Universitat de Lleida

Soil organic carbon dynamics and carbon sequestration in a semiarid Mediterranean agroecosystem: effects of conservation tillage and nitrogen fertilization

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TESIS DOCTORAL

Soil organic carbon dynamics and carbon sequestration in a semiarid Mediterranean agroecosystem: effects of conservation tillage and nitrogen fertilization

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SUMMARY

Soil organic carbon (SOC) is the largest terrestrial reservoir of carbon. The balance between C inputs (from plant residues) and C outputs (mainly as CO_2 from SOC decomposition) determines the content of SOC. Under agricultural systems, this balance is also affected by agronomical practices. Under semiarid Mediterranean agroecosystems, water limitation restrains plant growth and the return of crop residues to the soil. Under these conditions, SOC content is moreover negatively affected by high decomposition rates due to high temperatures, however limited by water availability. Alternative agronomical practices improving water conservation and water use efficiency, such as conservation tillage systems and crop nutrition, may improve crop growth and increase return of crop residue (C inputs) under these systems. However the actual response of SOC content will depend on its balance with C outputs.

This work studied the effects of long term adoption of tillage practices (NT, no-tillage; MT, minimum tillage; CT, conventional tillage) and nitrogen (N) fertilization level (zero; medium, 60 kg N ha^{-1}; high, 120 kg N ha^{-1}) on the SOC balance and the content of SOC. Below- and above-ground C inputs were quantified during three growing seasons, 10 to 13 years after the establishment of the experiment. During the same period, soil CO_2 flux was evaluated, together with the assessment of its components and the determination of C outputs. During the study period, C inputs were increased under NT and with medium and high N fertilization. Meanwhile C outputs were increased under NT, but less affected by N fertilization. Determination of the stock of SOC at the end of the evaluation period suggests that increased C outputs under NT were offset with increased C inputs, allowing the content of SOC to increase by 4.3 and 3.9 Mg C ha^{-1} in comparison to MT and CT respectively. Long-term medium and high N fertilization increased the stock of SOC by 3.4 and 4.5 Mg C ha^{-1} in contrast to unfertilized plots. Long-term adoption of conservation tillage practices (no-tillage) together with adequate N fertilizer use, proved to be effective tools to improve sustainability of semiarid Mediterranean drylands and to store C in the soil.

RESUMEN

El carbono orgánico del suelo (SOC) es el mayor depósito terrestre de C. El balance entre la entrada de C (de los residuos vegetales) y salidas de C (principalmente como CO_2 de la descomposición del SOC), determina el contenido de SOC. En los sistemas agrícolas el balance también está afectado por las prácticas agronómicas. En agroecosistemas semiáridos Mediterráneos, el agua es el principal factor limitante del crecimiento del cultivo y de la entrada de residuos en el suelo. Bajo estas condiciones, el contenido de SOC está además negativamente afectado por elevadas tasas de descomposición debido a las altas temperaturas, aunque limitadas por la disponibilidad de agua. Las prácticas agronómicas alternativas que mejoren la conservación del agua y la eficiencia del uso del agua, como el laboreo de conservación y la nutrición del cultivo, pueden mejorar el crecimiento vegetal y aumentar la cantidad de residuos (entrada de C) en estos sistemas. Sin embargo la respuesta del contenido de SOC dependerá del balance de las entradas con las salidas de C.

Este trabajo estudió los efectos de la adopción a largo plazo de sistemas de laboreo (NT, no-laboreo; MT, laboreo minimo; CT, laboreo convencional) y del nivel de fertilización nitrogenada (cero; medio, 60 kg N ha^{-1}; alto, 120 kg N ha^{-1}) en el balance de C del suelo y el contenido de SOC. Se cuantificaron las entradas de C en los residuos de la parte aérea y subterránea del cultivo durante tres ciclos de cultivo, entre 10 y 13 años después del establecimiento del experimento. Durante este mismo período, se evaluó el flujo de CO_2 del suelo, junto con el estudio de sus componentes y la determinación de las salidas de C. Durante el período de estudio, las entradas de C aumentaron bajo NT y con niveles medios y altos de fertilización nitrogenada. Simultáneamente las salidas de C aumentaron bajo NT y fueron ligeramente afectadas por la fertilización nitrogenada. La determinación del contenido de SOC al final del período de estudio, sugiere que el aumento de las salidas de C fue compensado con mayores entradas de C, permitiendo que el contenido de SOC aumentara en 4.3 y 3.9 Mg C ha^{-1} bajo NT con respecto a MT y CT. Niveles medios y altos de fertilización nitrogenada aumentaron el contenido de SOC en 3.4 y 4.5 Mg C ha^{-1} con respecto al contenido en las parcelas no fertilizadas. La adopción a largo plazo de prácticas de laboreo de conservación (no-laboreo o siembra directa), junto con el uso adecuado de la fertilització n nitrogenada demostraron ser herramientas para mejorar la sostenibilidad de los secanos semiáridos Mediterráneos, y almacenar C en el suelo.

RESUM

El carboni orgànic del sòl (SOC) és el depòsit de C més voluminós a la superfície terrestre. El balanç entre la entrada de C (dels residus vegetals) i sortides de C (principalment com CO_2 de la descomposició del SOC), determina el contingut de SOC. Als sistemes agrícoles el balanç també està afectat per les pràctiques agronòmiques. Als agroecosistemes semiàrids Mediterránis, l'aigua és el principal factor limitant del creixement del cultiu i de l´entrada de residus al sòl. Sota aquestes condicions, el contingut de SOC està a mes a mes negativament afectat per altes taxes de descomposició degut a las altes temperatures, tot i que limitat per la disponibilitat d'aigua. Les pràctiques agronòmiques alternatives que milloren la conservació de l'aigua i l'eficiència de l'ús de l'aigua, com ara el conreu de conservació i la nutrició del cultiu, poden millorar el creixement vegetal i augmentar la quantitat de residus (entrada de C) en aquestos sistemes. No obstant això la resposta del contingut de SOC dependrà del balanç de les entrades amb les sortides de C.

Aquest treball estudia els efectes de l'adopció a llarg termini de sistemes de conreu (NT, sembra directa; MT, mínim conreu CT, conreu convencional) i del nivell de fertilizació nitrogenada (zero; mitjà, 60 kg N ha^{-1}; alt, 120 kg N ha^{-1}) al balanç de C del sòl i el contingut de SOC. Es van quantificar les entrades de C en els residus del cultiu (foliar i radicular) durant tres cicles de cultiu, entre 10 i 13 anys després del establiment de l'experiment. Durant aquest període, es va avaluar el flux de CO_2 del sòl, juntament amb l'estudi dels seus components i la determinació de les sortides de C. Durant el període de estudi, les entrades de C van augmentar amb NT i amb nivells mitjos i alts de fertilització nitrogenada. Simultàniament les sortides de C van augmentar amb NT i van ser lleugerament afectades per la fertilització nitrogenada. La determinació del contingut de SOC al final del període d´estudi, suggereix que l´augment de les entrades C inputs estigués compensat amb majors entrades de C, permetent que el contingut de SOC augmentara finalment 4.3 i 3.9 Mg C ha^{-1} sota NT repecte a MT i CT. Nivells mitjans i alts de fertilització nitrogenada augmentaren el contingut de SOC en 3.4 i 4.5 Mg C ha^{-1} respecte al contingut a les parcel•les no fertilitzades. L´adopció a llarg termini de pràctiques de conreu de conservació (sembra directa), juntament amb l'ús adequat de la fertilització nitrogenada van demostrar ser eines per a millorar la sostenibilitat dels nostres secans, i emmagatzemar C al sòl.

ACKNOWLEDGEMENTS

To the Spanish Ministry of Science and Innovation, Comision Interministerial de Ciencia y Tecnologia of Spain (Grants AGL 2004-07763-C02-02 and AGL2007-66320-CO2-02/AGR), for sponsoring a grant for my doctorate studies.

To the people in the laboratory of extensive crops, at the Departament de Producció Vegetal i Ciència Forestal, of the University of Lleida: The field and laboratory technicians Carlos Cortes and Silvia Marti; undergraduate and master students, Dani Plaza, Josep Margalef, Jordi Tugues, Albert Masgoret, Xavier Penella, Cristina Maján and Judit Moncunill. As well as Sebastián Cela, the other phD student at the lab during that period of time, for the so much time and debates, and for his friendship.

To the farmers in the area, Albareda family and Josep Mª Penella, as well as Jaume Gregori from the regional extension service (Oficina Comarcal de lUrgell, DARP), for their visits to the field and support.

To Keith Parkinson, from PPSystems, Hitchin. For welcoming in his headquarters in October 2008 and for technical advices.

To the people at IRNAS (CSIC, Sevilla), Engracia Madejón, José Manuel Murillo, Sebastiana Melero, Rosa López Garrido, and Félix Moreno, for wellcoming and support for the study of soil biochemical properties.

To the people at the Soil Science department at Rothamsted Research, UK, with especial thanks to Andy Whitmore, for his collaboration to the research in this thesis, and support at all times.

And, to my advisors, Carlos Cantero-Martínez, Jorge Álvaro-Fuentes, and Jorge Lampurlanés, who were constantly next to me.

This thesis is dedicated to Maria Jesús, who enjoyed this research as part of our life together.

INDICE

INTRODUCCION

1. Crop management in rainfed Mediterranean agroecosystems

Under semiarid Mediterranean climate, rainfed agriculture is usually water limited. Water limitation reduces crop growth and grain yield. Yield varies markedly from year to year depending on the amount and distribution of precipitation, which are both highly variable (Austin *et al*., 1998). Therefore, in order to increase both the productivity and the sustainability of these agroecosystems it is essential to capture the water from precipitation into the soil and to use it efficiently for optimum yield production. Thus, in these areas, the design of adequate agronomical practices, such as conservation tillage and adequate nutrient management, is a key priority.

Conservation tillage system, including no-tillage (NT) and minimum tillage (MT) systems, maintains at least 30% of the soil surface covered after planting to reduce water erosion (Blevins and Frye, 1993). Under semiarid conditions, conservation tillage systems improve water capture through increased water infiltration and reduced evaporation of the water in the soil (Unger and McCalla, 1980; Loomis and Connor, 1992; Peterson *et al*, 2006). Improved water conservation is explained by the protective effect of crop residues left on soil surface, which protect from soil water losses. In Mediterranean Spain, several studies have observed not only beneficial effect of NT and MT on soil water conservation but also on water use efficiency (Moreno *et al*., 1997; Lampurlanés *et al*., 2002; Cantero-Martínez *et al*., 2007; Moret *et al*., 2007). Furthermore, in semiarid areas, conservation tillage has been recognized as a suitable system to increase soil organic carbon (SOC; Peterson *et al*., 1998; Halvorson *et al*., 2002). Increasing SOC stocks is associated with improvements in soil quality, productivity and positive environmental effects through atmospheric carbon (C) sequestration (Paustian *et al*., 1997; Lal, 2008).

Nitrogen (N) fertilization is usually the second key factor for crop yield under semiarid Mediterranean conditions. Nitrogen fertilization can help to increase water productivity by stimulating dry matter production and improving transpiration efficiency (Loomis and Connor, 1992). However, limited water availability reduces the response to N fertilizer addition (Ryan *et al*, 2009). It is important to mention that N fertilization implies an elevated cost, which has a direct effect on farm benefit. Furthermore, excessive N fertilization can lead to negative environmental effects such as subsurface water contamination by nitrates or the contribution to global warming by increasing the emission of certain greenhouse gasses (Del Grosso *et al*., 2000; Delgado *et al*., 2005). Consequently, N application rates should reach equilibrium among cost, environment and crop productivity.

Water limitation under semiarid Mediterranean conditions restricts crop growth and yields and also crop response to N fertilization. In the transition from intensive tillage to conservation tillage systems, the increase in soil water content may allow for increased uptake of soil nutrients by the crop. Moreover, conservation tillage practices may affect soil N dynamics increasing N immobilization in the soil organic matter and, thus, reducing mineral N available for crop growth (Thompson, 1992). For this reason, conservation tillage systems could increase the crop response to N fertilization in the long-term (Angás *et al*., 2006). However, to date, in Mediterranean dryland conditions, little information exists about the interactive long-term effects of tillage and N fertilization on crop growth and yield.

In semiarid Mediterranean conditions of NE Spain and during the early (i.e., 1-3 years) adoption of tillage and N fertilization, previous studies showed no significant interaction between both factors (Angás *et al*., 2006, Cantero-Martínez *et al*., 2003). However, as commented previously, the interaction is expected to occur in the long-term due to several factors. Conservation tillage systems are expected to improve soil physical and chemical quality. Improvements in soil aggregate stability, soil hydraulic properties and soil fertility have been reported in dryland conditions (e.g., Moret *et al*., 2007; Bescansa *et al*., 2006; Álvaro-Fuentes *et al*., 2007). However, soil strength could be increased under conservation tillage system (Lampurlanés and Cantero-Martínez, 2003). Such increase in soil strength may negatively affect root growth, reducing crop access to soil resources (Pabin *et al*., 1998).

In semiarid northeast Spain precipitation pattern is highly variable and there is a high probability (25%) of low rainfall in the spring (< 50 mm). In this area, as in other Mediterranean agroecosystems, soils have low content of SOC due to high mineralization rates, reduced return of crop residues due to water scarcity, and intensive tillage systems that have been used for decades or even centuries.

Under these rainfed conditions, barley (*Hordeum vulgare*, L.) monoculture is the most extended cropping system, since rotations with leguminous or other crops have been demonstrated not to be feasible because of the lack of economical benefits (Álvaro-Fuentes *et al*., 2009). In some parts of the region, conservation tillage systems have been adopted over 80% of the surface for more than 25 years. However, there are still others parts where the adoption of conservation systems is almost nil (Agracon, personal communication). Furthermore, in rainfed Mediterranean areas, it has been a common use excessive amounts of N fertilizer for barley copping (Cantero-Martínez *et al*., 2003). As commented before, excessive N additions negatively affect the economical benefit of the farms and they can lead to negative environmental effects. Therefore, under rainfed Mediterranean conditions, long-term experiments are essential to determine the effects tillage and N fertilization not only on crop productivity but also on the environmental effects associated to these practices.

2. Soil organic matter and soil organic carbon

Soil organic matter (SOM) is a key parameter for soil quality and productivity (Karlen *et al*. 1994). Furthermore, the stock of carbon in SOM represents the largest terrestrial reservoir of C (Lal and Kimble, 1997). Increasing soil organic carbon (SOC) stocks implies the removal of atmospheric CO_2 throughout the fixation of this CO_2 during the photosynthesis process and the incorporation of this atmospheric C into the soil as crop residues. This process is also known as C sequestration.

SOC content is the result of the balance between C inputs and C outputs which is regulated by soil microbial biomass (Fig. I.1). In agroecosystems, C inputs into the soil are mostly coming from crop residues (i.e., above- and below-ground) and rhizodepositions. Contrary, C outputs from the soil come mostly from the mineralization of SOC to CO_2 which emerges from the soil together with the CO_2 coming from autotrophic respiration (root respiration) in a process called soil respiration or soil CO_2 flux. The quantification of C inputs (i.e., crop residues), C outputs (i.e., soil CO_2 flux and its components), and the content of SOC are essential to understand SOC dynamics and to provide reliable assessments of changes on the stock of SOC. Soil microbial biomass is a pool of SOC but it is also an active driver of SOC dynamics highly responsive to management practices (Fig. I.1).

Adequate management practices on croplands, such as conservation tillage systems and adequate nutrient supply can sequester SOC about 0.74 to 1 Pg C per year worldwide (Bruce *et al*., 1999). These sequestration rates vary according to different determining factors, such as: climate, soil type and residue quality (Paul *et al*., 1999). Conservation tillage promotes lower decomposition rates. The reduction in tillage intensity slows down the incorporation of residues at deeper layers of the soil. Furthermore, it reduces aggregation turnover leading to the formation of greater number of stable soil aggregates, which physically protect SOC to microbial accessibility (Six *et al*., 1999). Besides conservation tillage can increase SOC stocks due to the increase in crop growth and thus C inputs. Under semiarid conditions, which are characterized by the presence of water limitation periods for crop growth, conservation tillage systems improve water use efficiency (Cantero-Martínez *et al*., 2007). As a response, the amount of biomass and yield produced per unit of water is increased, as well as the amount of crop residues, and hence C inputs.

At the same time, adequate nutrient supply can also increase C inputs and SOC stocks. N addition is a key factor for crop growth. However, the effects of N addition on SOC stocks are not clear (Alvarez, 2005; Khan *et al*., 2007). Alvarez (2005) reviewed the effects of N on SOC sequestration, compiling published data from more than one hundred sites around the world. This review showed the disparity in the responses of SOC to N fertilization. Two significant conclusions of this study were that the response of SOC to N fertilization was site-specific and also that the increase in C inputs due to higher N rates resulted in SOC storage. However, some studies have reported no effect of N applications on SOC, even when measuring increases in C inputs (Halvorson *et al*., 2002; Poirier *et al*., 2009).

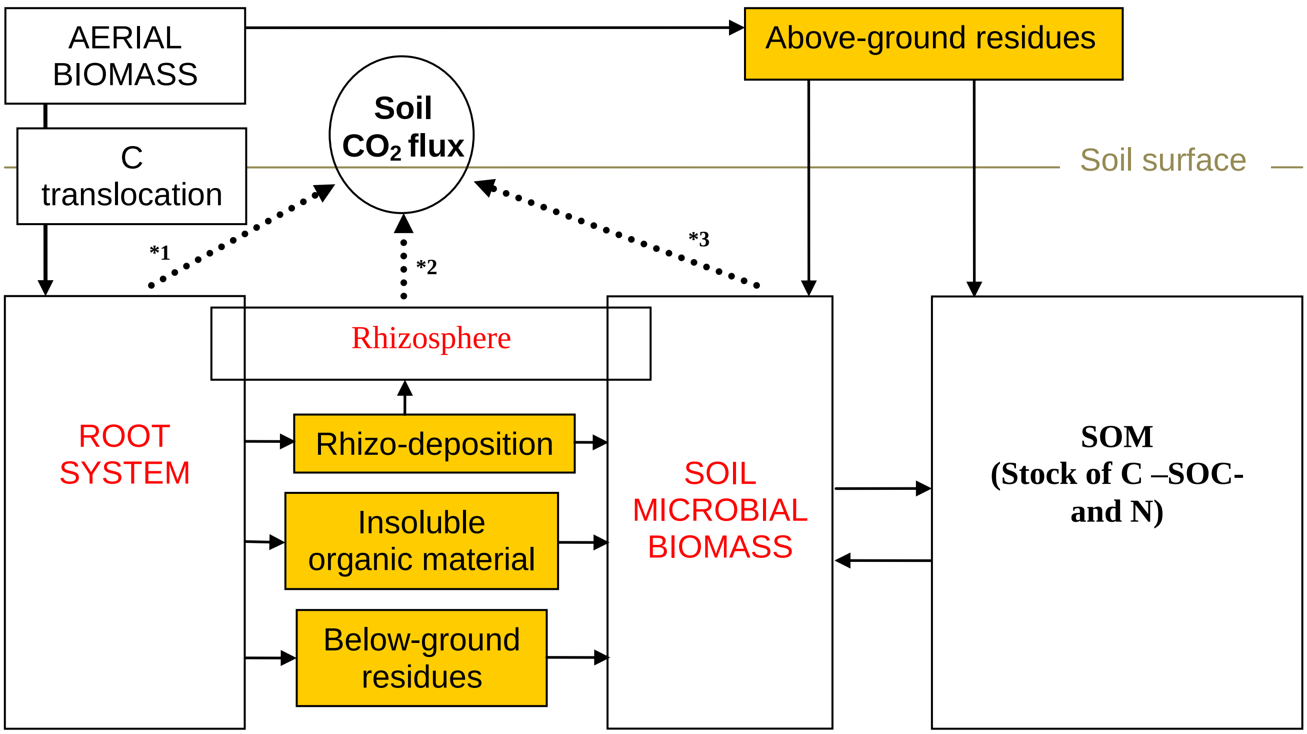

Figure I.1. Schematic representation of C inputs (in yellow boxes) and sources of CO_2 (in red letters) of soil CO_2 flux. Root tissue respiration (autotrophic respiration) (^{*1}), respiration of immediately available substances from mycorrhiza and rhizosphere (^{*2}), basal respiration of microbial decomposition of SOM (^{*3}), adapted from (Lambers, 1987; Smucker, 1984).

Mediterranean semiarid agroecosystems are water-limited environments with crop yields restrictions. Reduced crop growth results in a low return of crop residues into the soil and thus low C input and SOC content and soil fertility (Lal, 2004). As commented before, conservation tillage can increase crop biomass due to greater water conservation and water use efficiency by crop compared to intensive tillage. In northeast Spain, Cantero-Martínez *et al*. (2007) in an analysis with 15 years of data observed about 5-15% increase in crop yield in conservation tillage systems compared to intensive tillage. This increase in crop yield has reported increases in SOC stocks, particularly in soil surface, as observed in several experiments carried out in Spanish Mediterranean conditions (e.g., Moreno *et al*., 2006; Virto *et al*., 2007; Álvaro-Fuentes *et al*., 2008; Hernanz *et al*., 2009; Melero *et al*., 2009). In a national meta-analysis, Álvaro-Fuentes and Cantero-Martínez (2010) estimated that under a hypothetical scenario with the entire Mediterranean rainfed Spain under NT would offset the 17% of the total CO_2 equivalent emissions generated from agricultural soils in Spain.

In rainfed Mediterranean Spain, despite the effects of tillage on SOC dynamics have been extensively studied, little information exists about the effects of N fertilization. The effects of N fertilization on crop growth have been reported for different regions of Mediterranean Spain (e.g., in northeast Spain, Cantero-Martínez *et al*., 2003, and Pardo *et al*., 2009; in Andalucia, López-Bellido *et al*., 2000). In all these experiments, the increase of N fertilization resulted in an increase in crop growth and yields. However, the effects of N addition on SOC content have been scarcely studied. López-Bellido *et al*. (2010), in a vertisol located in Andalucia, showed no effect of N fertilization on SOC stocks. This study reported no significant interaction between tillage system and N fertilization.

In northeast Spain, no information exists about the interaction of tillage and N fertilization on SOC dynamics. The study carried out by Angás and Cantero-Martínez (Cantero-Martínez *et al*., 2003; Angás *et al*., 2006), in three locations of the Ebro river valley: Agramunt and Guissona (Lleida) and Candasnos (Huesca), showed positive effects of conservation tillage systems and N on crop growth during the three first years after the establishment of the experiment. However the interaction between these two factors was not clear because of the earliness of the experiment (i.e., 1-3 years after establishment). In this same experiment, a simulation study carried out with the CERES model, predicted that long-term interaction response was expected to occur (Cantero-Martínez *et al*., 2008). This experiment location in Agramunt was chosen for a long-term study, since it represents a medium soil-climate condition highly representative of the Mediterranean dryland agroecosystems. This thesis was carried out in this same tillage and N fertilization experiment located in Agramunt (Lleida) from year 10 to 13 after its establishment.

OBJECTIVES

The main objective of this thesis was to study the long-term effect of N fertilization and soil management on the C input/output dynamics in the soil - crop system under semiarid Mediterranean conditions. According to this objective, the main hypotheses of this thesis are that conservation tillage systems and N fertilization:

(i) increase C inputs into the soil due to an increase of the water use efficiency;

(ii) modify the CO_2 emission pattern over the short term after events such as tillage operations or rainfall, and long-term seasonal variations;

(iii) increase both SOC stocks and soil C pools

To achieve the main objective of the Thesis, the next specific objectives have been established:

- To Quantify C inputs into the soil relative to crop growth and water productivity (*Objective 1*).
- To determine the effects of soil management system and N fertilization on C outputs from the soil-crop system (*Objective 2*).
- To quantify changes in the total SOC content and in the different soil C pools after 13 years of the differential soil management system and N fertilization (*Objective 3*).

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DOCUMENT STRUCTURE

The document has been structured in 5 chapters all of them dealing with the long-term effects of tillage systems and N fertilization in different processes related to the soil C balance during three consecutive cropping seasons from October 2006 to July 2009:

Chapter 1: Crop growth and water use efficiency. The responses of crop growth and soil water dynamics were studied over the experimental, including information from the previous cropping season, 2005-2006.

Chapter 2: Root growth and soil physical properties. This study included the determination of root length density at main crop stages over three consecutive seasons, from 2006-2007 to 2008-2009 season. On the last season, soil physical properties were also evaluated.

Chapter 3: Evaluation of seasonal variation of soil CO_2 flux. Including quantification of soil carbon inputs, and determination of stock of soil organic carbon.

Chapter 4: Short-term dynamics of soil CO_2 flux: response to rainfall and tillage operations. A study on day by day variations of soil CO_2 fluxes after rainfall events and tillage operations.

Chapter 5: Root respiration: field and modelling approaches. A final study on the contribution of root respiration to soil CO_2 flux, and the treatment effects.

These five chapters are providing the basis for a discussion on the balance of soil organic C system and the response to long-term tillage systems and N fertilization in a semiarid Mediterranean agroescosystem which is finally presented.

Chapter 1: Crop growth and water use efficiency

Published in Soil and Tillage Research:
Morell, F.J., Lampurlanés, J., Álvaro-Fuentes, J., Cantero-Martínez, C. 2011. Yield and water use efficiency of barley in a semiarid Mediterranean agroecosystem: Long-term effects of tillage and N fertilization. 117, pp. 76-84.

Abstract

Conservation tillage systems (no-tillage, NT; and minimum tillage, MT) are being adopted in rainfed agroecosystems of the Mediterranean basin where water availability is the main limiting factor for crop productivity. We hypothesized that long-term adoption of conservation tillage systems would increase water use efficiency (WUE) and its response to N fertilizer additions due to improved soil water content.

A field experiment was established in 1996 on a loamy Xerofluvent Typic in the Ebro river valley (NE Spain). The experiment compared three nitrogen (N) fertilization levels (zero, 0 kg N ha^{-1}, medium, 60 kg N ha^{-1}, and high, 120 kg N ha^{-1}), under three tillage systems (CT, conventional tillage; MT and NT), annually cropped to barley (*Hordeum vulgare*, L.) as is usual in the region. Ten years after the experiment establishment, during four consecutive growing seasons, 2005-2006 to 2008-2009, we evaluated the response of soil water content, soil nitrate, above-ground dry matter, grain yield and yield components to long-term (>10 years) tillage and N fertilization treatments.

The long-term sustainability of NT and MT was confirmed. Mean yield and WUE under long-term conservation tillage systems were 66% and 57% higher than under CT, respectively. This improvement was mainly attributed to improved soil water usage under conservation tillage, mainly due to reduced water use during the pre-anthesis period. However, in a wet year yield did not significantly differ among tillage systems. The improvement of WUE with N fertilization was confirmed under NT, which medium and high N fertilizer level increased 98% mean grain yield and 77% mean WUE compared to CT. The increased response of crop and

yield to N fertilization under NT was due to improved soil water content. Soil N accumulation together with the lower water accumulation explained the lack of response to N fertilization under CT, even on a wet growing season (i.e., 2008-2009).

Long-term NT adoption was a sustainable practice for barley monoculture in the region, allowing for reduced costs and yield increase with N fertilizer additions. N fertilizer rates on rainfed Mediterranean croplands should be adjusted depending on the reduction of tillage intensity and rainfall of the year. In our system and as an example for this agroecosystems, N fertilizer rates should be kept at or below 60 kg N ha^{-1}, and should be further reduced on intensively cultivated soils.

Keywords: Dryland, winter cereal, soil mineral nitrogen, water productivity, direct seeding, biomass.

Abbreviations

NT, no-tillage; MT, minimum tillage; CT, conventional tillage; ZN, zero nitrogen; MN, medium level of N fertilization (60 kg N ha^{-1}); HN, high level of N fertilization (120 kg N ha^{-1}); GWC, gravimetric water content; SWC, soil water content; SN, soil nitrate N content; WU, water use; WU_{pre}, WU during pre-anthesis period; WU_{post}, WU during post-anthesis period; WUE, water use efficiency; WUE_{pre}, water use efficiency during pre-anthesis period; WUE_{post}, water use efficiency during post-anthesis period; WUE_b, WUE of above-ground biomass production; WUE_y, of grain production.

1. INTRODUCTION

Dryland agriculture in the Mediterranean region is mostly water limited, and yields vary markedly from year to year depending on the amount and distribution of precipitation, which are both highly variable (Austin *et al*., 1998). Water from precipitation must be captured and retained in soil and used efficiently for optimum yield production. Adequate management practices, such as conservation tillage and adequate N fertilization may increase water productivity, and limit environmental problems as soil erosion and N losses.

Conservation tillage practices include reduced soil tillage systems, such as minimum tillage (MT) and no-tillage (NT) systems, aimed at increasing the soil cover with the crop residues from the previous crop (CTIC, 2010). Improved soil surface cover usually improves water capture and retention (Unger *et al*., 1991).

NT is a promising practice for croplands on the Mediterranean basin, where it can improve water use efficiency (WUE) (Cooper and Gregory, 1987; Mrabet, 2000). NT and MT are mainly used for winter cereals, and the adoption in European Mediterranean countries is greater than in North African countries (Arrúe, 2006). In Spain, conservation tillage is adopted on over 4% of the surface and in some areas of Spain, it has been adopted over 80% of the surface and for more than 25 years (Cantero-Martínez *et al*., 2008).

Under semiarid Mediterranean conditions, N fertilization may also increase WUE by stimulating dry matter production (Latiri-Souki 1998), through a more rapid growth and improved transpiration efficiency. However, yields and WUE may be reduced when excessive N fertilizer is applied (Bladenopoulos and Koutroubas, 2003; Cantero-Martínez *et al*., 1995a). Moreover, N fertilization has to be adjusted because excessive N fertilization is an economical loss and leads to negative environmental consequences (Shepherd *et al*., 1993). In the Ebro river valley, as in other regions in the Mediterranean basin, N fertilizer rates for barley (*Hordeum vulgare*, L.) production has been usually applied between 100 and 200 kg of N ha^{-1} without agronomical control in many cases (Cantero-Martínez *et al*., 2003). These rates must be reduced to reach equilibrium among cost, environment and productivity.

The study of soil and crop responses to N fertilization under different soil tillage systems is useful to understand the interaction between these two factors, and to define best practices for improved N fertilization. Conservation tillage systems may improve water capture and retention, thus increasing crop growth and N uptake. This may reduce the availability of soil mineral N and may require increased N fertilization (Malhi *et al*., 2001; McConkey *et al*., 2002). In our

Mediterranean dryland systems, a previous study in the area found that the interaction between these two practices in the short-term (1 to 3 years) was not significant and no additional fertilizer was needed when MT and NT were adopted. However long-term adoption (>10 years) may lead to a different response.

We hypothesized that long-term adoption of conservation tillage systems and reduced N fertilization would be a sustainable strategy in the region 10 years after their adoption, and that WUE and response to N fertilization would be increased under conservation tillage systems due to improved soil water conservation. Consequently, the objective of this study was to evaluate long-term effects of tillage and N fertilization on crop response and WUE in a rainfed Mediterranean agroecosystem.

2. MATERIAL AND METHODS

2.1. Site, tillage and N fertilization

A long-term experiment on tillage and N fertilization of winter-barley was initiated in 1996 in Agramunt (41º 48'N, 1º 07'E; Lleida, Spain) (Cantero-Martínez *et al*., 2003). The experiment consisted of a factorial combination of three levels of N fertilization (zero, ZN; medium -60 kg N ha^{-1}-, MN; and high -120 kg N ha^{-1}-; HN), and three tillage systems with two conservation tillage systems (NT and MT) and one intensive tillage system (conventional tillage, CT). The experimental design was a randomized complete block design with three repetitions and a plot size of 50 m x 6 m. The mean annual rainfall in the area is 435 mm, and the soil was classified as Xerofluvent Typic (Soil Survey Staff, 1994). Main soil characteristics in the plough layer were the following: soil pH was 8.5; sand, silt and clay content were 465, 417 and 118 g kg^{-1}, respectively; and soil organic carbon content (SOC) in the soil surface (0-5 cm) was around 16 g kg^{-1} under NT, around 13 g kg^{-1} under MT and around 8 g kg^{-1} under CT, while SOC at deeper layers (10-25 cm) was similar among tillage systems, around 7 g kg^{-1}. Such increase in SOC at surface level under conservation tillage systems led to increased stock of C within the SOC

(Morell *et al*., 2011a). Water storage capacity of the soil at the beginning of the experiment was 215 mm within the top 1 m soil depth (Cantero-Martínez *et al*., 2003).

On average, rainfall has a bimodal distribution, with the major part occurring in autumn and late spring and little precipitation in winter and summer. However the pattern is highly variable and there is a high probability (25%) of low rainfall in the spring (< 50 mm). Barley is the main crop in the region, and it is widely adopted as a monoculture crop, with the growing season between November and June. Barley monoculture is the most extended cropping system in most of the areas within the region, where rotations with leguminous or other crops have demonstrated not feasible because the lack of economical benefits (Álvaro-Fuentes *et al*., 2009).

The CT treatment consisted of intensive tillage with a mouldboard plough to a depth of 25-30 cm with almost 100% of the residue incorporated in the soil. The mouldboard plough consisted of three bottoms of 0.50 m width. The MT treatment consisted of a cultivator pass to a depth of 10-15 cm with an incorporation of approximately 50% of the crop residue. The cultivator plough consisted of 5 rigid shanks spaced 20 cm apart and with a shank width of 5 cm. In the NT treatment, no soil disturbances occurred, and sowing was done by direct drilling after spraying with herbicide. Tillage operations were annually conducted between the end of October and the beginning of November. Barley cv. Hispanic was annually sown at a rate of 450 seeds m^{-2}, in rows spaced 17 cm apart with a no-till disc drill.

N fertilizer was split into two applications, with one-third being broadcast before tillage as ammonium sulphate (21% N) and two-thirds at the beginning of tillering as ammonium nitrate (33.5% N). Split application between sowing and tillering, with a major portion at tillering, have shown to improve the recovery of the applied N under semiarid Mediterranean conditions (Ramos *et al*., 1995).

Harvesting was done by the end of June with a standard medium-sized combine. The straw was chopped and spread over the plots by the combine machine. The field was kept free of vegetation for three to four months each summer. Additional details of the experimental site and cropping practices are given in Angás *et al*. (2006) and Cantero-Martínez *et al*. (2003).

2.2. Measurements

This study was conducted over a 4-yr period, during the cropping seasons 2005-2006, 2006-2007, 2007-2008 and 2008-2009, hereafter referred to as 2006, 2007, 2008 and 2009, respectively, and after ten years of the initiation of the experiment. During the four cropping seasons under study, we evaluated the long-term effects of tillage and N fertilizer on above-ground growth and yield of barley and on water productivity. Additionally we determined the soil water content (SWC) at significant growth stages and residual soil nitrate content at sowing (SN).

2.2.1. Weather conditions and SWC

An automated weather station at the experimental site registered daily maximum and minimum temperatures, precipitation and air humidity.

In every cropping season, soil samples were collected at sowing, tillering, beginning of stem extension, anthesis and harvest for determinations of SWC. The soil samples were taken using a 4 cm diameter soil auger. At each sampling, two samples per plot were taken at 25 cm increments to a depth of 100 cm. To reduce the effects of spatial variability on successive samplings, soil samplings were conducted on two regions of 10 m^2, 15 meters away from each end of the plot.

Gravimetric water content (GWC) was determined for every depth interval by drying a soil sub-sample in a forced-air oven at 105ºC for 48 hours (Campbell and Mulla, 1990) in a % basis. SWC up to 1 m depth was computed from GWC and bulk density (BD) of each depth, assuming a 250 mm (25 cm) depth interval (Eq. 1)

$$SWC = \sum_{i=1}^{4} GWC_i \times BD_i \times 250 \quad \text{[Eq. 1]}$$

2.2.2. Residual SN

Before sowing, the residual SN was determined on the same soil samples used for determinations of SWC. NO_3^- concentration was determined in the laboratory by Nitrachek® (reflectometry based instrument) on a solution of 100 g of soil in 100 ml of deionized water.

NH_4^+ concentration was determined in a solution of 100 g of soil in 100 ml of 1M solution of potassium chloride at several growth stages in 2007 cropping season. RQflex® (reflectometry based instrument) measurements were corrected with periodic standard soil analysis of NH_4^+.

Nitrachek® and RQflex® were used because they are faster and cheaper. Measurements of the instruments were corrected with periodic measurements with standard soil analysis of NO_3^- (Bremner, 1965). As in previous studies (Angás *et al*., 2006), the equipment were periodically calibrated in a certified laboratory, LAF-Applus+ laboratories, as well as measurements were calibrated with those obtained with official methods (colorimetric method with auto-analyzer) in the mentioned laboratory. R^2 of the calibrations were always higher than 0.98 and without any significant bias.

2.2.3. Above-ground growth and yield

Phenological stages were determined weekly following the BBCH scale (Lancashire *et al*., 1991). Crop biomass was sampled to quantify the total dry matter at the following developmental stages: tillering, beginning of stem extension, anthesis and maturity. Three samples per plot were taken by cutting the plants at the soil surface level on 50 cm along the seeding line. The samples were oven dried at 65-70º C for 48 hours, and then weighed. Yield components were determined at maturity. Ears were counted and, after oven drying, threshed to determine the number of grains per ear and the mean grain weight. In 2006, no samples of above-ground biomass at tillering and at beginning of stem extension were taken.

2.2.4. Water use and WUE

Water losses of due to runoff and leaching were assumed to be negligible (Cantero-Martínez *et al*., 1995b and 2003). Water use (WU) in mm, including soil water evaporation and crop transpiration, was calculated as the difference in SWC between two soil samplings plus the amount of rainfall during the interval of time. WU was calculated for the period between sowing and anthesis (pre-anthesis period, WU_{pre}), the period between anthesis and harvest (post-anthesis period, WU_{post}), and for the whole growing season (WU_{total}), between sowing and harvest.

WUE, in kg mm^{-1} ha^{-1}, was calculated as the amount of either above-ground biomass (WUE_b) or grain yield (WUE_y) per mm of water used. WUE for the above-ground biomass was calculated for the pre-anthesis (WUE_{pre}) and the post-anthesis (WUE_{post}) periods considering the WU and above-ground biomass produced in those periods.

2.3. Statistical analysis

Statistical analysis were performed using the SAS software (SAS Institute, 1990). A global analysis of variance using the PROG GLM option was performed for SN, total above-ground biomass at maturity (M), yield and yield components, considering cropping season, tillage system and N fertilization level as the main factors, and the interaction terms. Secondly, analyses of variance were conducted for each cropping season and for each variable, including total above-ground biomass at tillering, stem extension and anthesis, and SWC at different growth stages. Means separation of the main effects and/or the interaction terms were conducted by the Tukey adjustment. Error bars, indicating the standard deviation of the means, were presented in the plots.

In 2008 cropping season, CT was not included in the statistical analyses of crop variables due to crop failure. Under MT we omitted data for one block due to patchy distribution of crop growth, and hence type III sums of squares and mean squares were used instead type I.

3. RESULTS

3.1. Weather conditions

Precipitation during the growing seasons was 157, 307, 266 and 380 mm, in 2006, 2007, 2008 and 2009, respectively. Moreover the patterns of rainfall distribution greatly differed among seasons (Fig. 1.1). Precipitation during the summer-fallow periods was 84, 63, 31 and 50 mm in 2006, 2007, 2008 and 2009, respectively, and provided slight recharging of the soil water before sowing (Fig. 1). Monthly means of minimum and maximum temperatures of each growing season and means of the last thirty years are plotted in Figure 1.2.

3.2. Yield and yield components

Grain yields varied from 0 kg ha-1 (due to crop failure in CT 2008) to 4500 kg ha-1 in response to weather conditions and treatments (Table 1.1). Mean yields were 1075, 1519, 495 and 3680 kg ha-1 for 2006, 2007, 2008 and 2009 cropping seasons, respectively. In contrast to the mean annual yield of the last 30 years in this area (2800 kg ha^{-1}), we had two years of low production (2006 and 2007), one extremely low (2008), and a final year with high production.

Over four cropping seasons, and under a range of conditions, mean yields during the study period were 2062 kg ha^{-1} under NT, 1791 kg ha^{-1}, under MT , and 1155 kg ha^{-1}, under CT (Table 1.1). The improvement of grain yield with conservation tillage systems was greatest during the extremely dry year (i.e., 2008). In dry years (i.e., 2006 and 2007) yields were double under conservation tillage systems than under CT. In a wet year, grain yields did not significantly differ. N fertilization increased grain yields by 19% on average (Table 1.1), and it significantly interacted with tillage system and year (Table 1.2). The increase of grain yield with increasing N fertilization was significant under NT, but not under MT or CT, and this response was significant in 2006, 2007 and 2009, but not in 2008 (Table 1.1).

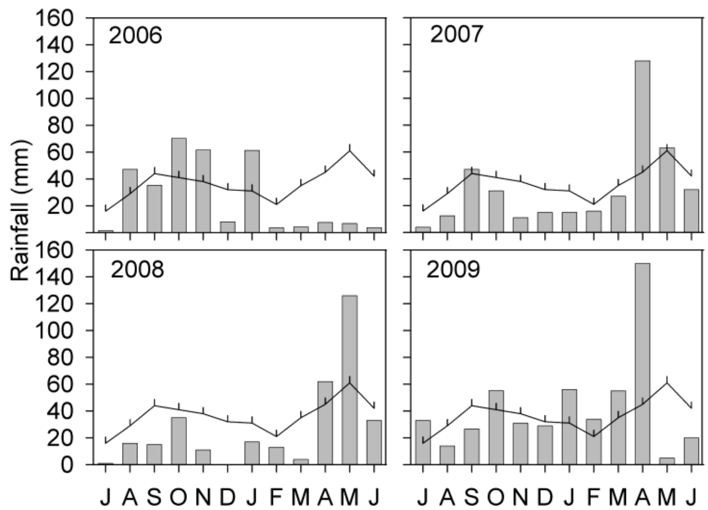

Figure 1.1. Precipitation. Bars indicate monthly rainfall during the four cropping seasons under study: 2006, 2007, 2008 and 2009, starting in July (J), after harvest of the previous year. Continued line with vertical ticks indicates average monthly rainfall (30 years) (line).

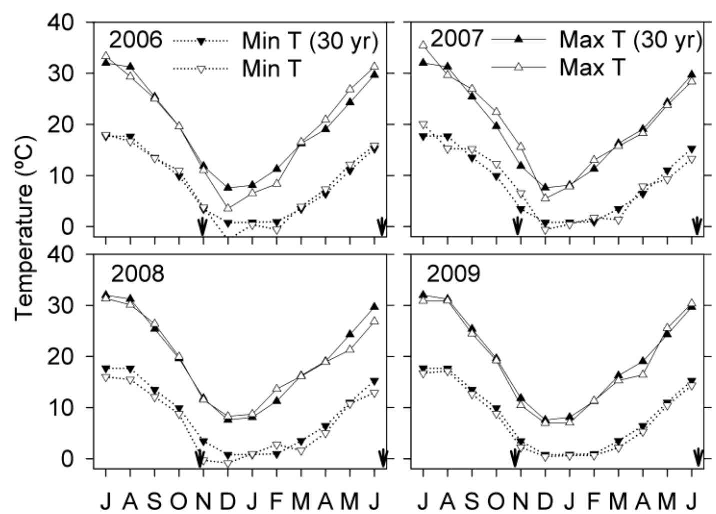

*Figure 1.2. Air temperature***.** *White triangles indicate monthly mean of the daily minimum (dotted line) and maximum (continued line) temperatures. Black triangles indicate average monthly mean of minimum (dotted line) and maximum (continued line) temperatures over the last 30 years. Small vertical arrows indicate sowing (by mid November, N) and harvest (by the end of June, J) dates on each cropping season.*

Table 1.1. Yields (kg ha^{-1}) in 2006, 2007, 2008 and 2009 cropping seasons. Comparison of N fertilization levels: zero N –ZN- (no fertilizer application); medium N -MN- (60 kg N ha^{-1}), and high N –HN- (120 kg N ha^{-1}), under different tillage systems, no-tillage (NT), minimum tillage (MT) and conventional tillage (CT). C.f., crop failure.

		Grain yield (kg ha^{-1})			**FNT**
		NT	MT	CT	
2006	ZN	1348 b	1101 b	306 d	919 b
	MN	1653 ab	1041 b	490 d	1061 b
	HN	1964 a	1103 b	669 cd	1246 a
TIL		1655 a	1082 b	488 c	
2007	ZN	1563 b	1944 ab	638 c	1382 b
	MN	2521 a	1972 ab	708 c	1734 a
	HN	2091 ab	1792 ab	443 c	1442 ab
TIL		2058 a	1903 a	596 b	
2008	ZN	480	462	c.f.	314 a
	MN	685	544	c.f.	410 a
	HN	861	609	c.f.	490 a
TIL		675 a	539 a	c.f.	
2009	ZN	2970 b	3346 ab	3646 ab	3321 b
	MN	4415 a	4089 a	3529 ab	4011 a
	HN	4195 a	3497 ab	3434 ab	3709 a
TIL		3860 a	3644 a	3536 a	
2006-2009	ZN	1590 bc	1714b	1148 c	1484 b
	MN	2319 a	1912 b	1182 c	1804 a
	HN	2278 a	1751 b	1137 c	1722 a
TIL		2062 a	1792 b	1155 c	

Note on table 1.1: Significant differences of the means according to Tukey´s adjustment ($P<0.05$) are indicated with different letters.TIL refers to the the mean value of N fertilization levels for each tillage system. FNT refers to the mean value of each N fertilization level.

The response of yield components to tillage systems depended on the cropping season (Table 1.2 and table 1.3). NT increased the number of ears on dry years (i.e., 2006 and 2007), but the opposite response occurred during the wettest year (i.e., 2009). The number of grains per ear tended to increase under NT, with significant increases in 2006 and 2009. The grain weight tended to be higher under NT, with a significant response in 2006. N fertilization increased the number of ears and the number of grains per ear (Table 1.3). The mean grain weight was also increased in response to N fertilization in 2009, but the opposite response occurred in 2006.

Table 1.2. Probability of significance of main factors (YEAR, cropping seasons, TIL, tillage system and FNT, N fertilization level) and their interaction at the global analyses of variance of: soil water content (SWC) at sowing (s), tillering (t), anthesis (a), and harvest (h); soil nitrate (SN); on above-ground biomass at maturity (M); yield components (number of ears unit area, Ears, number of grains per ear, GpE, mean weight of grain, Gw); yield and harvest index (HI); water use (WU) during different periods: WU_{pre}, WU in the pre-anthesis period; WU_{post}, WU in the post-anthesis period; and WU_{total}, WU for the whole growing season; and water use efficiency (WUE) during different periods: WUE_{pre}, WUE of total biomass in the pre-anthesis period; WUE_{post}, WUE of the total biomass in the post-anthesis period; WUE_b, WUE on the whole growing season and total above-ground biomass; and WUE_y, WUE on grain yield. n.s., non-significant effect, P>0.05.

	SWCs	SWCt	SWCa	SWCh	SN	M	Ears	GpE	Gw
Y	<0.001	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001	<0.001	0.003
T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.03	<0.001	<0.001
Y*T	0.05	<0.001	<0.001	<0.001	n.s.	0.002	<0.001	<0.001	n.s.
F	0.004	0.001	n.s.	n.s.	<0.001	<0.001	0.017	<0.001	n.s.
Y*F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001
T*F	n.s.	n.s.	<0.001	0.007	0.013	0.009	0.018	n.s.	0.026
Y*T*F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001
	HI	Yield	WUpre	WUpo	WUtotal	WUEpre	WUEpo	WUEb	WUEy
YEA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	<0.001	<0.001
TIL	<0.001	<0.001	<0.001	<0.001	n.s.	<0.001	n.s.	<0.001	<0.001
YEA	0.017	<0.001	<0.001	n.s.	<0.001	<0.001	0.03	0.004	<0.001
FNT	n.s.	<0.001	0.005	n.s.	0.002	0.003	n.s.	0.003	0.002
YEA	0.044	0.014	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02
TIL*F	n.s.	<0.001	n.s.	n.s.	n.s.	0.01	n.s.	0.01	<0.001
Y*T*F	n.s.	n.s.	n.s.	n.s.	0.04	n.s.	n.s.	n.s.	n.s.

3.3. Above-ground growth

Dry matter production and its growth pattern varied among cropping seasons (Fig. 1.3). In 2006 and 2007, mean dry matter weights at maturity were similar, around 600 g m^{-2}, with different patterns of crop growth. Post-anthesis growth in 2006 was severely reduced due to the lack of rainfall, while in 2007, the crop growth was reduced during pre-anthesis period and high growth during post-anthesis period. In 2008, crop growth was 400 g m^{-2} on average under conservation tillage systems, and the crop failed to grow under CT. In 2009, crop growth was the greatest of all seasons, and mean weights at maturity were up to 1000 g m^{-2}.

Table 1.3. Average of yield components during four cropping seasons: 2006, 2007, 2008, and 2009. C.f., crop failure. HI, harvest index; Ears, number of ears per square meter; GpE, grains per ear; and Gw, mean grain weight. N fertilization levels: zero (ZN), medium (MN), and high (HN). Tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). TIL are mean values for N fertilization levels. FNT are mean valuse of each N fertilization level.

		Ears (m^{-2})			**FNT**	GpE			**FNT**	Gw (mg)			**FNT**
		NT	MT	CT		NT	MT	CT		NT	MT	CT	
2006	ZN	354	317	316	**329 b**	13.9	11.5	8.9	**11.4 a**	41.5	39.2	35.8	**38.8 a**
	MN	474	390	321	**395 a**	14.8	12.2	9.8	**12.3 a**	39.1	38.0	33.7	**36.9 ab**
	HN	490	394	393	**426 a**	14.5	12.8	9.4	**12.2 a**	37.2	36.5	36.3	**36.7 b**
TIL		**440 a**	**367 b**	**343 b**		**14.4 a**	**12.2 b**	**9.3 c**		**39.3 a**	**37.9 a**	**35.3 b**	
2007	ZN	609	753	366	**576 b**	9.8	11.6	12.5	**11.3 a**	38.8	37.2	33.7	**36.6 a**
	MN	954	789	419	**721 a**	12.2	12.1	12.9	**12.4 a**	36.5	32.9	34.2	**34.6 a**
	HN	745	640	473	**619 ab**	12.4	13.6	12.6	**12.9 a**	34.4	33.7	33.6	**33.9 a**
TIL		**769 a**	**727 a**	**419 b**		**11.5 a**	**12.4 a**	**12.7 a**		**36.6 a**	**34.6 a**	**33.8 a**	
2008	ZN	502	827	C.f.	**665 a**	6.9	8.5	C.f.	**7.5 a**	32.0	35.1	C.f.	**33.2 a**
	MN	488	629	C.f.	**559 a**	7.9	8.3	C.f.	**8.1 a**	39.4	33.0	C.f.	**36.9 a**
	HN	558	655	C.f.	**607 a**	9.9	8.1	C.f.	**9.2 a**	36.7	29.4	C.f.	**33.8 a**
TIL		**516 b**	**704 a**			**8.2 a**	**8.3 a**			**36.0 a**	**32.5 a**		
2009	ZN	481 b	749 ab	1065 a	**765 b**	14.5	13.1	12.9	**13.5 c**	29.0 b	34.1 ab	38.3 ab	**33.8 a**
	MN	751 ab	856 a	994 a	**868 ab**	17.2	13.9	13.4	**14.8 b**	47.7 a	37.7 ab	33.0 ab	**39.5 ab**
	HN	783 ab	992 a	980 a	**919 a**	17.8	15,0	14.9	**15.9 a**	47.5 a	41.5 ab	34.7 ab	**41.2 b**
TIL		**672 c**	**866 b**	**1014 a**		**16.5 a**	**14.0 b**	**13.7 b**		**41.4 a**	**37.8 a**	**35.3 a**	

Note on table 1.3: For TIL and FNT, different letters indicate significant differences among means on each cropping season, and means when interaction effect was significant (P<0.05).

Tillage systems significantly affected dry matter production in 2006, 2007 and 2008, but not in 2009, the wettest year (Fig. 1.3). N fertilization significantly increased dry matter production at several growth stages in 2006, 2007 and 2009, but not in 2008, the driest year. In 2008, the interaction between N fertilization and tillage system was significant at various growth stages. N fertilization usually increased crop growth under NT, by an average of 50% on ZN, while it did not lead to any response under CT.

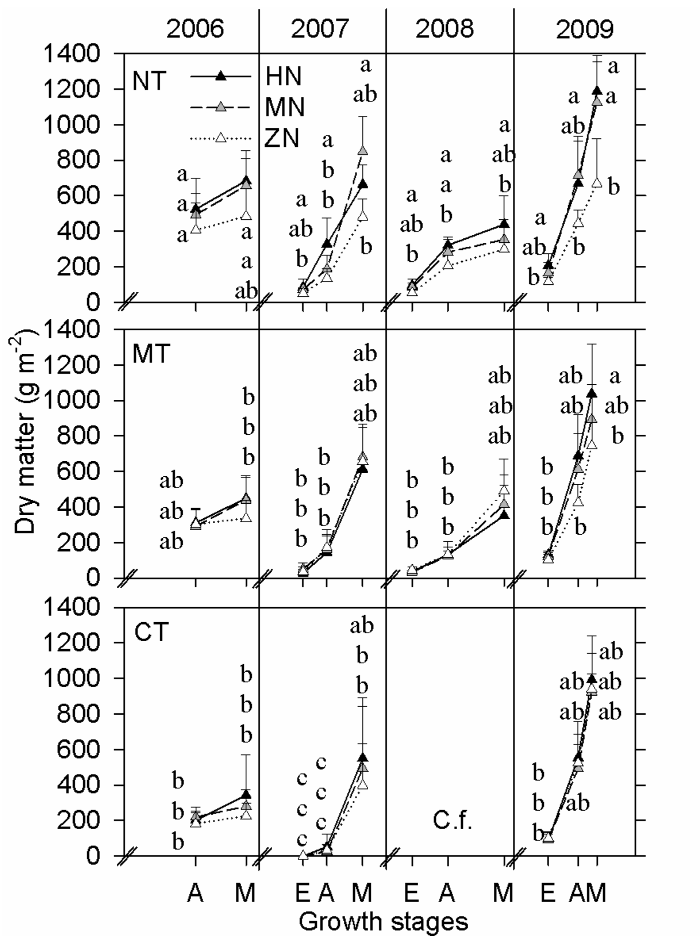

Figure 1.3. Total above-ground biomass production in 2005-2006, 2006-2007, 2007-2008 and 2008-2009 cropping seasons, for different tillage systems, no-tillage (NT), minimum tillage (MT) and conventional tillage (CT), and different N fertilization levels: zero N –ZN- (no fertilizer application); medium N -MN- (60 kg N ha^{-1}), and high N –HN- (120 kg N ha^{-1}), at different periods: beginning of stem extension (E), anthesis (A) and crop maturity (M). Error bars indicate standard deviation of the means. C.f., crop failure. In each cropping season and each period significant differences of the means according to Tukey´s adjustment (P<0.05) are indicated with different letters.

3.4. SWC, WU and WUE

Total WU by the crop (WU_{total}) ranged between 255 and 460 mm (Table 1.4) and was related to the precipitation during the growing season. Differences on the distribution and quantity of rainfall led to differences on the pattern of water use between pre- and post-anthesis periods. On average, pre-anthesis WU (WU_{pre}) was 50 and 60% of WU_{total} in 2007 and 2009. However, WU_{pre} was 85% of the WU_{total} in 2006 due to the low precipitation during spring, and 31% of the WU_{total} 2008 due to heavy precipitation during May.

Differences on SWC among tillage systems tended to occur at anthesis (Fig 1.4). This pattern of differences on SWC among tillage systems was also reflected in the pattern of water use in which increased WUpre under CT (Table 1.4), thus leading to reduced amount of SWC at anthesis (Fig. 1.4).

SWC was slightly improved with long-term N fertilization (data not shown) which may have partly contributed to improving crop growth. This improvement of SWC with N fertilization can be related to greater soil cover with increased residue production. Improved SWC also led to slight increases of WU_{total}, such as in 2006 and 2009 when WU_{total} on HN was 16 and 23 mm more than that on ZN (Table 1.4).

WUE_b and WUE_y, were between 11.3 and 22.3 kg biomass ha^{-1} mm^{-1}, and between 1.2 and 9.7 kg grain ha^{-1} mm^{-1} in all four years (Table 1.5). WUE_y was significantly affected by tillage system in the 2006 and 2007 seasons, when NT doubled the efficiency compared to CT. In this study, the improvement of WUE_y under NT in dry years compared to CT was due to improved WUE_{pre} (Table 1.5).
WUE_b was significantly increased by N fertilization in three out of the four seasons under study (i.e., 2006, 2007 and 2009), when WUE_b of MN and HN were between 30 and 40% greater than that of ZN. WUE_{pre} was greatest under NT in all the cropping seasons under study. WUE_y was also significantly affected by N fertilization level (Table 1.5). In 2007 and 2009, WUE_y was highest on MN where it ranged between 17 and 23% higher than that of ZN. In 2006, it was highest on HN.

Table 1.4. Water use (WU) in mm, in response to different N fertilization levels under different tillage systems; at four cropping seasons: 2006, 2007, 2008, and 2009. WU_{pre}, WU in the pre-anthesis period; WU_{post}, WU in the post-anthesis period; and WU_{total}, WU for the whole growing season. N fertilization levels: zero (ZN), medium (MN), and high (HN). Tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). TIL refers to the the mean value of N fertilization levels for each tillage system. FNT refers to the mean value of each N fertilization level.

		WU_{pre}			**FNT**	WU_{post}			**FNT**	WU_{total}			**FNT**
		NT	MT	CT		NT	MT	CT		NT	MT	CT	
2006	ZN	217 b	199 b	209 b	**208 b**	43	40	31	**38 a**	259	239	240	**246 b**
	MN	240 ab	207 b	206 b	**218 ab**	44	39	35	**40 a**	284	246	241	**257 ab**
	HN	248 a	225 ab	206 b	**226 b**	37	31	37	**35 a**	285	257	243	**262 a**
TIL		**235 a**	**210 b**	**207 b**		**41 a**	**37 a**	**34 a**		**276 a**	**247 b**	**241 b**	
2007	ZN	190	181	209	**194 a**	170	176	147	**164 a**	360	357	356	**358 a**
	MN	175	169	235	**193 a**	172	185	129	**162 a**	347	354	364	**355 a**
	HN	173	205	224	**201 a**	173	157	150	**160 a**	346	362	374	**360 a**
TIL		**180 b**	**185 b**	**223 a**		**171 a**	**173 a**	**141 b**		**351 a**	**358 a**	**365 a**	
2008	ZN	102	101	131	**102 a**	190	209	193	**197 a**	292 b	309 ab	323 ab	**308 a**
	MN	102	88	112	**95 a**	224	222	189	**212 a**	326 a	313 ab	301 ab	**313 a**
	HN	116	87	124	**102 a**	210	207	192	**203 a**	326 a	306 ab	316 ab	**316 a**
TIL		**107 b**	**93 b**	**122 a**		**208 a**	**213 a**	**191 b**		**315 a**	**309 a**	**313 a**	
2009	ZN	270	290	308	**289 a**	180	152	154	**162 a**	450	442	462	**451 b**
	MN	270	272	298	**280 a**	186	179	163	**176 a**	456	451	461	**456 b**
	HN	288	303	301	**297 a**	177	185	167	**176 a**	465	488	469	**474 a**
TIL		**276 b**	**288 ab**	**302 a**		**181 a**	**171 a**	**161 a**		**457 a**	**460 a**	**464 a**	

Note on table 1.4: For TIL and FNT, different letters indicate significant differences among means on each cropping season ($P<0.05$). Means of all treatments on each cropping season are separated with different letters when interaction effect was significant ($P<0.05$).

Table 1.5. Water use efficiency (WUE) in kg mm^{-1}, under different N fertilization levels: zero (ZN), medium (MN), and high (HN); under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT); at four cropping seasons: 2006, 2007, 2008, and 2009. WUE_{pre}, WUE of the total biomass in the pre-anthesis period; WUE_{post}, WUE of the total biomass in the post-anthesis period; WUE_b, WUE on the whole growing season and total above-ground biomass; and WUE_y, WUE on grain yield. C.f., crop failure.

		WUE_{pre}			**FNT**	WUE_{post}			**FNT**
		NT	MT	CT		NT	MT	CT	
2006	ZN	14.9	15.6	9.4	**15.1 a**	42.9	5.1	6.4	**18.2 a**
	MN	21.9	12.8	10.3	**15.0 a**	48.9	28.3	21.5	**32.9 a**
	HN	22.0	14.2	9.3	**13.3 a**	61.1	43.5	48.6	**51.1 a**
TIL		**19.6 a**	**14.2 b**	**9.7 c**		**51.0 a**	**26.5 a**	**25.5 a**	
2007	ZN	7.7 b	10.0 b	2.2 c	**6.6 b**	18.2	29.4	26.2	**24.6 a**
	MN	12.4 ab	11.0 b	1.5 c	**8.3 ab**	36.2	30.2	42.8	**36.4 a**
	HN	23.2 a	8.7 b	3.2 c	**11.7 a**	18.1	25.4	38.0	**27.2 a**
TIL		**14.4 a**	**9.9 b**	**2.3 c**		**24.2 a**	**28.3 a**	**35.7 a**	
2008	ZN	22.1	10.7	C.f.	**16.7 a**	7.4	24.1	C.f.	**15.0 a**
	MN	30.8	9.8	C.f.	**20.6 a**	5.4	19.2	C.f.	**11.6 a**
	HN	29.4	12.7	C.f.	**21.4 a**	9.0	13.4	C.f.	**10.5 a**
TIL		**27.4 a**	**11.7 b**	**C.f.**		**7.3 b**	**17.4 a**	**C.f.**	
2009	ZN	16.7	15.0	16.5	**16.1 b**	14.2	9.8	29.5	**17.8 a**
	MN	25.5	23.2	15.9	**21.5 a**	26.5	16.8	28.9	**24.1 a**
	HN	23.1	21.6	18.0	**20.9 ab**	30.7	19.7	27.6	**26.0 a**
TIL		**21.8 a**	**19.9 ab**	**16.6 b**		**23.8 a**	**15.4 a**	**28.7 a**	
		WUE_b			**FNT**	WUE_y			**FNT**
		NT	MT	CT		NT	MT	CT	
2006	ZN	17.2	11.9	9.0	**12.7 b**	5.3 b	4.6 b	1.3 c	**3.7 b**
	MN	22.5	15.5	10.7	**16.2 ab**	5.7 ab	4.3 b	1.9 c	**4.0 b**
	HN	24.4	17.4	14.9	**18.9 a**	7.0 a	4.0 b	2.9 c	**4.6 a**
TIL		**21.4 a**	**14.9 b**	**11.3 b**		**6.0 a**	**4.3 b**	**2.1 c**	
2007	ZN	12.6	19.5	12.1	**14.7 a**	4.1 b	5.4 ab	1.9 c	**3.8 b**
	MN	24.0	20.4	16.5	**20.3 a**	7.1 a	5.4 ab	1.9 c	**4.8 a**
	HN	20.1	15.5	17.2	**17.6 a**	5.7 ab	4.8 a	1.2 c	**4.1 ab**
TIL		**18.9 a**	**18.5 a**	**15.3 a**		**5.7 a**	**5.2 a**	**1.8 b**	
2008	ZN	11.7	18.7	C.f.	**14.7 a**	1.6	2.3	C.f.	**1.7 a**
	MN	12.5	16.0	C.f.	**13.8 a**	2.0	2.7	C.f.	**2.2 a**
	HN	16.0	10.4	C.f.	**12.8 a**	2.7	3.1	C.f.	**2.7 a**
TIL		**13.4 a**	**14.1 a**	**C.f.**		**2.1 a**	**2.3 a**	**C.f.**	
2009	ZN	15.4	13.2	21.3	**16.6 b**	6.7 b	7.4 b	8.0 ab	**7.4 b**
	MN	26.0	20.5	19.9	**22.1 a**	9.7 a	9.2 a	7.7 ab	**8.7 a**
	HN	25.6	20.2	20.5	**22.1 a**	9.0 a	6.9 b	7.5 b	**7.8 b**
TIL		**22.3 a**	**17.9 b**	**20.6 ab**		**8.5 a**	**7.9 a**	**7.7 a**	

Note on table 1.5: Different letters on each year indicate significant differences, $P<0.05$).

3.5. Residual soil mineral nitrogen

The quantity of nitrate N was between 150 and 1200 kg N-NO_3^- ha^{-1} (Table 1.6). The main effects were consistent in different years (Table 1.6). In all the cropping seasons, SN was highest under CT, medium under MT and lowest under NT. The interaction between tillage system and N fertilization was not significant in any of the individual year, (close to the significance level in 2008; P = 0.07).

However over the four years experimental period, a significant quantitative interaction occurred between tillage system and N fertilization (Table 1.2 and 1.6). Differences among N fertilization levels were higher under CT and MT than those under NT

Table 1.6. Soil nitrate content (SN). Comparison among N fertilization levels: zero (ZN), medium (MN), and high (HN); under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT); at four cropping seasons: 2006, 2007, 2008, and 2009.

		SN (kg N-NO_3 ha^{-1})			**FNT**
		NT	MT	CT	
2006	ZN	184	287	603	358 b
	MN	317	307	752	459 b
	HN	578	554	1264	799 b
TIL		360 b	383 b	873 a	
2007	ZN	134	274	630	346 b
	MN	351	382	913	549 ab
	HN	425	757	1065	749 a
TIL		303 b	471 b	869 a	
2008	ZN	172	262	514	316 b
	MN	230	458	838	508 b
	HN	276	843	1126	748 a
TIL		226 c	521 b	826 a	
2009	ZN	91	136	498	241 b
	MN	227	581	921	576 a
	HN	256	694	1097	682 a
TIL		191 c	470 b	839 a	
2006-2009	ZN	145 f	240 ef	561 cd	315 b
	MN	281 ef	432 de	856 b	523 ab
	HN	384 de	712 bc	1138 a	745 a
TIL		270 c	461 b	852 a	

Note on table 1.6: Different letters indicate significant differences among means on each cropping season (P<0.05). Means of all treatments are separated with different letters when interaction effect was significant (P<0.05).

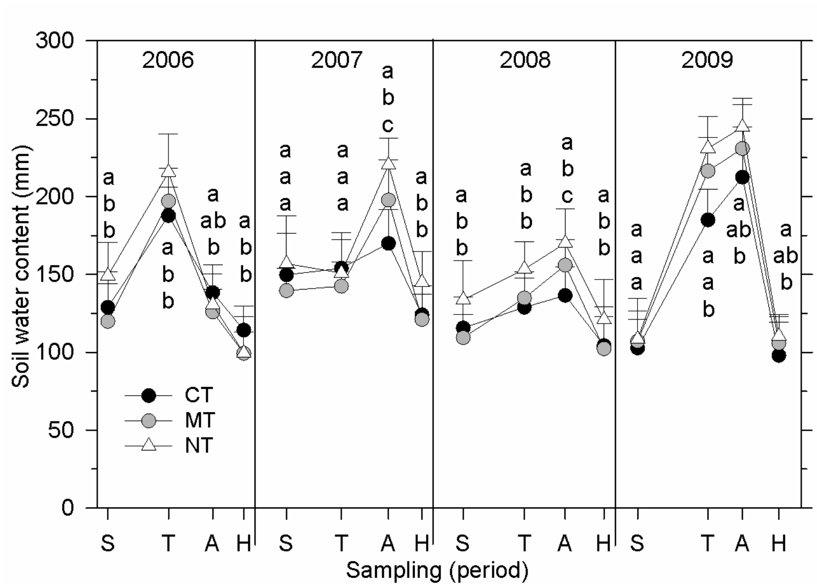

Figure 1.4. Soil water content in 2006-2005, 2006-2007, 2007-2008 and 2008-2009 cropping seasons, for different tillage systems, no-tillage (NT), minimum tillage (MT) and conventional tillage (CT), at sowing (S), tillering (T), anthesis (A) and harvest (H). Error bars indicate standard deviation of the means. In each cropping season and each period significant differences of the means according to Tukey´s adjustment (P<0.05) are indicated with different letters.

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4. DISCUSSION

4.1. Long-term effect of tillage

In this study and after ten years of the initiation of the experiment, there was a positive effect of NT on crop performance during most of the years. Furthermore, there were no detrimental effects of the NT system during wet years, which is in contrast to that described in other semiarid regions (Azooz and Arshad, 1998; Moret *et al*., 2007), and in spite of increased soil strength and reduced root growth under NT (Morell *et al*., 2011b).

The positive response of yield and crop growth to long-term conservation tillage systems contrasted with that observed during its early adoption, when crop growth and yields were slightly affected (Cantero-Martínez *et al*., 2003; López-Fando and Almendros, 1995). In our study, conservation tillage systems improved

SWC and thus more water was available for crop growth leading to better WU, specially in the post-antesis period (WU_{post}). This effect was also shown by Bescansa *et al*., (2006) and Cantero-Martínez *et al*. (2007) under the same conditions. In semiarid Mediterranean conditions, the improvement on soil water conservation due to conservation tillage adoption is increased in the medium to long term. This could be related to the fact that crop residue production under semiarid conditions is low, and thus it may take a few cropping seasons for the soil cover to improve with conservation tillage. In subhumid Mediterranean environments or in wet years, crop residue production may be excessive. When excessive crop residues are produced, it may be appropriate to remove part of the residues to avoid problems when seeding or allelopaties.

Under NT, greater soil water available for crop growth resulted in higher biomass, ears per square meter and grains per ear compared to CT. Differences between tillage systems were higher as much drier was the year. Consequently, WUE in biomass and yield were improved, showing the more efficient use of water by the crop under conservation tillage systems.

During the four years, soil mineral nitrogen showed high differences between tillage systems. The lower SN content in NT compared to CT did not limited crop yield. During the four years, in the NT treatment grain yields ranged between 1300 and 4400 kg ha^{-1} representing an uptake requirement of 80 kg N ha^{-1}. This uptake value is considerable lower than the amount of residual nitrogen observed in the study. Therefore, in our experiment, yields have been limited by soil water availability. Furthermore, under CT soil mineral nitrogen was greatly accumulated up to one meter soil depth. Two explanations could be given to this accumulation. Firstly, less water was available during the growing season and thus less N uptake was done. Secondly, leaching losses were negligible due to the non-percolate regimen trough the soil under such that lower rainfall conditions.

4.2. Long-term effect of N fertilization level

N fertilization did not considerably increase crop yield and biomass. Even a slightly reduction was observed in some years between high and medium levels.

Nevertheless, the increase of N fertilization significantly affected the soil mineral N content. However, the increase in SN did not affect crop yield and WUE in such a way. In our conditions, soil water continues being the most important limiting factor. Increases in SN could lead to higher biomass and thus WU in the pre-anthesis period. But this increase did not result in higher grain yield because water was mainly transpired and lost out of the grain-filling period when yield is produced. MN was better level of N fertilizer than HN which could decrease WUE_y, as observed in 2007 and 2009 (Table 1.5). Increased number of ears per unit area with increased N fertilization may be due to the increased tiller survival at later stages (Ramos *et al*., 1995). Reduced grain weight with HN may be the responsible of the reduced yields (Herwaarden *et al*., 1998), as observed in 2006 (Table 1.1).

These results suggest that N fertilizer addition in the region should be kept at 60 kg N ha^{-1} or below. Ramos *et al*. (1995), recommended 60 kg N ha^{-1} rates for barley in southern Spain, with an even split application between sowing and tillering with the greater proportion applied at tillering. Similarly, in other semiarid Mediterranean conditions (e.g., Greece and Italy), high doses of N fertilization showed no positive responses of crop growth (Bladenopoulos and Koutroubas, 2003; De Giorgio and Montemurro, 2006) and 50 kg of N ha^{-1} was found as the best compromise for the yield capacity of winter cereal production. Future fertilizer experiments in the region should also consider doses lower than 60 kg N ha^{-1}. However, the fertilizer recommendation may depend on the tillage system due to the observed significant interactions occurring in the long-term.

4.3. Tillage and N fertilization interaction

The tillage by N fertilization interaction was observed in above-ground dry matter (Fig. 1.3), yield and associated WUE (Table 1.1 and 1.5, respectively), and SN (Table 1.6). Crop growth and yield usually responded positively to N fertilization under NT, as well as under MT during the wettest year (i.e., 2009), but not under CT. The response to N fertilization under NT can be partly related to greater SWC under this system (Fig. 1.4) and to lower but not limiting SN content (Table 1.6). The lack of response under CT, even during a wet cropping season could be related to the accumulation of SN (Table 1.6). As commented in the Results section, in some sampling moments, soil nitrate content was higher than 1000 kg $N\text{-}NO_3$ ha^{-1}. After more than 10 years with medium and high N fertilizer applications, this significant accumulation of SN was mainly attributed to: i) the accumulation of fertilizer exceeding the uptake by the crop; ii) the N mineralization from soil organic matter; and iii) the lack of leaching. It was assumed that in this experiment SN leaching was insignificant since the SWC (Fig. 1.4) plus rainfall (Fig. 1.1) slightly exceeded the soil water storage capacity of the soil in the top 1 m (215 mm approximated) only in certain moments. It is worth mentioning that in other experiments located in the same semiarid area we have found similar levels of soil mineral N (data not shown). Furthermore, high amount of mineral N in the top 1 m of the soil has been previously reported in another areas in the Mediterranean region with high levels of N fertilizer additions (Abad *et al*., 2004).

The quantity of ammonium N to one meter depth was around 15 and 20 kg $N\text{-}NH_4^+$ ha^{-1} with similar amounts on different treatments and through the season (data not shown; Margalef-Garcia, 2010). Soil samplings were performed at least 1 month after fertilizer applications. Consequently, applied ammonium in the ammonium sulphate and in the ammonium nitrate had been transformed to nitrate or volatilized before soil sampling. The ammonium fraction represented a small fraction of the soil mineral N (less than 3% in our soil) and showed practically no variation during the growing season, as observed in Vázquez *et al*. (2006), indicating that, under semiarid conditions, ammonium N is a low and rather stable fraction of the soil mineral N.

Reduced amounts of SN under conservation tillage systems could be mainly attributed to two reasons. First, the increase of SOC stock under NT (Morell *et al*., 2011a) and the concomitant increase of organic N stored in the soil organic matter, which may account of up to 500 kg of N stored in soil organic matter with the increase of 4 Mg ha^{-1} of the SOC stock under NT. Second, increased exportation of N with the grain under conservation tillage systems due to increased yields (Table 1.1). As a rough estimation, given a mean increase of 600 kg ha^{-1} of yield under NT compared to CT and with a 2% of N in the grain, the N exportation with grain under NT over the 14 years period may be of up to 170 kg of N.

Mediterranean agroecosystems are characterized by poorly developed soils prone to erosion and reduced available water, and hence low yields and crop residue production, which are leading to soil degradation and economical limitations. The present study demonstrates an increase of the benefits of conservation tillage systems over the long-term. For this reason, conservation tillage adoption, and especially NT, should be encouraged in other semiarid Mediterranean countries, where NT is in its early stage of adoption (Derpsch *et al*., 2010). Our results show that the long-term adoption of NT and adjustment of N fertilization improve productivity and reduces the risk of desertification in the area.

5. CONCLUSIONS

In Mediterranean dryland agroecosystems, long-term adoption of conservation tillage systems is an agronomical sustainable strategy to improve soil water conservation that leads to increase yields and improve WUE. Long-term addition of N fertilizer only under NT improves WUE and grain yield. Application of N fertilizer under CT leads to the accumulation of high amounts of nitrate in the soil. Long-term adoption of NT with adjusted N fertilization improves the agronomical and economical sustainability of cereal based rainfed agroecosystem under Mediterranean conditions.

ACKNOWLEDGEMENTS

This work was supported with by the Comisión Interministerial de Ciencia y Tecnología of Spain (Grants AGL 2004-07763-C02-02 and AGL2007-66320-CO2-02/AGR). We thank Carlos Cortés and Silvia Martí for their technical assistance. We acknowledge the Spanish Ministry of Science and Innovation for funding the doctorate studies of F.J. Morell and the Consejo Superior de Investigaciones Científicas (CSIC) for the contract of Jorge Álvaro-Fuentes within the Junta Ampliación Estudios (JAE-DOC) program co-founded by the FSE. We acknowledge Alexander Pswarayi for language correction.

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Chapter 2: Root growth and soil physical properties

Published in Agronomy Journal:
Morell, F.J., Cantero-Martínez, C., Álvaro-Fuentes, J., Lampurlanés, J. 2011. Root growth of barley as affected by tillage systems and nitrogen fertilization in a semiarid Mediterranean agroecosystem. 103 (4), pp. 1270-1275.

Abstract

Conservation tillage systems are being widely adopted in the Mediterranean region. A long-term field experiment was established in 1996 comparing three N fertilization levels (zero, 0 kg ha^{-1}; medium, 60 kg ha^{-1}; and high, 120 kg ha^{-1}), under three tillage systems (conventional tillage CT; minimum tillage, MT; and no-tillage, NT) in a semiarid Mediterranean agroecosystem annually cropped with barley (*Hordeum vulgare,* L., cv. Hispanic), to study the response of root growth. During four consecutive growing seasons, from 2005 to 2009, root length density (RLD) and soil water content were evaluated. Penetration resistance (PR) and soil bulk density were only evaluated in the fourth and last year of this experiment. In dry years, root growth was similar under NT and MT systems but highly reduced under CT due to reduced water availability, which, in the surface 25 cm of soil was 7 % and 18 % lower than under MT and NT systems, respectively. However, in a wet year (i.e., 2009), RLD was double under CT than under NT due to reduced soil strength. PR at 5 to 15 cm soil depth under NT was 1MPa greater than under MT or CT. Root growth was not affected by nitrogen fertilization, in contrast to the response of grain yield that showed a significant interaction between N fertilization and tillage system. In spite of moderate soil compaction, which may reduce root growth in wet years, long-term NT adoption does not reduce grain yields for monoculture of barley under semiarid Mediterranean conditions.

Abbreviations:

NT, no-tillage; MT, minimum tillage; CT, conventional tillage; SWC, soil water content; RLD, root length density; RL, root length; PR, soil penetration resistance; BD, soil bulk density; AGB, above-ground dry matter at anthesis;

TIL, tillage system factor; NFL, N fertilization level factor; TIL x NFL , interaction term.

1. INTRODUCTION

Conservation tillage practices, that includes no-tillage (NT) and minimum tillage (MT) systems, are being adopted in the Mediterranean basin. Adoption of NT and MT is higher in European Mediterranean countries than in North African countries (Arrúe, 2006). In Spain, conservation tillage is adopted on over 4% of the surface, although in some areas it has been adopted on over 80% of the surface and for more than 25 years (Cantero-Martínez *et al*, 2008). Increased soil cover with crop residues usually leads to improved water capture and retention (Unger *et al*., 1991), and increased water use efficiency (Cooper *et al*., 1987; Cantero-Martínez *et al*., 2007). However little is known on the effects of conservation tillage systems on root growth under Mediterranean conditions, especially after their long-term adoption (> 10 years).

Early adoption of conservation tillage systems may increase the water storage capacity of the soil which improves root growth and grain yield (Lampurlanés *et al*., 2002; Lampurlanés and Cantero-Martínez, 2003). Long-term adoption of conservation tillage systems further increases the water storage capacity of the soil (Bescansa *et al*., 2006), thus it may improve root growth. However, long-term adoption of conservation tillage systems, and especially NT, could increase soil compaction. Soil compaction determines the soil mechanical impedance to root growth, and increased soil compaction may reduce the size of the root system and it decrease grain yield (Qin *et al*., 2006; Pietola, 2005; Montagu *et al*., 2001). Long-term NT adoption on a vertisol under semiarid Mediterranean conditions has been found to reduce root growth in spring wheat (Muñoz-Romero *et al*., 2010).

In Mediterranean conditions, reduced water availability limits the response to N fertilizer additions (Ryan *et al*., 2009). Increased water storage conservation tillage systems may increase the response of crop growth to N fertilizer addition. Rates of N fertilizer must be adjusted to reach equilibrium among cost, environment, and productivity. Crop growth and grain yield may show significant interactions between long-term N fertilizer additions and tillage systems, with higher response of crop growth to N fertilizer under conservation tillage systems (Fox and Bandel, 1986). The response of root growth is expected to show a similar interactive response.

The objective of this study was to investigate the response of barley root growth to tillage systems and N fertilization over four consecutive cropping seasons in a long-term (>10 years) tillage and fertilization study.

2. MATERIALS AND METHODS

2.1. Site, Tillage, and N Fertilization

The experiment was located in Agramunt (41º 48'N, 1º 07'E; Lleida, Spain). The average annual rainfall at the experimental site (30-yr average) is 435 mm. Rainfall usually has a bimodal distribution, with the majority occurring in autumn and late spring, and the least in winter and summer (Fig. 2.1). However the distribution pattern of rainfall is highly variable. There is a high probability (25%) of low rainfall (< 70 mm) in the spring. Barley is the main crop in the region, and it is widely adopted as a monoculture with the growing season between November and June for optimization of water use (Álvaro-Fuentes *et al*., 2009).

The experiment began in 1996 on a Typic Xerofluvent (Soil Survey Staff, 1994). The Ap horizon (0-28 cm) contained: 465 g kg^{-1} sand, 417 g kg^{-1} silt, and 118 g kg^{-1} clay (gravel content < 20 g kg^{-1}). SOC between 6 and 9 g kg^{-1}, pH between 7.8 to 8.1; gravimetric content of water in the soil at field capacity (-33 kPa matrix potential) was 160 g kg^{-1}, and at wilting point (-1500 kPa matric potential) was 50 g kg^{-1} (Cantero-Martínez *et al*., 2003). This study reports on the growing seasons 2005-2006, 2006-2007, 2007-2008, and 2008-2009, hereafter referred to as 2006, 2007, 2008, and 2009, respectively.

The experiment consisted of a factorial comparison of three levels of N fertilization (zero; medium, 60 kg N ha^{-1}; and high, 120 kg N ha^{-1}) and three tillage systems: two conservation tillage systems (NT and minimum tillage, MT), and one intensive tillage system (conventional tillage, CT) in a randomized block design with three replications and a plot size of 50 m x 6 m. Tillage operations were conducted at the end of October to the beginning of November.

The CT treatment consisted of an intensive tillage with a moldboard plow to a depth of 25 to 30 cm with almost 100% of the residue incorporated into the soil. The MT treatment consisted of a cultivator pass to a depth of 10 to 15 cm with an incorporation of approximately 50% of the crop residue. On the NT treatment, herbicide (0.54 L a.i. N-(phosphonomethyl)glycine ha^{-1}) was sprayed one to two weeks before sowing. Sowing was done with a disc direct driller (17 cm row spacing; Maquinaria Agrícola Solá S.L. manufacturer), maintaining the same sowing depth across the tillage systems. Herbicide for broadleaf weed control was applied at tillering over the whole experiment.

Since the beginning of the experiment, winter barley was annually cropped under rainfed conditions. Sowing was done by mid November, around one to two weeks after tillage, at a rate of 450 seeds m^{-2}. N fertilizer was supplied in two applications: one-third was broadcast before tillage as ammonium sulfate (21% N) and the other two-thirds at the beginning of tillering as ammonium nitrate (33.5% N). Harvesting was done by the end of June with a standard medium-sized combine (5.4 m wide). Straw was chopped and spread over the plots by the harvester machine. The field was kept for three/four months in summer-fallow until soil preparation. Additional details of the experimental site and cropping practices are given in Angás *et al*. (2006) and in Cantero-Martínez *et al*. (2003).

2.2. Root and Soil Measurements

Soil samples were collected each year at tillering, beginning of stem extension, anthesis, and harvest (Lancashire *et al*., 1991) for measurement of SWC and RLD. The soil samples were taken using a 4 cm diameter soil auger. At each sampling, two samples per plot were taken between the seeded rows at 25 cm depth to a depth of 100 cm. In 2007, RLD were not taken under CT because the crop failed to grow due to dry conditions. Gravimetric SWC was determined by drying a soil sub-sample in a forced-air oven at 105ºC for 48 hours (Campbell and Mulla, 1990). A 100 g sub-sample of fresh soil was washed by elutriation (Pearcy *et al*., 1989) to extract the roots that were stained with a 1% Congo red solution following the procedure of Ward *et al*. (1978). Root length was determined by the line intersection method (Newman, 1966):

by counting the number of intersections of red-stained roots spread over a surface of 80 cm^2 with a 1 cm mesh. We calculated RLD as the quotient between root length and the volume of the soil sub-sample. The volume of the samples was calculated as the quotient between the sample dry weight and the soil bulk density at each 25 cm depth interval. The volume of water in the soil profile (0-100 cm) was calculated as the sum of the amount of water in each 25 cm depth interval of soil. The water use between two stages, including both crop transpiration and soil water evaporation, was calculated as the difference between the SWC at a later stage and that at an earlier stage, added to the amount of rainfall during that period. Percolation was not considered because it is usually negligible in semiarid conditions (Cantero-Martínez *et al*., 1995 and 2003).

Penetration resistance was evaluated at tillering and at anthesis during the 2009 cropping season. We used a hand-held penetrograph (Stiboka penetrograph, Eijkelkamp Agrisearch Equipment®, Giesbeek, Netherlands) with a range of measurements between 0 and 5 MPa. It draws a graph of the soil resistance to penetration against soil depth up to 80 cm soil depth. We used a conical point of 1 cm^2 and 60º point angle to make two measurements per plot. In the results section, values at every 5 cm depth are presented, starting at 2.5 cm.

Bulk density (BD) was evaluated after the 2009 harvest. After digging the soil to 50 cm depth, the soil cores by hammering stainless steel cutter edge cylinders at the following depth intervals; 0-5, 5-10, 10-20, 20-30, and 30-40 cm. Cylinders were 50 mm high and with a 60 mm inner diameter (141 cm^3 inner volume). The soil in the cores was dried and weighed. Bulk density was calculated as the quotient between the dry soil weight and the cylinder inner volume.

2.3. Statistical Analysis

Statistical analyses were performed using SAS software (SAS Institute, 1990). Root length density data required transformation, which was done following the Box-Cox transformation (Box and Cox, 1964). Analyses of variance were conducted with PROC GLM, considering as fixed factors tillage system (TIL), N fertilization level (NFL), year, development stage and depth interval, and the interactions among them. Orthogonal contrasts were used for mean separation of statistically significant ($P<0.05$) main factors, while multiple comparisons of least-squares means according to Tukey´s adjustment were used for significant interactions. Additionally, PR was analyzed on a second instance including soil moisture as a covariate in the ANOVA model. Graphs were done with Sigmaplot 11 (Systat Software, Inc., 2008).

3. RESULTS

3.1. Rainfall Patterns, SWC, and Grain Yield

The monthly rainfall at the experimental site during each cropping season is shown compared to the 30-yr average at the site (Fig. 2.1). Precipitation during growing seasons in 2006, 2007, 2008, and 2009 was: 157, 307, 266 and 380 mm; of which 139 (88%), 207 (67%), 109 (41%), and 355 mm (93%) occurred during the pre-anthesis period. In 2006, rainfall was high during winter and it was low during spring (< 59 mm). In 2007 and 2008, monthly rainfalls during both winters were lower than interannual mean values, and higher in April 2007 and May 2008, respectively (Fig. 2.1). In 2009, rainfall was adequate during winter, high in April and low in May. Soil water content depended on rainfall distribution and quantities in different cropping seasons (Figs. 2.2 and 2.3). The amount of water in the top 100 cm of soil was significantly increased with long-term adoption, especially during anthesis (Fig. 2.2). At the top 25 cm soil layer, SWC was usually greater under NT than under CT (Fig. 2.4). The value of SWC under MT was usually between that under NT and CT (Fig. 2.3). SWC at tillering in 2007 and 2008 was between 6 and 14 g kg^{-1} greater on 120 than on 0, and medium on 60. Increased residue production from N fertilizer additions (data not shown) was likely the major reason for this slight increase of SWC with N fertilizer additions.

Grain yield from the experimental plots varied from 0 kg ha^{-1} (due to crop failure under CT in 2008) to 4500 kg ha^{-1}. Mean yields in 2006, 2007, 2008, and 2009 cropping seasons were: 1075, 1519, 495, and 3680 kg ha^{-1} (2.1). In contrast to the mean annual yield of the last 30 years (2800 kg ha^{-1}), we had two years of low production (2006 and 2007), one of extremely low production (2008), and a final year with high production (2009). In low and extremely low yielding years, grain yields under conservation tillage systems were greater than under conventional tillage system, while in a wet year (2009), yields were similar among tillage systems. Moreover, there was a significant interaction between tillage system and N fertilization level. Under NT, 60 and 120 of N fertilization had greater grain yield than 0 (Table 2.1). However no significant responses to N fertilizer addition occurred under MT and CT.

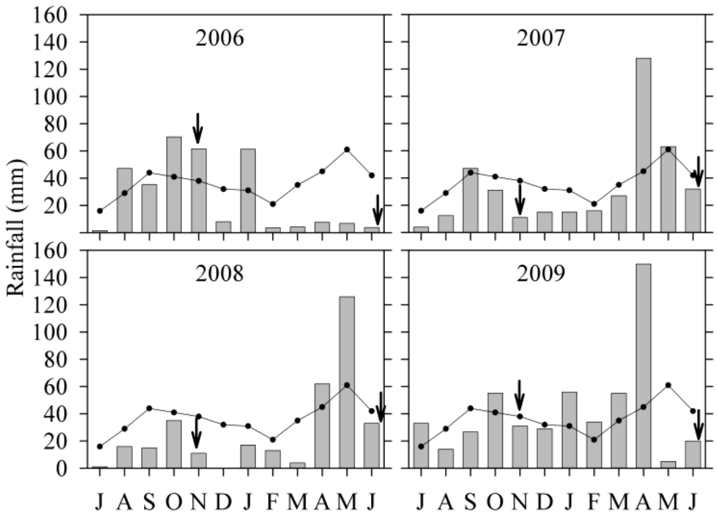

Figure 2.1. Monthly rainfall during the four cropping seasons under study (vertical bar chart), 2006, 2007, 2008 and 2009, starting in July (J), after harvest of the previous year. Average monthly rainfall (30 years) (line) for Agramunt. Vertical arrows indicate sowing and harvest times.

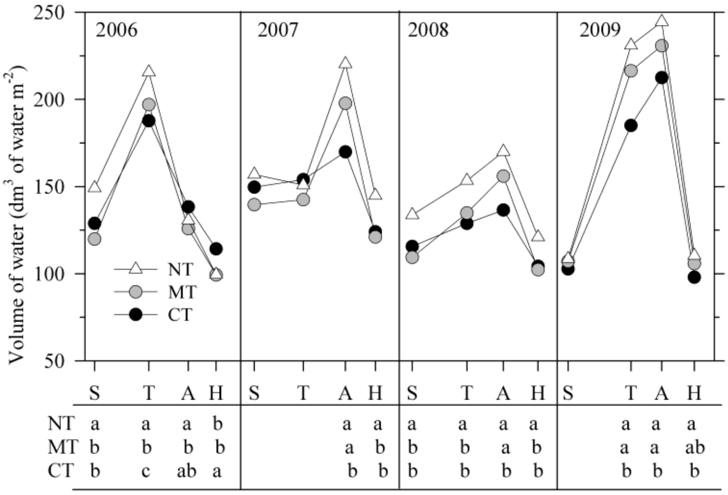

Figure 2.2. Volumetric soil water content at 0-100 cm soil depth layer, at sowing (S) and significant growing stages: tillering (T), anthesis (A), and harvest (H); during four cropping seasons: 2006, 2007, 2008, and 2009; under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). The values are the average of three N fertilization levels. Significant differences (P<0.05) among tillage system at each sampling are indicated below the graph with different letters.

3.2. Root Growth

Root length densities were between 0 and 2.7 cm cm^{-3} in the top 25 cm soil depth (Fig. 2.4). In all years values of RLD were greater at anthesis, and the value of RLD at maturity was between half to one third the value at anthesis.

Root length density was significantly affected by tillage systems (P-value < 0.001), in significant interaction with year and development stage (P-value < 0.001). In 2007, RLD in the surface 25 cm soil under conservation tillage was greater than CT at anthesis and harvest (Fig. 2.4). Root length density was greater under MT at the beginning of stem extension (Fig. 2.4). In 2008, the crop failed to grow under CT due to lower water availability (Fig. 2.3), and RLD was low and similar under NT and MT, with values below 1.2 cm cm^{-3} (Fig. 2.4). In 2009, RLD was higher under CT and MT than under NT (Fig. 2.4).

Table 2.1. Grain yield in each growing season (2006, 2007, 2008 and 2009), under different tillage systems and different N fertilization rates: 0, 60 and 120 kg N ha^{-1}. Different letters indicate significant differences among N fertilizer levels and tillage systems in each growing season (Tukey, P<0.05).

		No-tillage	Minimum tillage	Conventional tillage
Growing season	**Fertilizer rate (kg N ha^{-1})**	—— kg ha^{-1} ——		
2006	0	1350 b	1100 b	300 d
	60	1650 ab	1050 b	500 cd
	120	1960 a	1100 b	650 c
2007	0	1550 b	1950 ab	650 c
	60	2500 a	1950 ab	700 c
	120	2100 ab	1800 ab	450 c
2008	0	500 a	700 a	crop failure
	60	700 a	800 a	crop failure
	120	850 a	900 a	crop failure
2009	0	2950 b	3350 ab	3650 ab
	60	4400 a	4100 a	3550 ab
	120	4200 a	3500 ab	3450 ab

N fertilization significantly affected RLD in interaction with tillage system and year (P-value = 0.004), and in interaction with development stage and year (P-value = 0.015). *A posteriori* analyses of variance by year and phenological stage, found a significant reduction of RLD at the 25-50 cm soil depth layer under NT at the beginning of stem extension in 2009: RLD was 0.56 cm cm^{-3} under unfertilized plots to 0.12 cm cm^{-3} with medium and high N fertilizer additions. The negative effect of N fertilization on root growth has also been found during early development in another study (Rajkai Végh, 1991).

We found a significant relationship between root length per unit area (km m^{-2}) at anthesis and total water use during the pre-anthesis (Fig. 2.5). Significant linear and quadratic relationships were found under MT and NT, repectively (Fig. 2.5). Correlation was not significant under CT. Significant relationship between the size of the root length, RL (km m^{-2}); and above-ground growth at anthesis, AGB (g m^{-2}); was found under MT (AGB = 111.9 x RL.- 245, R^2=0.68, P<0.05), which means that every additional kilometer of root growth per square meter, implied additional 112 g of above-ground dry matter at anthesis.

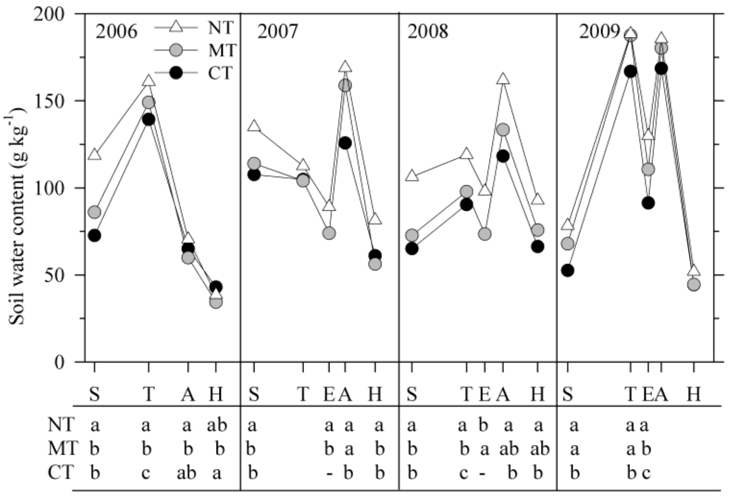

Figure 2.3. Gravimetric soil water content at 0-25 cm soil depth layer, at sowing (S) and significant growing stages: tillering (T), beginning of stem extension (E), anthesis (A), and harvest (H); during four cropping seasons: 2006, 2007, 2008, and 2009; under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). The values are the average of three N fertilization levels. Data under conventional tillage at stem extension in 2007 and 2008 is missing. Significant differences (P<0.05) among tillage system at each sampling are indicated below the graph with different letters.

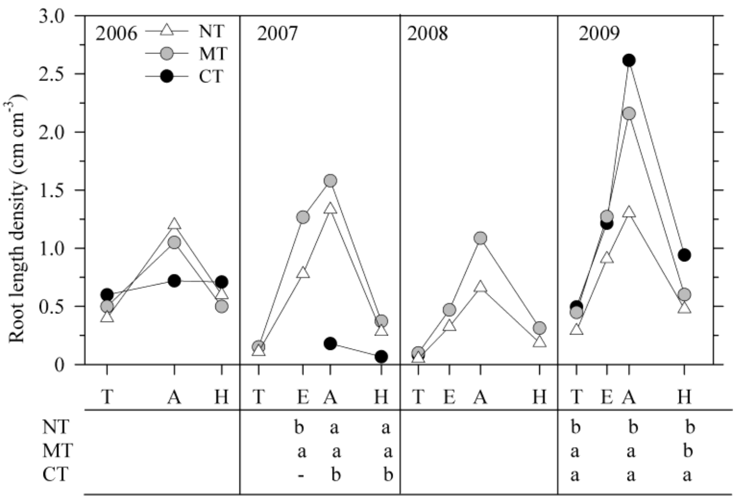

Figure 2.4. Root length density at 0-25 cm soil depth layer at significant growing stages: tillering (T), beginning of stem extension (E), anthesis (A), and harvest (H); during four cropping seasons: 2006, 2007, 2008, and 2009; under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). The values are the average of three N fertilization levels. Data under conventional tillage at stem extension in 2007 and 2008 is missing. Significant

differences (P<0.05) among tillage system at each sampling are indicated below the graph with different letters.

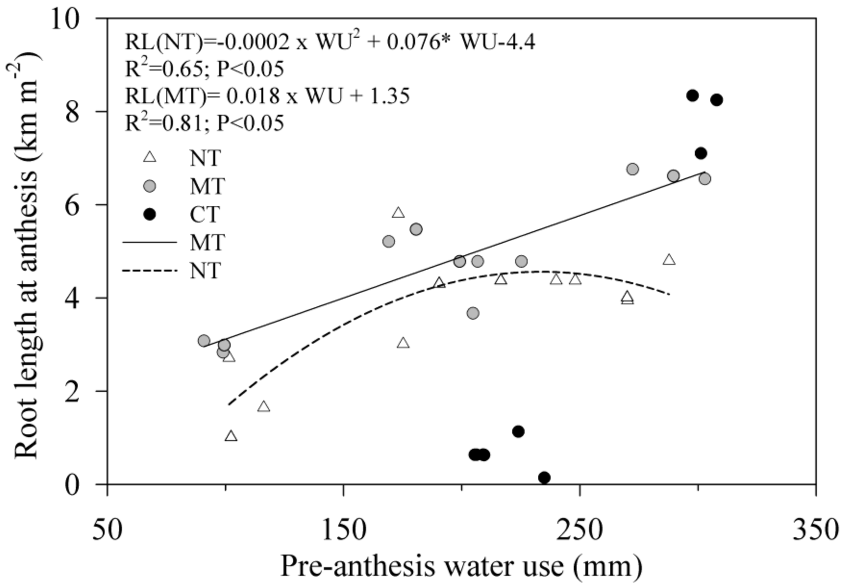

Figure 2.5. Relationship between water use during the pre-anthesis period and the root length achieved at anthesis. The linear of the adjustment between root length (RL) and pre-anthesis water use (WU) is presented for MT treatment.

3.3. Soil Strength and Soil Compaction

Penetration resistance significantly differed among tillage systems in the upper 45 cm soil (Fig. 2.6). It was higher under NT than under CT and MT in the top 15 cm soil depth. At 15 to 20 cm, PR was similar under MT and NT, and lower under CT. Penetration resistance was lower under NT than under CT and MT at tillering and at anthesis below the 30 cm soil depth.

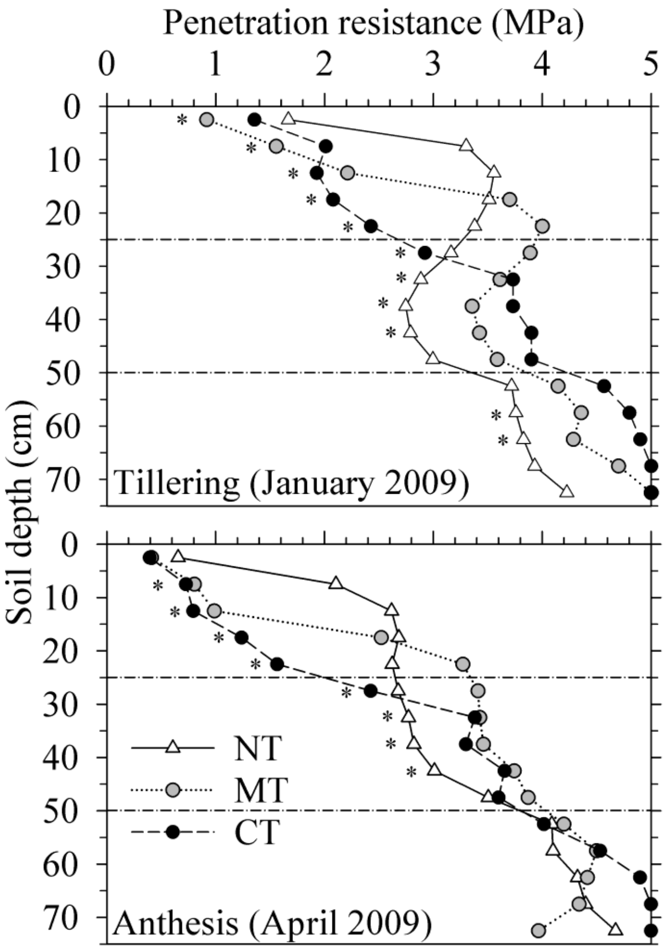

Figure 2.6. Soil penetration resistance at tillering and at anthesis in 2009 cropping season, for different tillage systems: no-tillage (NT); minimum tillage (MT); conventional tillage (CT); average of three N fertilization levels. At each depth interval, asterisks indicate significant differences among means (P<0.05).

Bulk density was on average 1.43 g cm^{-3} under NT, 1.41 g cm^{-3} under MT and 1.40 g cm^{-3} under CT. At 0-5 cm, BD was lower under NT and MT than under CT (Table 2.2). However at 5-10 cm, BD was higher under NT than under MT and under CT. At 10-20 cm, BD was higher under MT and under CT, and medium under NT. Below 20 cm depth, differences were not significant.

During the wet year (i.e., 2009), there was a significant negative linear relationship among treatments between PR and mean RLD in the top 25 cm. There was a significant linear decrease of RLD of almost 1 cm cm^{-3} for every 1 MPa increase of PR with long-term conservation tillage systems.

Table 2.2. Soil bulk density after harvest in July 2009, for different tillage systems: no-tillage (NT); minimum tillage (MT); conventional tillage (CT); average of three N fertilization levels. At each soil depth interval, means with significant difference (P<0.05) are indicated with different letters, where a indicates the highest value.

	Soil bulk density (g cm^{-3})		
Soil depth (cm)	NT	MT	CT
0-5	1.32 b	1.33 b	1.42 a
5-10	1.52 a	1.39 b	1.41 a
10-20	1.44 ba	1.49 a	1.39 b
20-30	1.45	1.46	1.41
30-40	1.42	1.39	1.38

4. DISCUSSION

Total root length per unit area was up to 6.3 km m^{-2}, as reported under similar conditions (Brown *et al*., 1987; Gregory *et al*., 1992). The decrease of RLD between anthesis and maturity was due to the senescence of roots during this period. According to the methodology (Ward *et al*., 1978), we measured living (respiring) root tissue which, according to Hansson *et al*., (1991), rapidly declines as the crop senesces.

The response of root growth to tillage systems depended on the growing season, and it was greatly related to soil water content, and water use, and to soil strength in 2009. In dry years, increased RLD under conservation tillage systems was attributed to higher SWC than under CT (Figs. 2.2 and 2.3). Increased SWC (Fig. 2.3) was likely the result of increased soil cover from crop residues from the preceding cropping season due to reduced residue incorporation (Lampurlanés and Cantero-Martínez, 2006).

Over the four years of this study, the root growth under MT and NT was positively related to the pre-anthesis water use (Fig. 2.5). Increased water availability under MT led to a linear increase of root length. The quadratic relationship under NT (Fig. 2.5), indicated a limited response of root growth to increased water availability in the soil, due to soil compaction and increased PR of the soil (Fig. 2.6 and Table 2.2). However, it can be also observed that under NT the crop was able to absorb the same amount of water as MT with a lower root length (Fig. 2.5). As reported by Barraclough *et al*. (1989) a root density of 1 cm cm^{-3} was enough for extracting all of the potentially available water in a drying soil.

The reduction of RLD in 2009 under NT was related to the higher PR in the top 25 cm (Fig. 2.6). Penetration resistance under NT was higher than 2 MPa, which is generally reported to produce a significant reduction of root growth (Atwell, 1993; Martino and Shaykewich, 1994). Higher PR at 15-20 cm under MT (Fig. 2.6) was due to the depth of tillage operations. The effect of tillage systems on PR between 30-35, 40-45, and 55-65 cm soil depth were not significant if SWC was used as a covariate in the analyses of variance of PR. For this reason, decreased PR under NT at these deeper layers could be attributed to wetter conditions (Fig. 2.3).

An improvement of soil structure may occur in response to long-term adoption of NT (Plaza-Bonilla *et al*., 2010). Improved soil structural stability has been related to increased RLD of wheat in a NT system under Mediterranean semiarid conditions (Martínez *et al*., 2008). In this experiment, earthworm populations (Lumbricidae family) were active at the experimental site and higher under NT (Ojeda *et al*., 1997; Cantero-Martínez, 2005), which may have contributed to the development of a system of biopores allowing root growth under NT in spite of soil compaction.

Higher root elongation during the whole cropping season is expected in response to fertilizer addition (Brown *et al*., 1987; Hansson *et al*., 1991), however observations in this study did not lead us to this conclusion. Response of crop growth and grain yield to N fertilization was hindered under CT and high levels of N under MT due to accumulations of mineral N in the soil under long-term CT, and under 120 under MT of more than 400 kg of N-NO_3 ha^{-1}, in contrast to NT, where mineral N content remained between 100 and 300 kg of

$N\text{-}NO_3$ ha^{-1}. Reduced SWC and accumulation of mineral nitrogen in the soil limited the response of root growth to N fertilizer additions.

5. CONCLUSIONS

Long-term conservation tillage system increased root length density in dry years due to increased soil water content compared to CT, which positively affected grain yields. In a wet year, root length density was lower under no-tillage than MT and CT due to increased soil strength. However, grain yield was not negatively affected. The response of root growth to N fertilization was slight, and only occurred under conservation tillage system.

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Chapter 3: Seasonal dynamics of soil CO_2 flux

Published in Journal of the Soil Science Society of America:
Morell, F.J., Cantero-Martínez, C., Lampurlanés, J., Plaza-Bonilla, D., Álvaro-Fuentes, J. 2011. Soil carbon dioxide flux and organic carbon content: Effects of tillage and nitrogen fertilization. 75 (5), pp. 1874-1884.

Abstract: The response of soil carbon dioxide (CO_2) flux to long-term tillage practices (NT, no-tillage; MT, minimum tillage; CT, conventional tillage) and nitrogen (N) fertilization level (zero; medium, 60 kg N ha^{-1}; high, 120 kg N ha^{-1}) was studied during three growing season in a rainfed Mediterranean agroecosystem. Soil CO_2 flux was related to the crop growth, with the highest flux during spring (i.e., March-May). Tillage and N fertilization effects on soil CO_2 flux during growing seasons depended on weather conditions: greater soil CO_2 flux under MT and NT on dry years, greater under CT and MT on a wet year. N fertilization affected soil CO_2 flux during this wet growing season: flux with N fertilizer additions was higher than flux on the unfertilized plots. Thirteen years after establishment of the experiment, the soil organic carbon (SOC) stock under long-term NT was 3.9 Mg C ha^{-1} greater than under CT and 4.3 Mg C ha^{-1} greater than under MT. The SOC stocks with N fertilizer additions were 4 Mg C ha^{-1} greater than the stock on unfertilized plots. The increase of C inputs with N fertilization was more pronounced under NT than under MT or CT. For this reason the increased response of SOC stock to N fertilization is expected under NT in a longer period of time. The product between soil water content and soil temperature explained 75 and 94% of the seasonal variability of soil CO_2 flux. However, soil CO_2 flux and SOC stock were hardly related.

Abbreviations: GS, growing season; SF, summer/autumn fallow period; C, carbon; N, nitrogen; CO_2, carbon dioxide; NT, no tillage; MT, minimum tillage; CT, conventional tillage; ZN, zero N fertilization; MN, medium N fertilization level; HN, high N fertilization level; SOC, soil organic carbon; SWC, soil water content; TIL, tillage system factor; FNT, nitrogen level factor.

1. INTRODUCTION

Carbon dioxide is produced in soils by mineralization of soil organic carbon (SOC) and plant residues, and autotrophic respiration of roots. Most of the CO_2 produced in the soil is released to the atmosphere as a flux of CO_2 from the soil to the atmosphere. Annual gross CO_2 flux to the atmosphere has been estimated to be 80.4 Pg C-CO_2 worldwide (Raich *et al*, 2002), which is ten times the annual CO_2 emission by fossil fuel combustion. The study of soil CO_2 flux is useful for documenting total metabolic activity, as well as for investigating the soil carbon balance and management impacts (Raich and Schlesinger, 1992, Fortin *et al*., 1996).

Natural and anthropogenic disturbances of soil CO_2 flux have been widely evaluated (e.g., Reicosky and Lindstrom, 1993; Bremer *et al*., 2002; Xu and Wan, 2008). Under semiarid Mediterranean conditions, soil CO_2 flux has been evaluated on forest ecosystem (e.g., Casals *et al*., 2000), in steppe ecosystem (e.g., Maestre and Cortina, 2003) and in agroecosystems (e.g., Sanchez *et al*., 2003), showing a similar range of values but differences on seasonality and dependence on the soil temperature and moisture. In agroecosystems, conservation tillage systems have shown to reduce soil CO_2 flux (Sanchez *et al*., 2002; Álvaro-Fuentes *et al*., 2008; Akbolat *et al*., 2009). The response of soil CO_2 flux to nitrogen (N) fertilization and its interaction with tillage systems have been previously studied under semiarid Mediterranean conditions (Morell *et al*., 2010). However, under these conditions, no data has been reported on the effects of N fertilization and tillage systems on soil CO_2 flux during the overall growing season.

On the other hand, the stock of C in SOC represents the largest terrestrial reservoir of C (Lal and Kimble, 1997). Adequate management practices on croplands, such as conservation tillage systems and adequate nutrient supply, may increase the stock of SOC about 0.74 to 1 Pg C per year worldwide (Lal and Bruce, 1999). The content of SOC is determined by the soil carbon (C) balance between C inputs mainly in plant residues and C outputs, mainly as part of the soil CO_2 flux. Soil CO_2 flux and quantification of C inputs provide insights to the processes that regulate soil C sequestration, however the analyses of soil C content is required for reliable assessments of changes on soil C storage (Ellert and Janzen, 1999).

Conservation agricultural systems have shown to increase the stock of SOC under a wide range of conditions (e.g., Follet, 2001; Franzluebbers, 2010). Also under semiarid Mediterranean conditions several studies have reported significant increases of the SOC stock (Hernanz *et al*., 2009; Farina *et al*, 2010; Plaza-Bonilla, 2010). On the other hand, N fertilization may increase the SOC stock due to increased C inputs from improved crop productivity (Lee *et al*., 2007; Luo *et al*., 2010). However, there are a number of controversial results (Khan *et al*., 2007; Mulvaney *et al*., 2009; Powlson *et al*., 2010) that require further evaluations of the effects of N fertilization on soil C stocks under different environmental conditions. Numerous studies have shown positive effects with adequate nutrient management on SOC content under semi-arid conditions (Rudrappa *et al*, 2006; Bakht *et al*, 2009). Nevertheless, there is a gap of information under semiarid Mediterranean conditions and on its interaction with different tillage systems.

Our aim was to study the long-term effect of N fertilizer application under different tillage systems on soil CO_2 flux and on the stock of SOC. In this manuscript, we present the evaluation of soil CO_2 flux during three years, including three consecutive growing seasons and measurements during summer-fallow periods, ten to thirteen years after the establishment of the experiment.

2. MATERIAL AND METHODS

2.1. Site, tillage and N fertilization

The study was carried out in a long-term field experiment established in 1996 in Agramunt (41º 48'N, 1º 07'E; Lleida, Spain). Mean annual precipitation is 430 mm and mean temperature is 13.8 ºC. The experiment consisted of a long-term comparison of three tillage systems and three N fertilization levels on a factorial randomized complete-block design with three repetitions (plot size 50 m x 6 m). The tillage systems were two conservation tillage systems (no-tillage, NT and minimum tillage, MT) and one intensive tillage system (conventional tillage, CT). The three levels of N fertilization were: zero (ZN), medium (MN), 60 kg N ha^{-1}, and high (HN), 120 kg N ha^{-1}. The soil was classified as Typic Xerofluvent (Soil Survey Staff, 1994). The soil in the Ap horizon (0-28cm) contained 465 g kg^{-1} sand, 417 g kg^{-1} silt and 118 g kg^{-1} clay, with a pH of 8.5. Soil organic carbon (SOC) concentration at the beginning of the experiment was 11 g kg^{-1} at top 5 cm.

The cropping system consisted of a barley (*Hordeum vulgare*, L.) cv. Hispanic monoculture under rainfed conditions. By the end of June, at grain harvesting, the straw residue was spread over the plot in all the treatments. Following, the soil remained on a summer-fallow period. During this period, the field was mostly free of vegetation due to dry conditions. Tillage operations were conducted by the end of October or beginning of November. The CT treatment was accomplished with a mouldboard ploughing to a depth of 25-30 cm soil surface with almost 100% of the residue incorporated in the soil. The MT treatment consisted of a cultivator pass to a depth of 10-15 cm with an incorporation of approximately 50% of the crop residue. In the NT treatment, sowing was done by direct drilling after spraying with herbicide: 1.5 L per hectare, 36% glyphosate [N-(phosphonomethyl)glycine]. N fertilizer was split in two applications. One-third of the fertilizer was broadcasted previous to tillage as ammonium sulphate (21% N) and two-thirds at the beginning of tillering as ammonium nitrate (33.5% N). Barley was seeded at a sowing rate of 450 seeds m^{-2} in mid November, around two weeks after tillage operations. The present study was carried out between October 2006 and July 2009.

2.2. Measurements of soil CO_2 fluxes

2.2.1. Seasonal variation of soil CO_2 fluxes

Soil CO_2 fluxes were measured every 7-14 days. Measurements were carried out during three growing seasons (GS), 2006-2007, 2007-2008 and 2008-2009 hereafter referred to as GS07, GS08 and GS09, and summer-fallow periods (SF), except for July and August 2008 when no measurements were taken due to equipment maintenance.

An open chamber system (model CFX-1, PPSystems, Hitchin, Hertfordshire, UK) connected to an infrared gas analyzer (model EGM-4, PPSystems, Hitchin, Hertfordshire, UK) determined soil CO_2 flux by calculating the difference in CO_2 concentration between air entering and leaving the chamber. The chamber had a 21 cm diameter cylindrical shape, covering a soil surface of 346 cm^2. The chamber was directly inserted 2 cm deep in the soil. Flow rate of the chamber was adjusted to 900 mL min^{-1}. Flux readings were taken 3 to 4 minutes after the chamber had been inserted into the soil, when readings of CO_2 flux were stable.

Two regions of 6 m^2 were defined on each plot, and one measurement within each region was taken on each sampling day. Measurements at each region were carried out at the same time of the day, between 10:00 and 14:00 hours. During growing season, the above ground biomass was removed by cutting 25 cm of the seeding line without altering the soil surface, providing an area where the chamber could be comfortably inserted in the soil. Removal of the aerial part without altering the soil surface has been proven not to modify significantly soil CO_2 flux for a time of one hour or more (Liu and Li, 2006).

Soil environmental conditions were determined at each sampling point. Soil temperature at 5 cm soil depth was determined with a hand-held probe (TM65, Crison). Gravimetric soil water content (0-5 cm depth) was determined by oven drying a soil sample at 105 ºC. Daily air temperature and precipitation observations were recorded at the experimental site using an automated weather station.

We pooled measurements of soil CO_2 flux in four periods. One period grouped those measurements during fallow (28 measurements; SF) and the other three periods grouped measurements during each growing season: GS07, GS08 and GS09, with 21, 19 and 16 measurements respectively.

2.2.2. Diurnal variation of soil CO_2 fluxes

Diurnal variations of soil CO_2 flux were evaluated several times in winter (D1-D3) and in spring (D4-D6) in 2008 and 2009 (Fig. 3.1). Six PVC collars (Ø20 cm, height 5 cm) were inserted on the medium N fertilization level of each tillage systems. The collars were inserted between seeding lines without removing any plant from the surroundings area, since plant removal would have reduced the soil CO_2 flux on the subsequent hours due to a decrease on root respiration (Liu and Li, 2006). The part of the collars entering the soil was sharpened and a wood cover with the inner shape of the collar was used to introduce it 2-3 cm in the soil. Seven samplings were conducted between midnight and dusk at given times to capture the diurnal variation.

Diurnal variations of soil CO_2 flux were adjusted to a sinusoidal function: $F = 1+ [a \cdot \sin(2\pi \cdot T/24+ e)]$ (Equation 1); where F is the soil CO_2 flux, T, is the time of the day, between 0 and 24, and values of a and e are the adjustment parameters (Chatskikh and Olesen, 2007).

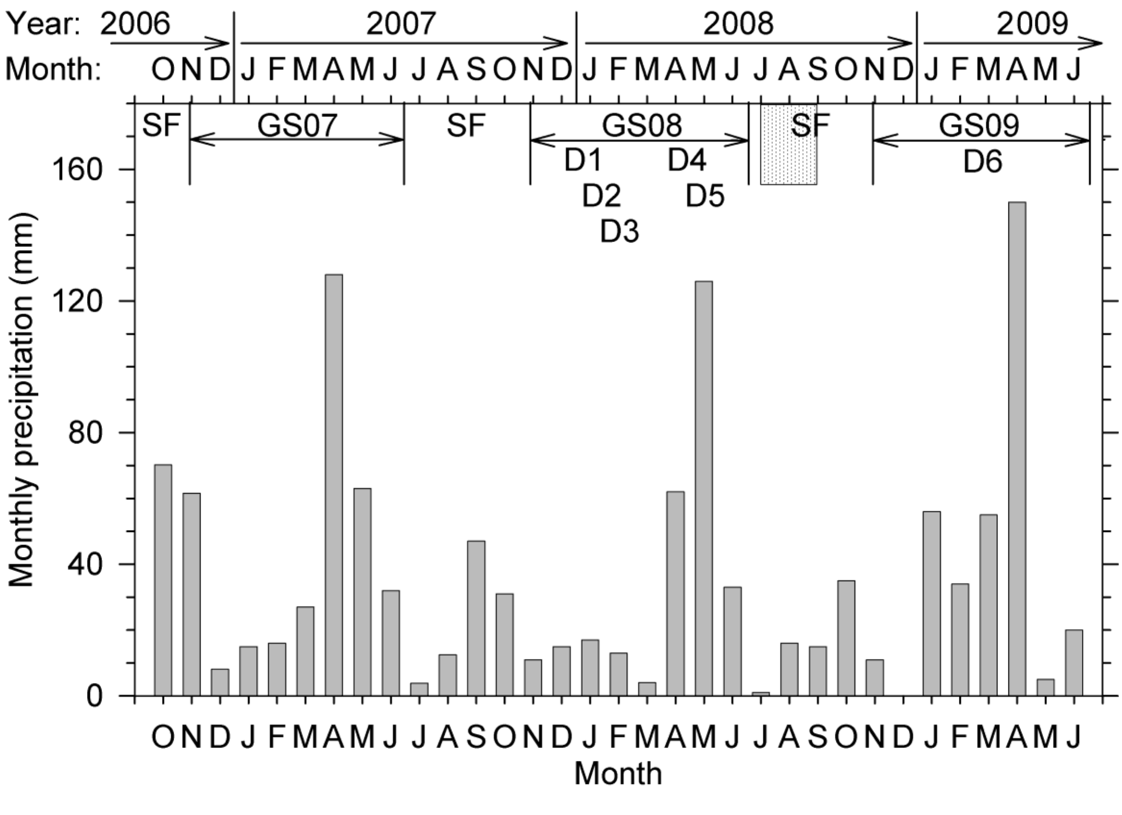


Figure 3.1. Monthly precipitation during the experimental period, starting in October 2006 until end of June 2009. Including three consecutive growing seasons 2006-2007 (GS07), 2007-2008 (GS08), 2008-2009 (GS09), and measurements during summer fallow periods. D, indicates evaluation of diurnal variations, evaluated 6 times on different days (D1-D6). Grey box indicates a two month period with a lack of measurements.

The functions obtained in winter (D1-D3), and those obtained in spring (D4-D6) were averaged ($\bar{a}$ and $\bar{e}$) and then normalized, providing winter and spring functions with the relative hourly contributions under different tillage systems.

2.3. Crop growth: C inputs

The annual C input was approximated as the sum of the C content in the straw left at harvest and that in the root biomass. The crop return with straw was estimated from harvest indexes at maturity and grain yields of the plots. Harvest indexes were determined at crop maturity. Three samples per plot were taken by cutting the plants at the soil surface level on 50 cm along the seeding line. Grains were separated from the rest of the plant, oven dried at 65-70º C for 48 hours, and then weighted, and the harvest index determined. Harvesting was done by the end of June with a standard medium-sized combine. The straw was chopped and spread over the plots by the harvester machine, and the grain yield of each plot determined.

Root dry matter was determined at maturity. Soil cores from the row and the interrow were collected to 50 cm depth. Roots were separated by washing the soil on a 0.5-mm sieve, and weighed after oven drying. For the calculation of the C input, we considered 45 % of C on the above-ground crop residues and 40 % of C in the root dry matter (Álvaro-Fuentes *et al*., 2008).

2.4. SOC stock

Soil was sampled in July 2009, one month after harvest, to determine the SOC stock. Two areas were sampled in each plot at 10 m distance from the plot extremes. Soil samples were taken at five depth intervals: 0-5, 5-10, 10-20, 20-30, and 30-40 cm soil depth. After air-drying soil samples, SOC concentration was determined by oxidation with potassium dichromate (Walkley and Black, 1934).

Soil bulk density was determined at the same soil depth intervals by core sampling. The SOC stock (Mg ha^{-1}) was calculated in terms of equivalent soil mass (esm) following the procedure of Ellert and Bettany (1995) for the 0-10 and 10-40 cm soil depth interval. The standard reference soil mass used was that of the heaviest soil layer analyzed (832 Mg ha^{-1} for 0-5 and 5-10 cm layers, and 1656 Mg ha^{-1} for 10-20, 20-30 and 30-40 cm layers).

2.5. Data analysis

Each experimental period was analyzed as repeated measures analyses of variance (General linear model; SAS Institute 1990). Tillage system (TIL), N fertilization level (FNT) and their interaction (TILxFNT) were considered as the between subject effects. Sampling time (date) and its interaction with the between subjects effects were considered as the within subject effects. The cumulative CO_2-C emission in each period was calculated from linear interpolation between consecutive samplings using the trapezoid rule.

The analyses of variance of carbon inputs in above-ground crop residues, root biomass and total C input, considered tillage system (TIL), N fertilization level (FNT), cropping season (Year) and their interactions (TILxFNT, TILxYear, FNTxYear, and TILxFNTxYear). The analyses of variance of the SOC concentration considered the following factors: tillage system (TIL), N fertilization level (FNT), soil depth interval (Depth) and their interactions (TILxFNT, TILxDepth, FNTxDepth, and TILxFNTxDepth).

Simple regression analyses of daily soil CO_2 flux and root length per unit area (RL), soil water content (W), soil temperature (T) and their product (WT), were performed with Sigmaplot 11 (Systat Software, Inc., 2008) for each tillage systems (average of the three N fertilization levels) and each period considered (SF, GS07, GS08, and GS09).

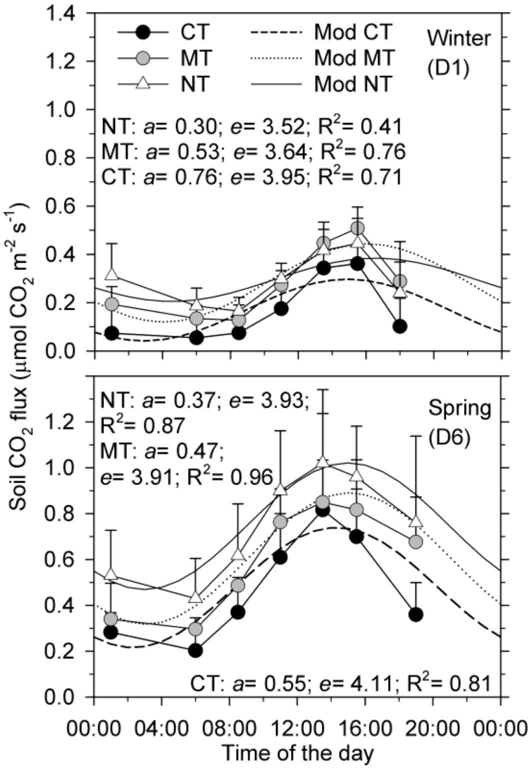
3. RESULTS AND DISCUSSION

3.1. Soil CO_2 flux

3.1.1. Diurnal variation of soil CO_2 flux

Soil CO_2 flux showed a marked diurnal patterns in winter and spring (Fig. 3.2). The diurnal variation of soil CO_2 flux was related to the same of soil temperature (data not shown) as found in other works (Eriksen and Jensen, 2001; Šimek and Hynšt, 2008). Soil temperature variations are related with the CO_2 production due to its effect on soil microbial activity. Moreover, CO_2 production from rhizosphere respiration follows a similar diurnal variation due to changing photosynthetic activity with irradiation (Kuzyakov and Cheng, 2001). Diurnal variation under CT was more pronounced than those under MT or NT (Figs. 3.2 and 3.3). This was partly related with greater variations of soil temperature under CT than under MT or NT, due to drier conditions during growing season, as it is later discussed.

Average winter and spring functions (Fig. 3.3) were obtained from evaluations in GS08 and GS09. Variations during GS07 were assumed to be similar to in figure 3.3. Average winter and spring functions were used for the correction of winter and spring diurnal variations of all three growing seasons. The value of the measured flux was divided by its corresponding hourly contribution, according to the time of the day when it had been measured. In this way the daily emission was better estimated (Shi *et al*., 2006) and the bias among tillage system was partly corrected.



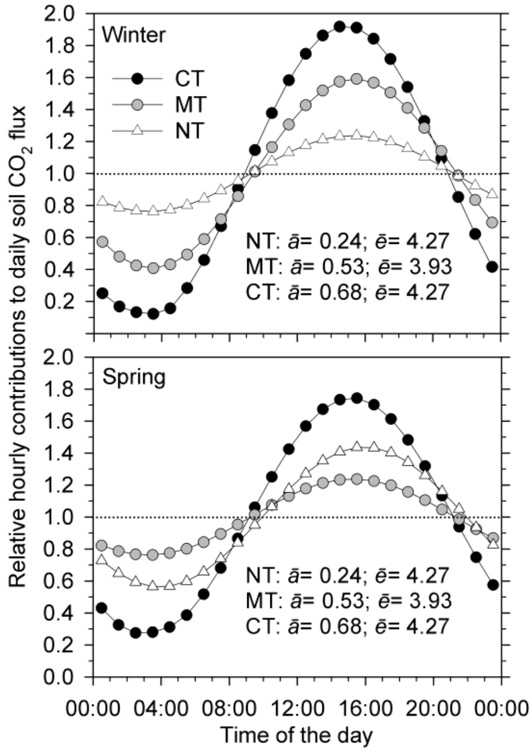


Figure 3.2 (left). Diurnal variation and adjusted sinusoidal functions under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT); in one day in winter and in spring. Error bars indicate standard deviation. Figure 3.3 (right). Relative hourly contributions to daily soil CO_2 emission in winter and in spring under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT).

Diurnal variations in summer and autumn were neglected in this study, and the mean of measurements between 10.00 and 14.00 in summer and autumn was considered as representative of the daily emission. Morning to afternoon variation of soil CO_2 flux during summer and autumn was slight or nil (data not shown). Possible explanation could be that root respiration was absent during these periods, which may be one of the causes of the diurnal variations of soil CO_2 flux. Moreover, microbial activity was the main source of CO_2 flux and it was probably limited due to dry conditions, and less by soil temperature.

3.1.2. Seasonal variation

Soil CO_2 fluxes during summer fallow period were below 0.5 g CO_2-C m^{-2} d^{-1} when the soil was dry, but greater than 1 g CO_2-C m^{-2} d^{-1} when the soil was wetter (Fig. 3.4). Soil CO_2 fluxes during growing seasons were lowest in December and beginning of January, with fluxes below 0.2 g CO_2-C m^{-2} d^{-1}, and highest during April and May, with fluxes between 1.5 and 7 g CO_2-C m^{-2} d^{-1} (Fig. 3.5). This range of values of soil CO_2 flux agreed with those previously reported in a semiarid steppe of south Spain (Maestre and Cortina, 2003) and beneath barley in semiarid central Spain (Sánchez *et al*., 2003).

The distribution and amounts of precipitation (Fig. 3.1), can partly explain the contrasting ranks of soil CO_2 flux among growing seasons (Fig. 3.5). Limited rainfall in GS08 reduced crop growth, which probably reduced root respiration.

Reduced SWC probably reduced microbial respiration as well. The seasonal patterns of soil CO_2 flux has been related to plant vitality (Sanchez *et al*., 2003) and to leaf area indexes (Bremer *et al*., 1998), which can be considered as surrogates of root respiration. The cumulative soil CO_2 flux during the pre-anthesis period over the years under study was significantly related to the size of the active root length (Fig. 3.6; data on root length at anthesis from Morell *et al*, 2011), thus indicating that root respiration was a dominant factor during growing season.

GS07 and the following summer fallow provided a full year measurements that allowed the estimation of the annual soil CO_2 emission. The estimation of the soil CO_2 flux between sowing 2006 and 2007 was 135 under

CT, 264 under NT and 311 g C m^{-2} under MT. Such estimates are half the value obtained by Sanchez *et al*. (2003) beneath barley in central Spain, but agree with others estimates in semiarid agroecosystem in Argentina (Bono *et al*., 2008). Raich and Schlensinger (1992) suggested that the annual soil CO_2 emission (SR, standing for soil respiration) is related to the net primary productivity (NPP) in different terrestrial vegetation biomes, in a linear relation (SR = 1.24 NPP + 24.5), with a difference between SR and NPP that represents an estimate of the root respiration. The annual C input in GS07 was close to the estimated soil CO_2 emission, giving little space for root respiration. However, the cumulative annual soil CO_2 emission in this study may be an underestimation, since soil CO_2 flux after rainfall events were not considered, and this may significantly contribute to the annual soil CO_2 emission (Parkin and Kaspar, 2004; Morell *et al*., 2010).

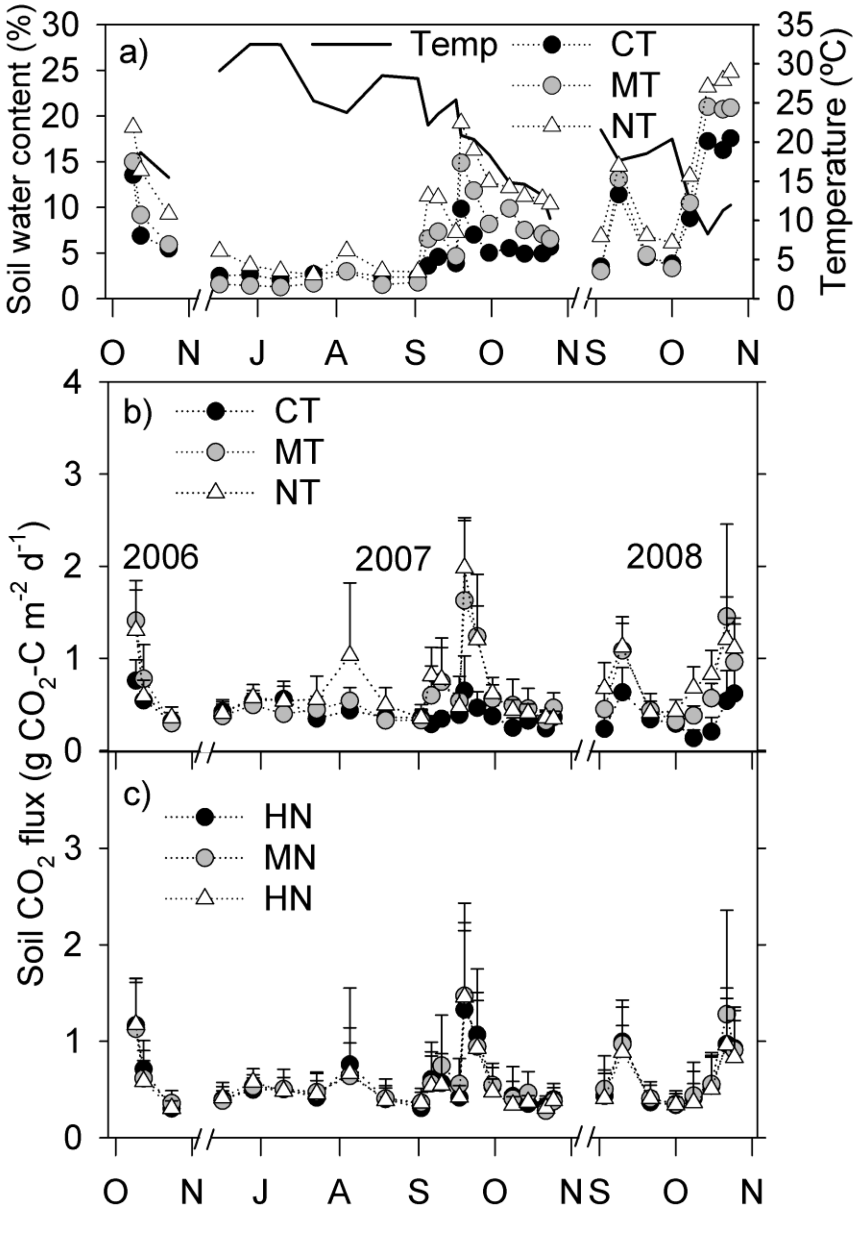


Figure 3.4. Soil CO_2 fluxes and soil environmental conditions during summer fallow periods (SF) in three consecutive years: 2006, 2007, and 2008. Month ticks are placed on the mid of the month. a) Mean soil water content under different tillage systems: no-tillage (NT); minimum tillage (MT); and conventional tillage (CT). b) Mean soil CO_2 fluxes under different tillage systems: NT, MT, and CT. c) Mean soil CO_2 fluxes under different N fertilizer levels: zero (ZN), medium level (MN), and high level (HN). Error bars indicate standard deviation in graphs b and c.

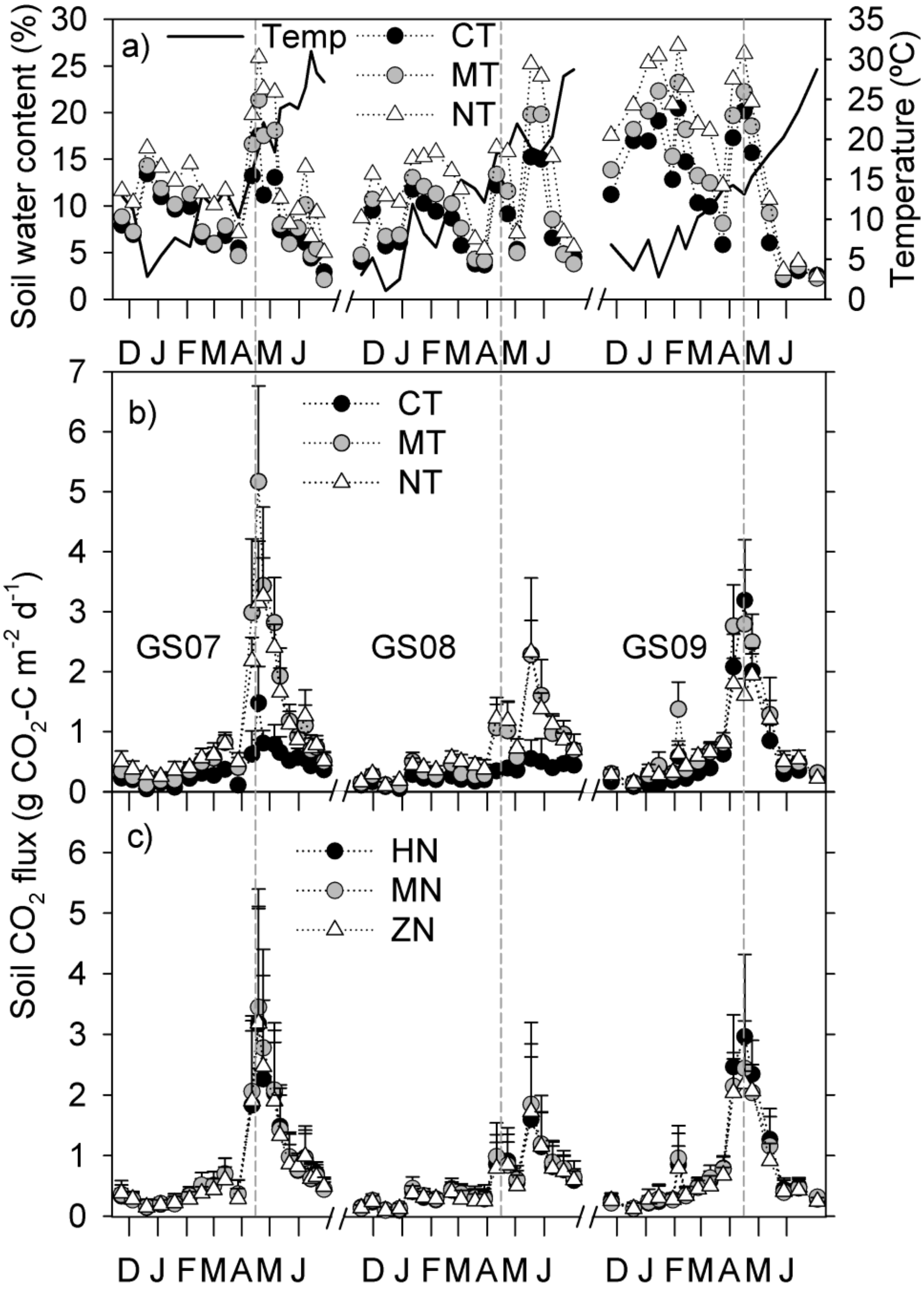


Figure 3.5. Soil CO_2 fluxes and soil environmental conditions during three consecutive growing seasons 2006-2007 (GS07), 2007-2008 (GS08) and 2008-2009 (GS09). Month ticks are placed in the mid of the month. Gray vertical dash lines, at the end of April in each growing season, indicate anthesis. a) Mean soil water content under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). b) Mean soil CO_2 fluxes under different tillage systems: NT, MT, and CT. c) Mean soil CO_2 fluxes under different N fertilizer levels: zero (ZN), medium level (MN), and high level (HN). Error bars indicate standard deviation in graphs b and c.

C fixation was numerically greater in GS09 and GS07 than in GS08 (Table 3.1) due to improved crop productivity. However, under NT, cumulative soil CO_2 emissions through the growing season in GS08 and GS09 were similar, 154 and 152 g CO_2-C m^{-2} respectively, with the limitations of this calculations mentioned before. Leaving aside the contribution of root respiration to soil CO_2 flux, which was probably lower in GS08 than in GS09 due to reduced crop growth (Table 3.1), these estimations indicate important disproportions in the soil C balance in these two growing seasons. As proposed before by Ciais *et al*. (2005), extreme drought events could reverse an ecosystem from C sink to a transient net C source, due to imbalances between C inputs and losses. For instance, during 2007-2008 cropping season, negative soil C balance may have occurred due to decreased C inputs, and increased C outputs with wet conditions in May (Fig. 3.1). Cropping seasons with negative soil C balance may be frequent in semiarid Mediterranean agroecosystems, where the precipitation pattern greatly affects C inputs and C outputs.

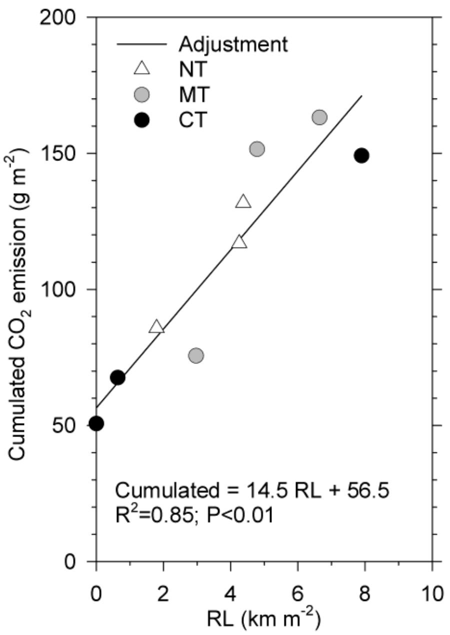


Figure 3.*6. Relationship between root length at anthesis (RL) and cumulative soil CO_2 emission from sowing to anthesis. Nine points corresponding to the observations under different tillage systems (average of three N fertilization levels): no-tillage (NT), minimum tillage (MT), and conventional tillage (CT); on three consecutive growing seasons; and linear adjustment among all the observations.*

3.1.3. Effects of tillage systems and N fertilization

Soil CO_2 flux were significantly affected by tillage systems in all three growing seasons and during fallow period (Table 3.2). During GS07 and GS08, conservation tillage systems (NT and MT) had higher soil CO_2 fluxes than CT (Fig. 3.5b), as well as during fallow period (Fig. 3.4b). These differences can be related to the response of crop growth and soil properties, which were significantly affected by long-term adoption of different tillage systems.

Conservation tillage systems improved crop growth, as reflected on increased C fixation under NT and MT than under CT (Tables 3.1 and 3.3). The improvement of crop growth under conservation tillage systems on dry seasons is commonly found in the semiarid Mediterranean region due to improved water conservation (Cantero-Martínez *et al*., 2007; Mrabet, 2000). Improved crop growth under conservation tillage systems increased C inputs (Table 3.1), which was responsible for the increased soil CO_2 flux during some periods (Figs. 3.4b and 3.5b). Long-term adoption of conservation tillage systems significantly increased SOC concentration as it is later presented. Higher SOC concentration and increased amount of crop residues did probably increase the amount of mineralizable organic C and thus microbial respiration, and thus soil CO_2 flux.
N fertilization only affected soil CO_2 flux during GS09 (Table 3.2), when it slightly increased soil CO_2 flux (Fig. 3.5c). The significant interactions between TILxFNT, and those between Date, and TIL, FNT and their interaction (TILxFNT), were considered *a posteriori* by analyzing soil CO_2 flux by sampling date (data not shown).

According to this analysis, medium and high N fertilization levels increased soil CO_2 flux on the samplings during the period between mid March and end of April, and this increase occurred on different sampling days on each tillage system. The lack of a significant response of soil CO_2 flux to N fertilization during GS07 and GS08, was in part due to reduced availability of water during these two growing seasons (Fig. 3.5a). Reduced water availability limited the response of crop growth to N fertilization, as it tends to occur in dry years in the region (Cantero-Martínez *et al*., 1995 and 2003).

Table 3.1. C inputs (g m^{-2}) with crop residue return under different tillage systems and N fertilization levels: zero (ZN), medium (MN), and high (HN).

† Straw is the estimated amount of above-ground crop residues (g m-2) calculated from grain yield of the plots and harvest index. ‡ Root, is the root biomass (g m-2). § C inputs were calculated assuming 45% of C in straw and 40% in root dry matter (40% of C).

		No-tillage			Minimum tillage			Conventional tillage		
		ZN	MN	HN	ZN	MN	HN	ZN	MN	HN
2006-2007	Straw †	321b	511a	451a	456a	484a	425a	210b	242b	404ab
	Root ‡	37b	94a	96a	80ab	71ab	71ab	44ab	41b	39b
	C input §	159cb	267a	241ab	237ab	246ab	220ab	112c	125cb	197b
2007-2008	Straw †	261a	292a	368a	284a	209a	174a	0b	0b	0b
	Root ‡	26a	33a	33a	24a	20a	15a	0b	0b	0b
	C input §	128a	145a	179a	137a	102a	84a	0b	0b	0b
2008-2009	Straw †	332c	679ab	752a	414bc	463b	667ab	541b	570b	642ab
	Root ‡	77a	81a	113a	93a	102a	104a	116a	86a	91[a]
	C input §	180c	338a	383a	223bc	249b	342a	290ab	291ab	326[a]

Note on table 3.1: Different letters indicate significant differences (P<0.05) among tillage systems and N fertilization levels.

Table 3.2. Soil CO_2 flux ANOVA: Significance of between subject effects (tillage system, TIL; N fertilization level, FNT; and their interaction) and within subject effects (Date) during three growing seasons (GS), and summer fallow measurements (SF). n.s. stands for non-significant (P<0.05).

		Fallow	Growing seasons		
	Effect	SF	GS07	GS08	GS09
Between subject effects	TIL	<.0001	<.0001	<.0001	<.0001
	FNT	n.s.	n.s.	n.s.	<.0001
	TILxFNT	n.s.	n.s.	n.s.	0.0022
Within subject effects	Date	<.0001	<.0001	<.0001	<.0001
	DatexTIL	<.0001	<.0001	<.0001	<.0001
	DatexFNT	n.s.	n.s.	n.s.	<.0001
	DatexTILxFNT	n.s.	n.s.	n.s.	0.014

The effect of FNT on C input was significant in GS07 and in GS09, when it significantly interacted with the tillage system (Table 3.3). The increase of C input with N fertilization was more pronounced under NT, and reduced under CT, especially in 2008-2009 growing season (Table 3.1). However in GS09, differences on soil CO_2 flux between tillage systems appeared over time, as indicated by the significant interaction between TIL and Date (Table 3.2), with differences around anthesis (Fig. 3.5b). The response of soil CO_2 flux to N fertilization occurred in GS09, when crop growth and C input were significantly increased by N fertilization. It supports the idea that the effects of N fertilization on soil CO_2 flux are due to stimulated rhizosphere respiration (Kou *et al*., 2008; Xu and Wan, 2008; Iqbal *et al*., 2009). However, to confirm the hypothesis, the relative contributions of root respiration and soil C mineralization (microbial respiration) to the soil CO_2 flux needs to be considered in future experiments.

3.1.4. Soil environmental factors and seasonal variation of soil CO_2 flux

During the summer fallow periods, SWC at top 5 cm experienced great seasonal changes (Fig. 3.4), which accounted for 42 and 51% of the variability of daily soil CO_2 flux under NT and MT respectively (Fig. 3.7). Soil moisture is a major driver of soil CO_2 flux under semiarid conditions (Jin *et al*., 2010), contrarily to areas with small ranges of soil seasonal change in soil moisture where soil temperature is the major driver of coil CO_2 flux (Lee *et al*., 2007). The lack of adjustment under CT indicates the contribution of other factors such as soil moisture at deeper layers, since potential microbial activity is evenly distributed through the soil profile (Madejón *et al*., 2009).

Table 3.3. Total C inputs ANOVA: Significance of main factors: tillage system, TIL; N fertilization level, FNT; and year or cropping season (Year); and their interaction during three cropping seasons. n.s. stands for non-significant (P<0.05).

		By cropping season (Year)		
Effect	2006-2009	2006-2007	2007-2008	2008-2009
TIL	<.0001	<.0001	<.0001	0.0131
FNT	<.0001	0.0005	n.s.	<.0001
TILxFNT	0.0005	0.0002	n.s.	<.0001
Year	<.0001			
YearxTIL	<.0001			
YearxFNT	0.0007			
YearxTILxFNT	0.0412			

Soil CO_2 flux was increased during warm periods (soil temperature >20 ºC) only during periods of high soil moisture (Fig. 3.4). The interaction between SWC and soil temperature, considered as the relation with the product between them (Fig. 3.7), provided a significant (P<0.05) quadratic adjustment under NT (Fig. 3.6). In semiarid ecosystems, increases of soil CO_2 flux with high soil temperature (> 20 ºC) are constrained by low soil moisture (Conant *et al*., 2000), but the increase of soil CO_2 flux occurs when the soil is both wet and warm, and hence the quadratic relation found under NT (Fig. 3.6). The increase of soil CO_2 flux with summer and autumn rainfall will later decrease as the soil dries (Morell *et al*, 2010), which duration will depend on the prevailing temperatures.

During GS09, SWC was high during most of the season (Fig. 3.5a), and for these reasons the linear relationships between soil CO_2 flux and SWC or soil temperature were not significant (Fig. 3.8). During GS07 and GS08, variations of soil CO_2 flux were partly explained by variations of SWC under NT and MT, and by variations of soil temperature under CT (Fig. 3.8 and Table 3.4). The linear regression of soil CO_2 flux with the product of SWC and soil temperature (WT) was significant in all cases, and R^2 values were between 0.75 and 0.92 (Table 3.4). However, the relationship with WT was significant, thus indicating their interactive relationship between SWC and soil temperature: high temperatures tended to occur when SWC was low, and SWC tended to be high when soil temperature was low. Late in the growing season (May-June) and during summer fallow period, high soil temperatures will keep soil moisture low. This is an important effect that limits the increase of soil CO_2 flux with high temperatures late in the growing season and during summer fallow in semiarid ecosystems (Conant *et al*., 2000).

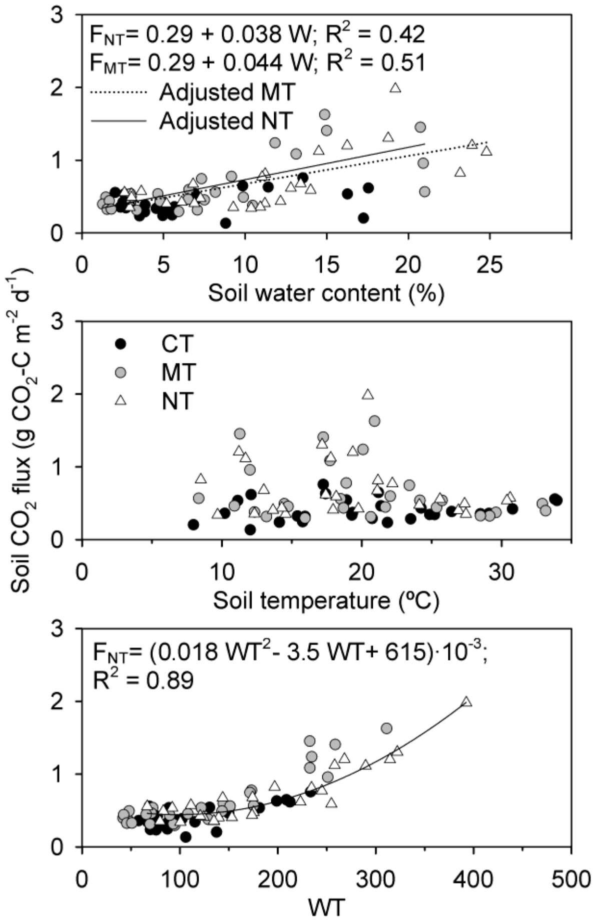


Figure 3.7. Relationships between soil CO_2 flux (F) during summer fallow periods and soil water content at the 0 to 5 cm soil depth (W), soil temperature at 5 cm soil depth (T), and the product of soil water content and soil temperature (WT); from observations under different tillage systems: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). Lines represent significant linear adjustments (P<0.05) under NT and MT.

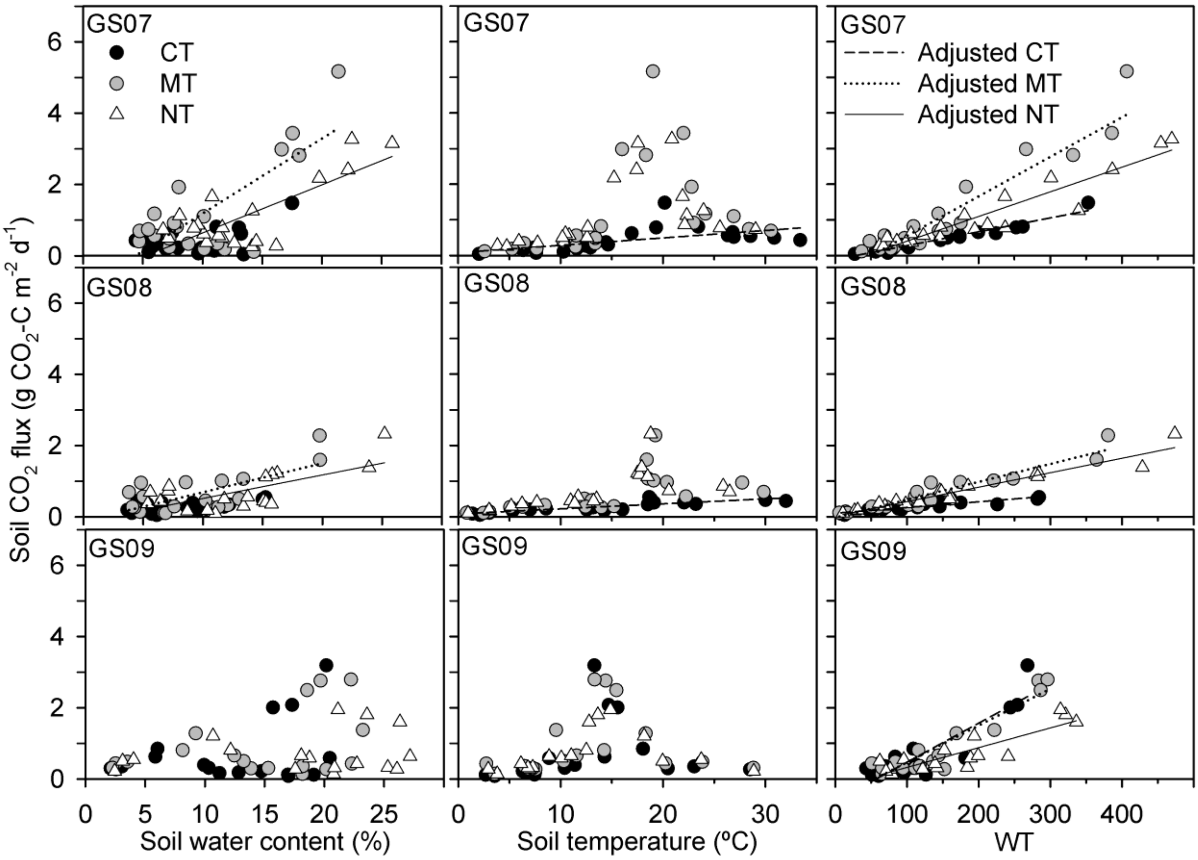


Figure 3.8. Relationships between soil CO_2 flux (F) and soil water content at the 0 to 5 cm soil depth (W), soil temperature at 5 cm soil depth (T), and the product of soil water content and soil temperature (WT), in three consecutive growing seasons: 2006-2007 (GS07), 2007-2008 (GS08), and 2008-2009 (GS09). Lines represent significant linear adjustments (P<0.05) under: no-tillage (NT), minimum tillage (MT), and conventional tillage (CT). Equations are presented in a separated table (Table 3.4).

Table 3.4. Equations of the linear adjustment of soil CO_2 flux, under different tillage systems: no-tillage (FNT), minimum tillage (FMT), and conventional tillage (FCT); during three growing seasons: 2006-2007 (GS07), 2007-2008 (GS08), and 2008-2009 (GS09); with soil water content in the soil surface (0-5 cm) and soil temperature at 5 cm depth. n.s. stands for non-significant (P<0.05).

	Soil water content (W)	Soil temperature	W x Soil temperature (WT)
GS07	F_{NT}= -0.89 + 0.165 W, R^2= 0.59	n.s.	F_{NT}= (6.9 WT- 290)·10^{-3}; R^2= 0.89
	F_{MT}= -1.10 + 0.256 W, R^2= 0.58	n.s.	F_{MT}= (11.8 WT- 600)·10^{-3}; R^2= 0.91
	n.s.	F_{CT}= 0.15 + 0.03 T, R^2= 0.33	F_{CT}= (3.9 WT- 113)·10^{-3}; R^2= 0.94
GS08	F_{NT}= 0.066 W - 0.14;R^2= 0.45	n.s.	F_{NT}=(4.1 WT +15.9)·10^{-3};R^2= 0.92
	F_{MT}= 0.081 W - 0.12;R^2= 0.49	n.s.	F_{MT}=(5 WT - 26.8)·10^{-3};R^2= 0.90
	n.s.	F_{CT}= 0.014 T+ 0.08; R^2= 0.71	F_{CT}=(1.6 WT + 98.1)·10^{-3};R^2= 0.83
GS09	n.s.	n.s.	F_{NT}=(5.5 WT - 228)·10^{-3};R^2= 0.75
	n.s.	n.s.	F_{MT}=(10.3 WT - 560)·10^{-3};R^2= 0.87
	n.s.	n.s.	F_{CT}=(10.9 WT - 620)·10^{-3};R^2= 0.80

3.2. Soil organic carbon stock

SOC concentration was significantly affected by both tillage systems (TIL) and N fertilization (FNT), but there was no significant interaction TILxFNT (Table 3.5). The response to soil tillage systems was different at each soil depth layer, thus the significant interaction between TIL and depth of the soil layer (Depth) (Table 3.5). At the 0-5 cm depth layer, SOC concentration was higher under NT than under MT, and lowest under CT (Table 3.6). At 5-10 cm depth, SOC concentration was higher under MT than under NT (Table 3.6). At the 10-20 cm and 20-30 cm depth layers, SOC concentration was higher under CT than under MT and NT (Table 3.6). Greater SOC concentration at 5-10 cm under MT, and at 10-30 cm under CT may be related to depth of tillage operations and residue incorporation (Angers and Erizsen-Hamel, 2008; Plaza-Bonilla *et al*., 2010). There were no-significant differences among tillage systems at the 30-40 cm depth layer (Table 3.6). SOC concentration with medium and high N fertilizer levels was significantly higher than that on unfertilized plots. Differences were significant at the 0-5 cm depth layer (Table 3.6), and the same trend was observed at deeper depth intervals, although no-significant ($P<0.05$).

Effect	SOC		SOC stock (0-10)	SOC stock (10-40)	SOC stock (0-40)
TIL	<0.0001		<0.0001	<0.0001	0.005
FNT	<0.0001		0.004	n.s.	0.007
TILxFNT	n.s.		n.s.	n.s.	n.s.
Depth	<0.0001				
DepthxTIL	<0.0001				
DepthxFNT	n.s.				
DepthxTILxFNT	n.s.				

Table 3.5. Soil organic carbon (SOC) concentration and stock ANOVA: Significance of factor effects: tillage system; TIL, N fertilization level; FNT, soil depth interval (Depth) and their interaction in July 2009. n.s. stands for non-significant (P<0.05).

Table 3.6. Soil organic carbon (mg g^{-1}) and SOC content in equivalent soil mass (esm SOC; Mg C ha^{-1}) at the experimental site in July 2009 under different tillage systems and N fertilization levels: zero (ZN), medium (MN), and high (HN). Different letters into a row indicate significant differences (P<0.05) among tillage systems and N fertilization levels.

† The standard reference soil mass was 832 Mg ha^{-1} for the 5 cm layer. ‡ The standard reference soil mass was 1656 Mg ha^{-1} for the 10 cm layers. § Sum of the C stock in the 0-10 cm and 10-40 cm depth intervals

	Depth Interval (cm)	No-tillage				Minimum tillage				Conventional tillage					N fertilization levels		
		ZN	MN	HN	*Mean*	ZN	MN	HN	*Mean*	ZN	MN	HN	*Mean*		ZN	MN	HN
SOC (g C kg^{-1})	0-5	15.0	17.2	17.8	*16.7a*	11.5	12.9	12.6	*12.3b*	7.4	7.7	8.4	*7.8c*		11.3b	12.6a	12.9a
	5-10	8.3	9.6	9.9	*9.3b*	10.3	11.4	10.4	*10.7a*	7.5	7.8	8.2	*7.8c*		8.7a	9.6a	9.5a
	10-20	5.8	6.9	6.2	*6.3b*	5.5	5.9	6.4	*5.9b*	7.0	7.8	8.3	*7.7a*		6.1a	6.9a	7.0a
	20-30	6.2	5.3	5.7	*5.7ab*	4.8	5.3	5.6	*5.2b*	6.4	7.1	7.0	*6.8a*		5.8a	5.9a	6.1a
	30-40	5.0	5.3	5.3	*5.2a*	4.8	5.2	5.1	*5.0a*	5.3	5.6	6.0	*5.6a*		5.0a	5.3a	5.5a
SOC_{esm} Stock (Mg C ha^{-1})	0-10 †	19.4	22.0	22.6	*21.3a*	18.0	19.8	18.8	*18.9b*	12.3	12.8	13.7	*12.9c*		16.6B	18.2A	18.4A
	10-40 ‡	28.2	29.0	28.4	*28.5b*	25.1	27.0	28.2	*26.7b*	30.7	33.5	34.9	*33.1a*		28.0	29.8	30.5
	0-40 §	47.6	51.0	51.0	*49.9a*	43.1	46.7	47.0	*45.6b*	43.0	46.3	48.6	*46.0b*		44.6B	48.0A	48.9A

The stock of SOC in the top 0-40 cm profile, in equivalent soil mass, was between 43.0 and 51.0 Mg C ha^{-1}. Long-term NT had higher soil C stock than MT and CT, and long-term medium and high N fertilization levels higher than unfertilized plots (Table 3.6). These observations confirmed the hypothesis that N fertilization tends to increase or maintain SOC content (Powlson *et al*., 2010), contrarily to the opposite trend suggested in other works (Khan *et al*., 2007, Mulvaney *et al*., 2009). Increases of crop residue production and C inputs with N fertilization are expected to increase SOC content, though it does not always occur (e.g., Halvorson *et al*, 2002). The response of the SOC stock to N fertilization in our experiment could be related with that of C inputs (Table 3.1). Response of C inputs to N fertilization under semiarid conditions is limited due to reduced water availability (Cantero-Martínez *et al*., 1995 and 2003; Luo *et al*., 2010) and it will need several years for significant increase of SOC content to occur.

Above-ground crop residue incorporation of above-ground crop residues may be essential to increase of SOC content with adequate N fertilization (Lugato *et al*., 2006). N fertilization increased C input from above-ground residues but not C inputs on root biomass (Table 3.1). Additionally, the increase of C inputs with N fertilization was more pronounced under NT than under MT, and least under CT (Table 3.1). Long periods of time may allow cumulative effects of increased C inputs to increase the SOC content. We expect that in a longer period of time, differences of SOC between N fertilizer levels will tend to be higher under NT than under MT and CT.

Relationships between soil CO_2 flux and SOC stocks were not found, probably due to the differential contributions of root respiration to soil CO_2 flux under different treatments. This was emphasized by the relationship of soil CO_2 emission with increased root growth (Fig. 3.6). The study of soil CO_2 flux was useful for comparison of total soil metabolic activity (including microbial and root respiration) and its relation with soil environmental factors under different tillage systems. The relation of active root length with the relative contributions of root respiration to soil CO_2 flux should be considered in future experiments.

4. CONCLUSIONS

In rainfed Mediterranean agroecosystems, long-term N fertilization and tillage affected soil CO_2 emissions. However, differences among treatments were not observed in the all three growing seasons studied. Variations in rainfall and crop growth among cropping seasons and treatments led to changes on the soil CO_2 fluxes measured. Thus, during dry years (i.e., 2007 and 2008), soil CO_2 flux was higher under NT and RT than under CT. Soil water conservation under NT and RT treatments during dry seasons allowed for higher C inputs and better conditions for microbial decomposition compared to CT. Similarly, soil CO_2 flux increased with N fertilization during the wettest season (i.e., 2009) due to higher N uptake and crop growth during wet years. However, similar emissions were observed during the other two drier seasons. Related with this, the product between soil water content and soil temperature explained between 75 and 94% of the seasonal variability of soil CO_2. Differences in C inputs were also responsible of the differences found on SOC stocks among treatments. Despite long-term NT and N fertilization led to significant SOC sequestration, soil CO_2 flux and SOC stocks were not related. Different contributions of both microbial-derived CO_2 and root-derived CO_2 to the total CO_2 flux among treatments could be the main responsible of the lack of relationship between soil CO_2 flux and SOC stocks.

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Chapter 4: Short-term dynamics of soil CO_2 flux: response to rainfall and tillage operations

Published in Agriculture, Ecosystems and Environment:
Morell, F.J., Álvaro-Fuentes, J., Lampurlanés, J., Cantero-Martínez, C. 2010. Soil CO_2 fluxes following tillage and rainfall events in a semiarid Mediterranean agroecosystem: Effects of tillage systems and nitrogen fertilization. 139 (1-2), pp. 167-173.

Abstract

The response of soil CO_2 flux to tillage operations and rainfall events was studied under semiarid Mediterranean conditions. The study was conducted on a barley monoculture agro-ecosystem during summer/autumn fallow periods in four consecutive years (2005-2008). The study compared three N fertilization levels (zero, medium; 60 kg N ha^{-1}; high, 120 kg N ha^{-1}) under three tillage systems (NT, no-tillage; MT, minimum tillage; CT, conventional tillage). Tillage operations led to a pulse of soil CO_2 flux. This pulse was linearly related to soil CO_2 flux on the day before tillage operations under MT (slope=4.22) and CT (slope=16.7), indicating the extent of soil disturbances. However, the associated soil CO_2-C losses during tillage operations were reduced and similar among different tillage systems. The soil CO_2 flux after rainfall was higher under NT, where it was linearly related to soil temperatures (0.15*soil temperature – 1.06). Soil CO_2 fluxes decreased on the following days as the soil dried. N fertilization affected CO_2 flux in 5 out of 35 observation days, with higher fluxes with N fertilization under conservation tillage systems. Emissions after rainfall events led to large soil CO_2-C losses, and these were of higher magnitude under conservation tillage systems (NT and MT).

Abbreviations: TIL, tillage system factor; NIT, N fertilizer level factor; NT, no tillage; MT, minimum tillage; CT, conventional tillage; SOC, soil organic carbon; SOM, soil organic matter; SWC, soil water content; ZN, no (zero) N fertilizer application; MN, medium N fertilizer level (60 kg N/ha); HN, high N fertilizer level (120 kg N/ha); *Fb*, soil CO_2 flux on the day before tillage operations; *Fa*, soil CO_2 flux immediately after tillage operations; rainfall events: D06, December 2006; S07, September 2007; O07, October 2007; J09, July 2009.

Key words: semiarid, precipitation, long-term experiment, soil CO_2 emission, soil CO_2 flux

1. INTRODUCTION

Soil organic carbon (SOC) content determines soil quality and fertility. Moreover SOC is the greatest terrestrial reservoir of carbon. Upon conversion to agriculture, most soils lose one-third to one-half of their SOC content (Lal and Bruce, 1999). Part of this SOC can be restored with adequate crop management practices (i.e. cropping systems, tillage practices and cover crops) and agricultural inputs (fertilizers and irrigation). The use of adequate management practices can help to offset the increase of atmospheric CO_2 concentration while improving soil quality and productivity (Lal, 2004; Johnson *et al*., 2007). The SOC content is determined by the balance between the rate of C inputs from crop production and the rate of C outputs as CO_2 efflux from decomposition of crop residues and soil organic matter (Paustian *et al*., 1997). Soil microbial activity transforms plant-assimilated C (input) to SOC or CO_2 that returns to the atmosphere (output). This process leads to a flux of CO_2 from the soil to the atmosphere.

The process of soil CO_2 flux is altered by management practices (e.g. soil tillage operations) and by meteorology (e.g. rainfall events). Additionally, long-term management practices (e.g. tillage systems, N fertilization) will affect soil characteristics and hence soil CO_2 production and flux. Most studies under different environments agree that soil microbial activity is usually limited by C availability and strongly modulated by soil environmental conditions.

Tillage operations lead to different responses that may be related to the cultivation type and history and with the soil type (Calderón *et al*., 2000), as well as soil conditions when tillage is implemented (Prior *et al*., 1997; Kessavalou *et al*., 1998a). Tillage of previously untilled soil under some agroecosystems led to an increase of CO_2 efflux from the soil. This increase starts after tillage operation and it extends for a period of time. This response may be due to aggregate disruption and exposition of protected organic matter to decomposition (La Scala *et al*., 2008). Tillage systems modify soil environmental conditions and the SOC content and stratification applied after medium- to long-term periods. Soil CO_2 flux may be affected as a response (Franzluebbers *et al*., 1995; Bono *et al*., 2008). In cultivated soils, tillage operations lead to a release of gases entrapped in the soil pores (Reicosky *et al*., 1997): soil CO_2 fluxes occurred as an initial burst of CO_2 emission that is followed by a stabilization of CO_2 flux within hours. The associated CO_2-C loss from the soil during these periods after tillage may be important depending on the characteristics of each system (Alvarez *et al*., 2001). Rainfall has also great effects on soil CO_2 fluxes that may lead to significant CO_2-C loss (Rochette *et al*., 1991).

In agroecosystems, soil CO_2 flux follows a seasonal trend in relation to soil environmental conditions and crop phenology. During drought periods, soil CO_2 flux is mainly driven by soil water content (Akinremi *et al*., 1999). In these ecosystems, precipitation events lead to increased CO_2 emissions from activation of heterotrophic respiration (Inglima *et al*., 2009), due to a recovery of microbial activity (Borken and Matzner, 2009). Temperature limits the response of soil CO_2 emission to rainfall events (Almagro *et al*., 2009). However soil tillage operations and precipitations modify the seasonal trends of soil CO_2 emissions (Sanchez *et al*., 2003). CO_2 fluxes that occur after tillage and precipitation events under these agroecosystems are fairly understood. In Mediterranean agroecosystems, short-term tillage-induced events have been studied in response to different tillage system (Álvaro-Fuentes *et al*., 2007; López-Garrido *et al*., 2009).

Nitrogen management impacts SOC dynamics (Lal, 2009). On the one hand N fertilization has proven to increase the SOC through increasing biomass production and hence C inputs to the soil (Luo *et al*., 2010). On the other hand, N fertilization affects soil CO_2 flux and hence C outputs from the soil (Sainju *et al*., 2008; Ding *et al*., 2007). As a result, N fertilization may affect the SOC content. Khan *et al*., 2007 and Mulvaney *et al*., 2009 suggested that the loss of SOC from soils may occur in response to synthetic fertilizers. The interpretations of these studies were rather controversial (Powlson *et al*., 2009; Reid, 2007). The measurement of *in situ* soil CO_2 fluxes can also contribute to this discussion of the effects of N fertilization on SOC dynamics. In Mediterranean conditions, to the best of our knowledge, no previous work has explored the effects of N fertilization on soil CO_2 flux.

In this work we studied soil CO_2 fluxes induced by tillage implementation and rainfall events after 10 years of different tillage systems and N fertilization rates. This study is part of a wider study of the long-term effects of N fertilization on SOC dynamics under different tillage systems in a semiarid Mediterranean agroecosystem.

2. MATERIALS AND METHODS

2.1. Site, tillage and N fertilization

A long-term experiment on tillage and N fertilization in winter-barley was initiated in 1996 in Agramunt (41º 48'N, 1º 07'E; Lleida, Spain) (Cantero-Martínez *et al*., 2003). Three levels of N fertilization: zero (ZN), medium (MN) -60 kg N ha^{-1}- and high (HN) -120 kg N ha^{-1}-, were compared in a factorial design with three tillage systems (no-tillage, NT and minimum tillage, MT -conservation tillage systems-; and conventional tillage, CT –intensive tillage system-) in a randomized block design with three repetitions and a plot 50 x 6 m size.

The soil is Xerofluvent typic (Soil Survey Staff, 1994) with a mean annual precipitation of 430 mm. In 1996 the soil in the Ap horizon (0-28cm) contained 465 g kg^{-1} of sand, 417 g kg^{-1} of silt and 118 g kg^{-1} of clay, an organic carbon concentration ranging from 6 to 9 g kg^{-1} and pH, from 7.8 to 8.1.

In this long-term experiment, winter-barley was cropped every year under rainfed conditions. By the end of June, after harvest, straw residue was spread over the plot in all the treatments. During the summer-autumn fallow period, the field was mostly free from vegetation for three-four months due to dry conditions. In 2008 summer weeds appeared, and a herbicide treatment (glyphosate) was applied at the beginning of September over all the plots to keep the soil free of vegetation. Tillage operations were annually conducted by the end of October or beginning of November. The CT treatment consisted on full inversion tillage operation with moldboard plough to a soil depth of 25-30 cm with almost 100% of the residue incorporated into the soil. The moldboard plow consisted of three bottoms of 0.50 m width.

Intensive tillage operations in 2007 and 2008 were replaced with disk ploughing to a depth of 25-30cm instead of the one mentioned before. This was due to the soil water conditions at the moment of tillage implementation, too dry in 2007 and too wet in 2008. Disk ploughing consisted of full inversion tillage similar to moldboard ploughing in terms of depth of disturbance, soil loosening and residue incorporation.

The MT treatment consisted of a cultivator pass to a depth of 10-15 cm, with an incorporation of approximately 50% of the crop residue. The plough consisted of 5 rigid shanks spaced 20 cm and a shank width of 5 cm. No soil disturbances were produced in the NT plots. Barley was annually seeded in mid November two-three weeks after the tillage operations. N fertilizer was split into two applications: one-third of the dose previous to tillage as ammonium sulfate (21% N) and two-thirds of the dose at the beginning of tillering as ammonium nitrate (33.5% N).

2.2. Measurements

Soil CO_2 fluxes were measured during autumn tillage operations in four consecutive years (2005-2008 period). An open chamber system (model CFX-1, PPSystems) connected to an infrared gas analyzer (model EGM-4, PPSystems) was used. The chamber has a cylindrical diameter of 21 cm, which covers a soil surface of 346 cm^2. Two regions of 6 m^2 were defined on each plot, and one measurement within each region was taken on each sampling day. The chamber was directly inserted about 1-2 cm deep in the soil. The air flow rate of the chamber was adjusted to 900 mL min^{-1}. Flux readings were taken 3 to 4 min after the chamber had been inserted into the soil, when readings of CO_2 flux were stable. All measurements were carried out between 09:00 and 13:00 hours.

Soil CO_2 fluxes were evaluated by successive measurements over the same regions. For each tillage system, the soil CO_2 flux was measured five times each year; 24 hours prior to tillage (-1d), immediately after tillage implementation (0h), two hours after second measurement (2h), 24 hours after tillage (1d) and 48 hours after tillage (2d). For NT plots, the 2h sampling was suppressed since the difference in gas fluxes between 0h and 2h was negligible.

The effects of rainfall events on soil CO_2 flux were evaluated in December 2006, in September and October 2007 and in July 2009. Soil CO_2 flux was measured on the day before and after rainfall events. In September and October 2007, we extended the experimental period after each rainfall event for the observation of the evolution of soil CO_2 flux with subsequent soil drying. CO_2 flux was also affected by 8 mm rainfall during the study of the effects of tillage operations in 2006. We also consider this event on the effects of rainfall on soil CO_2 flux.

Environmental conditions in the soil surface were determined at each sampling point. Soil temperature at 5 cm depth was determined with a had-held probe (TM65, Crison). Gravimetric soil water content (SWC) in the soil surface (0-5 cm depth) was determined by oven drying a soil sample from each sampling point at 105ºC. Daily air temperature and precipitation were recorded at the experimental site in an automated weather station.

The measurements during tillage and during rainfalls were conducted during the fallow periods, when the soil was free from living plants and there was not any live plant root. In December 2006, measurements were conducted at crop emergence, when the contribution of root respiration was supposed to be minimal (Rochette *et al*., 1999). Emerging green area was trimmed without altering the soil surface, before inserting the soil chamber. For these reasons, the CO_2 efflux during these events was mainly attributed to microbial respiration and it was therefore accounted as net C loss from the soil.

In previous observations, diurnal variations in summer and autumn were reduced. Measurements at the given time were assumed to provide estimations of daily soil CO_2 flux. Cumulative soil CO_2-C emission was calculated by linearly interpolating CO_2 flux measurements on consecutive days (trapezoid rule). The accumulated flux of CO_2 was divided by the duration of the experimental period: 2 days for tillage periods, and 1, 3 or 6 days for rainfall events. The soil C loss is reported in kg CO_2-C $ha^{-1}d^{-1}$. This calculation provides rough estimates of cumulative soil CO_2-C emission and the associated soil C loss.

2.3. Data analysis

Statistical analyses of data were performed with SAS (SAS Institute, 1990). Analyses of variance (ANOVA) were used to detect the significance of the main factor effects: tillage system (TIL) and N fertilizer dose (NIT), and the interaction factor effect (TILxNIT) on each sampling day. Separation of means was determined by multiple comparisons of least-squares means where the effects were statistically significant (P<0.05).

3. RESULTS

3.1. Tillage effects on short-term soil CO_2 fluxes

The soil CO_2 flux on the day before tillage operations was significantly affected by tillage systems, with higher soil CO_2 flux under NT and MT than under CT, especially in 2006 and 2008 (-1 in Fig. 4.1). The coefficients of variation at this time were between 27 and 47 %.

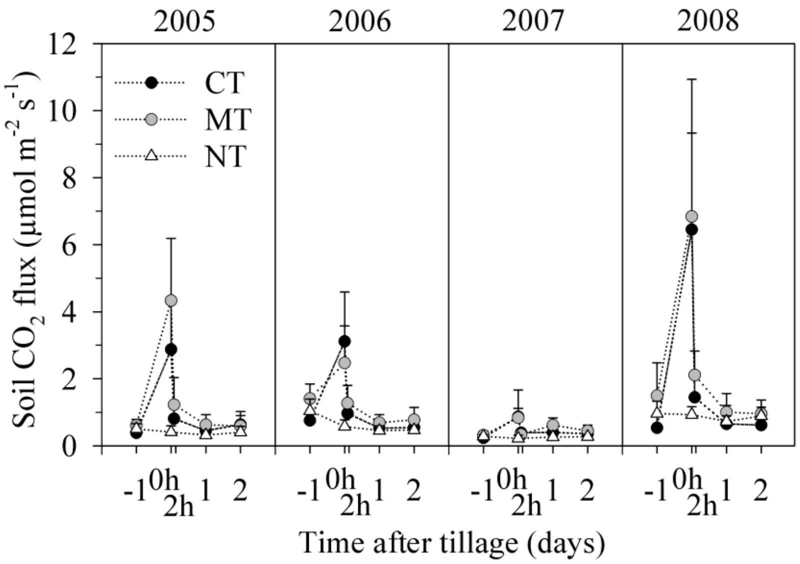

Figure 4.1. Soil CO_2 fluxes during tillage operations (CT, conventional tillage; MT, minimum tillage; NT, no-tillage) in four consecutive years. 0h and 2h indicate measurements immediately after tillage and 2 hours after tillage. Vertical bars indicate standard deviations of the means for each tillage system.

Immediately after tillage operations, under MT and CT, the soil CO_2 flux increased several times the flux rate on the day before (Fig. 4.1). The CO_2 flux after tillage operations was similar under MT and CT in all years, with no significant differences between these two systems, and significantly higher than those under NT. Under NT, soil CO_2 flux was similar to the flux on the day before (-1) (Fig. 4.1). Considering the years 2005, 2007 and 2008, soil CO_2 fluxes were similar on these two successive days (slope= 1.03; R^2=0.87; $P<0.05$) (Fig. 4.2), indicating steady fluxes under NT during this period. However, fluxes in 2006 were not steady due to the effects of a rainfall event and subsequent soil drying.

The observed burst of the CO_2 flux after tillage operations was linearly related to the flux on the previous day (Fig. 4.2). The fluxes on the previous day (*Fb*) were related to the fluxes immediately after tillage operations (*Fa*). The slope of the linear adjustment was 4.22 under MT and 16.7 under CT.

Two hours after tillage operations, the soil CO_2 flux under MT and CT sharply decreased in comparison to the flux immediately after tillage operations (Fig. 4.1). However, differences among tillage system were still significant in three out of the four years studied. In 2007, under driest conditions, differences among tillage systems were no longer significant at this moment. The coefficients of variation on the samplings the days of tillage operations were up to 65%. During the two following days, fluxes were stable, while significant differences among tillage systems still occurred. The coefficients of variation during these two days were similar to those on the day before tillage operation, between 29 and 46 %.

The effect of N fertilization was significant twice on the samplings after tillage operations, once in 2006 and once in 2007. In 2007, immediately after tillage operations, fluxes were 0.40 µmol m^{-2} s^{-1} on ZN and 0.98 and 0.61 µmol m^{-2} s^{-1} on MN and HN respectively. In 2006, two hours after tillage operations, fluxes were 0.61 µmol m^{-2} s^{-1} on ZN and 1.30 and 1.36 µmol m^{-2} s^{-1} on MN and HN respectively. Again in 2006, two days after tillage operations, the interaction between tillage and N fertilizer was significant, with significant effect of N fertilizer level under MT, where the soil CO_2 fluxes were 0.49, 0.84 and 1.01 µmol m^{-2} s^{-1} under ZN, MN and HN, respectively.

SWC was higher under NT, followed by MT, and drier under CT, with significant differences during most samplings: mean SWC over all samplings were 16.4, 12.4 and 10.4 g·g^{-1} (g H_2O g^{-1} dry soil) under NT, MT and CT respectively, with differences among years (Fig. 4.3). At 0h and at 2h measurements, in 2006 and 2007, SWC significantly differed among N fertilizer levels: SWC in ZN was on average 0.01-0.02 g·g^{-1} drier than the fertilized treatments (MN and HN) (data not shown). Soil temperatures during the experimental periods ranged between 10 and 20 ºC, and temperatures were similar among tillage systems. Temperatures in 2006 were between 17 and 20º C, and led to a subsequent soil drying after an 8 mm precipitation event on the day before -1. Average soil temperatures for the samplings in the other years were between 10 and 17º C.

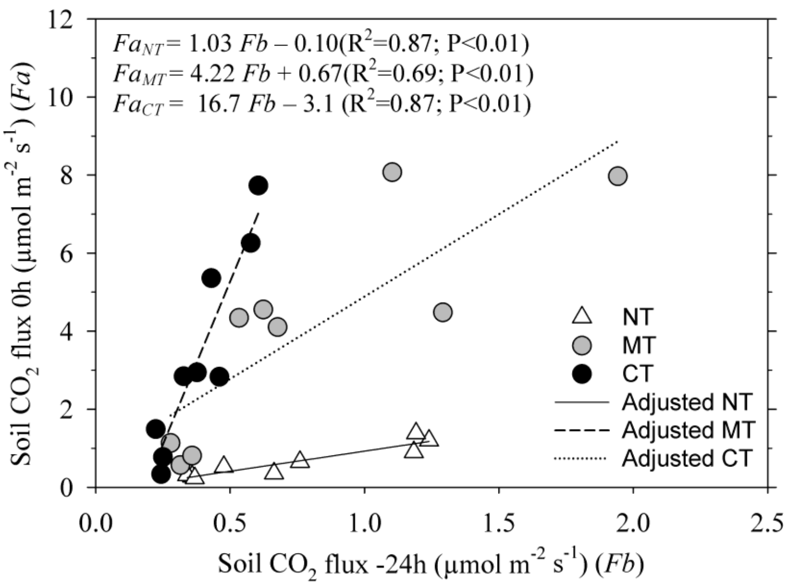

Figure 4.2. Relation of soil CO_2 efflux the day before tillage (-24h) and immediately after tillage implementation (0h), for each tillage system (CT, conventional tillage; MT, minimum tillage; NT, no-tillage). Nine points per treatment corresponding to observations in three years (2005, 2007 and 2008) and three N fertilization levels for each year and tillage system. Lines were obtained from linear adjustments for each tillage system. Fb and Fa are fluxes before and after tillage operations.

3.2. Rainfall effects on soil CO_2 fluxes

On the day before rainfalls (-1 d), the soil CO_2 fluxes were lower than 1 μmol m^{-2} s^{-1}, with non-significant differences in July 2009 (J09) (Fig. 4.4). After rainfalls, mean CO_2 flux (in µmol m^{-2} s^{-1}) were 0.33 after 6 mm in December 2006 (D06), 1.68 after 4 mm in September 2007 (S07), 1.42 after 14 mm in October 2007 (O07) and 2.77 after 29 mm in J09 (Fig. 4.4). The coefficients of variation were similar among sampling days during the experimental periods, and were between 20 and 45%.

Soil rewetting led to high increases in soil CO_2 flux in three out of the four periods studied. The soil CO_2 flux after rainfalls was significantly affected by tillage systems, with higher fluxes under MT and NT than under CT (Fig. 4.4). In D06, the response was negative under CT and almost nothing under conservation tillage systems (NT and MT). In S07 and O07, the CO_2 flux after rainfall was up to 3 µmol m^{-2} s^{-1} and the differences among tillage systems were evident. Additional rewetting between day 1 and 2 in O07 (Fig. 4.5) further increased CO_2 flux on day 2 (Fig. 4.4). In J09, after 23 mm rainfall, soil CO_2 flux increased 8 times those on the day before. CO_2 flux was up to 3.4 µmol m^{-2} s^{-1}, and soil CO_2 flux was higher under conservation tillage systems had under CT.

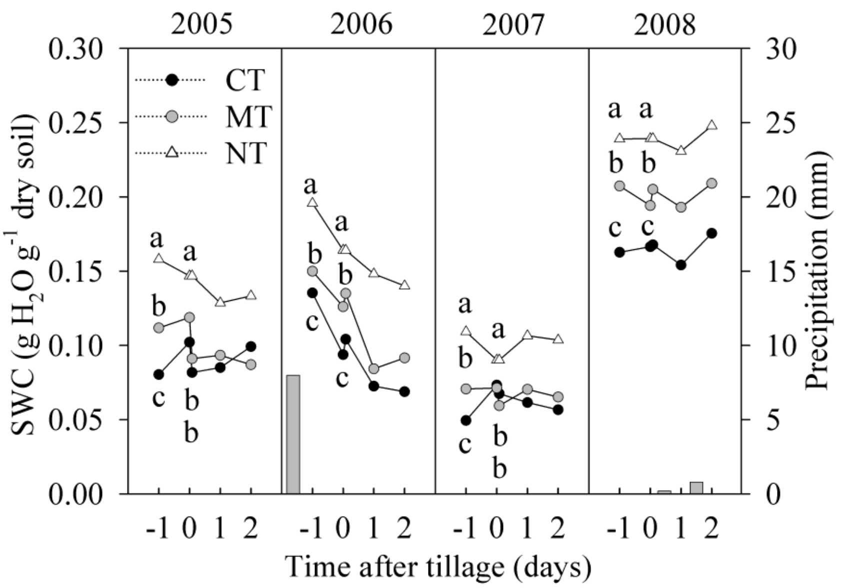

Figure 4.3. Gravimetric soil water content (SWC) in the top 5 cm during the tillage periods studied under different tillage treatments (CT, conventional tillage; MT, minimum tillage; NT, no-tillage), line plot. Different letters indicate mean separation among tillage systems (P<0.05). Mean separation on the following days was the same as indicated for day 1. Grey bars indicate precipitation (mm).

In S07 and O07, the soil CO_2 fluxes on the following days decreased as the soil dried (Figs. 4.4 and 4.5). A similar trend was observed in 2006 under NT during tillage operations: as the soil was drying after precipitation (Fig. 4.3), soil CO_2 fluxes decreased as much as a 44% decrease between day -1 and day 0 under this system (Fig. 4.1).

The response of soil CO_2 flux to N application was infrequent, and it was only detected in two samplings out of 15, and in both cases, in interaction with tillage system. In S07, 3 days after precipitation, and in O07, on the previous day to rainfall event, differences were significant among N fertilization levels under MT, where average flux was 0.55 µmol m^{-2} s^{-1} under ZN and 0.66 µmol m^{-2} s^{-1} on fertilized soil (i.e., MN and HN), however differences were not significant under NT or CT.

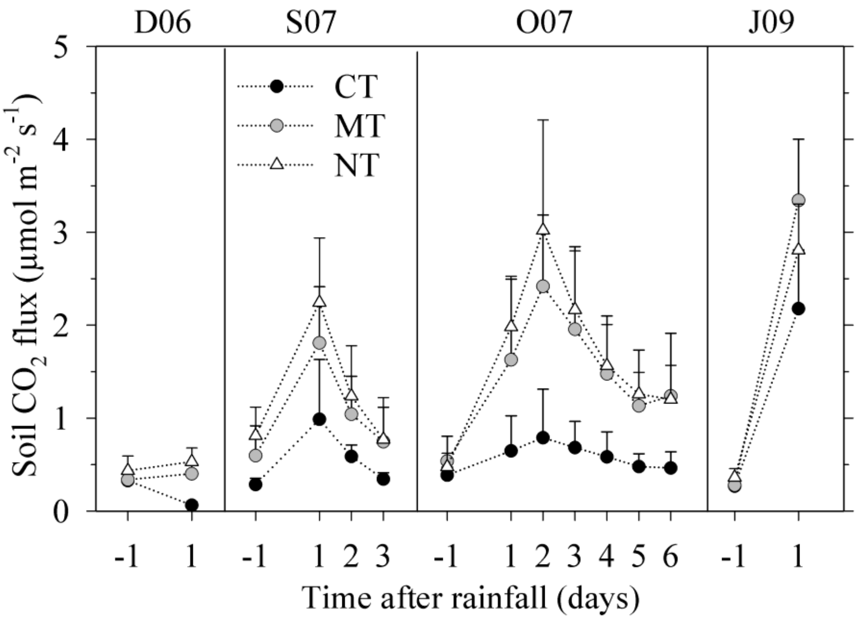

Figure 4.4. Soil CO_2 fluxes before and after rainfall events (D06, December 2006; S07, September 2007; O08, October 2007; 09, July 2009) under different tillage systems (CT, conventional tillage; MT, minimum tillage; NT, no-tillage). Vertical bars indicate standard deviations of the means for each tillage system.

As found during the tillage implementation periods, SWC after the rainfall events differed among tillage systems. SWC was higher under NT higher than under CT, and medium under MT (Fig. 4.5). Soil temperature was between 1 and 4 ºC higher under CT than under NT, and intermediate under MT system, and ranged between 29 and 9º C.

Soil CO_2 flux after the observed rainfall events were related to the soil temperatures. Under NT the soil CO_2 flux after the rainfall event showed a significant linear relationship to soil temperatures (slope=0.15, R^2=0.96, P<0.01) (Fig. 4.6), for temperatures between 10.3 and 24.2º C.

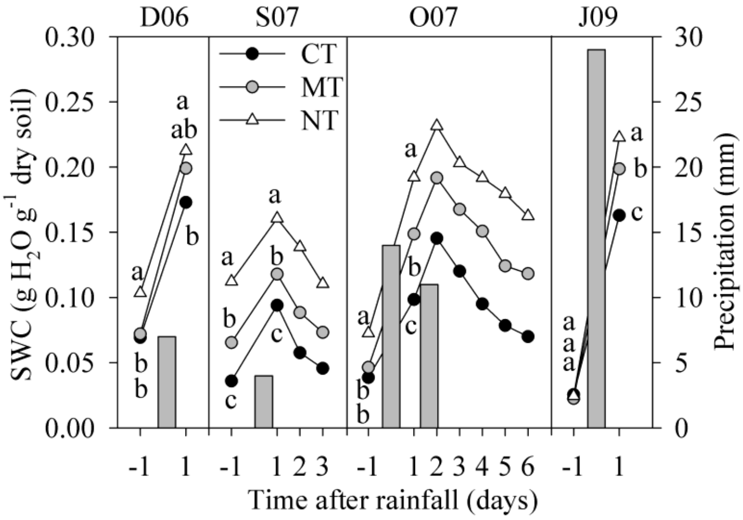

Figure 4.5. Gravimetric soil water content (SWC) in the top 5 cm previous and after rainfall events under CT, conventional tillage; MT, minimum tillage; and NT, no-tillage. In -1, values on the previous day are indicated. The periods are D06, December 2006; S07, September 2007; O08, October 2007; 09, July 2009. Different letters indicate mean separation among tillage systems (P<0.05). In S07 and O07 mean separation on the following days was the same as indicated for day 1. Grey bars indicate precipitation (mm).

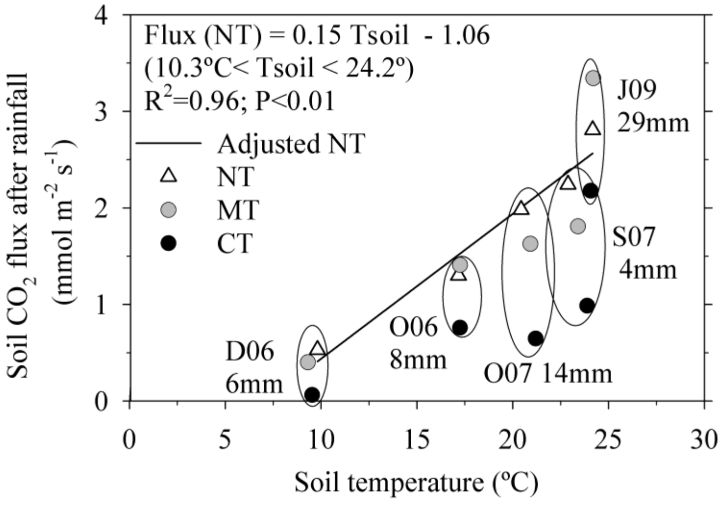

Figure 4.6. Relation of soil CO_2 flux after rainfall (1) and temperature, for each tillage system (CT, conventional tillage; MT, minimum tillage; NT, no-tillage) on the day after rainfall events. Ellipses indicate the rainfall events considered: O06, October 2006; D06, December 2006; S07, September 2007; O07, October 2007; J09, July 2009. The amount precipitated (in mm) is also indicated for each event. The adjusted line is drawn for NT system. The adjustments were not significant under MT and CT (P<0.05).

Table 4.1. Estimations of daily soil CO_2-C loss from tillage-implementation periods (Tillage) and rainfall events (Rainfall).
[a] CT, conventional tillage; MT, minimum tillage; NT, no-tillage.
[b] zero, ZN; medium, MN, 60 kg N ha^{-1}; HN, 120 kg N ha^{-1}.
[c] The number in brackets indicates the duration in days of each experimental period.

		Cumulative CO_2 (kg CO_2-C ha^{-1} d^{-1})					
		Tillage system [a]			N fertilization level [b]		
	Period [c]	NT	MT	CT	ZN	MN	HN
Tillage operations	2005 (2)	4.7	8.8	6.6	6.7	7.6	7.1
	2006 (2)	6.4	9.3	7.2	6.2	8.2	9.2
	2007 (2)	3.3	5.3	4.1	4.2	4.5	4.3
	2008 (2)	10.7	14.4	10.0	12.6	13.0	11.0
Rainfall events	Dec 06 (1)	5.5	4.2	0.7	3.6	3.5	3.3
	Sep 07 (3)	14.7	12.4	6.6	10.0	12.9	10.9
	Oct 07 (6)	19.3	17.0	6.3	14.4	14.9	13.7
	Jul 09 (1)	28.1	33.4	21.8	25.9	27.0	30.7

4. DISCUSSION

4.1. Tillage events

The soil CO_2 immediately after tillage ranged from 1 to 8 µmol m^{-2} s^{-1} (Fig. 4.1), with major differences among years in relation with differences on SWC (Fig. 4.3). Rochette and Angers (1999) in a prairie under cultivation observed pulses between 7 and 20 µmol m^{-2} s^{-1}. Reicosky *et al*. (1997) observed pulses between 1.6 and 3.2 µmol m^{-2} s^{-1} in a cultivated land. As previously described, tillage operations led to an immediate burst in the soil CO_2 flux due to an increase of the air transport coefficient from soil loosening (Reicosky and Lindstrom, 1993; Reicosky *et al*., 1997). The subsequent response on the following days will depend on the soil environmental conditions when the soil is tilled (Prior *et al*., 1997; Kessavalou *et al*., 1998a) and cultivation history and soil type (Calderón *et al*., 2000).

Differences among tillage systems agree the previous studies in the region (Álvaro-Fuentes *et al*., 2007; López-Garrido *et al*., 2009). We report a relation between the release of CO_2 after tillage operations and the CO_2 flux before tillage (Fig. 4.2). The higher slope obtained under CT (16.7), compared to that obtained under MT (4.22), indicates that the higher soil disturbance leads to a higher gas release. Previous work on the region reports an increase immediately after tillage operations from 3 to 15 times greater than the fluxes observed on the day before (Álvaro-Fuentes *et al*., 2007). In other studies in other agroecosystems, it has been reported an increase of soil CO_2 efflux of 3.8, 6.7, 8.2, and 10.3 times larger than the no-tilled soil for 10, 15, 20 and 25 cm plowing depths (Reicosky and Archer, 2007).

4.2. Rainfall events

The soil CO_2 flux during rainfall events as well as on the day before tillage operations was higher under conservation tillage systems (NT and MT) than under CT (Fig. 4.1 and 4.3). Soil water status limited soil CO_2 production and emission. As observed after rainfall events, once rewetting occurred, temperature determined the extent of the activation of soil CO_2 flux, and soil drying determined the duration of the activation. Hence during summer and autumn, water limited-seasons in Mediterranean conditions, the effect of rainfalls on soil CO_2 flux lasts for a period of time as long as the soil remains moist (Fierer *et al*., 2003), and this is not a pulse response, but a period of time during which water-limitation is overcome. The responses of CO_2 fluxes under undisturbed soil have been observed in contrast to disturbed soil in other experiments under field conditions. In these experiments, the response of CO_2 flux is higher under no-tillage systems or on undisturbed soils in comparison with that on tilled soils (Kessavalou *et al*., 1998a; Jackson *et al*., 2003).

The relationship obtained under NT (Rsoil= 0.15 Tsoil -1.06, for Tsoil between 10.3 and 24.2ºC) (Fig. 4.6), is similar as the one obtained by Rochette *et al*., 1991 under a maize crop and considering data with volumetric soil water content between 10 and 35 % (Rsoil= 0.2 Tsoil -0.74; for Tsoil between 13 and 33ºC). Under MT and under CT there was no clear relation between soil CO_2 flux and soil temperature, indicating that other factors may have been of greater concern during the observed events. Under NT, the activation CO_2 flux has been related to soil temperature. Further rewetting (Fig. 4.5) led to further increase of soil CO_2 flux (Fig. 4.4), hence providing evidence that the extenxt soil rewetting will also influence this activation.

4.3. Tillage systems effects

The effects of TIL on soil CO_2 flux could be related to the differences in soil environmental conditions (SWC and soil temperature) and to the organic C availability due to changes on SOC concentration and crop residue production. Annual biomass production differed among tillage systems. Crop residues production was 30 and 23 % higher under NT and MT than under CT, due to improved water use efficiency under conservation tillage systems in this region (data not shown). Due to of tillage operations and the residue incorporation into deeper layers, SOC significantly responded to tillage systems and in 2006 the SOC concentration was: 12.1, 17.6 and 20.3 g kg^{-1}, in the soil surface (0-5cm soil depth) under CT, MT and NT respectively. No significant differences were shown at deeper soil layers, and this pattern on SOC stratification led to a similar pattern on soluble carbon availability, microbial biomass and biochemical activity (Madejón *et al*., 2009). SOC accumulation and crop residue production enhanced substrate availability. Substrate availability is one major control of the response of soil CO_2 flux to rainfall events (Casals *et al*., 2009) from the effects on the soil microbial activity. Moreover, the increases in SOC concentration leads to an increase of the capacity of the soil to retain water, as observed on the SWC after rainfall events (Fig. 4.5). Under semiarid Mediterranean conditions, the soil CO_2 fluxes were more responsive to rainfall events under NT and MT than under CT.

4.4. N fertilization effects

The effect of N fertilization level on soil CO_2 flux was significant in 4 out of 35 samplings. Soil CO_2 flux on soil with fertilization (MN and HN) was up to double the flux on unfertilized soil. In two samplings the interaction term

(TILxNIT) was also significant and in an additional sampling on the second day after tillage operations in 2006. The interaction indicated that the effect of N fertilization increased soil CO_2 flux under conservation tillage systems (NT and MT), but not under CT.

The response of soil CO_2 flux to N application and the interaction with the tillage systems can be related with similar response and interaction on residue production. Medium and high N fertilization levels increased 10 to 20% the residue production under conservation tillage systems (MT and NT), but not under CT. The response of biomass production was due to increased available water under conservation tillage systems during growing seasons (work in process). The increased residue production may have led to increased substrate availability and increased soil microbial activity that would hence increase soil CO_2 flux, as observed in some samplings.

The balance between C inputs and outputs occurs towards an equilibrium value of SOC (Johnston *et al*., 2009). When C inputs and/or outputs are modified, a new steady state will tend to occur towards a new SOC level. Adequate fertilization contributes to the increase in SOC by promoting crop dry matter production and does not alter the decomposition of native SOC (Snyder, 2010). However, Khan *et al*., 2007 and Mulvaney *et al*., 2009 suggested that N fertilization may increase the rate of decomposition, and that SOC may decline in response to N fertilization. On the contrary, the role of N in chemically stabilizing the C in the soil was early proposed (Paustian *et al*., 1992: cited by Snyder, 2010). According to other authors, Khan *et al*., 2007 and Mulvaney *et al*., 2009, did a wrong interpretation of the observations (Powlson *et al*., 2009) and there is no evidence that the decline in SOC concentrations in that study was due N fertilization (Reid, 2007).

Proper N fertilization in a in sub-humid region has proven an 11% increase on SOC in top 15 cm of after 20 years of no-tillage adoption (Lemke *et al*., 2010). As evaluated in 2006 (unpublished data), SOC in our experiment was not significantly affected by N fertilization. However, SOC concentration is expected to increase in response increase of biomass production with N fertilization under conservation tillage systems. The effects of N fertilization under conservation tillage systems on SOC in this experiment are expected in a longer period of time due to little increase of N fertilization on residue production (i.e., 10-20%). Moreover, the increased CO_2 emissions during some periods seemed to partly offset the increase on C inputs with N fertilization under conservation tillage systems. As previously commented on this section, the increase on C inputs may have led to increased substrate availability and increased soil microbial activity that increased soil CO_2 flux, as observed in some samplings.

4.5. Cumulative soil CO_2 emissions

The daily CO_2 -C loss after tillage is within the range of values obtained by Ellert and Janzen, 1999, that reported 2.7 kg C ha^{-1} d^{-1}, during two days; Prior, 1997, that reported 5.0 kg C ha^{-1} d^{-1}, during 8 days after tillage implementation and McGinn *et al*., 1998, that reported 8.4 kg C ha^{-1} d^{-1} during 11 days after tillage. Short-term losses of CO_2-C after tillage are small in terms of soil C balance (Roberts and Chan, 1990; Álvaro-Fuentes *et al*., 2008) even after initial cultivation of no-tilled soil (Quincke *et al*., 2007). Despite the initial flush, tillage operations produced a pulse of CO_2 emission of short duration that has little effect on the cumulative CO_2-C losses (Table 4.1).

From long-term observations (unpublished data), mean annual soil CO_2 flux rates in this experimental area are around 10 kg CO_2-C ha^{-1} d^{-1}, and those during summer and autumn are 4 and 7 kg CO_2-C ha^{-1} d^{-1}, in agreement with similar agroecosystems in semiarid Spain (Sanchez *et al*., 2003; López-Garrido *et al*., 2009). The calculated rates after rainfall were up to 6 times the mean rates during summer and autumn (Table 4.1). The general pattern of soil CO_2 flux during the summer/autumn fallow period may occur as a low CO_2 emission, with increases after rainfall, and subsequent decrease as the soil dries.

According to the meteorological data on the experimental site (since 1995), during the summer-autumn fallow period, rainfall (> 0.2 mm) occurred once every 7.4 days, and once every 14 days if rainfall higher than 4 mm are considered. This means that over the around 120 days of the summer-autumn fallow period there are on average 8.6 rainfalls events, considering events higher than 4 mm which we evaluated in this study. According to the rainfall frequency and their associated CO_2-C losses (Table 4.1), soil CO_2 emissions after rainfalls during summer fallow are of greater importance than those after tillage operations. This is due to a more persisting effect (Kessavalou *et al*., 1998b; Ball *et al*., 1999).

Tillage system had substantial effects on CO_2 emissions after rainfalls (Table 4.1) that must be taken into account when comparing emissions under different tillage systems. Hence, CO_2 emission after rainfalls during the fallow period, especially under semiarid conditions, must be taken into account to accurately estimate the annual soil CO_2 emissions and for the evaluation of different tillage systems.

5. CONCLUSIONS

This experiment highlights the extent of changes on the dynamics of soil CO_2 emission with conservation tillage systems as well as the contributions of tillage operations and precipitation events on the soil CO_2 emissions in semiarid Mediterranean conditions. Tillage operations led to pulse emissions that were infrequently affected by N fertilization, however these pulse had little effect on cumulated CO_2 emissions. Fluctuation of soil CO_2 flux occurred with soil rewetting and soil drying. Under NT, the soil CO_2 flux after rainfalls could be related to the soil temperature. Emissions after rainfall events are high and must be taken into account when estimating the soil CO_2-C loss in semiarid Mediterranean agroecosystems. During these events, conservation tillage systems (NT and MT) increased the soil CO_2 emission.

ACKNOWLEDGEMENTS

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

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Chapter 5. Root respiration: field and modelling approaches.

Published in Plant and Soil:

Morell, F.J., Whitmore, A.P., Álvaro-Fuentes, J., Lampurlanés, J., Cantero-Martínez, C. 2012. Root respiration of barley in a semiarid Mediterranean agroecosystem: Field and modelling approaches. 351 (1-2), pp. 135-147.

Abstract

Aims: Root respiration is a major contributor to soil CO_2 flux, and its response to management practices needs to be evaluated. The aim was to determine the effect of management practices (tillage systems and nitrogen fertilization levels) on root respiration and to develop a model able to simulate root respiration and its components.

Methods: T The study was carried out during two contrasting growing seasons (2007-2008 and 2008-2009). Root respiration, including root tissue respiration (R_{ts}) and rhizomicrobial respiration of exudates (R_{rz}), was estimated as the difference between the soil CO_2 flux of cropped and bare soil (the so-called root exclusion technique). Additionally a novel sub-model of R_{ts}, was used to simulate root respiration based on root growth and specific root respiration rates.

Results: Root respiration was reduced under no-tillage. The model agreed well with the patterns and the amounts of the observed values of root respiration, although prior calibration was needed.

Conclusions: Root respiration was reduced by the long-term adoption of no-tillage, but was increased by N fertilizer. The root exclusion technique and the model were useful means to estimate root respiration on cropland under semiarid Mediterranean conditions. Additionally the model successfully separated out the theoretical contributions of R_{ts} and R_{rz} to root respiration.

Abbreviations: NT, no-tillage; MT, minimum tillage; CT, conventional intensive tillage;BSP, bare sub-plot; CSP, cropped sub-plot; *RR*, root respiration; R_{ts}, root tissue respiration; R_{rz}, respiration of rhizodepositions; R_m, root mass.

1. INTRODUCTION

The global annual flux of soil carbon dioxide (CO_2) has been estimated to be 75-80 Pg CO_2-C, which is ten times the annual CO_2 emission by fossil fuel combustion (Raich *et al*., 2002). Soil CO_2 flux out of soil is an issue of major importance in the study of the biogeochemistry of soil carbon (Del Grosso *et al*., 2005, Li *et al*., 1994), and on the effects of management practices that, in the end, may affect the soil carbon content (SOC) of agroecosystems and soil carbon sequestration. Soil CO_2 fluxes have been evaluated in many different ecosystems but less is known about arid and semiarid regions (Raich and Schlesinger, 1992).

The CO_2 flux from the soil integrates all sources of CO_2 production in the soil, including residue decomposition, turnover of native soil organic matter (SOM) and the root derived CO_2, or root respiration (*RR*) (Lundergärdh, 1926). *RR* integrates root tissue respiration (R_{ts}) and microbial respiration of root exudates and rhizodeposits (R_{rz}). Both R_{ts} and R_{rz} are tied to the consumption of organic compounds supplied by the above-ground to below-ground biomass. The quantities R_{ts} and R_{rz} make similar contributions to *RR* in crops and grasses (Kuyakov and Larionova, 2005). The effects of management practices on soil CO_2 flux may occur through the response of *RR* (Curtin *et al*., 2002; Xu and Wan, 2008). The contribution made by *RR* to the total soil CO_2 flux is needed for a full understanding of the carbon balance in agroecosystems, and the study of the effects of management on soil carbon sequestration.

Estimates of *RR* can be obtained by the root exclusion technique or from mechanistic models. The root exclusion technique is used to determine RR with the difference between soil CO_2 flux from bare soils (i.e. the loss of C from SOM mineralization only) and soil CO_2 flux from cropped soil (Hanson *et al*., 2000). It has been successfully used in pot studies to estimate *RR* (Gavrichkova and Kuzyakov, 2008; Martin and Merckx, 1992). Rochette *et al*. (1999), reported the utility of the root exclusion technique to estimate *RR* in a field experiment on a maize crop. However, it has not been used in croplands under semiarid Mediterranean conditions. This technique for estimating *RR* could be valuable in semiarid Mediterranean agroecosystems because these soils are poor in SOC and the soil basal respiration is low (< 1 µmol m^{-2} s^{-1}).

Mechanistic models describing the flux of soil CO_2 can distinguish the contribution of different sources of CO_2 (Del Grosso *et al*., 2005). An approach known as component integration measures the separate constituent components contributing to soil CO_2 flux, including crop growth and specific root respiration rates (Hanson *et al*., 2000). Based on the component integration method, we developed a new sub-model for the simulation of root tissue respiration within the MOTOR system (Whitmore, 2007; Whitmore *et al*., 2011). The MOTOR system simulates soil CO_2 flux from microbial respiration and SOM decomposition, above-ground and root growth and rhizodeposition. Here we add explicit routines to simulate root respiration which were then integrated with the root growth and soil respiration sub-models.

Previous studies have shown the effects of no-tillage systems (NT) on soil CO_2 flux, and have usually found a reduction of soil CO_2 flux under NT or minimum tillage (MT) in comparison with conventional intensive tillage systems (CT) (Fortin *et al*., 1996; Franzluebbers *et al*., 1995; Sanchez *et al*., 2002). Recent work has shown that soil CO_2 flux is reduced under NT even in the long-term (Álvaro-Fuentes *et al*., 2008; Sainju *et al*., 2008). However, the response of root respiration needs to be determined; as it may be affected by tillage systems and it may be responsible for the observed response of soil CO_2 flux. Under semiarid Mediterranean conditions, NT reduces root growth and root proliferation in the soil (Muñoz *et al*., 2010), which may affect root respiration.

Nitrogen (N) fertilization may cause slight increases in soil CO_2 flux during the growing season (Chen *et al*., 2004; Ding *et al*., 2007). Soil CO_2 fluxes may be increased due to greater C input from enhanced plant productivity (Paustian *et al*., 1997) and/or stimulated rhizosphere respiration (Iqbal *et al*., 2009; Xu and Wan, 2008). Under semiarid conditions, the response of soil CO_2 flux to N fertilization may be small (Curtin *et al*., 2002). However, during the growing season, one might expect to see an increase in soil CO_2 flux with N fertilization due to increased crop growth and below-ground C allocation.

The aim of this work was to evaluate the effects of tillage systems and N fertilization on soil and root CO_2 flux, and to compare *RR* values with those obtained with a model, which was used to establish the relative contributions of R_{rz} and R_{ts} to *RR*.

2. MATERIALS AND METHODS

2.1. Experimental data

2.1.1. Study site and crop growth

This study was conducted in a long-term field experiment established in 1996 in Agramunt (Ebro Valley, NE Spain). The mean annual rainfall in the area is 435 mm, and the soil was classified as a Xerofluvent Typic (Soil Survey Staff, 1994). The soil in the Ap horizon, 0-28 cm soil depth, contained 465 g kg^{-1} sand, 417 g kg^{-1} silt and 118 g kg^{-1} clay, with a pH of 8.5. Soil organic carbon concentration in 2008 at different soil depths is presented in Table 5.1.

Table 5.1. SOC concentration under different tillage systems (no-tillage, NT; minimum tillage, MT; conventional tillage, CT) at the experimental site in 2008.

	Depth (cm)	NT	MT	CT
SOC ($mg\ g^{-1}$)	0-5	15.8	12.3	7.9
	5-10	9.4	10.7	8.1
	10-25	6.6	6.5	7.8
	25-50	5.3	5.1	5.6

The experiment consisted of a factorial combination of three levels of N fertilization: zero, medium (kg N ha^{-1}), and high (120 kg N ha^{-1}); and three tillage systems: two conservation tillage systems (no-tillage, NT and minimum tillage, MT) and one intensive tillage system (conventional or inversion tillage, CT). The experimental design was a randomized block with three replicates and a plot size of 50 m x 6 m, oriented south to north (Fig. 5.1).

Since 1996, weeds have been treated with herbicide prior to seeding before the end of October. Tillage operations were conducted between the end of October and the beginning of November, to 30 cm soil depth under CT, and to 15 cm soil depth under MT. The CT treatment consisted of full inversion tillage with a moldboard plough to a soil depth of 25-30 cm with almost 100% of the residue incorporated into the soil. The moldboard plow consisted of three bottoms of 0.50 m width. The MT treatment consisted tilling the soil to a depth of 10-15 cm. The plough consisted of a tilling operation to 30 cm soil depth. No soil disturbances were produced in the NT plots, apart from that of the sowing machine. Barley (*Hordeum vulgare*, L., cv. Hispanic) was cropped each year under rainfed conditions (sown by the middle of November and harvested by the end of June). It is the most extended cropping system in the region, and ensures optimization of water use (Álvaro-Fuentes *et al*., 2009). N fertilizer was broadcasted, and its application was split between sowing (1/3) and tillering (2/3).

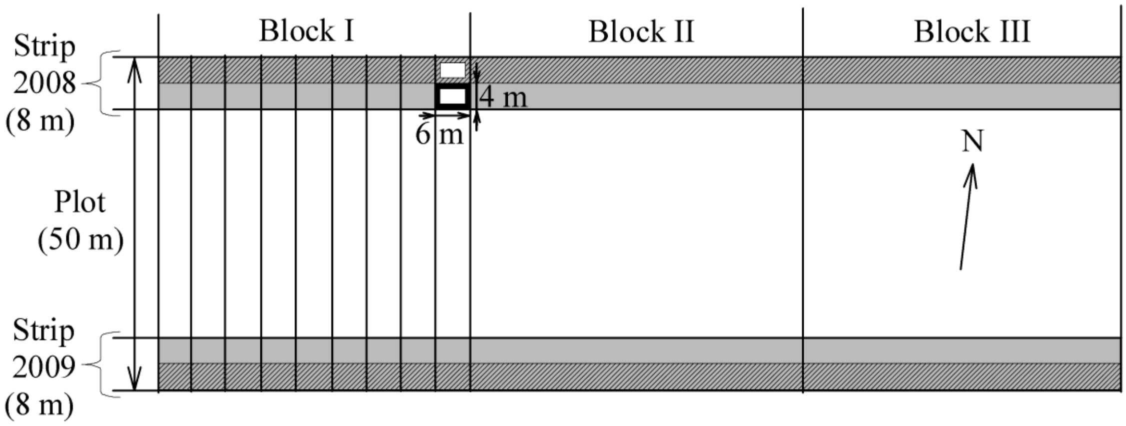

Figure 5.1. Map of the experimental field. The division in Block I corresponds to the 9 plots per block, omitted in blocks II and III. Gray strips are the study areas in 2007-2008 (Strip 2008) and 2008-2009 (Strip 2009) cropping seasons. The darker grey fill indicates the soil strip that was kept bare. Black square indicates a sub-plot (6 x 4 m), and the white inner squares indicate the sampling areas (4 x 2 m).

The respiration study was conducted during the 2007-2008 and 2008-2009 cropping seasons, hereafter referred to as 2008 and 2009 respectively. For this study we used 8 m wide strips at the north and south extremes of the plots in alternate years. The north strip was used in 2008 and the south strip in 2009 (Fig. 5.1).

Each 8 m strip was further divided into two 4 m wide strips (Fig. 5.1). One of the 4 m strips was treated with herbicide, glyphosate (dose 1L ha^{-1}), after the crop had grown enough for the herbicide treatment to be effective (three-four leaves stage), on day 67 after seeding in 2008 and on day 97 in 2009. The crop was allowed to continue growing on the other strip as on the rest of the plot. As a result, for every 24 m^2 (6 x 4 m) bare sub-plot (BSP), there was next to it another sub-plot (CSP) cropped with barley (Fig. 5.1). A second herbicide treatment was applied on BSP in 2008 by mid-May to stop the growth of weeds after May rainfalls (Fig. 5.2).

Above-ground dry matter was sampled at major development stages: tillering, beginning of stem extension, anthesis and maturity by cutting three samples of the plants at the surface level along 50 cm strips on each plot. Samples were oven-dried at 65-70ºC for 48 hours, and then weighted. Above-ground dry matter production was used to evaluate the performance of a crop growth model (Fig. 5.3).

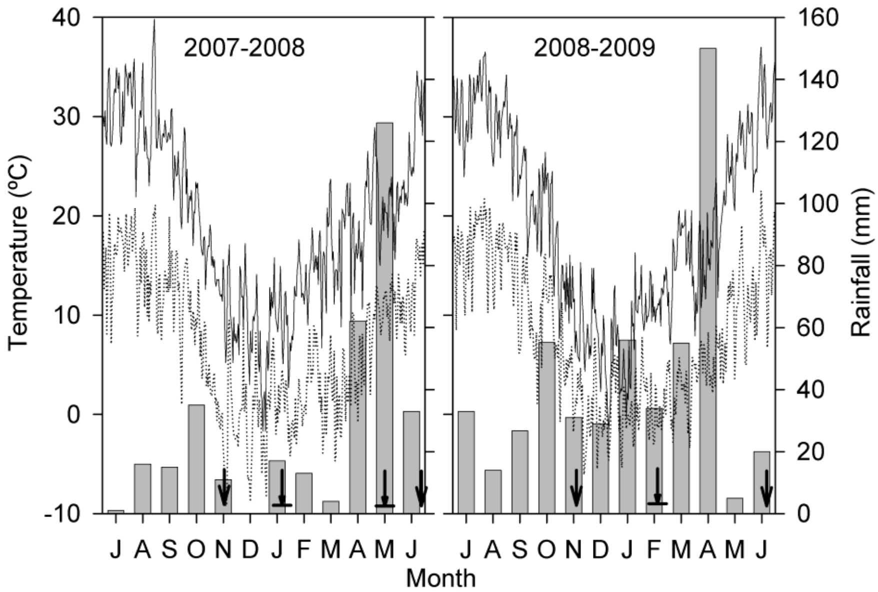

Figure 5.2. Meteorological conditions at the experimental site during 2007-2008 and 2008-2009 cropping seasons. Monthly rainfall (vertical bar chart), starting in July (J), after harvest of the previous year. Maximum and minimum daily temperatures (line plots). Small vertical arrows indicate sowing (by mid November, N) and harvest (by the end of June, J) dates on each cropping season. Arrows with line at the end indicate dates of herbicide application on bare soil subplots.

Anthesis occurred by the end of April, and the grain was harvested at the beginning of July in 2008 and one week earlier in 2009. 2008 was a dry year (47% less rainfall during the pre-anthesis period than the interannual mean rainfall during the same period) with wet conditions by May (double the interannual mean rainfall in that month) (Fig. 5.2), and 2009 was a wet year (76% more rainfall during the pre-anthesis period than the interannual mean rainfall during the same period) with dry conditions in May (Fig. 5.2). In 2008, crop growth was greatly reduced because of the shortage of water. The crop had a patchy distribution in some of the plots under MT and failed under CT. For this reason, the study of soil CO_2 flux and *RR* in 2008 was limited to NT.

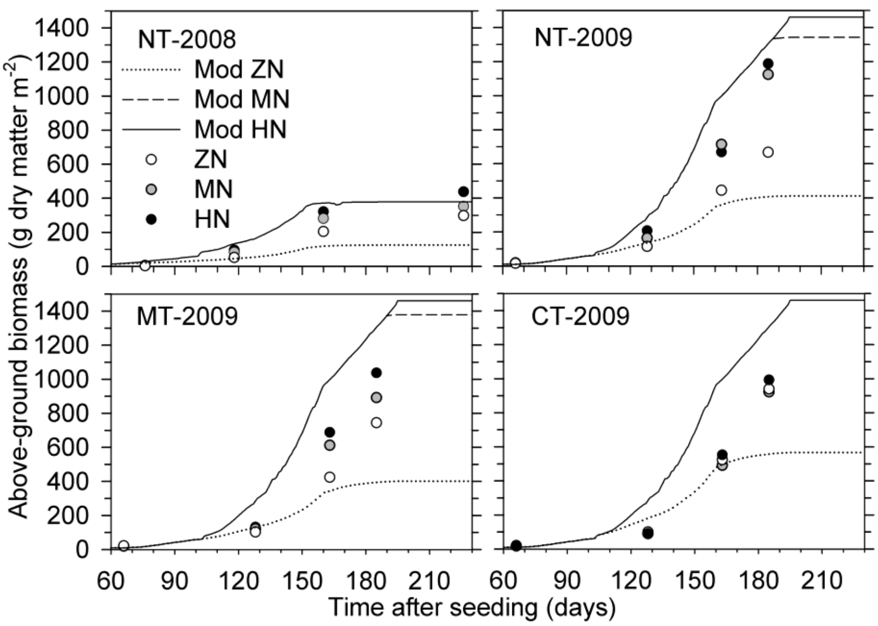

Figure 5.3. Total above-ground biomass production under no-tillage (NT), in 2007-2008 and 2008-2009 growing seasons, and under minimum tillage (MT) and conventional tillage (CT) in 2008-2009 growing season, on different N fertilization levels: zero (ZN); medium (MN), 60 kg N ha^{-1}; high (HN), 120 kg N ha^{-1}). Points correspond to observations at tillering, beginning of stem extension, anthesis and crop maturity. Simulations for MN and HN are alike.

Below-ground growth was determined to allow comparison with the modelled root growth. Root biomass (dry weight) was determined on soil samples up to 50 cm depth taken with a 4 cm soil auger. At crop maturity, four samples per plot were taken, two within the row and two between rows, and roots in each sample were recovered by rinsing the soil with water on a 0.5 mm sieve and hand picking the root fragments. Roots were oven dried before weighing.

2.1.2. Soil CO_2 flux measurements and estimation of root respiration

Soil CO_2 flux was measured with an open chamber system 21 cm in diameter (model CFX-1, PPSystems) covering a surface of 341 cm^2 of soil and with a flow rate adjusted to 900 mL min^{-1}. The chamber was connected to an infrared gas analyzer (model EGM-4, PPSystems). On the CSP, above-ground biomass was removed by cutting 25cm of a crop row without altering the soil surface and the chamber placed on the soil area where the crop had been removed. Removal of the crop without altering the soil surface has been proven not to modify soil CO_2 flux for a time of one hour or more (Liu and Li, 2006). The chamber was inserted into a different place each sampling time, to sample an area with respiring roots. The chamber was inserted into the soil to a depth between 1 and 2 cm. Flux readings were taken when values stabilized, normally 3 to 4 minutes after inserting the chamber into the soil. Measurements at each plot, one on the CSP and another in the BSP, were carried out within the 8 m^2 in the middle of each subplot to avoid edge-effects. Measurements were carried out between 8 a.m. and midday every day. Samplings were collected every 7 to 10 days, between the day before herbicide application (67 and 97 DAS in 2008 and 2009) until harvest giving a total of 17 samplings in 2008 and 19 samplings in 2009.

Soil CO_2 flux in the BSP was mainly due to microbial respiration and could be ascribed to the loss of C from SOM mineralization. Field estimates of *RR* between tillering and maturity were obtained as the difference between soil CO_2 flux in CSP and BSP for each treatment and sampling day, corrected for the diurnal variation and expressed as g of CO_2-C m^{-2} d^{-1}.

Soil temperature at 5 and 10 cm depth and gravimetric soil water content, 0-5 cm depth, were also determined at each sampling site. The main objective of these measurements was to be aware of any difference in soil environmental conditions between CSP and BSP.

2.2. Model approach

2.2.1. Model description

The model consists of a series of integrated sub-models that provide daily estimates of processes relating to soil N and crop growth (Whitmore, 2007; Whitmore *et al*., 2011). Models describing nitrogen mineralization from soil (Whitmore, 2007), growth of barley (Whitmore, 1988) and its development (Whitmore, 1995), root growth and the whole soil-crop system (Addiscott and

Whitmore, 1987; Whitmore *et al*., 2011), were modified to partition the respiration between root and soil as described below. The model needs weather, agronomy and soil characteristic data. The meteorological data required for the model are daily values of: precipitation, maximum and minimum temperatures, solar radiation, sunshine hours, atmospheric humidity and wind speed. The agronomic information required is sowing and harvest dates, N fertilizer application (dose and application date) and yield potential. Data on the following soil properties is required by the model: SOC content, bulk density, texture and depth of tillage. The N uptake was limited in the model in 2008 to reduce crop growth to the levels of actual growth observed in the field.

2.2.2 Root respiration model

A novel sub-model of root tissue respiration (R_{ts}), together with modelled respiration of rhizodeposits (R_{rz}), was used to simulate *RR* under the experimental conditions in this study. *RR* was modeled in relation to the modeled root growth and specific root respiration rates. The amount of CO_2 released by root respiration (R_{ts}) was modeled as a function of the standing root tissue biomass plus a modification for soil temperature. Osman (1971) reported rate of wheat root respiration at 10, 20 and 30^{o}C and at three different stages of plant growth (Eq 1). The Q_{10} value, which defines the temperature dependence or sensitivity of root tissue respiration to temperature variation, was obtained by fitting lines to his data as follows:

$Q_{10} = 0, \quad T < 0;$

$Q_{10} = (Q_{12}(i)\ T) / 12, \quad 0 < T < 12;$

$Q_{10} = Q_{12}(i) + (T – 12) / 4, \quad T > 12$ (Eq 1)

Where *i* denotes one of six stages of growth based on the accumulation of photo-thermal time: emergence, double ridge, anthesis, ripening, maturity and death (Whitmore, 1995). Q_{12} is a parameter for the temperature sensitivity of root respiration at different growth stages, and its value changes with growth stage as described by Osman (1971) taking the values 5 until emergence, 2.2 between emergence and double ridge stage, and 1.6 after that.

Root tissue respiration R_{ts} is then described by equation 2:

$R_{ts} = R_m\ \varphi\ Q_{10}(i)\ R_F(i)$ (Eq 2)

Where R_m is the root mass in each layer in the model, φ is a constant reflecting the average rate of respiration in mg CO_2-C per g dry matter per day and takes the value of 26.18 based on Osman (1971), R_F is the proportion of photosynthate that is diverted to roots at growth stage *i* following van Keulen and Seligman (1987) and takes values of: 1 until emergence, 0.5 until double ridge, 0.2 until anthesis, 0.01 until ripening, and 0 from ripening to death.

Microbial respiration was obtained from the soil organic matter turnover module directly (Whitmore, 2007; Whitmore *et al*., 1997), which included microbial maintenance respiration, decomposition of SOM, and decomposition of rhizodeposits. The bare soil carried a crop until day 67 after seeding in 2008 and until day 97 in 2009, when the crop growth was stopped in the model. After crop growth ceases, rhizodeposits appear no longer to be produced, reducing substrate availability in the model and hence microbial respiration. The difference in modelled microbial respiration between cropped and bare soil provided the estimate of rhizosphere respiration (R_{rz}). The sum of modelled R_{ts} and R_{rz} provided a model-based estimate of the *RR*.

Input data

Daily precipitation and minimum and maximum temperatures were monitored with a meteorological station at the experimental site, while solar radiation, air humidity and mean wind speed (height: 2 m) were obtained from the weather station from Oliola (Servei de Meteorologia de la Generalitat de Catalunya) at 7 km distance from the experimental site. The agronomic characteristics have been previously presented with the description of the experiment (above).

2.3. Statistical analyses

Soil CO_2 flux on CSP and BSP, and estimates of RR were tested with the SAS statistical package (SAS Institute, 1990). Measurements showed little correlation between sampling days, as observed in the comparison of individual measurements of any two successive days (data not shown). For this reason we took sampling day as an independent factor. We performed analysis of variance for each cropping season considering the effects of tillage system, N fertilizer level and sampling day as well as its interactions. Where the factor or interaction were statistically significant ($P<0.05$), we used a multiple comparisons of least-squares means according to Tukey´s test to separate treatments means. The goodness of fit of the modelled *RR* to the observed *RR* was statistically tested using the methodology in Smith *et al*., (1997).

3. RESULTS

3.1. Soil CO_2 flux under cropped and bare soil

On the CSP, mean soil CO_2 flux was 0.87 µmol CO_2 m^{-2} h^{-1} in 2008 and 1.32 µmol CO_2 m^{-2} h^{-1} in 2009 (Fig. 5.4). On the BSP, mean soil CO_2 flux was 0.49 µmol CO_2 m^{-2} h^{-1} in 2008 and 0.56 µmol CO_2 m^{-2} h^{-1} in 2009 (Fig. 5.4).

In 2008, soil CO_2 flux on the BSP decreased in response to N fertilization (Table 5.2). Mean fluxes were 0.64, 0.62 and 0.55 µmol CO_2 m^{-2} h^{-1} on zero, medium and high N fertilization levels respectively (Fig. 5.4). On the CSP, soil CO_2 flux was slightly increased with medium and high fertilizer additions but not significantly different (Table 5.2), and mean fluxes were 0.99, 1.06 and 1.11 µmol CO_2 m^{-2} h^{-1} on zero, medium and high N fertilization levels respectively. This trend was especially marked during the pre-anthesis period (Fig. 5.4).

In 2009, the effects of tillage and N fertilization, the interaction between them, and the interaction between tillage system and sampling day were significant for CO_2 flux on the CSP (Table 5.2). The significance between tillage systems and sampling day means that differences among tillage systems appeared over time. Soil CO_2 flux under CT was greatest, modest under MT and least under NT on the 5 of the 6 samplings between 142 and 173 DAS. This period corresponded to the period of greatest soil CO_2 fluxes (Fig. 5.4) and the fastest period of crop growth (Fig. 5.3) lasting from stem elongation to a few days after anthesis. Differences between zero and medium N fertilization levels were significant under MT, but not under NT or CT, although a similar trend exists under NT (Fig. 5.4). Soil CO_2 flux on BSP in 2009 was significantly greater under MT, 0.60 µmol CO_2 m^{-2} h^{-1}, than under CT, 0.51 µmol CO_2 m^{-2} h^{-1}, despite the fact that these differences are hard to see (Fig. 5.4). Values under NT were in between those under MT and CT.

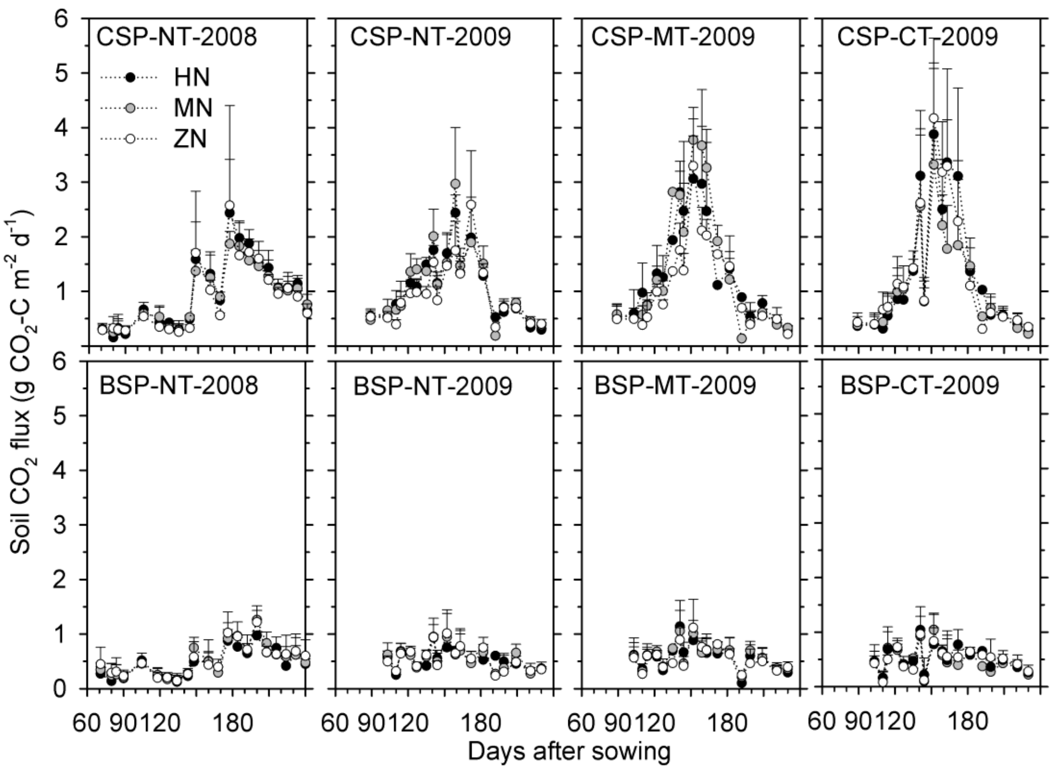

Figure 5.4. Soil CO_2 fluxes in 2007-2008 (2008) and 2008-2009 (2009) growing seasons, under different tillage systems (NT, no-tillage; MT, minimum tillage; CT, conventional tillage) and three N fertilizer levels (ZN, zero; MN, medium level; HN, high level), on bare sup-plot (BSP) and cropped sub-plot (CSP). Error bars indicate standard deviation

Table 5.2. Probabilities of the main effects: sampling (Day), tillage system (TIL), N fertilization level (FNT), and their interactions, in the analyses of variance of soil CO_2 flux and RR. Soil CO_2 flux, on cropped sub-plots (CSP) and bare sub-plots (BSP) in 2007-2008 (-08) and 2008-2009 (-09) growing seasons. RR, in 2007-2008 (2008) and 2008-2009 (2009) growing seasons. NS, non-significant (P<0.05).

	Soil CO_2 flux				*RR*	
Effect	CSP-08	BSP-08	CSP-09	BSP-09	2008	2009
Day	<.0001	<.0001	<.0001	<.0001	<0.0001	<0.0001
TIL	-	-	<.0001	0.0039	-	<0.0001
TILxDay	-	-	<.0001	NS	-	<0.0001
FNT	NS	0.039	0.042	NS	0.0270	NS
FNTxDay	NS	NS	NS	NS	NS	NS
TILxFNT	-	-	0.0051	NS	-	0.0217
TILxFNTxDay	-	-	NS	NS	-	NS

There was little difference in the soil water content and soil temperature between CSP and BSP (data not shown), thus it is fair to assume that differences in soil environmental conditions had little effect on decomposition rates of soil organic matter (Hanson *et al*., 2000). Moreover, soil CO_2 fluxes on the BSP were small (< 1 µmol CO_2 m^{-2} h^{-1}; Fig. 5.4), in contrast to wetter conditions (Moyano *et al*., 2007), where basal soil respiration is greater, and root respiration only represents a minor part of the soil CO_2 flux from cropped soil. Smaller CO_2 fluxes from bare soil of this experiment ensured more reliable estimates of RR. Finally, priming effects may occur in the rhizosphere (Kuzyakov and Larionova, 2005). However, we neglected priming effects since they are usually not large and their nature is in dispute (Brookes *et al*., 2009; Kemmit *et al*., 2008, Kuzyakov *et al*., 2009). For these reasons, it was reasonable to assume little differences on microbial respiration and to estimate *RR* rates as the difference between soil CO_2 flux of CSP and BSP in this experiment.

3.2. Estimating of root respiration rates

3.2.1. Root exclusion technique

Field estimates of *RR* were obtained for the period between tillering and anthesis. Root respiration before tillering was not evaluated, but it was assumed to be low due to reduced above-ground dry matter during that period (Rochette *et al*., 1999). Root respiration during growing season was a major contributor to soil CO_2 flux. Cumulative soil respiration of cropped soil during three growing seasons was linearly related to the size of the root system as presented in another study (Morell *et al*, 2011a). According to the estimates of *RR* presented in this study, the contribution of RR to the total soil CO_2 flux was up to 80% around anthesis (Figs. 5.4 and 5.5).

In 2008, estimated *RR* was less than 0.5 g CO_2-C m^{-2} d^{-1} until day 150 (Fig. 5.5), increasing up to 1 g of CO_2-C m^{-2} d^{-1} by day 160. In 2009, the *RR* showed a pattern similar to the growth rate of the crop (Fig. 5.3), with higher values during the most active growth period (150-170 DAS), and then a decrease between days 170 and 200. Values of *RR* were up to 3.8 g of CO_2-C

m^{-2} d^{-1} under CT on day 152. There were strong two-ways interactions between tillage system, N fertilization and sampling day in *RR* (Table 5.2). In 2008, *RR* was significantly affected by N fertilization. Mean values over the growing season were 0.37, 0.39 and 0.49 g of CO_2-C m^{-2} d^{-1} on zero, medium and high N fertilization levels respectively (Fig. 5.5).

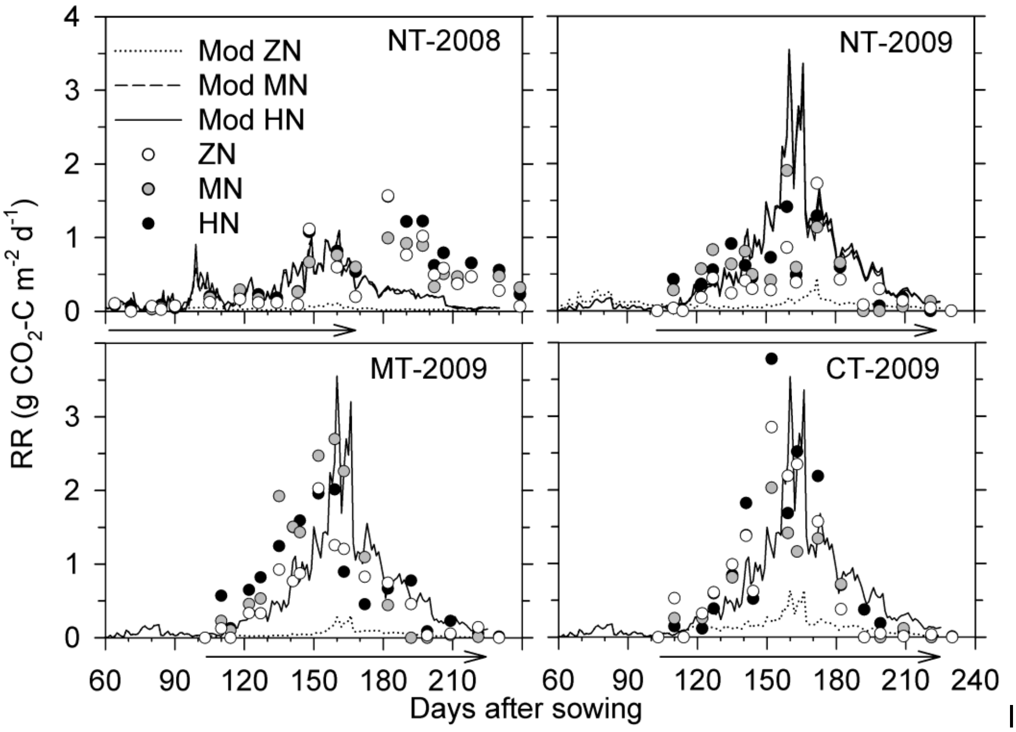

Figure 5.5. Modeled and observed RR under different tillage systems (NT, no-tillage; MT, minimum tillage; CT, conventional tillage) and three N fertilizer levels (ZN, zero; MN, medium level; HN, high level) in 2007-2008 (2008) and 2008-2009 (2009) growing seasons. Arrow at the bottom of the plots indicates the periods considered for the calculations of cumulative respirations.

In 2009, mean *RR* was 0.56 g of CO_2-C m^{-2} d^{-1} under NT, 0.82 g of CO_2-C m^{-2} d^{-1} under MT and 0.88 g of CO_2-C m^{-2} d^{-1} under CT. Differences among tillage systems were significant between days 142 and 173. Differences among N fertilization levels were only significant under MT (Fig. 5.5). Mean *RR* under MT was 0.66 g of CO_2-C m^{-2} d^{-1} on zero N fertilization level, 45% greater on medium than on zero N fertilization level and 32% greater on high than on zero N fertilization level.

3.2.2. Model approach

According to the model, and in agreement with our assumptions, *RR* rate before tillering was less than 0.1 g CO_2-C m^{-2} d^{-1} in both cropping seasons. The maximum *RR* calculated by the model was 1.1 g CO_2-C m^{-2} d^{-1} 2008, and 3.6 g CO_2-C m^{-2} d^{-1} in 2009. In both years, simulated *RR* was similar under different tillage systems and on medium and high N fertilization levels (Fig. 5.5). However the modelled *RR* on the unfertilized treatments was much less than the observed *RR* and than *RR* calculated for medium and high N fertilization levels (Fig. 5.5) due to a reduction in the simulated crop growth that did not occur in practice (Fig. 5.3). As a result the zero N fertilization level has not been considered further in the comparison of measured and modelled *RR*.

In 2008, we made comparisons between model and measurement until day 168 only, because measured *RR* rates after day 168 were three times higher than the rates calculated by the model probably due to decomposition of root tissue. In 2009, 5 of the 17 estimations had to be excluded from the statistical analyses because there were insufficient replicates (3). The relationship between modelled and observed *RR* was significant in 5 out of the 8 comparisons (Table 5.3), with no significant bias in any instance. The model allowed the separation of both sources of root respiration, root tissue respiration and rhizodeposits respiration (Fig. 5.6), as well as estimating cumulative values (Table 5.4).

Cumulative values of root respiration during the pre-anthesis period in 2008 and during the whole growing seasons in 2009 (Table 5.4) were calculated from linear interpolation between consecutive samplings using the trapezoid rule. Cumulative values could be only roughly calculated with the observed *RR* rates, because of the lack of estimates between samplings. Preanthesis cumulative values of cumulative of *RR* in 2008 were 23.5 and 27.9 g C m^{-2} in the medium and high N fertilization levels, respectively, compared to 32.8 g C m^{-2} predicted by the model. Cumulative values were between 60.8 and 105.4 g C m^{-2} in 2009 compared with values between 84.5 and 89.2 g C m^{-2} predicted by the model (Table 5.4).

Table 5.3. Adjustment of modeled and estimated RR in 2008 and 2009, under different tillage systems (no-tillage, NT; minimum tillage, MT; conventional tillage, CT) and N fertilization levels (medium level, MN; high level, HN).
[1] error in RMSE based on SE of observations see Smith et al 1997. [2] F test for lack of fit (Whitmore 1991) values of 1.44 and above indicate a difference between model and measurement at 5% significance level

		RMSE (%)	Error[1]	F Lofit[2]
2008	NT-MN	81	494	0,04
	NT-HN	53	574	0,02
2009	NT-MN	79	379	0,05
	NT-HN	104	243	0,1
	MT-MN	56	330	0,02
	MT-HN	66	482	0,04
	CT-MN	69	711	0,04
	CT-HN	92	636	0,04

4. DISCUSSION

4.1. Response to tillage and N fertilization

Soil respiration coming from microbial respiration, as estimated from soil CO_2 flux on the bare soil (Fig. 5.4), was slightly decreased by N fertilization in 2008 but not in 2009 (Table 5.2). Previous studies have also observed a slight suppression of microbial activity in response to increased N fertilizer application to unplanted soil in a paddy rice field (Iqbal *et al*, 2009). N fertilization may have a negative effect on the decomposition of soil organic matter partly as a result of lower pH (Kowalenko *et al*, 1978). On the other hand, soil respiration on the bare soil was greater under NT and MT than under CT in 2009 (Fig. 5.4), as has been also observed after rainfall events during fallow periods (Morell *et al*., 2010), and it can be attributed to increased return of crop residues under NT, leading to increased substrate availability and microbial respiration.

Table 5.4. Observed and modeled root biomass at maturity as dry matter weight (DM), and cumulative values of root respiration (RR) from field and modeled data, under three tillage systems (no-tillage (NT), minimum tillage (MT) and conventional tillage (CT)) and three N fertilizer levels (zero nitrogen, ZN; medium level, 60 kg N/ha, MN; and high level, 120 kg N/ha, HN). NT 08: cumulated RR between days 100 and 168 DAS.*

		Root biomass (g DM m^{-2})		Cum. *RR* respiration (g C m^{-2})	
		Observed	Modeled	Observed	Modeled
NT 08*	ZN	26.2	10.9	23.4	4.3
	MN	33.2	83.4	23.5	32.8
	HN	33.2	83.4	27.9	32.9
NT 09	ZN	76.7	19.1	53.4	10.3
	MN	81.0	167.1	66.0	84.5
	HN	113.3	180.0	60.8	88.2
MT 09	ZN	92.7	16.9	65.2	6.5
	MN	102.0	170.1	95.2	86.1
	HN	103.6	180.1	84.9	88.8
CT 09	ZN	116.0	36.3	95.3	17.9
	MN	85.9	180.1	76.5	89.2
	HN	91.1	180.1	105.4	89.2

Root respiration rates were greater under MT and CT (Fig. 5.5) than under NT in 2009. There was little difference between treatments in root biomass (Table 5.4). However, observations of root length density in another study in this same experiment and in 2009 growing season, showed significant reductions of root growth, from more than 2 cm cm^{-3} under CT and MT in the top 25 cm to 1.5 cm cm^{-3} under NT, due to increased bulk density and penetration resistance (Morell *et al*., 2011b). Hence, it can be concluded that reduced root growth under NT led to a reduction in *RR* in 2009 growing season.

N fertilization significantly increased root respiration (*RR*) under NT in 2008 and under MT in 2009 (Table 5.2 and Fig. 5.5), which may be attributed to increased crop growth (Fig. 5.3) and C translocation below-ground. Kou *et al*. (2008) also described increased root respiration concomitant with increased above- and below-ground growth. However, the proportional allocation to roots may be reduced at greater N fertilization levels (Johansson, 1992), and the positive response of soil CO_2 due to increased crop growth may be partly counteracted. As suggested by previous authors (Iqbal *et al*., 2009; Sainju *et al*., 2008; Xu and Wan, 2008), increased soil CO_2 fluxes in response to N fertilization can be attributed to stimulated root respiration, partly as a result of an indirect effect of N fertilization on the decomposition of the root materials released (Liljeroth, 1990) or directly from increased root tissue respiration. The response of crop growth and hence of root respiration to N fertilization under semiarid conditions was limited due to reduced water availability.

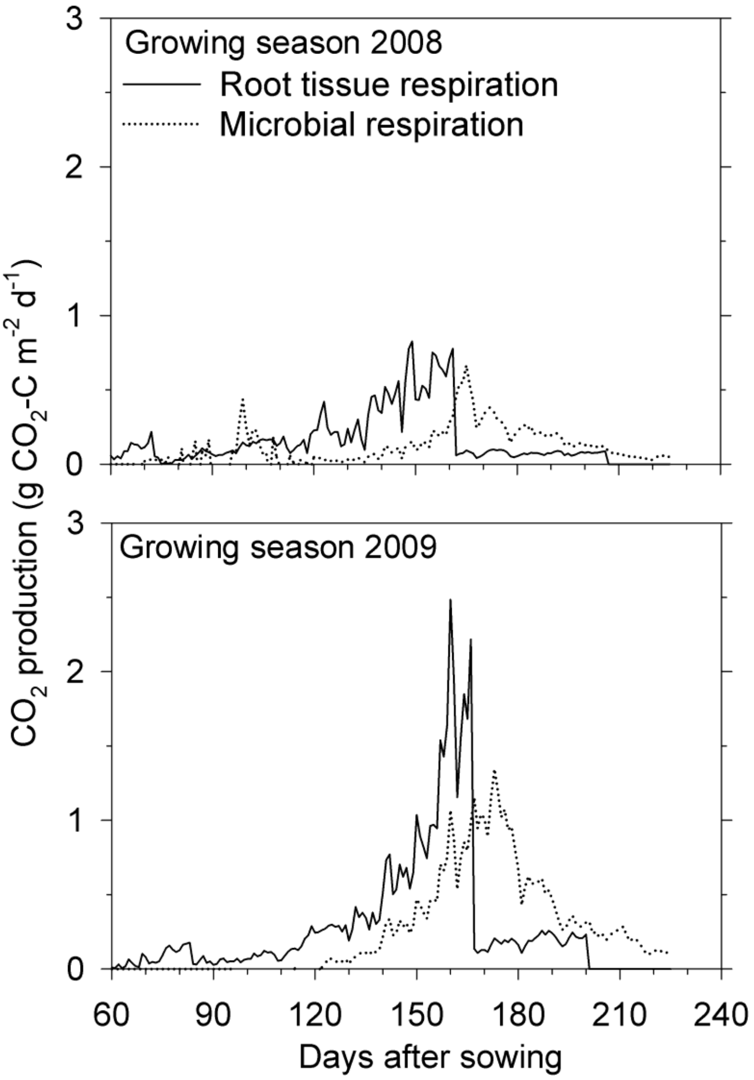

Figure 5.6. Modeled root tissue respiration and root induced microbial respiration on medium nitrogen fertilizer treatment under no-tillage in 2008 and 2009.

4.2. Performance of the model

The modelled crop growth had to be limited in 2008 to predict the reduction of crop growth due to the water limitation that occurred in that year. After this calibration in 2008, the model simulated well the pattern of the crop growth, and root growth and respiration in both years, including the higher values between days 150 and 170. Based on the results of this experiment (Fig. 5.5), the model confirmed the estimates of *RR* obtained by the root exclusion technique in many cases (Table 5.3). *RR* rates in a wet year (i.e., 2009) were three times those in a dry year (i.e., 2008; Fig. 5.5). Greater *RR* rates were observed at the stages of greater rate of crop growth, between days 120 and 170 (Fig. 5.5). During this period, C translocation to the root system is lager, and C substrates are used for root growth, root maintenance respiration and active transport processes (Lambers *et al*., 1987).

The root respiration model predicted little difference in root growth and root respiration (Fig. 5.5 and Table 5.4) between tillage systems. The reduction of the modelled crop growth on zero N fertilization level was due to a predicted reduction of the soil mineral N content that did not occur in the field (data not shown). Reduced crop growth in the model led to reduced root respiration on this treatment.

The lack of agreement between observed and modelled data during the post-anthesis period in 2009 was attributed to root decomposition. Root biomass is usually reduced between anthesis and crop harvest (Hansson *et al*., 1991). However during May 2008, heavy rainfall (Fig. 5.2) may have accelerated root decomposition of the senescing crop, thus increasing the *RR* rates determined as the differences between cropped and bare soil. The partial decomposition could partly explain the lack of agreement between observed and modelled root biomass (Table 5.4), since a great portion of the root biomass may have been decomposed, while the model only predicts decomposition of a small portion of the root tissue before harvest. Root biomass was only sampled at maturity in this experiment (Table 5.4), and we cannot confirm the extent of root decomposition between anthesis, maturity and harvest. Additional samplings of root biomass should be ideally conducted at different growth stages.

Previous work on C allocation with isotopic ^{14}C pulse-labelling, has reported that half of the C fixed in above-ground dry matter is translocated belowground, of which 62% can be accounted as C input to the soil (rhizodeposits and root biomass), and the remaining 38% would be root respiration (Swinnen *et al*., 1994). Taking account of the observed above-ground dry matter in 2009 (Fig. 5.3), and the estimates from Swinnen *et al* (1994), we would expect a below-ground C translocation between 134 and 238 g C m^{-2} (with half of the C in the above-ground dry-matter at maturity and assuming 40% of dry matter is carbon) (Fig. 5.2), of which between 50.9 and 90.4 would be used in root tissue respiration. These values are close to the estimates obtained in our study with the root exclusion technique (i.e., 53.4 and 105.4 g C m^{-2}; Table 5.4). At the same time, according to our simulations, 260 g C m^{-2} would be translocated below-ground. Considering Swinnen *et al*.'s (1994) estimates, 98.8 g C m^{-2} would be root respiration compared to the 89 g C m^{-2} estimated in the model (Table 5.4).

The model estimated the relative contributions of root tissue respiration (R_{ts}) and rhizomicrobial respiration (R_{rz}) to *RR*. The modelled *RR* is the sum of the activity predicted in two processes, R_{ts} and R_{rz} (Fig. 5.6). The model approach provided a compartmentalization between these two sources of C (Fig. 5.6), with 52% assigned to root tissue respiration (R_{ts}) and 48% to rhizomicrobial respiration (R_{rz}), in agreement with those obtained with combination of isotopic and non-isotopic methods (Kuyakov and Larionova, 2005). A further advantage of the modeling approach is that it is also able to predict day to day variations in *RR* depending on daily meteorological conditions, photosynthesis, and temperature effects on root and microbial respiration (Fig. 5.6), and thus allowing for more precise calculation of cumulative respiration (Table 5.4).

5. CONCLUSIONS

Under Mediterranean areas, long-term no-tillage adoption reduced root respiration due to reduced root growth. The increase of root respiration in response to N fertilization was related to the increase in crop growth. However, reduced water availability in Mediterranean conditions limits the response of crop growth and root respiration to N fertilizer addition. Field and modeled root respiration rates were in good agreement in patterns and absolute terms. The model estimated the contribution of root respiration to total soil CO_2 flux, though previous calibration of the pattern and amount of root growth was required. The model establishes a theoretical contribution of rhizomicrobial and root tissue contributions to root respiration.

ACKNOWLEDGEMENTS

This work was supported with by the Comision Interministerial de Ciencia y Tecnologia of Spain (Grants AGL 2004-07763-C02-02 and AGL2007-66320-CO2-02/AGR). The Servei de Meteorologia (Generalitat de Catalunya) is acknowledged for providing meteorological data. The field and laboratory technicians Silvia Martí and Carlos Cortés are gratefully acknowledged. F.J. Morell received a grant for his doctorate studies from the Spanish Ministry of Science and Innovation, and wishes to acknowledge the Soil Science Department at Rothamsted Research for hosting during a research period in autumn 2009. Rothamsted Research is an institute of the UK Biotechnology and Biological Sciences Research Council. A.P. Whitmore acknowledges the support of the Institute Strategic Programme grant on Sustainable Soil Function.

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GENERAL DISCUSSION

Under the semiarid Mediterranean conditions of this study, water availability was the main limiting factor for crop growth and productivity. Throughout the four years studied, rainfall quantities and its distribution determined grain yields and biomass production (Table 1.1, and Fig. 2.1), as it usually occurs in drylands under semiarid Mediterranean conditions (Austin *et al*., 1998), and it limited the annual C input from crop residues (Table 3.1). Nevertheless, conservation tillage systems improved soil water conservation and thus crop growth, grain yields, and C inputs compared to conventional tillage (CT) systems. The improvement of water availability due to increased water conservation under conservation tillage systems resulted in the positive response of crop growth and grain yield to N fertilization under no-tillage (NT), as well as under minimum tillage (MT), but not under CT (Figure 3, chapter 1; Table 1, chapter 1). This result contrasted with the lack of interaction during the early adoption (1-3 years; Angás *et al*., 2006, Cantero-Martínez *et al*., 2003)of these management practices. The improvement on soil water conservation in the long-term was related to the fact that crop residue production is low, and thus it took a few growing seasons for the residue to improve the soil surface, leading to increased soil water infiltration and reduced soil water evaporation.

Improvement of crop growth under conservation tillage systems, and with N fertilization under NT, lead to increased above-ground C inputs to the soil (Table 3.1). Below-ground C inputs to the soil, as determined by root biomass, were similarly affected by conservation tillage systems, but less affected by N fertilization (Table 3.1). Conservation tillage systems increased below-ground C inputs especially on dry years. In wet years, root length density was found to be negatively affected by long-term adoption of conservation tillage practices due to increased soil penetration resistance (Fig. 2.6). In spite of reduced root growth, long-term NT adoption did not reduced grain yields or C inputs, and thus the process should be regarded as a soil consolidation process rather than soil compaction.

Differences in root dynamics, particularly root density, among tillage systems impacted soil CO_2 emissions (Fig. 3.6). Additional study of soil CO_2 flux confirmed that the differences among tillage systems were mostly due to differences on root respiration (Fig. 5.5). Contribution of root respiration to soil

CO_2 flux was highest between stem elongation and anthesis (Fig. 5.4), and this contribution was lower under NT than under MT or CT, in relation with its reduced root length density (Fig. 2.4). Enzyme laboratory assays on soil samples of this experiment, found a slight increase of dehydrogenase activity in the cropped soil in comparison to the bare soil (Fig. D.1). This activation was attributed to respiration of rhizodeposits, and hence the difference between cropped and bare soil was attributed to two main sources: root tissue respiration and respiration of rhizodeposits (Morell, 2008).

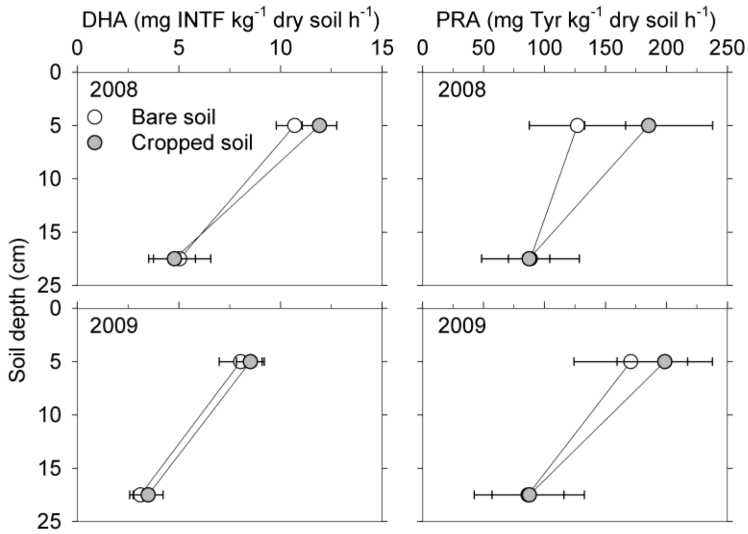

Figure D.1. Dehydrogenase activity (DHA) and Protease activity (PRA), in April 2008 and 2009 in soil samples from the experimental site in Agramunt.

A model study allowed a theoretical separation of the contribution of root tissue respiration and rhizomicrobial respiration, which were attributed 52% and 48% of the root respiration respectively (Fig. 5.6).

The increase of soil CO_2 flux with N fertilization during 2008-2009 growing season occurred in interaction with tillage systems, as well as interaction with the measurement day (Table 3.2). The increase occurred under NT and RT, and during the period around anthesis (Fig. 3.5), corresponding to the period of higher crop activity and root respiration. Additional study of root respiration confirmed that this response to N fertilization was attributed to differences on root respiration (Table 5.2).

On the other hand, the remaining part of the soil CO_2 flux is attributed to microbial respiration and it can be accounted as a net C loss, or C output.

According to the soil respiration data obtained during the fallow periods and after rainfall events, we can presume that NT and MT showed higher C outputs than CT (Fig 3.4.b, Table 4.1).

No clear evidence was found of N fertilization on increasing microbial respiration and C outputs, which could be expected from the higher return of crop residues in fertilized plots. Moreover there was no trend of differences on soil biochemical properties related to C dynamics among N fertilizer levels, such as dehydrogenase activity (data not shown) or microbial biomass carbon (Fig. D.2).

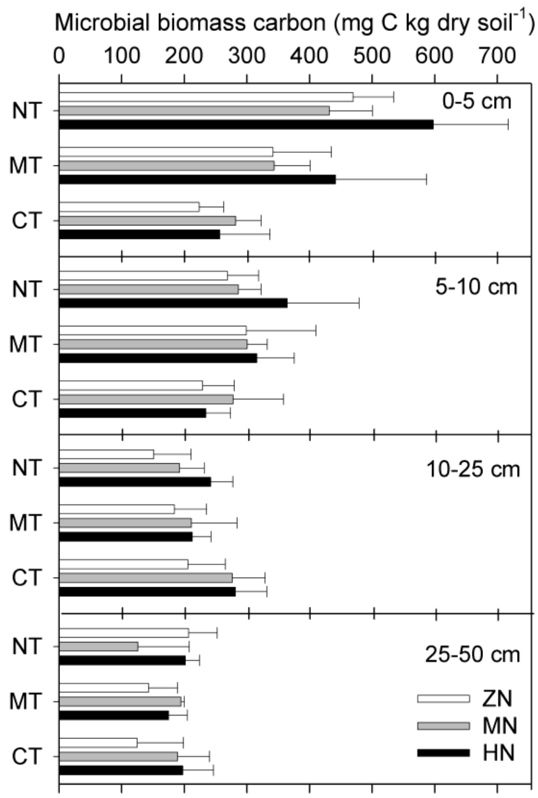

Figure D.2. Microbial biomass carbon in October 2008, for different tillage systems, no-tillage (NT), minimum tillage (MT) and conventional tillage (CT), and different N fertilization levels: zero N –ZN- (no fertilizer application); medium N -MN- (60 kg N ha^{-1}), and high N –HN- (120 kg N ha^{-1}) from the experimental site in Agramunt. Error bars indicate standard deviation of the means.

Decreased C outputs under the CT system was a sign of substrate and soil organic matter depletion in the soil for microbial respiration despite the significant short-term soil CO_2 peaks observed during tillage implementation (Fig. 4.1), which had little effects on the annual CO_2 cumulative fluxes (Table 4.1). For this reason, as occurred in this case, reducing soil CO_2 flux should not be seen as benefit of a given management practice, but the balance between C inputs and C outputs, should be regarded instead.

Soil CO_2 flux was positivively related to SOC stock. Increased C outputs under NT, were partly compensated with increased C inputs (Table 3.1) thus allowing for a positive balance in the stock of SOC. The positive balance between C inputs and C outputs was confirmed in the determination of the SOC stock, with 4.3 and 3.9 Mg C ha^{-1} more under NT than under MT and CT respectively over the 0-40 cm soil depth layer (Table 3.6). Furthermore, not only tillage affected SOC levels but also N fertilization in a lesser extent. Long-term N fertilization led to a significant increase of the SOC concentration in the 0-5 cm soil depth interval as detected in July 2009 (Tables 3.5 and 3.6). Mean SOC concentrations over the 0-5 cm soil depth interval were 12.9 g C kg^{-1} and 12.6 g C kg^{-1} with high and medium N fertilizer additions, while it was 11.3 without N fertilizer. Similarly, the stock of SOC was 3.4 and 4.5 Mg C ha^{-1} more with medium and high N fertilization level than on unfertilized plots for the entire 0-40 cm soil layer. This increase confirmed the hypothesis that N fertilization tends to increase or maintain SOC concentration, contrarily to the idea presented in other papers (Khan *et al*., 2007, Mulvaney *et al*., 2009). Model predictions suggest that medium and high N fertilization rates under no-tillage system of this experiment could lead to significant SOC sequestration in the next 20 years (Álvaro-Fuentes *et al*., 2012).

Long-term NT system and adequate N fertilizer rates led to a win-win-win situation where soil productivity was coupled with the improvement of soil quality and the sequestration of SOC in a winter cereal system under the semiarid Mediterranean conditions of this experiment.

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CONCLUSIONS

1. In semiarid Mediterranean dryland agroecosystems, long-term adoption of conservation tillage systems was a sustainable agronomical strategy to improve soil water conservation leading to the increase of yields and improved water-use efficieny (WUE). Long-term addition of N fertilizer only under no-tillage (NT) improved WUE and grain yield.

2. Long-term conservation tillage system increased root length density in dry years due to increased soil water content compared to conventional tillage (CT). Contrarily, in a wet year, root length density was lower under NT than minimum tillage (MT) and CT due to increased soil strength. The response of root growth to N fertilization was slight, and only occurred under conservation tillage system.

3. Reduced root growth under NT led to a reduction of root respiration. The increase of root respiration in response to N fertilization was related to the increase in crop growth.

4. Field and modeled root respiration rates were in good agreement in patterns and absolute terms. The model built in this thesis, estimated the contribution of root respiration to total soil CO_2 flux, though previous calibration of the pattern and amount of root growth was required. The model establishes a theoretical contribution of rhizo-microbial and root tissue contributions to root respiration.

5. Variations in rainfall and crop growth among cropping seasons and treatments led to changes on the soil CO_2 fluxes measured. The product between soil water content and soil temperature explained between 75 and 94% of the seasonal variability of soil CO_2. Root respiration was a major contributor to soil CO_2 flux which determines its seasonal pattern.

6. Tillage operations had little effect on cumulated CO_2 emission. However emissions after rainfall events were high and must be taken

into account when estimating the soil CO_2-C loss in semiarid Mediterranean agroecosystems.

7. No-tillage system increased the stock of SOC, with 4.3 and 3.9 Mg C ha^{-1} more than under minimum and conventional tillage systems respectively over the 0-40 cm soil depth layer.

8. Long-term N fertilization also increased the stock of SOC, with 3.4 and 4.5 Mg C ha^{-1} more with medium and high N fertilization levels than on unfertilized plots.

CONCLUSIONES

1. En agroecosistemas de secano semiáridos Mediterráneos, la adopción a largo plazo de sistemas de laboreo de conservación resultó ser una estrategia agronómica sostenible para la mejora de la conservación del agua dando lugar a mayores rendimientos y mayor eficiencia de uso del agua (EUA). La fertilización nitrogenada a largo plazo solo mejoró la EUA y el rendimiento de grano bajo no laboreo (NL).

2. La adopción a largo plazo de los sistemas de laboreo de conservación aumentó la densidad de longitud radicular en años secos debido a un aumento de la cantidad de agua en el suelo en comparación con sistemas de laboreo convencional. De manera contraria, en un año húmedo, la densidad de longitud radicular fue menor bajo NL que bajo mínimo laboreo (ML) y laboreo convencional (LC) debido a una mayor resistencia a la penetración del suelo. La respuesta del crecimiento radicular a la fertilización nitrogenada fue ligera, y únicamente ocurrió en sistemas de laboreo de conservación.

3. El menor crecimiento radicular bajo NL dió lugar a una reducción de la respiración radicular. El aumento de la respiración radicular con la fertilización nitrogenada se relacionó con aumento del crecimiento del cultivo.

4. Las tasas de respiración radicular en campo y modelizadas concordaron tanto en su patron como en términos absolutos. El modelo construido en esta tesis, determinó la contribución de respiración radicular al flujo de CO_2 del suelo, aunque requirió la calibración previa del patrón de crecimiento y cantidad radicular.

5. La variación de la distribución de la lluvia y del crecimiento del cultivo entre ciclos de cultivo llevó a cambios en los flujos de CO_2 del suelo. El producto entre el contenido de agua del suelo y la temperatura del suelo explicaron entre un 75 y un 94% de la variación estacional del CO_2 del suelo. La respiración radicular fue el mayor contribuyente del flujo de CO_2 del suelo, y determina su variación dentro del ciclo de cultivo.

6. Las operaciones de laboreo tuvieron un ligero efecto sobre las emisiones acumuladas de CO_2. Sin embargo las emisiones después de eventos de lluvia fueron elevadas y debe tenerse en cuenta cuando se estime las pérdidas de CO_2-C del suelo en agroecosistemas Mediterráneos.

7. El sistema de no laboreo aumentó la cantidad de carbono orgánico en el suelo (COS) en 4.3 y 3.9 Mg C ha^{-1} mas que bajo mínimo laboreo y laboreo convencional respectivamente en el intervalo de profundidad de 0 a 40 cm del suelo.

8. La fertilización nitrogenada a largo plazo también aumentó el stock de COS en 3.4 y 4.5 C ha^{-1} con niveles medios y altos de fertilización nitrogenada con respecto a las parcelas no fertilizadas.