $\Delta\text{SOC}_{\text{rate}}$  was greater in NT (46%) compared to CT, while  $\Delta\text{SOC}_{\text{rate}}$  was greater under the high N application rate with respect to the control rate, as previously reported by Morell *et al.*, (2011). The last 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 soil compared to CT.

In P2-R,  $\Delta\text{SOC}_{\text{rate}}$  became 55% lower than in P1-R with an average value of  $222 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  and did not show differences between treatments, despite the fact that C-inputs were similar to P1-R. It is known that SOC sequestration is not an endless process (Powlson *et al.*, 2011). Therefore, if the amount of C-inputs returned to a soil is maintained over time, a new equilibrium on SOC will be reached depending on soil properties and environmental conditions (von Lützow *et al.*, 2006). For instance, in a NT chronosequence experiment formerly managed under CT in Mediterranean conditions, Álvaro-Fuentes *et al.*, 2014, observed that more than 75% of the total SOC sequestered was gained during the first 11 years after NT adoption, with the highest SOC sequestration rate during the first 5 years after NT adoption. From the 11<sup>th</sup> year onwards, the change in the annual rate of SOC sequestration decreased significantly and SOC reached a new equilibrium. Therefore, the greatest differences in SOC sequestration rates occur at the first years after NT adoption, while rates tend to converge after the first decade. That process would explain the strong decrease in SOC sequestration rate under NT and RT observed in our experiment in P2-R. Moreover, the greater SOC content under NT and RT at the beginning of P2-R could have

provided better conditions for soil biological processes enhancing mineralization of SOC (De Bona *et al.*, 2008).

In P3-I, the conversion from rainfed to irrigated land allowed the cultivation of a more productive summer crop such as maize resulting in higher amounts of crop residues returned to the soil and a mean SOC increase of  $969 \text{ kg C ha}^{-1} \text{ year}^{-1}$ . An 88% increase in SOC (0-40 cm depth) was found with the introduction of irrigated maize in NT with respect to the rainfed period (P1-R and P2-R). In southwestern Nebraska, Gillabel *et al.* (2007) reported that center-pivot sprinkler irrigated fields under a corn–winter wheat –soybean rotation stored more SOC than adjacent non-irrigated fields ( $33.01 \pm 0.88$  vs.  $26.64 \pm 1.06 \text{ Mg C ha}^{-1}$ ) (0-20 depth) and estimated a rate of SOC sequestered of  $0.19 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  during 33 years of irrigation. They attributed C storage could be linked to a greater C-input under irrigation. Similarly, Adviento-Borbe *et al.* (2007) showed an increment in SOC sequestration ( $440$  and  $662 \text{ kg ha}^{-1} \text{ yr}^{-1}$  for RT and CT, respectively of 0-30 cm depth) due to increased biomass inputs in two continuous maize systems, six years after the introduction of changes in the intensity of management in irrigated agroecosystems in Nebraska.

Interestingly, our study showed that the conversion from rainfed to irrigated land led to a strong increase of a labile soil carbon fraction such as POC. Particulate organic carbon was extremely sensitive to the conversion of rainfed to irrigated conditions with a 75% increase as an average of 0-40 cm soil depth. This fraction was identified as one of the most sensitive to changes in tillage and N fertilization management practices in Mediterranean soils (Plaza-Bonilla *et al.*, 2014). Labile soil C fractions such as POC play a key role in the formation of microaggregates and macroaggregates acting as binding agents (Elliott, 1986), allowing the persistence of SOC (Puget *et al.*, 2000). Conversion of rainfed into irrigated land may have important effects on soil aggregation, given the feedback mechanism between soil aggregation and SOC dynamics (Six *et al.*, 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), and the increase in soil microbial activity (Denef *et al.*, 2008). In addition, POC can be modulated by tillage, with an improvement in C-physical protection within aggregates when NT is used. Differently to POC, POxC showed a small 6% increase after the conversion from rainfed to irrigated conditions. This last fraction is usually related to a more active C cycle, which in turn, favours microbial activity (Haynes, 2005). Opposite to the more labile fractions, C-min remained stable given its recalcitrant nature. The C fractions studied were modulated by tillage, with a greater accumulation of POC, POxC and C-Min at the soil surface soil 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 of an enhancement of

POC. Tillage disrupts soil aggregation, reducing physical protection of POC occluded within aggregates (Grandy and Robertson, 2006) reducing the opportunity for SOC sequestration.

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 results of greater crop productivity under irrigation. Our data showed that under NT and RT the crop made a more efficient use of the nitrogen fertilizer applied compared to CT. The lower use of N under CT would be due to poor soil structure and surface crusting which reduced water availability limiting the response of the crop to N fertilization. The occurrence of soil crusting under CT reduced the infiltration of water into the soil profile compromising plantlet establishment (Pareja-Sánchez *et al.*, 2017). As a consequence of reduced water infiltration under CT crop growth was limited and C-inputs as crop residues were 32% lower than under NT at the highest N rate. In this line, Follett *et al.* (2013) suggested that increasing N fertilization rates may increase SOC stocks depending on soil depth considered and tillage practice. For example, Halvorson *et al.*, (2004) showed in irrigated continuous maize systems that rate of SOC sequestration (0–15 cm depth) under NT with high N fertilization rate was 1.4–2.0 Mg C ha<sup>-1</sup> yr<sup>-1</sup>. Meanwhile CT treatment that showed a rate of 0.2 Mg C ha<sup>-1</sup> yr<sup>-1</sup> even with about the same level of crop residues that was produced under the no-till treatment. Similarly, Follett *et al.* (2005) observed an increase in SOC under NT production systems with high N fertilization rate (1 and 1.9 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in the 0–15- and 15–30-cm depths respectively), compared to CT in the same rate (0.2 and 0.6 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in the 0–15- and 15–30-cm depths, respectively) under rotation systems wheat–maize in irrigated conditions in Mexico. They suggested that greater SOC sequestration under NT was due to higher aboveground crop-residue. In our study, no-till on N-fertilized maize systems can potentially increase SOC sequestration on large areas converted into irrigated under Mediterranean conditions.

## 5. Conclusion

This study shows that 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 soil water conservation compared to conventional tillage leading to higher C-inputs and allowing the response N applications increasing soil carbon sequestration rate. However, 13 years after the implementation of these practices, SOC sequestration rates were reduced from 492 to 222 kg C ha<sup>-1</sup> yr<sup>-1</sup>, as average of treatments. When Mediterranean rainfed lands are converted into irrigation an increase of SOC sequestration rates occurs reaching a value of 969 kg C ha<sup>-1</sup> yr<sup>-1</sup>

as an average of treatments. Water allows more biomass production and more C-inputs from crop residues. Main lever of SOC sequestration due to irrigation conversion is particulate organic carbon which shows a great sensitivity to irrigation conversion. If NT is used in this newly irrigated systems SOC sequestration rates can attain  $1959 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  applying high N fertilizer rates in comparison to  $731 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ , which would be attained when using conventional tillage.

Our results indicate that the introduction of irrigation and crop management practice based in NT and medium N fertilizer rate can contribute to the sequestration of significant amounts of atmospheric  $\text{CO}_2$ .

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# **General discussion**



## General discussion

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### *1. Contribution of tillage systems and N fertilization on soil quality and reduction of GHG emissions in newly irrigated Mediterranean agroecosystems.*

This study has demonstrated that tillage and N fertilization rate exert a significant impact on soil GHG emissions under Mediterranean agroecosystems recently converted to irrigation. In this work, previous soil management of the experiments had a great impact on the results obtained. Two decades of contrasting tillage systems under rainfed conditions led to different initial soil state before the conversion of the area into irrigation. Soil inversion with moldboard plough for the previous 21 years (CT) led to a deterioration of the soil physical properties. This worsening was due to a lower structural stability, causing soil surface crusting, which resulted in reduced water infiltration. Soil crusting in CT could be the consequence of aggregate breakdown due to tillage operations and lower SOC concentration at the soil surface compared to NT, which would have provided less resiliency to soil aggregates. In this line, *Álvaro-Fuentes et al.*, (2008) and *Plaza-Bonilla et al.*, (2013) showed that NT promotes soil aggregate stability by increasing the percentage of stable macroaggregates and the proportion of carbon in micro-aggregates compared to CT. Differently, NT for 21 years provided greater resilience to soil degradation and crust formation, enhancing water infiltration, with almost two-fold greater water infiltration in NT compared to CT.

The impact of tillage systems on soil structure combined with the use of N fertilization played a major role on crop productivity. When we considered the long-term maintenance of the different soil management systems (LTE experiment), the lack of enough water available in the long-term CT led to a decrease in maize grain yields compared to NT and RT, due to lower water infiltration (1.70, 2.40 and 3.14 mm h<sup>-1</sup> for CT, RT and NT, respectively). The same pattern happened in the maintenance of rates of N fertilization (medium and high), and in this case for maize crop the medium rate of 200 kg N ha<sup>-1</sup> increased maize aboveground biomass and grain yield, compared to the control (no N application), without further increases when applying 400 kg N ha<sup>-1</sup>. These data suggest that the N rate could be reduced by half without compromising maize grain yield. Moreover, that reduction would decrease the risk of N losses such as N<sub>2</sub>O emissions to atmosphere. Furthermore, the application of high rates of nitrogen under CT leads to a greater

content of mineral N in the soil than under NT or RT. This result would be explained by lower N uptake maize biomass in CT causing lower NUE. When we considered the return to intensive tillage after several long-term no tillage (STE), maize grain yields and yield components did not show differences between soil management treatments. Likewise, 3 years of CT after 20 year of no-till are not enough to break down the soil structure to affect negatively the crop performance.

Under these circumstances, the impact of tillage systems on soil structure combined with the N fertilization rate played a major role on GHG emissions. In this regard, high N fertilization rate combined with NT system had the largest impact on CO<sub>2</sub> emission compared to CT at the same rate of N fertilization. In this work, the cumulative CO<sub>2</sub> emissions in NT were 37% greater compared to CT (3856 vs. 2824 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). Meanwhile, NT appeared with the averaged increase of SOC: 80% (in the 0–40 cm layer) higher in NT than in CT (1255 vs. 698 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). These data show that in spite of the higher CO<sub>2</sub> emissions in NT, these are compensated by its high capacity of C sequestration. The use of high rate of N fertilization also leads to greater aboveground biomass and consequently greater SOC susceptible to be mineralized and therefore greater CO<sub>2</sub> emissions under NT or RT. In turn, it is expected that the increase in SOC and the concomitant improvement of soil biological activity in NT result in higher soil CO<sub>2</sub> emissions to the atmosphere (Reicosky, 2007).

With respect to the CH<sub>4</sub>, the soil acted as a net sink of CH<sub>4</sub> in all treatments, being greater accumulation the oxidation of CH<sub>4</sub> under RT and NT than CT. Methane is oxidized by bacteria in the aerobic surface layer of soils. Since these bacteria are sensitive to disturbance, NT or RT practices may be beneficial to the capacity of soils to oxidize CH<sub>4</sub> (Hütsch, 2001).

In this study the application of N was an important contributor of N<sub>2</sub>O emission, being in agreement with those of previous studies in irrigated Mediterranean areas (Álvaro-Fuentes *et al.*, 2016). The present work demonstrates the effect of N fertilizer on N<sub>2</sub>O emissions in Mediterranean irrigated areas is determined by soil tillage. The different tillage systems studied influenced N<sub>2</sub>O emissions through variations in the WFPS and mineral nitrogen content, which play a substantial role in N<sub>2</sub>O emissions, by influencing microbial activity and the behavior of water in the soil matrix (Rees *et al.*, 2013). Regarding this, nitrification rather denitrification has been demonstrated to be the main source of N<sub>2</sub>O production in Mediterranean soil, even in irrigated fields (Guardia *et al.*, 2018). Our study confirms this fact, since it likely that a fast nitrification of the ammonium to nitrate could have been the main N<sub>2</sub>O production process which is justified by the low levels of soil NH<sub>4</sub><sup>+</sup> and the low WFPS, especially in CT and RT treatments.

Differently, under NT, in some periods, denitrification could have also produced N<sub>2</sub>O emissions due to the higher WFPS as observed by other authors (Venterea *et al.*, 2005). This last assumption would be supported by the greater denitrification potential of NT treatment compared CT and RT observed in the study.

In all three maize growing seasons, the greatest cumulative soil N<sub>2</sub>O emissions were obtained with the highest N rates and declined as the rate of N decreased. Therefore, less N fertilizer applied in low rates could result in less cumulative N<sub>2</sub>O emissions leading to a lower EF. Our results in the Ebro valley showed that the EF (range from 0.09%- 0.33%) was much lower than in other Mediterranean areas observed in a meta-analysis of the N<sub>2</sub>O emissions by Cayuela *et al.* (2017). These differences could be due to several causes as soil texture or management of irrigation which influenced in the emissions. Therefore, the results of this work like those of Cayuela *et al.* (2017) confirm that the IPCC default EF often overestimates the emissions of N<sub>2</sub>O in Mediterranean areas.

## 2. Effect of rainfed land conversion to irrigation on greenhouse gas emissions.

Conversion from rainfed land to irrigation may lead to increases of GHG emissions (Aguilera *et al.*, 2013). This transformation causes an increase in water available in the soil, which allows the cultivation of more productive summer crops such as maize which requires high rates of N fertilization. Both irrigation and N fertilization increase crop total biomass production, with higher crop residue amounts than under rainfed conditions, resulting in SOC increase (Khan *et al.* 2007). More available water can accelerate the decomposition of soil organic matter due to the impact of moisture on the activity of microorganisms (Almagro *et al.*, 2009).

In our work, irrigation increased the amount of crop residues incorporated to the soil impacting soil CO<sub>2</sub> emissions as other studies have described after irrigation (Mariko *et al.*, 2007) or a period of precipitation (Tenesaca and Al-Kaisi, 2015). Greater levels of SOC and the application of N fertilizers could also lead greater N<sub>2</sub>O emissions by nitrification and denitrification. In this regard, in the previous barley rainfed conditions of the same experimental field, cumulative annual N<sub>2</sub>O emissions were 34% lower (Plaza-Bonilla *et al.*, 2014 a), than the values found in our study in irrigated conditions. These data suggest that the increase in soil moisture and the use of higher N fertilization rates when transforming to irrigation, where the main causes behind the increase in N<sub>2</sub>O emissions. However, these emissions can be compensated with SOC sequestration. In our experiment N<sub>2</sub>O emissions corresponded to 167 kg CO<sub>2</sub> equivalent ha<sup>-1</sup> year<sup>-1</sup>, as an average of treatments and years, while SOC sequestration



amounted 3553 kg CO<sub>2</sub> equivalent ha<sup>-1</sup> year<sup>-1</sup>, in irrigated period. Although the use of N fertilizer would continue over time according to crop needs, the greater emission of N<sub>2</sub>O is probably compensated with the SOC sequestration as our data showed.

Conversion from rainfed land to irrigation decreased methane uptake by soil. While average CH<sub>4</sub> uptake was -1.25 kg CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> under rainfed barley production (Plaza-Bonilla *et al.*, 2014 b) it only reached an average value of -0.60 kg CH<sub>4</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> under irrigated conditions. One possible cause explaining the decrease in soil CH<sub>4</sub> uptake under irrigated conditions would be the increase in soil water content, which would have reduced soil air-filled porosity, partially restricting the activity of methanotrophs.

Besides in the conversion from rainfed land to irrigation, it is important to highlight the impact on yield-scaled N<sub>2</sub>O emissions. In this work, an increase in the N fertilizer rate led to an increase in the yield-scaled N<sub>2</sub>O emissions in the first out three years, although a similar trend was observed in the subsequent two years. But, as explained before, similar yields were obtained with both 400 kg N ha<sup>-1</sup> and 200 kg N ha<sup>-1</sup>. These results suggest that optimal N rates can produce maximum yields while reducing annual yield-scaled emissions by 40%. Moreover, although the yield-scaled N<sub>2</sub>O emissions did not differ significantly between tillage treatments, a marked trend existed in the rates found between tillage systems, in the order CT > RT > NT. These results suggest that although cumulative GHG emissions under CT are lower, the reduction in crop yield in CT led to an increase in yield-scaled N<sub>2</sub>O emissions compared to NT.

### *3. Identification of the best agricultural practices for SOC sequestration after conversion of a rainfed land into irrigated.*

Time series analyses should allow an accurate estimation of the rate and duration of SOC sequestration and provide clues to understand its determinism (Dimassi *et al.*, 2014). Our work demonstrates that under Mediterranean conditions, there is potential for SOC sequestration when there are changes in management practices. At the beginning of the long-term experiment the traditional practices of the area consisted of intensive tillage and crop residue removal. In the first period (1996 to 2009) under rainfed a moderate rate of N fertilization and the specially the maintenance of crop residues in the soil surface under NT, have favored the increase of SOC. Therefore, during this period there were significant differences between treatments on SOC sequestration (394, 508 and 574 kg C ha<sup>-1</sup> yr<sup>-1</sup> for CT, RT and NT respectively and 358, 509 and 610 kg C ha<sup>-1</sup> yr<sup>-1</sup> for 0, Medium and Hight, respectively). In a second period (2009-2015) under rainfed conditions also there was still SOC sequestration, the increase became 55% lower (with an

average value of 222 kg C ha<sup>-1</sup> yr<sup>-1</sup>) suggestion a reduction in the sequestration rate reaching a certain equilibrium condition and was not showed differences between treatments. The third period (2015-2017), new management practices such as the implementation of irrigation had led to an increase in SOC with an average value of 969 kg C ha<sup>-1</sup> year<sup>-1</sup>. In this irrigated period, N fertilization rate played an important role on SOC sequestration, since high N rates combined with NT led to increase SOC sequestration. This was due to increased C-inputs as result of greater crop productivity under irrigation. However, under CT the lack of enough water available for the production of biomass, due to soil surface crusting, generated minor C-inputs leading to lower SOC sequestration compared to NT or RT. Clearly this study showed that the amount of C-input was the main factor determining the changes in SOC stocks, which explained 70% of the observed differences in SOC sequestration.

Interestingly, this study showed that the conversion from rainfed to irrigated land led to a strong increase of a labile soil carbon fraction such as POC. Particulate organic carbon was extremely sensitive to the conversion of rainfed to irrigated conditions with a 75% increase as an average of 0-40 cm soil depth. Labile soil C fractions such as POC play a key role in the formation of microaggregates and macroaggregates acting as binding agents (Elliott, 1986), allowing the persistence of SOC. In addition, POC can be modulated by tillage, with an improvement in C-physical protection within aggregates when NT is used.

Therefore, in these systems transformed to irrigated land, a N fertilization rate adequate to crop needs together with a reduction in tillage system are promising practices, not only because of the impact on crop yields but also because of the impact of GHG emissions, soil protection and C sequestration. If some problems arise under the implementation of no-tillage, such as those related to crop residue management and/or soil compactation in the planting row, the implementation of new strategies of reduced tillage as strip-tillage would be a key alternative.

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## **General conclusions**



## General conclusions

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Based on the research objectives described, the conclusions obtained in this thesis are the following:

1. Long-term (21 years) intensive tillage led to soil surface crusting reducing the infiltration of irrigation water. As a consequence, crop water stress occurred under this tillage treatment, causing lower maize biomass and grain yields and reducing water and nitrogen use efficiency, compared to reduced and no-tillage systems.
2. The main process behind soil crusting under intensive tillage was the lower water-stability of aggregates. Differences in the stability of aggregates between tillage treatments were explained by different SOC levels as a result of long-term (21 years) use of contrasted tillage systems during the previous rainfed conditions.
3. By contrast, if the soil presented a long history (21 years) of continued management under no-tillage before the transformation from rainfed to irrigation, soil structural degradation was minor since, no-tillage provides higher resilience to soil crusting.
4. The traditional application of rates  $400 \text{ kg N ha}^{-1}$  did not bring improvements in grain yield, WUE and NUE compared to rates of  $200 \text{ kg N ha}^{-1}$ .
5. The use of no-tillage with high rates of N resulted in greater  $\text{N}_2\text{O}$  and  $\text{CO}_2$  fluxes compared to conventional tillage, as result of enhanced maize growth promoting root respiration and greater soil organic C availability to decomposers.
6. The increase of soil WFPS under no-tillage had a major effect on emission rates especially when combined with the high rate of N fertilization that increased soil mineral N.
7. In this cropping system and climate regime, the mean  $\text{N}_2\text{O}$  EF measured was 0.19%, much lower than the 1% factor currently default by the IPCC.
8. The yield-scaled  $\text{N}_2\text{O}$  emissions did not differ significantly between tillage treatments and were increased by the use of a high N rate only in the first out of three years of study.



9. The soil acted as a CH<sub>4</sub> sink in all treatments. However, CH<sub>4</sub> oxidation was lower in conventional tillage compared with reduced tillage and no-tillage, which could be the result of the effect of tillage on gas diffusivity. Moreover, CH<sub>4</sub> uptake decreased after the transformation into irrigation, as a result of the wetter soil conditions which could have affected the methanotrophic communities by reducing soil CH<sub>4</sub> oxidation

10. In the rainfed Mediterranean conditions the use of no-tillage increases soil water conservation compared to conventional tillage leading to higher C-inputs and allowing the response N applications increasing SOC sequestration rate. However, 13 years after the implementation of these practices, SOC sequestration rates were reduced from 492 to 222 kg C ha<sup>-1</sup> yr<sup>-1</sup>, as average of treatments.

11. When Mediterranean rainfed lands are converted into irrigation an increase of SOC sequestration rates occurs reaching a value of 969 kg C ha<sup>-1</sup> yr<sup>-1</sup> as an average of treatments, showing a great sensitivity to irrigation conversion the particulate organic carbon.

12. In this newly irrigated systems, if no-tillage is used, SOC sequestration rates can attain 1959 kg C ha<sup>-1</sup> yr<sup>-1</sup> applying high N fertilizer rates in comparison to 731 kg C ha<sup>-1</sup> yr<sup>-1</sup>, which would be attained when using conventional tillage.

Based on the results obtained in this thesis, it can be concluded that in Mediterranean agroecosystems recently transformed into irrigation the reduction in tillage intensity and N fertilization rates, are adequate strategies maintain or even increase crop productivity. Although the reduction in tillage can lead to higher emissions of N<sub>2</sub>O and CO<sub>2</sub> from the soil to the atmosphere, these are compensated by a greater yield and higher soil organic carbon sequestration rates.



