Universitat de Lleida

Emisiones de gases de efecto invernadero y protección del carbono orgánico del suelo en secanos Mediterráneos: efectos del laboreo y de la estrategia de fertilización

Daniel Plaza Bonilla

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A la família

A la Mar

Humanity, disdainful of what was created without it, believes…that it can develop (the planet) by destroying the slow accumulation of plant wealth that collaboration between the atmosphere and the earth had produced over thousands of centuries. Will the large… … forest, this huge laboratory of climates, this humid and war velvet belt of plant from which rhythmic spirals of atmospheric waver harmoniously soar, be transformed wisely, exploited with respect for humanity and nature, by taking into account its relationship with the soil and the atmosphere, or will humanity give in to the temptation to assault the earth, attack the forest quickly and without thought? In the latter case, if one thinks about it, it is humanity itself which would be endangered, … because the atmosphere would be unbalanced and instability would be introduced into climate around the whole world.

F. Schrader, *Atlas de Géographie Historique*, 1896.

Agraïments

Si... la secció dels agraïments, la que no es sotmet a revisió per parells, aquella que, tot i semblar senzilla, presenta el nivell de dificultat més elevat de tot el document i és que... com agrair tota la ajuda, consells, opinions i recolzament de tanta i tanta gent i durant tantes i tantes llargues jornades? Resulta impossible plasmar en unes breus línies tota la col·laboració i ànims rebuts durant els darrers quatre anys. Les meves més sinceres disculpes per tots els que m'he pogut deixar, no sou menys imprescindibles que la resta.

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Durante los primeros 12 meses de desarrollo de la tesis doctoral recibí una ayuda para personal predoctoral en formación de la Universitat de Lleida. Posteriormente, recibí una beca del Programa de Formación de Profesorado Universitario (FPU) del Ministerio de Educación (referencia AP2009-4408) que cubrió los 36 meses siguientes de la tesis.

Resum

L'objectiu principal d'aquest estudi fou la quantificació de l'efecte de diferents tipus de conreu del sòl i tipus i dosis de fertilització nitrogenada sobre l'emissió de gasos d'efecte hivernacle (CH_4, CO_2 i N_2O) del sòl a l'atmosfera i l'agregació del sòl com a mecanisme de protecció del carboni orgànic en agroecosistemes de secà semiàrid Mediterranis. Per tal d'assolir aquest objectiu es varen plantejar cinc camps experimentals on s'hi comparaven sistemes de conreu (sembra directa i conreu intensiu) i tipus (minerals i orgànics) i dosis de fertilitzants nitrogenats, localitzats al centre i l'est de la vall de l'Ebre. A Agramunt (Lleida, establert l'any 1996) i Senés de Alcubierre (Huesca, establert l'any 2010) s'hi van quantificar les emissions de CO_2, CH_4 i N_2O al llarg de dos i tres anys, respectivament, els estocs de C i N orgànics del sòl i el rendiment de collita i es va calcular l'emissió d'equivalents de CO_2 per kg de gra produït. Alhora, a Agramunt es va determinar la proporció de macroagregats i microagregats estables a l'aigua i la concentració de C d'ambdós així com la d'altres fraccions del sòl. D'altra banda, en un altre experiment a Agramunt es va establir una cronoseqüència de sembra directa de cinc fases amb 0, 1, 4, 11 i 20 anys, en les que es va determinar la distribució d'agregats estables a l'aigua i tamisats en sec i la seva concentració de C orgànic. A St. Martí Sesgueioles (Barcelona, establert l'any 2007) s'hi van comparar dosis creixents de fertilitzant mineral en un maneig del sòl en sembra directa. A Conill (Barcelona, establert l'any 2008) es va estudiar l'aplicació de purí porcí a dos dosis de N, gallinassa i un tractament control també sota sembra directa. En ambdós experiments es va realitzar un seguiment de la dinàmica temporal de l'agregació del sòl, la protecció del carboni en els agregats i el carboni de la biomassa microbiana durant dos campanyes de cultiu. Finalment, es va realitzar una incubació de macroagregats per a observar l'impacte del tipus de conreu i del tipus de fertilització (mineral i orgànica amb purí porcí) sobre la producció de CH_4, CO_2 i N_2O.

En general, els sistemes de conreu i el maneig de la fertilització nitrogenada van afectar les emissions de gasos d'efecte hivernacle del sòl a l'atmosfera i, en el cas del conreu, l'estabilització física del carboni orgànic del sòl. Així, per als sistemes de conreu, en l'experiment de curt termini, sota sembra directa es van generar unes majors emissions de N_2O i CO_2 i una menor oxidació del CH_4 que en conreu intensiu. No obstant, al comparar-los a llarg termini, ambdós sistemes de conreu van presentar la mateixa emissió de N_2O i la oxidació del CH_4 va ser major en sembra directa, fets que també es van observar a l'incubar els macroagregats. Alhora, a l'augmentar el nombre d'anys sota sembra directa, el sòl va presentar una major proporció de macroagregats estables i una major formació de microagregats d'elevada concentració de C. Pel que fa a l'impacte de la fertilització nitrogenada sobre l'emissió de gasos d'efecte hivernacle, l'aplicació de dosis creixents de N tan orgànic com mineral va provocar un augment de la emissió de N_2O del sòl. En canvi, per a una mateixa dosi, aquests dos tipus de fertilitzant van emetre una quantitat de N_2O similar. D'altra banda, la fertilització nitrogenada mineral no va millorar la protecció del C en els agregats del sòl mentre que la orgànica només va promoure un lleu increment en l'estabilitat d'aquests. Als secans semiàrids Mediterranis, la combinació de l'ús de la sembra directa i la fertilització amb dosis mitjanes de purí porcí és una estratègia de maneig òptima ja que minimitza les emissions de gases d'efecte hivernacle i manté la productivitat del cultiu. Alhora, ambdues pràctiques milloren l'estabilitat dels agregats, maximitzant la quantitat de carboni orgànic segrestat i millorant l'estat estructural del sòl.

Resumen

El objetivo principal de este estudio fue cuantificar el efecto de diferentes tipos de laboreo del suelo y dosis de fertilización nitrogenada sobre la emisión de gases de efecto invernadero (CH_4, CO_2 y N_2O) del suelo a la atmosfera y la agregación del suelo como mecanismo de protección del carbono orgánico en agroecosistemas de secano semiárido Mediterráneo. Para alcanzar este objetivo se plantearon cinco campos experimentales en los que se comparaban sistemas de laboreo (siembra directa y laboreo intensivo) y tipos (minerales y orgánicos) y dosis de fertilizantes nitrogenados, localizados en el centro y el este del valle del Ebro. En Agramunt (Lleida, establecido el año 1996) y Senés de Alcubierre (Huesca, establecido el año 2010) se cuantificaron las emisiones de CO_2, CH_4 y N_2O a lo largo de dos y tres años, respectivamente, los stocks de C y N orgánicos del suelo y el rendimiento de cosecha y se calculó la emisión de equivalentes de CO_2 per kg de grano producido. Además, en Agramunt se determinó la proporción de macroagregados y microagregados estables al agua y la concentración de C de ambos así como otras fracciones del suelo. Por otro lado, en otro experimento en Agramunt se estableció una cronosecuencia de siembra directa de cinco fases con 0, 1, 4, 11 y 20 años, en las que se determinó la distribución de agregados estables al agua y tamizados en seco y su concentración de C orgánico. En St. Martí Sesgueioles (Barcelona, establecido el año 2007) se compararon dosis crecientes de fertilizantes mineral en un manejo del suelo en siembra directa. En Conill (Barcelona, establecido el año 2008) se estudió la aplicación de purín a dos dosis de N, gallinaza y un tratamiento control también bajo siembra directa. En ambos experimentos se realizó un seguimiento de la dinámica temporal de la agregación del suelo, la protección del carbono en los agregados y el carbono de la biomasa microbiana durante dos campañas de cultivo. Finalmente, se realizó una incubación de macroagregados para observar el impacto del tipo de laboreo y del tipo de fertilización (mineral y orgánica con purín porcino) sobre la producción de CH_4, CO_2 y N_2O.

En general, los sistemas de laboreo y el manejo de la fertilización nitrogenada afectaron las emisiones de gases de efecto invernadero del suelo a la atmosfera y, en el caso del laboreo, la estabilización física del carbono orgánico del suelo. Así, para los sistemas de laboreo, en el experimento de corto plazo, bajo siembra directa se generaron unas mayores emisiones de N_2O y CO_2 y una menor oxidación del CH_4 que en laboreo intensivo. No obstante, al compararlos a largo plazo, ambos sistemas de laboreo presentaron la misma emisión de N_2O y la oxidación de CH_4 fue mayor en siembra directa, hechos que también se observaron al incubar los macroagregados. Además, al aumentar el número de años bajo siembra directa, el suelo presentó una mayor proporción de macroagregados estables y una mayor formación de microagregados de elevada concentración de C. Por lo que respecta al impacto de la fertilización nitrogenada sobre la emisión de gases de efecto invernadero, la aplicación de dosis crecientes de N tanto orgánico como mineral provocó un aumento de la emisión de N_2O del suelo. En cambio, para una misma dosis, estos dos tipos de fertilizante emitieron una cantidad de N_2O similar. Por otro lado, la fertilización nitrogenada mineral no mejoró la protección del C en los agregados del suelo mientras que la orgánica solo promovió un leve incremento en la estabilidad de éstos. En los secanos semiáridos Mediterráneos, la combinación del uso de la siembra directa y la fertilización con dosis medias de purín porcino es una estrategia de manejo óptima ya que minimiza las emisiones de gases de efecto invernadero y mantiene la productividad del cultivo. Además, ambas prácticas mejoran la estabilidad de los agregados, maximizando la cantidad de carbono orgánico secuestrado y mejorando el estado estructural del suelo.

Summary

The main objective of this study was the quantification of the effects of different types of soil tillage and types and rates of nitrogen fertilization on the emission of soil greenhouse gases (CH_4, CO_2 and N_2O) to the atmosphere and the soil aggregation as an organic carbon protection mechanism in the dryland semiarid Mediterranean agroecosystems. In order to achieve that objective five experimental fields were established comparing different tillage systems (no-tillage and intensive tillage) and types (mineral and organic) and rates of nitrogen fertilizers, localized in the center and East of the Ebro Valley. In Agramunt (Lleida, established in 1996) and Senés de Alcubierre (Huesca, established in 2010) CO_2, CH_4 and N_2O emissions were quantified during two and three years, respectively. Soil organic C and N stocks, crop yield and the emission of CO_2 equivalents per kg of grain produced were also determined. Moreover, in Agramunt, water-stable macroaggregates, microaggregates within macroaggregates and their C concentration and other different soil fractions were quantified. In turn, in another experiment in Agramunt a no-tillage chronosequence with 0, 1, 4, 11, 20 years was established and the water-stable and dry-sieved aggregates distributions and their C concentration were determined. In St. Martí Sesgueioles (Barcelona, established in 2007) increasing rates of mineral fertilizer under no-tillage were compared. In Conill (Barcelona, established in 2008) the application of pig slurry at two N rates, poultry manure and a control treatment under a no-tillage management were studied. In both experiments the soil aggregation dynamics, the C protection within aggregates and their C concentration and the microbial biomass carbon were analyzed during two cropping seasons. Finally, an incubation of macroaggregates was carried out in order to study the impact of the type of tillage and fertilizer (mineral and organic with pig slurry) on the production of CH_4, CO_2 and N_2O.

In general, tillage systems and nitrogen fertilizer management affected the emissions of soil greenhouse gases to the atmosphere while tillage also impacted the physical stabilization of organic carbon. In the case of tillage system, in the short-term experiment, greater N_2O and CO_2 emissions and lower CH_4 oxidation were observed. However, in the long-term, both tillage systems presented the same N_2O emission and the CH_4 oxidation was greater under no-tillage, aspects that were also observed in the macroaggregate incubation. Moreover, the soil presented a greater proportion of water-stable macroaggregates and greater C-enriched microaggregates within those macroaggregates when increasing the number of years under no-tillage. In the case of the impact of the nitrogen fertilization on the greenhouse gases emission, the application of increasing N rates by both organic and mineral sources increased the soil N_2O emission. As a difference, for a given N rate, similar amount of soil N_2O was quantified for both fertilizer types. The mineral nitrogen fertilization did not improve the C protection within soil aggregates while the organic fertilization caused a low increase in their stability.

In the dryland semiarid agroecosystems of the Mediterranean, the combination of no-tillage and fertilization with medium N rates of pig slurry is an optimum strategy in terms of greenhouse gases minimization and maintenance of crop productivity. Moreover, both practices improve the aggregate stability maximizing the amount of organic carbon protected and improving soil structure.

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Lista de abreviaturas

C	Carbon
C_2H_2	Acetylene
CH_4	Methane
CH_4-C	Carbon emitted as methane
CO_2	Carbon dioxide
CO_2-C	Carbon emitted as carbon dioxide
CT	Conventional tillage
D	Depth
ECD	Electrical conductivity detector
FID	Flame ionization detector
GHG	Greenhouse gases
GWP	Global warming potential
K	Potassium
M	Soil water-stable macroaggregates
M-C	Carbon concentration of the soil water-stable macroaggregates
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
mM	Microaggregates within soil water-stable macroaggregates
mM-C	Carbon concentration of the microaggregates within soil water-stable macroaggregates
M-POxC	Permanganate-oxidizable carbon concentration of the water-stable macroaggregates
MWD	Mean weight diameter of the dry-sieved aggregates
N	Nitrogen
N_2	Molecular nitrogen
N_2O	Nitrous oxide
N_2O-N	Nitrogen emitted as nitrous oxide
NH_4^+	Soil ammonium
NO	Nitric oxide
NO_3^-	Soil nitrate
NT	No-tillage
NT-1	No-tillage maintained for one year
NT-4	No-tillage maintained for four years
NT-11	No-tillage maintained for eleven years
NT-20	No-tillage maintained for twenty years
O_2	Oxygen
O_3	Ozone
OC	Organic carbon
P	Phosphorous
POC	Particulate organic carbon
POxC	Permanganate-oxidizable carbon concentration of the soil
PM	Poultry manure
PM100	Poultry manure applied at 100 kg N ha^{-1}
PS	Pig slurry
PS100	Pig slurry applied at 100 kg N ha^{-1}
PS200	Pig slurry applied at 200 kg N ha^{-1}
RT	Reduced tillage
s+cM-C	Carbon concentration of the silt-plus clay-sized fraction of the macroaggregates

SM	Soil moisture
SOC	Soil organic carbon
SR	Stratification ratio
STN	Soil total nitrogen
SWC	Soil water content
t	Sampling time
T	Tillage
WFPS	Soil water filled pore space
WSC	Water-soluble carbon

Introducción general

Introducción general

El aumento de la concentración de gases de efecto invernadero (dióxido de carbono, CO_2; metano, CH_4; y óxido nitroso, N_2O) en la atmosfera ha sido señalado como el principal agente causante del cambio climático (Bloomfield, 1992; Vitousek et al., 1997). Para el conjunto de España, el sector agrícola es el principal productor de CH_4 y N_2O, suponiendo el 10% de las emisiones totales de gases de efecto invernadero (MAGRAMA, 2012).

Desde el período pre-industrial la concentración atmosférica de N_2O ha aumentado alrededor de un 0,2-0,3% anual debido, principalmente, a la producción y aplicación de fertilizantes nitrogenados (Forster et al., 2007; Weiss, 1981). La emisión de N_2O desde el suelo es el resultado de los procesos biológicos de nitrificación y desnitrificación, aunque ciertos procesos químicos como la quemodesnitrificación también pueden generar cantidades importantes de ese gas bajo determinadas circunstancias (Bremner, 1997). Durante la nitrificación el amonio es oxidado biológicamente a nitrato, liberándose N_2O por parte de los microorganismos como resultado de la utilización del nitrito como aceptor de electrones (Poth y Focht, 1985). A su vez, la desnitrificación es la reducción del nitrato a óxidos de nitrógeno (NO y N_2O) y, posteriormente, a nitrógeno molecular (N_2) por microorganismos heterótrofos cuando se dan condiciones limitantes en la concentración de O_2 en el suelo (Bremner, 1997). No obstante, existe también la desnitrificación generada por microorganismos nitrificadores autótrofos basada en la oxidación del amoníaco a nitrito, seguida por la reducción de éste a NO, N_2O o N_2 bajo limitaciones puntuales de oxígeno (Wrage et al., 2001). Así, la fertilización nitrogenada influencia notablemente todos estos procesos y, por tanto, las emisiones de N_2O (Eichner, 1990). De esta manera, por ejemplo, el Panel Intergubernamental de Expertos sobre Cambio Climático (IPCC, en inglés) recomienda actualmente un factor de un 1% para calcular la cantidad de nitrógeno emitido en forma de N_2O en relación con el nitrógeno aplicado en suelos agrícolas (IPCC, 2006). A su vez, la producción de N_2O también se ve influenciada por la composición de los fertilizantes utilizados. Algunos autores han observado un incremento en las emisiones de dicho gas al aplicar fertilizantes orgánicos debido a los aportes de carbono orgánico fácilmente degradable, el cual actúa como fuente de energía para los microorganismos desnitrificadores (Petersen, 1999). No obstante, otros autores han obtenido resultados contrarios en los que la aplicación de subproductos orgánicos generó una menor

emisión de N_2O en comparación con los fertilizantes minerales (Meijide et al., 2009). El manejo del suelo también influye decisivamente en la producción y emisión de N_2O. Se ha sugerido que la mayor conservación de agua en el suelo en sistemas de siembra directa puede producir mayores emisiones de N_2O del suelo (Aulakh et al., 1984). En cambio, recientemente, van Kessel et al. (2013) han señalado que el mantenimiento de la siembra directa a lo largo del tiempo podría llevar a una menor emisión de N_2O en comparación con el laboreo convencional. En España, hasta la fecha, no existen estudios en los que se analice el impacto de los sistemas de laboreo sobre la emisión de N_2O a lo largo del ciclo de cultivo.

El CH_4, otro importante gas de efecto invernadero, presenta un potencial de calentamiento global 25 veces superior al del CO_2 (Forster et al., 2007). En la agricultura, la producción de metano se da en condiciones anaeróbicas como la fermentación entérica de los rumiantes, el cultivo de arroz en suelos encharcados o el procesamientos de estiércoles (Mosier et al., 1998). En cambio, en suelos aerobios pueden darse las condiciones adecuadas para que se produzca la oxidación del metano (Goulding et al., 1995). Durante este último proceso los microorganismos metanotrofos presentes en el suelo captan pequeñas cantidades de metano de la atmósfera y lo oxidan, utilizándolo como fuente de energía, liberando, finalmente, CO_2. Aunque en los suelos agrícolas las cantidades de CH_4 oxidadas por unidad de superficie son bajas, se estima que contribuyen a la destrucción de alrededor de un 15% de la cantidad global de este gas (Born et al., 1990). Las diferentes prácticas de manejo del suelo influyen decisivamente en la oxidación del metano. Así, Ball et al. (1999) sugirieron que el uso del laboreo puede reducir la oxidación de éste gas debido a la alteración de la comunidad de microorganismos metanotrofos del suelo y a los posibles cambios en la estructura del suelo que llevarían a una reducción de la tasa de difusión del CH_4 a través de la arquitectura porosa del suelo. Por otro lado, la fertilización nitrogenada también juega un papel muy importante sobre la oxidación de metano en los suelos agrícolas. La presencia de amonio (NH_4^+) en el suelo se relaciona con el establecimiento de relaciones de competencia entre los microorganismos metanotrofos y los nitrificadores por la enzima metano-monooxigenasa (Hütsch, 2001). Debido a las aplicaciones de fertilizantes basados en el amonio dicha competencia inhibe el proceso de oxidación de metano en el suelo, efecto que puede mantenerse a lo largo del tiempo al provocar cambios en la comunidad microbiana (Hütsch et al., 1994). Sin embargo, en España, no

existen cuantificaciones del impacto de las anteriores prácticas agrícolas sobre los flujos de metano entre el suelo y la atmosfera.

Los suelos agrícolas pueden representar una fuente importante de CO_2 debido a la mineralización de la materia orgánica contenida en éstos (Follett, 2001). No obstante, un manejo adecuado puede incrementar la cantidad de carbono orgánico del suelo (COS), reduciendo la concentración atmosférica de CO_2 y ayudando con ello a mitigar los efectos del cambio climático. A su vez, este incremento del COS conllevaría una mejoría en la calidad y la fertilidad de los suelos (Lal, 2004; Smith, 2004). El incremento o secuestro de carbono en el suelo se basa en la protección física, química y bioquímica de compuestos orgánicos de este elemento que provienen del proceso fotosintético vegetal (Hassink, 1997; Six et al., 2002). De ellos, la protección física mediante la formación de agregados en el suelo es el mecanismo que se ve más ampliamente afectado por las prácticas agrícolas. Entre otras, prácticas como el laboreo tienen un profundo efecto sobre la agregación del suelo. Por ejemplo, se ha observado que la utilización de la siembra directa, en comparación con el laboreo convencional, viene acompañada por una mayor formación de microagregados estables dentro de los macroagregados, proceso que incrementa la capacidad de protección del carbono orgánico en el suelo a lo largo del tiempo (Six et al., 1999; Six y Paustian, 2013). Los microagregados limitan el acceso de los microorganismos al carbono almacenado en estas estructuras resultando en un almacenamiento en el tiempo. No obstante, en sistemas de laboreo la mayor frecuencia de rotura de macroagregados no permite formar estos microagregados y, por tanto, limita el secuestro del carbono en el interior de éstos (Six et al., 1999). La fertilización nitrogenada también puede jugar un papel importante sobre la agregación del suelo y la protección del carbono orgánico. A pesar de que la información al respecto es escasa, algunos experimentos llevados a cabo en condiciones húmedas han observado una mejora en las propiedades físicas de los suelos después de la aplicación continuada de fertilizantes orgánicos (Abiven et al., 2009).

Los agregados del suelo no solo controlan el almacenamiento físico de carbono en el suelo sino que también intervienen en la arquitectura porosa de éste último. Esta arquitectura resulta de la diferente disposición de los agregados y regula el movimiento o difusión de los gases a través del suelo. Por lo tanto, la agregación del suelo juega un papel importante en la producción, consumo o transporte de gases hacia o desde la superficie y su posterior liberación a la atmosfera (Smith, 1990; Ball et al., 1999; Ball,

2013). Así, por ejemplo, tal y como se ha comentado anteriormente, el uso del laboreo puede reducir la oxidación del metano debido a la menor difusión de este gas a través de los poros del suelo (Ball et al., 1999). Además, también se han asociado pérdidas de N_2O por desnitrificación a la presencia de micrositios anaeróbicos en diferentes fracciones de agregados (Parkin, 1987) e incrementos de emisiones de CO_2 por rotura de agregados como consecuencia de prácticas de laboreo (Álvaro-Fuentes et al. 2007; Morell et al., 2010). No obstante, se necesitan más estudios con el fin de determinar con mayor exactitud las relaciones existentes entre la agregación del suelo, el manejo agrícola y la emisión de gases de efecto invernadero, así como determinar los factores que controlan estas posibles relaciones.

Los agroecosistemas de secano semiárido del valle del Ebro son representativos de la producción agrícola en la zona Mediterránea. El valle del Ebro presenta un clima Mediterráneo de tendencia continental con una pluviometría que oscila entre los 300 a 450 mm en el centro del valle y que puede alcanzar los 1000 mm en las áreas montañosas circundantes, caracterizada por una marcada estacionalidad e irregularidad interanual, típicamente mediterránea (Vicente-Serrano et al., 2003). Asimismo, la oscilación térmica es muy intensa, alcanzándose con relativa facilidad los 40ºC en verano y los -10ºC en invierno (Cuadrat, 1999). El viento dominante es de componente noroeste, conocido popularmente como *Cierzo*, de gran poder evaporante. Los suelos del valle se caracterizan por una escasa profundidad más acusada en las posiciones elevadas, la presencia de horizontes cálcicos y unos rangos texturales limosos y franco-arcillo-limosos. El principal sistema productivo en la zona es el monocultivo de cereales de invierno, mayoritariamente cebada y trigo, cuyo rendimiento presenta una elevada dependencia de la pluviometría estacional y la capacidad de almacenamiento de agua del suelo (Gibbon, 1981; Austin et al., 1998).

En estas zonas, la tecnología de cultivo se ha basado tradicionalmente en el laboreo intensivo del suelo mediante arados de vertedera, subsoladores o gradas de discos como labor primaria y sucesivos pases de cultivador o chisel como labor secundaria, hecho que ha llevado a una disminución acusada del contenido de carbono orgánico y la consiguiente degradación de los suelos (Álvaro-Fuentes et al., 2009; Fernández-Ugalde et al., 2009). No obstante, el área bajo agricultura de conservación, ya sea mediante técnicas de mínimo laboreo o de siembra directa, ha ido progresivamente en aumento durante las últimas dos décadas debido, en mayor medida, al encarecimiento de los

combustibles fósiles (Cantero-Martínez et al., 2003). La fertilización nitrogenada es otro de los factores productivos clave en las zonas Mediterráneas. A pesar de su importancia en términos económicos para los agricultores, su uso no suele basarse en criterios agronómicos, tendiéndose a la sobrefertilización (Angás et al., 2006). Por otro lado, la cabaña ganadera de la zona, especialmente la del porcino, ha aumentado exponencialmente durante las últimas décadas como complemento de las rentas agrarias, llegando a suponer más del 40% de la producción total de porcino en España (Daudén y Quílez, 2004). La mayor parte del subproducto obtenido, purín en el caso del porcino, se aplica en los suelos sin determinar de forma previa su riqueza en nutrientes, hecho que ha conllevado problemáticas ambientales como la contaminación de aguas por nitratos y, como consecuencia la declaración de zonas vulnerables (Yagüe y Quílez, 2010).

Por tanto, en los agroecosistemas de secano Mediterráneo del noreste español, existe una necesidad de documentar la sostenibilidad de diferentes prácticas de manejo agrícola, como el laboreo y la fertilización nitrogenada, en términos de emisión de gases de efecto invernadero y protección del C orgánico en los agregados del suelo.

Hipótesis de partida, objetivos y estructura de la tesis

La hipótesis de partida de este estudio es que el uso de diferentes tipos de laboreo y estrategias de fertilización nitrogenada afectarán a la agregación y protección de carbono orgánico en el suelo y, además, regularán la actividad biológica de éste, provocando, como consecuencia, variaciones en las pérdidas de C y N en forma de gases de efecto invernadero (CO_2, CH_4 y N_2O).

Para validar dicha hipótesis el objetivo general de la presente Tesis Doctoral es la cuantificación del efecto de diferentes tipos de laboreo y fertilización nitrogenada sobre las emisiones de gases de efecto invernadero y la agregación del suelo como mecanismo de protección del carbono orgánico. Con este trabajo se pretende identificar aquellas prácticas de laboreo y fertilización nitrogenada que garanticen la sostenibilidad de los sistemas de secano del valle del Ebro en base a unas menores emisiones de gases de efecto invernadero y una mayor protección del carbono orgánico en los agregados del suelo, sin menoscabo de la productividad del sistema.

Los objetivos específicos planteados fueron cinco:

(i) Analizar el efecto de la interacción entre el laboreo y la dosis de fertilizante nitrogenado mineral al largo plazo sobre los mecanismos de protección del carbono orgánico.

(ii) Cuantificar los efectos del mantenimiento de la siembra directa sobre la agregación del suelo y la protección del carbono orgánico.

(iii) Determinar el impacto de la aplicación de diferentes fertilizantes orgánicos y minerales sobre la dinámica estructural del suelo y la protección del carbono orgánico bajo siembra directa.

(iv) Cuantificar el efecto del laboreo y la fertilización nitrogenada sobre la producción de N_2O, CH_4 y CO_2 en la escala de agregado, para identificar las relaciones entre manejo agrícola, agregación y producción de estos gases.

(v) Cuantificar las emisiones de N_2O, CH_4 y CO_2 y la productividad del cultivo bajo diferentes tipos de laboreo y dosis y tipos de fertilización nitrogenada.

A continuación se describe brevemente el contenido de cada capítulo de la presente memoria de tesis:

Capítulo 1. Organic protection within soil aggregates: long-term effects of tillage and N fertilization.

En este capítulo se determina el efecto, al largo plazo, de la interacción de dos tipos de laboreo (laboreo convencional y siembre directa) y tres dosis de fertilizante nitrogenado mineral (0, 60 y 120 kg N ha^{-1}) sobre diferentes fracciones de carbono orgánico protegidas en agregados del suelo y el impacto de dichas prácticas sobre la proporción de microagregados dentro de macroagregados estables.

Capítulo 2. Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions.

Este capítulo se centra en el estudio de la evolución temporal de la agregación y de la protección física del carbono orgánico en una cronosecuencia de años bajo siembra directa.

Plaza-Bonilla, D., Cantero-Martínez, C., Viñas, P., Álvaro-Fuentes, J. 2013. Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions. Geoderma 193-194, 76-82.

Capítulo 3. Soil aggregate stability as affected by fertilization type under semiarid no-tillage conditions.

Este capítulo se estudia el efecto de la aplicación de (i) diferentes tipos y dosis de fertilizante orgánico y (ii) dosis de fertilizante nitrogenado mineral sobre la dinámica de la macroagregación y la protección del carbono orgánico en suelos bajo un manejo en siembra directa. El contenido del capítulo se corresponde con el artículo:

Plaza-Bonilla, D., Álvaro-Fuentes, J., Cantero-Martínez, C. 2013. Soil aggregate stability as affected by fertilization type under semiarid no-tillage conditions. Soil Science Society of America Journal 77, 284-292.

Capítulo 4. Soil management effects on greenhouse gases production at the macroaggregate scale.

En este capítulo se analiza el impacto de las prácticas de laboreo y fertilización nitrogenada sobre la producción de gases de efecto invernadero (CO_2, CH_4 y N_2O) en los macroagregados del suelo, así como la importancia de los procesos de nitrificación y desnitrificación sobre la producción de N_2O en esta misma escala al aplicar diferentes tipos de fertilizante. El contenido del capítulo se corresponde con el artículo:

Plaza-Bonilla, D., Cantero-Martínez, C., Álvaro-Fuentes, J. 2014. Soil management effects on greenhouse gases production at the macroaggregate scale. Soil Biology and Biochemistry 68, 471-481.

Capítulo 5. Soil gaseous-C fluxes, stocks and biomass-C inputs as affected by tillage and N fertilization in Mediterranean agroecosystems.

En este capítulo se cuantifican las emisiones de metano y dióxido de carbono, la productividad del cultivo, los aportes de C al suelo en forma de restos de cosecha y los stocks de C orgánico del suelo al utilizar diferentes tipos de laboreo y dosis y tipos de fertilizante nitrogenado así como las variables que regulan las emisiones de dichos gases. El contenido del capítulo se corresponde con el artículo:

Plaza-Bonilla, D., Cantero-Martínez, C., Bareche, J., Arrúe, J.L., Álvaro-Fuentes, J. Soil gaseous-C fluxes, stocks and biomass-C inputs as affected by tillage and N fertilization in Mediterranean agroecosystems. Plant and Soil. En revision.

Capítulo 6. Tillage and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed Mediterranean area.

En este capítulo se cuantifican las emisiones de óxido nitroso y las emisiones de este gas por kilogramo de grano producido bajo dos tipos de laboreo (siembra directa y laboreo convencional) y diferentes dosis y tipos de fertilizante nitrogenado en dos experimentos de campo bajo condiciones semiáridas de secano. Asimismo se estudia el impacto de dichas prácticas sobre las variables que regulan la producción del óxido nitroso. El contenido del capítulo se corresponde con el artículo:

Plaza-Bonilla, D., Álvaro-Fuentes, J., Arrúe, J.L., Cantero-Martínez, C. Tillage and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed Mediterranean area. Agriculture, Ecosystems and Environment. En revisión

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Capítulo 1

Organic protection within soil aggregates: long-term effects of tillage and N fertilization

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Pending of being submitted.

Organic protection within soil aggregates: long-term effects of tillage and N fertilization.

Abstract

Agricultural management practices play a major role in the control of the organic carbon protection mechanisms and its distribution within different soil fractions. However, the role of both N fertilizers and tillage systems on physical C protection is not well elucidated. Soil samples from three depths (0-5, 5-20 and 20-40 cm) were obtained in a long-term (16 years) tillage (conventional intensive tillage with moldboard plow, CT; no-tillage, NT) and mineral N fertilization (0, 60 and 120 kg N ha^{-1}) experiment. The water-stable macroaggregates (M) and their dichromate- and permanganate-oxidizable C concentrations (M-C and M-POxC, respectively), the microaggregates within macroaggregates (mM) and their C concentration (mM-C), and the C concentration of both the particulate organic matter (POC) and the silt-plus-clay-sized particles were measured (s+cM-C). In soil surface, tillage affected the majority of the soil aggregate fractions and C pools studied while N fertilization and the interaction between both factors showed a minor role. The proportion of water-stable macroaggregates was 4.4, 1.6 and 1.1 times greater under NT compared to CT for the 0-5, 5-20 and 20-40 cm depths. Also, the amount of microaggregates within macroaggregates was 6.9, 1.8 and 1.5 times greater under NT compared to CT for the same depths. The application of N increased slightly the amount of POC and M-POxC indicating that N only affected the most active C pools, without a significant effect on either the stability of the macroaggregates or the formation of microaggregates within macroaggregates. On average, the mM-C explained 70% of the differences in SOC demonstrating the role played by the microaggregates formed within macroaggregates on SOC protection. Our results show that C allocation and protection within soil macroaggregates and the concomitant formation of microaggregates within those macroaggregates were mainly influenced by tillage, with NT as the most efficient system when protecting C, whereas the application of N had minor effects.

1. Introduction

The protective capacity of soil aggregates against soil organic matter decomposition has been broadly documented. Balesdent et al. (2000) pointed out (i) the sorption of soil organic matter to solid surfaces, (ii) the sequestration of C in small pores, (iii) the control of microbial turnover by predators, and (iv) the O_2 limitation, as the main mechanisms of C physical protection in soils. Several studies have shown an increase of C mineralization and the concomitant enhanced CO_2 fluxes when soil aggregates are crushed (e.g., Aoyama et al. 1999b; Beare et al. 1994). Also it has been demonstrated that macroaggregates rapidly reflect changes in soil management. For instance, Elliott (1986) found a 25-50% reduction in soil macroaggregates (>0.250 mm) when cultivating a native soil. Other experiments showed that, when tillage is performed soil aggregates are broken and soil organic carbon (SOC) decomposition is accelerated (Balesdent et al. 2000; Peterson et al. 1998). The rapid responses to different management practices showed by soil macroaggregates reside in the changes produced on their binding agents, such as roots and hyphae (Tisdall and Oades, 1982). These authors proposed the concept of aggregate hierarchy and described the mechanisms involved in the formation of macro- and microaggregates. In turn, Oades (1984) slightly modified this theory postulating that microaggregates are formed within macroaggregates. The modification proposed by Oades (1984) was corroborated by Angers et al. (1997) who traced isotopically the fate of C and N added to soil in micro- and macroaggregates. The microaggregate formation within macroaggregates is the result of different processes such as the decomposition of organic debris, the deposition of microbial by-products, the reorientation of clay platelets and the physicochemical reaction between polyvalent cations, organic molecules and clays (Jastrow, 1996). The microaggregates bound inside macroaggregates protect the C in the long-term, especially under those types of soil management that promote a greater macroaggregate formation (Six et al., 2000). Therefore, it is essential to identify management practices that enhance the microaggregation process within soil macroaggregates.

Fertilizer application results in variable effects on aggregation. Although generally improve soil aggregation, under some conditions mineral fertilizers may decrease SOC concentration and aggregation (Bronick and Lal, 2005). Aoyama et al. (1999b) suggested that mineral fertilizer-derived N can accumulate in a relatively labile form, protected within macroaggregates. However, they found no effects of mineral N fertilizer on C protection within macroaggregates. Contrarily, Manna et al. (2006) found

a reduction of the proportion of macroaggregates when applying mineral fertilizers in a rice-wheat-jute (*Corchorus olitorius L.*) system under sub-tropical conditions possibly due to a faster decomposition of active C pools.

In the Mediterranean region, the role of tillage on soil C protection by aggregates has been recently studied. For instance, Álvaro-Fuentes et al. (2008) and Plaza-Bonilla et al. (2010) found greater aggregate stability and greater proportion of water-stable macroaggregates in soil surface when soil tillage is reduced or eliminated. In those areas the proportion of macroaggregates in the soil increases when no-tillage (NT) is maintained over time, maximizing the amount of C stabilized in the soil (Álvaro-Fuentes et al. 2012, Plaza-Bonilla et al. 2013a). Moreover, the same authors studied the short-term impact of the application of mineral and organic fertilizers in a no-tilled soil on the stability of macroaggregates (Plaza-Bonilla et al. 2013b). They observed that the use of different fertilization strategies had a minor effect on the water-stability of the macroaggregates hypothesizing that this finding was due to the high stability already obtained with the elimination of tillage. In the Mediterranean areas different studies have been carried out in order to study the effects of N fertilization on soil C sequestration. In the same experimental plots, Morell et al. (2011c) observed an increase in the soil C stock of 4 Mg C ha^{-1} in the fertilized plots compared to the control plots without N application. Moreover, Morell et al. (2011a) observed a better performance of the crop and greater C inputs to the soil when N fertilizers were applied in no-tilled plots. On the contrary, in the conventionally-tilled plots no effects on C inputs were found when applying N fertilizer due to limited soil moisture that restricted N uptake by the crop. Also in the same experiment, Álvaro-Fuentes et al. (2013) observed higher microbial biomass C in N fertilized plots than in the unfertilized ones. Those findings suggest that N fertilization could affect the mechanisms of soil physical C protection, for a given type of tillage. In an experiment located in Kansas, Mikha and Rice (2004) observed greater amount of stable macroaggregates and aggregate-associated C and N when NT and manure were used. In turn, in the Indian Himalayas, Bhattacharyya et al. (2012) observed an interactive effect between tillage and N fertilization combinations on soil macroaggregates. Unfortunately, in both cases, the authors did not compare the fertilizer application with a control treatment without N. In the Mediterranean areas, tillage and N fertilization are agricultural management practices that have a great potential of optimization. Although their effects on bulk soil C have been already documented, there is a lack of studies attempting to quantify the interactive effects of

both practices on soil aggregation and C physical protection mechanisms. Consequently, the objective of this study was to evaluate the long-term interactive effects of tillage and N fertilization on the protection of C within aggregates and the mechanisms that regulate this physical protection. Our hypotheses were (i) that NT plus the application of N fertilizer results in a greater proportion of microaggregates within water-stable macroaggregates and a greater concentration of C stabilized within these microaggregates, and (ii) that, contrarily, under conventional tillage (CT), the lack of response of crop biomass to the application of N fertilizers eliminates the possibility of greater aggregation.

2. Materials and Methods

2.1. Experimental site

A long-term experiment on tillage and N fertilization was established in 1996 in Agramunt (Lleida), in the semiarid zone of the Ebro river valley, NE Spain. The main edapho-climatic characteristics of the experiment are listed in Table 1. Two types of tillage (NT, no-tillage, and CT, conventional intensive tillage with moldboard plow) and three N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared in a randomized block design with three replications. N rates were chosen according the limited potential productivity of the area (60 kg N ha^{-1}) and the traditional rates applied by farmers (120 kg N ha^{-1}). Soil was analyzed each four years for phosphorous and potassium content. If needed, those nutrients were applied in the whole experimental area according to crop requirements. Plot size was 50 m x 6 m. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. The CT treatment consisted of one pass of a moldboard plow to 25 cm depth immediately followed by one or two passes with a cultivator to 15 cm depth, both in September (Fig. 1). Conventional tillage operations in 2007 and 2008 were replaced with disk plowing to a depth of 25–30 cm due to poor soil water conditions at the time of tillage operations (Morell et al. 2010).

Table 1 General characteristics of the experimental site. Soil properties were measured in the Ap horizon (0-28 cm depth) at the beginning of the experiment.

Site and soil characteristics	
Latitude	41º48'N
Longitude	1º07'E
Elevation (m)	330
Annual precipitation (mm)	430
Annual ETo (mm)	855
Annual water deficit (mm)[¶]	425
Soil classification[†]	Typic Xerofluvent
pH (H2O, 1:2.5)	8.5
EC1:5 (dS m^{-1})	0.15
CaCO3 eq. (%)	40
Water retention (kg kg^{-1})	
33 kPa	0.16
1500 kPa	0.05
Particle size distribution (%)	
Sand (2000-50 µm)	46.5
Silt (50-2 µm)	41.7
Clay (<2 µm)	11.8

[¶] Calculated as the difference between mean annual precipitation and mean annual ETo.

[†] According to the USDA classification (Soil Survey Staff 1975).

Figure 1 Tillage performed with a moldboard plow in the conventional tillage (CT) treatment.

Nitrogen fertilizer was manually-applied and split into two applications: one-third of the dose before tillage as ammonium sulphate (21% N) and the rest of the dose at the beginning of tillering, in February, as ammonium nitrate (33.5% N). Planting was performed in November with a disk direct drilling machine set to 2-4 cm (Fig. 2). The cropping system consisted of a barley (cv. Hispanic in the 1996-2010 period and cv. Cierzo in the 2010-2012 period) monocropping, which is the traditional cropping system of the area. Prior to the setting up of the experiment, the historical management of the field was based on conventional intensive tillage with moldboard plowing and small grain cereals monoculture.

Figure 2 Planting cereal with a disk direct drilling machine

2.2. Soil and biomass samplings and analyses

Soil sampling was performed in July 2012, right after crop harvest. For each plot, two soil pits of 0.5 m depth and 20 m apart were opened. In each pit, a composite sample was collected from three samples randomly selected. Soil samples were obtained using a flat spade in three soil layers from 0 to 40 cm depth (0-5, 5-20 and 20-40 cm) and stored in crush-resistant airtight containers. Once in the laboratory, soil samples were gently sieved with a 8 mm-sieve and air-dried at room temperature. For each sample, water-stable macroaggregates (> 0.250 mm) were obtained.

Macroaggregates separation was performed according to a modified wet sieving method adapted from Elliott (1986). Briefly, 100 g air-dried soil subsample was placed on the top of a 0.250 mm sieve and submerged for 5 min in deionized water at room temperature. The sample was manually-sieved 50 times for 2 min. The water-stable macroaggregates obtained were oven-dried at 50 ºC during 24 h and weighed. Microaggregates contained within stable macroaggregates were isolated with a modification of the methodology described by Six et al. (2000). A 10-g water-stable macroaggregates subsample was immersed in 50 mL deionized water into a beaker during 20 minutes. Afterwards, the macroaggregates subsample was placed on top of a 0.250 mm screen with 50 glass beads (dia. = 4 mm) in an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain) below a continuous flush of water. A sieving time of 10 min and the lowest power program of the machine were used. The continuous flow of water obtained with the use of a nozzle immediately washed the microaggregates and other particles smaller than <0.250 mm onto a 0.053 mm screen avoiding any further alteration. The material on the 0.053 mm sieve was sieved in order to separate the stable microaggregates from the silt-plus-clay-sized material. The material on the 0.250 mm sieve (considered coarse particulate organic matter), the microaggregates within macroaggregates and the silt-plus-clay-sized particles (<0.053 mm) were oven-dried at 50 ºC during 24 h and weighed. The sand content of the macroaggregates and the microaggregates within macroaggregates were determined by dispersing a 5 g subsample in sodium hexametaphosphate solution using a reciprocal shaker. After the dispersion, the subsample was sieved with a 0.053 mm sieve and the amount of sand was dried. The proportion of sand-free water-stable macroaggregates (g g^{-1} dry soil) was expressed as follows:

$$\text{Macroaggregates}_{\text{sand-free}} = \text{Water-stable macroaggregates weight} / [1 - (\text{sand proportion}_{\text{macroaggregates}})]$$

In turn, the proportion of sand-free microaggregates within water-stable macroaggregates (g g^{-1} dry soil) was expressed as:

$Microaggregates_{sand\text{-}free}$ =
= (microaggregate weight - weight of 0.053-0.250 mm sized sand) / (macroaggregate weight - weight of 0.250-2 mm sized sand).

The organic C concentration of the bulk soil (SOC), the water-stable macroaggregates (M-C), the microaggregates within macroaggregates (mM-C) and the silt-plus-clay-sized fractions (<0.053 mm) (s+cM-C) obtained after the microaggregate isolation were determined using the dichromate wet oxidation method of Walkley-Black described by Nelson and Sommers (1996). During the oxidation extensive heating at 150 ºC for 30 minutes was used in order to increase the digestion of SOC (Mebius, 1960). The coarse particulate organic C of the water-stable macroaggregates (POC) was calculated as substracting the amount of C in the microaggregates within macroaggregates (mM-C) and in the silt-plus-clay-sized fraction (s+cM-C) to the amount of C contained in the water-stable macroaggregates (M-C).
The permanganate oxidizable organic C of the bulk soil (POxC) and of the water-stable macroaggregates (M-POxC) were measured according to the method of Weil et al. (2003). Briefly, 2.5 g of air-dried and 0.5 mm grinded soil were introduced in 50-mL polypropylene centrifuge tubes. Then 18 mL of distilled water and 2 mL of 0.2 M $KMnO_4$ stock solution were added and the tubes were shaken for 2 min at 200 oscillations per min on an oscillating shaker. Afterwards, the tubes were allowed to settle for 10 min. After that time, 0.5 mL of the supernatant of every tube was transferred into a second tube and 49.5 mL of distilled water was added (during this operation care has to be taken in order to avoid the presence of soil in the 0.5 mL subsample). The tubes were closed and manually shaked to mix the subsample of supernatant and the water. Next, a volume of 3.5 mL of each sample was placed in polystyrene cuvettes and its absorbance was read at 550 nm with a Spencor 210 (Analytik-Jena, Jena, Germany) spectrophotometer. All the samples were replicated two times. Four standard stock solutions, a soil standard and a solution standard prepared in the same manner as the soil samples were also read. Permanganate oxidizable C was determined following Weil et al. (2003):

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

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

In order to quantify the aboveground C residue inputs, biomass samplings were performed at the maturity stage of the crop (i.e., mid June). Three 0.5-m-long rows were hand-harvested per plot. The grain was removed with a laboratory threshing machine and the straw was oven-dried at 65 ºC for 48 h and weighed. After that the biomass was transformed to Mg of C per hectare taking into account the distance between rows (0.17 m) and the mean C concentration in the straw during the experiment (40% C).

The data were analyzed using the SAS statistical software (SAS institute, 1990). To compare the effects of tillage, nitrogen and soil depth, analysis of variance (ANOVA) for a randomized block design was performed using the general linear model procedure. When significant, differences among treatments and depths were identified at the 0.05 probability level of significance using the LSD test. To determine the relationships between some variables, linear regression analyses were performed with Sigmaplot 11 (Systat Software, 2008).

3. Results

Tillage significantly affected the water-stable macroaggregates (M) and the microaggregates within macroaggregates (mM) proportions, the macroaggregate C concentration (M-C), the particulate organic carbon (POC), the C concentration of the silt-plus-clay-sized particles (s+cM-C) and the permanganate oxidizable C of the macroaggregates (M-POxC) (Table 2). However, the application of different rates of N fertilization only affected the POC and the M-POxC fraction. In turn, the interaction between tillage and nitrogen only affected significantly the SOC and the M-POxC (Table 2).

Table 2 Analysis of variance of main factors (T, tillage; N, nitrogen rate; D, depth of sampling) and their interaction.

Effects	M	mM	SOC	POxC	M-C	M-POxC	mM-C	POC	s+cM-C
T	***	***	ns	ns	***	***	ns	***	**
N	ns	ns	ns	ns	ns	*	ns	*	ns
D	***	ns	***	***	***	*	***	***	**
TxN	ns	ns	*	ns	ns	*	ns	ns	ns
TxD	***	*	***	***	**	ns	***	**	***
NxD	ns	ns	ns	ns	ns	ns	ns	ns	ns
TxNxD	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: not significant; *P<0.05; **P<0.01; *** P<0.001

T: tillage; N: nitrogen rate; D: depth

M: water-stable macroaggregates

mM: microaggregates within macroaggregates

SOC: bulk soil organic carbon

POxC: permanganate oxidizable organic carbon of the bulk soil

M-C: water-stable macroaggregates-C concentration

M-POxC: macroaggregate-permanganate oxidizable organic carbon

mM-C: microaggregates within macroaggregates-C concentration

POC: particulate organic matter-C concentration after the microaggregate isolation.

s+cM-C: C concentration of the silt-plus-clay-sized fraction after the microagreggate isolation

The amount of water-stable macroaggregates under NT was 26.5, 10.8 and 6.2 g 100 g^{-1} dry soil for the 0-5, 5-20 and 20-40 cm soil depths, respectively, while, under CT, the proportion was 6.0, 6.9 and 5.5 g 100 g^{-1} dry soil for the same depths (Fig. 3). A significant stratification of the water-stable macroaggregates over soil depth was found under NT (Fig. 3). Thus, under NT the lowest proportion of soil water-stable macroaggregates (>0.250 mm) was found in the 20-40 cm soil depth (Fig. 3). Moreover,

a greater proportion of microaggregates within macroaggregates was found under NT compared to CT in the 0-5 and the 20-40 cm depths (Fig. 4). Under NT the proportion of microaggregates within macroaggregates was 18.0, 14.5 and 14.3% for the 0-5, 5-20 and 20-40 cm soil depth, respectively, whereas under CT the proportion represented 11.6, 12.3 and 10.6% for the same soil depths (Fig. 4).

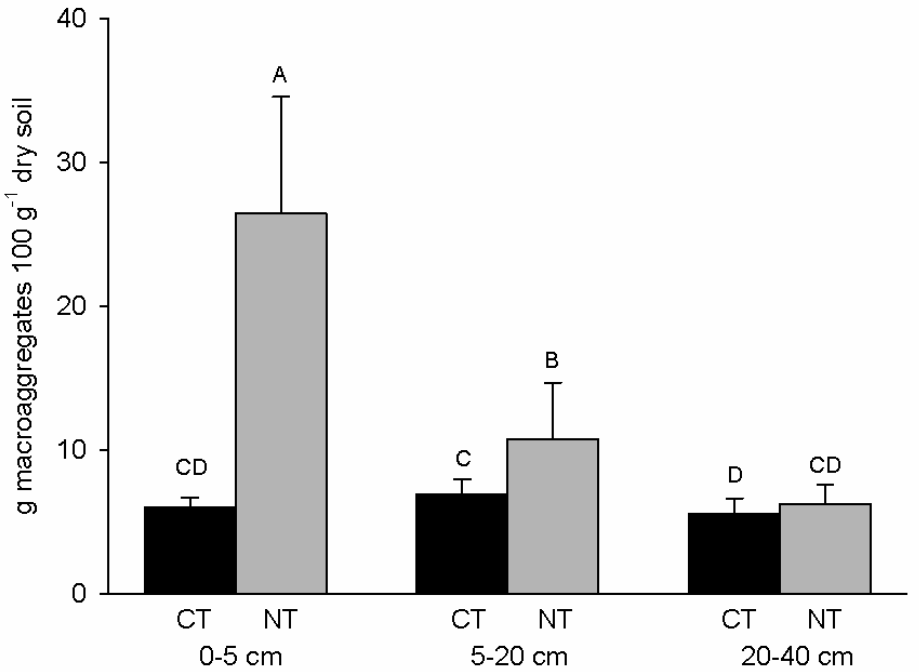

Figure 3 Proportion of water-stable macroaggregates as affected by tillage (CT, conventional tillage; NT, no-tillage) at 0-5, 5-20 and 20-40 cm soil depths. Upper-case letters indicate significant differences between tillage treatments and depths at $P<0.05$.

In the soil surface (0-5 cm), the long-term use of NT increased the total SOC concentration, the mM-C and the s+cM-C by about 59%, 30% and 30%, respectively, compared to CT (Table 3). As it has been previously commented, depth stratification of some variables was found in the NT treatment. For instance, the amount of SOC, M-C, mM-C and POC for the 0-5 cm soil depth was 2.3, 2.6, 1.7 and 5.8 times greater, respectively, than the values for the 20-40 cm depth (Table 3).

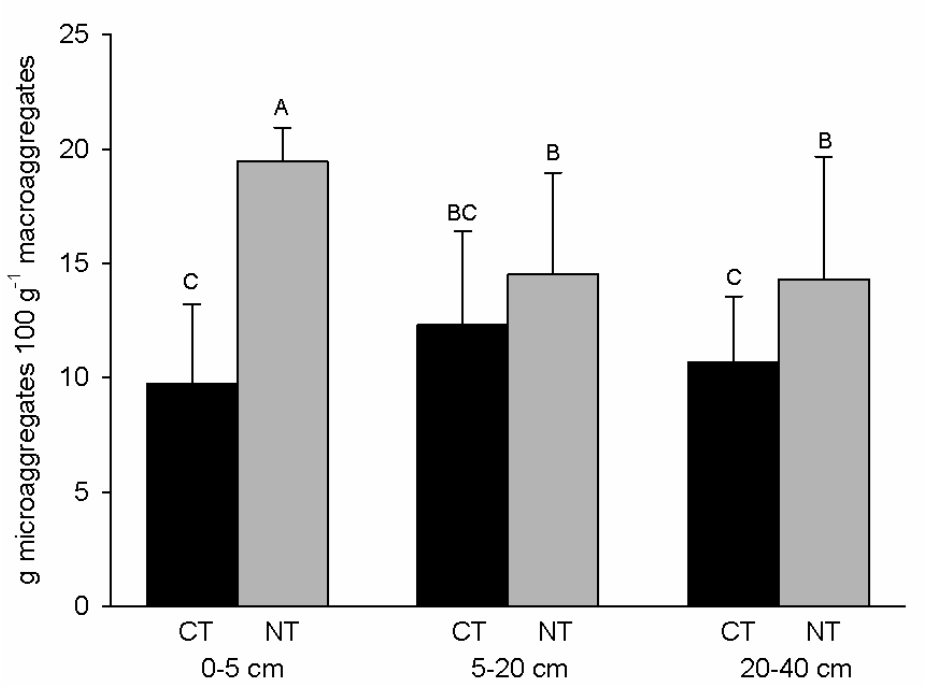

Figure 4 Proportion of microaggregates within water-stable macroaggregates as affected by tillage (CT, conventional tillage; NT, no-tillage) at 0-5, 5-20 and 20-40 cm soil depths. Upper-case letters indicate significant differences between tillage treatments and depths at *P*<0.05.

Table 3 Dichromate oxidizable organic C of the bulk soil (SOC), the water-stable macroaggregates (M-C), the microaggregates within macroaggregates (mM-C), the particulate organic matter (POC) and the silt-plus-clay-sized particles (s+cM-C) as affected by tillage (CT, conventional tillage; NT, no-tillage) at 0-5, 5-20 and 20-40 cm soil depths.

Depth (cm)	Dichromate oxidizable organic C (g C kg^{-1})									
	SOC		M-C		mM-C		POC		s+cM-C	
	CT	NT	CT	NT	CT	NT	CT	NT	CT	NT
0-5	9.3b¶	14.8a	28.4a	24.6ab	23.3b	30.4a	17.8ab	22.6a	13.9b	18.0a
5-20	8.9bc	8.2cd	23.9b	17.4c	23.0b	23.4b	14.2ab	7.7c	14.1b	14.5b
20-40	6.9d	6.5e	22.2b	13.6c	23.6b	18.1c	11.4bc	3.9d	14.1b	13.6b

¶ For each soil fraction, different lower-case letters indicate significant differences between tillage treatments and depths at *P*<0.05.

The permanganate oxidizable organic C of the bulk soil (POxC) ranged between 402.2 and 145.1 mg C kg^{-1} dry soil under NT in the 0-5 and the 20-40 cm soil depths, respectively, and between 251.5 and 207.6 mg C kg^{-1} dry soil under CT in the same depths (Table 4). In the 0-5 cm soil depth, the POxC was 60% greater under NT

compared to CT (Table 4), similarly to the increase observed in the SOC pool under NT for the same depth (Table 3).

Table 4 Permanganate oxidizable organic C of the bulk soil (POxC) and the water-stable macroaggregates (M-POxC) as affected by tillage (CT, conventional tillage; NT, no-tillage) at 0-5, 5-20 and 20-40 cm soil depths.

Depth (cm)	Permanganate oxidizable organic C (mg C kg^{1})			
	POxC		M-POxC	
	CT	NT	CT	NT
0-5	251.5b¶	402.2a	829.0	587.2
5-20	230.3b	213.7b	705.4	493.1
20-40	207.6b	145.1c	670.5	440.1

¶ Different lower-case letters indicate significant differences between tillage treatments and depths at $P<0.05$.

N fertilization affected significantly the POC, being greater when N was applied as compared to the control treatment (11.2, 14.2 and 13.5 g C kg^{-1} dry soil when applying 0, 60, and 120 kg N ha^{-1}, respectively). The average M-POxC values were 534.4, 707.3 and 614.5 mg C kg^{-1} dry soil for the 0, 60, and 120 kg N ha^{-1} treatments, respectively, with significant differences between the 0 and 60 kg N ha^{-1} treatments, and intermediate values for the 120 kg N ha^{-1} treatment (data not shown). The application of N under NT had no significant effects on the M-POxC. However, under CT, greater M-POxC values were found when applying N compared to the control (578.0, 909.5 and 717.5 mg C kg^{-1} dry soil for the 0, 60, and 120 kg N ha^{-1} treatments, respectively), with significant differences between the rate of 60 kg N ha^{-1} and the other two treatments.

Several significant linear relationships between SOC and the different C pools studied were found (Table 5). The C concentration of the microaggregates within water-stable macroaggregates (mM-C) was the only fraction that was linearly related with SOC for all the depths studied under both CT and NT (Table 5). As an average for the three depths studied, the mM-C explained about 70% of the differences in SOC. Also, the C concentration in the water-stable macroaggregates (M-C) was well related to SOC in the 0-5 and 20-40 cm depth under NT and only in the 5-20 cm soil depth under CT (Table 5). In addition, we also observed a very close correlation between SOC and the more active fraction (POxC) when pooling the results of all the samples (R^2: 0.87; $p<0.001$) (Fig. 5). A significant linear relationship was also observed between M-C and M-POxC. Nevertheless, in this case, the R square value was lower (R^2: 0.34; $p<0.001$) (Fig. 5).

Table 5 Determination coefficients (R^2) between the organic C of the bulk soil (SOC) and C concentration of macroaggregates (M-C), microaggregates within macroaggregates (mM-C), particulate organic carbon (POC) and silt-plus-clay-sized particles (s+cM-C) for different tillage systems (CT, conventional tillage; NT, no-tillage) and soil depths (0-5, 5-20 and 20-40 cm).

Variables	CT			NT		
	0-5	5-20	20-40	0-5	5-20	20-40
M-C	ns	0.50*	ns	0.46*	ns	0.64**
mM-C	0.84***	0.66**	0.72**	0.51*	0.57*	0.88***
POC	ns	0.53*	ns	ns	ns	ns
s+cM-C	ns	0.74**	ns	ns	0.45*	ns

ns: not significant; *P<0.05; **P<0.01; *** P<0.001

For the whole experimental period (1996-2012), the average aboveground C inputs were significantly different, with mean values of 1.36 and 1.76 Mg C ha^{-1} y^{-1} for the CT and the NT treatments, respectively (Table 6).

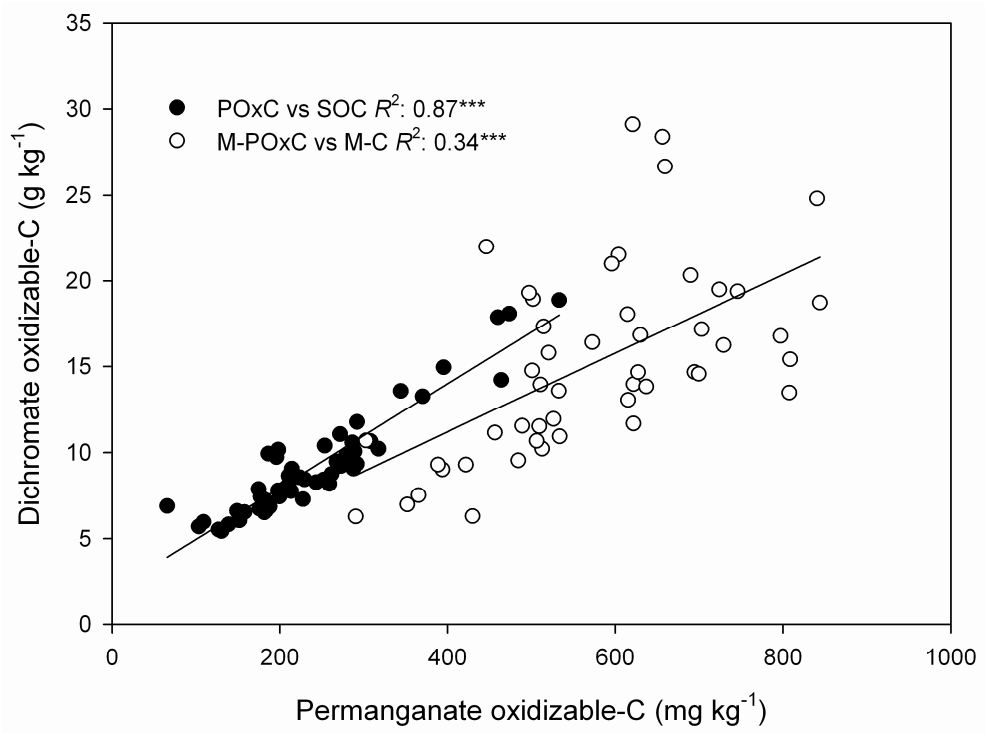

Figure 5 Linear regression between (i) the permanganate-oxidizable organic carbon (POxC) and the dichromate-oxidizable organic carbon of the bulk soil (SOC) and (ii) the permanganate-oxidizable organic C of the water-stable macroaggregates (M-POxC) and the dichromate-oxidizable organic C of the macroaggregates (M-C). ***P<0.001

Table 6 Carbon aboveground inputs for different tillage systems (CT, conventional tillage; NT, no-tillage) and N fertilization rates.

Tillage	N fertilization rate (kg N ha^{-1})	Aboveground C inputs (Mg C ha^{-1} y^{-1}) †
CT	0	1.18b‡
	60	1.38bc
	120	1.51b
	Mean	1.36§
NT	0	1.30bc
	60	1.91a
	120	2.08a
	Mean	1.76

†Mean aboveground C inputs during the 1996-2012 period. ‡Different letters indicate significant differences between treatments (P<0.05). §Indicates significant differences between tillage systems (P<0.05).

4. Discussion

While tillage affected significantly to the majority of the soil C pools studied, the application of N and the interaction between them had minor effects in our experiment. The application of 60 and 120 kg N ha^{-1} increased significantly the C inputs under NT compared to CT in which no greater C inputs were observed when increasing the rate of N applied. However, no differences in those inputs were found between the 60 and the 120 kg N ha^{-1} rates under NT (Table 6). Those results demonstrate that, under the semiarid Mediterranean conditions of our experiment, the responses to the long-term application of different rates of mineral N on C inputs, C pools and soil aggregation are rather limited. For instance, after thirteen years of differential fertilization, Morell et al. (2011c) observed an increase in the soil C stock of 4.3 Mg C ha^{-1} in N fertilized plots compared to the control treatment without fertilization. Under similar edaphoclimatic conditions, Plaza-Bonilla et al. (2013b) did not observe any increase in the water-stability of macroaggregates after short-term application of mineral N fertilizer under NT. However, these authors found a greater mean weight diameter of the dry-sieved aggregates when increasing N fertilizer rate, suggesting that the application of N promotes the formation of larger but unstable aggregates. Similarly, Aoyama et al. (1999a) observed no effects of mineral N application on the proportion of soil water-stable macroaggregates. In our experiment, the application of N increased the concentration of POC and M-POxC. Elliott and Coleman (1988) and Jastrow and Miller (1997) suggested that the accumulation of particulate organic matter and the deposition of microbial by-products are important mechanisms in the stabilization of soil macroaggregates. Moreover, in our experimental site, a greater amount of microbial biomass-C was found in the fertilized treatments compared to the control (Álvaro-Fuentes et al. 2013). As it has been pointed out, our results reflect a positive role of medium rates of N when sequestering C in active pools such as POC and M-POxC when compared to the control, although, according to the data, the use of the high N rate (i.e. 120 kg N ha^{-1}) could have increased the decomposition of those pools. However, their greater C concentration was not accompanied by a final effect on the stability of the macroaggregates nor in the formation of microaggregates within macroaggregates. Some studies have shown that the addition of N fertilizer enhances the turnover of the macroaggregates by maximizing the decomposition of the binding agents or decreasing their involvement (Bossuyt et al. 2001; Le Guillou et al. 2011). Nevertheless, our results

did not show a long-term deleterious effect on water-stable macroaggregates when applying mineral N to the soil, as observed in other studies (Aoyama et al. 1999a; N'Dayegamiye, 2009). However, it could be hypothesized that the greater amounts of the labile forms of C, as POC or M-POxC, that have been observed when applying N, could imply a positive trend in C accumulation in the soil in a longer term.

Concurrently, C inputs were affected by the N rate applied in the NT treatment, while no positive response was observed under CT (Table 6). Under NT, the medium rate applied (60 kg N ha^{-1}) produced the greatest amount of C inputs to the soil (Table 6). A slow or null C accumulation when applying mineral N fertilizers has already been documented in Mediterranean areas (López-Bellido et al. 2010; Morell et al. 2011c; Triberti et al. 2008) and related with the low amount of water available for the crop in these areas, mainly under tilled systems (Angás et al. 2006). Those hydric restrictions limit the effect of the N application on the amount of C inputs as aboveground and belowground biomass returned to the soil (Morell et al. 2011a, 2011b). Gale et al. (2000) demonstrated that the C derived from the roots is more important than the surface-derived C in the stabilization of macroaggregates. Thus, in our experiment, the little response of C inputs to N application resulted in a similar proportion of water-stable macroaggregates between N rates.

The greater SOC concentration found in soil surface (i.e. 0-5 cm) under NT was accompanied by both a higher proportion of water-stable macroaggregates and microaggregates within macroaggregates and a higher C concentration in these microaggregates. Several studies have shown a close relationship between the proportion of water-stable macroaggregates and SOC, thus demonstrating the major role played by soil macroaggregates when stabilizing C (Barthès et al. 2008; Benbi and Senapati, 2010; Mikha et al. 2010). A reduced macroaggregate turnover under NT and its relationship with the C stabilization process has been recognized (Beare et al. 1994). However, our results also show that SOC presents an important dependence on the C concentration of the microaggregates developed within macroaggregates (mM-C). Six et al. (2000) and Denef et al. (2004) proposed that an enhanced microaggregate formation within macroaggregates due to a reduced macroaggregate turnover would be the main mechanism of soil C stabilization under NT. Our data corroborates that the lack of disturbance and the greater C inputs associated with NT enhanced the formation of stable macroaggregates in the 0-5 and the 5-20 cm depths. Also, under NT, the higher proportion of C-enriched microaggregates within macroaggregates that we have found

could be released when the macroaggregates become unstable. This process would maximize the amount of C and its time of residence in the soil due to the greater protective capacity of the smaller soil fractions (Dungait et al. 2012).

Our data showed a close relationship between the dichromate oxidizable C and the permanganate-oxidizable C for the bulk soil and the water-stable macroaggregates as observed by Lucas and Weil (2012). Interestingly, for the permanganate-oxidizable C that relationship was closer for the bulk soil than for the macroaggregates (R^2: 0.87 and 0.34, respectively). Culman et al. (2012) studied the permanganate- and dichromate-oxidizable C in bulk soil and particulate organic matter fraction samples. They observed a closer relationship between the permanganate- and dichromate-oxidizable C in the bulk soil samples than in the particulate organic matter samples suggesting that permanganate-oxidizable organic C reflects a processed and stabilized labile pool of soil C. The importance of the particulate organic matter in the macroaggregate stabilization has been long recognized (Jastrow and Miller, 1997; Aoyama et al. 1999a; Six et al. 1999) and could explain the lower R^2 value that we have found for the relationship between M-POxC and M-C compared to the relationship between SOC and POxC. Also, the dichromate-oxidizable and permanganate-oxidizable C concentrations of the water-stable macroaggregates and the POC were greater under CT compared to NT. It has to be taken into account that our results are greatly affected by the correction of the proportion of sand-sized particles in the macroaggregates, which was greater under CT that involves soil inversion, a trend that we have also observed in previous studies (Plaza-Bonilla et al. 2010, 2013a). We hypothesize that inversion tillage could lead to a redistribution of soil particles within the soil profile, with an increase in the amount of sand-sized particles mainly in the soil surface.

5. Conclusions

The long-term use of NT significantly increased the proportion of water-stable macroaggregates, the microaggregates within macroaggregates and the C concentration of those microaggregates. The application of N fertilizers led to an increase in C active pools. However, the scarce response of C inputs to N application eliminated any further improvement either in the proportion of macroaggregates or in the formation of microaggregates within macroaggregates. Our study demonstrates that, under semiarid Mediterranean conditions, long-term use of NT enhances the microaggregation process within macroaggregates while the use of N fertilizers scarcely improves soil C protection perhaps due to the limitations imposed in those environments by water shortage in the soil profile.

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Capítulo 2

Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions

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Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions

Abstract

Low-intensity soil management systems like no-tillage (NT) are being increasingly accepted as an essential part of sustainable farming systems. The objective of this work was to study the effects of NT maintenance over time on soil aggregation and soil organic carbon (SOC) protection on a semiarid Mediterranean agroecosystem. A NT chronosequence was established with five phases: (i) conventional tillage (CT); (ii) NT for 1 year (NT-1); (iii) NT for 4 years (NT-4); (iv) NT for 11 years (NT-11) and (v) NT for 20 years (NT-20). N fertilization was based on pig slurry for the whole experimental area. Soil samples were collected from four depths (i.e., 0–5, 5–10, 10–20, 20–30 cm). Dry and water-stable aggregates, SOC concentration and C concentration of water stable aggregates were measured. SOC concentration reached its maximum value after 11 years under NT. However, the differences between NT phases were only found in the 0–5 cm soil depth. In soil surface (i.e., 0–5 cm), water-stable large macroaggregates (2–8 mm) were 0.02, 0.12, 0.32 and 0.31 g g−1 dry soil for the NT-1, NT-4, NT-11 and NT-20 phases, respectively. C concentration of microaggregates increased in relation with the number of years under NT. SOC and water-stable macroaggregate stratification were greatest with the increase in the years under NT, emphasizing the close relationship between SOC and aggregation. In Mediterranean semiarid agroecosystems, the increase in the proportion of stable macroaggregates and the enrichment of C concentration within microaggregates are two main mechanisms of SOC protection when NT is maintained over time.

1. Introduction

Soil aggregates are the arrangement of soil particles of different sizes joined by organic and inorganic materials (Amezketa, 1999) and their stability can be used as an index of soil structure (Bronick and Lal, 2005). Soil aggregates physically protect SOC from its degradation by soil microorganisms (Beare et al., 1994a; Tisdall and Oades, 1982) and it is evidenced by the flush of carbon dioxide observed upon soil aggregates disruption (Beare et al., 1994a).

Soil structure and SOC are extremely sensitive to crop and soil management (Blanco-Canqui and Lal, 2004). It is well established that NT adoption in a previously conventionally-tilled soil results in the physical stabilization of SOC within soil aggregates (e.g., Six et al., 1999; Álvaro-Fuentes et al., 2009). During the process of SOC stabilization within soil aggregates, plant roots and fungal-derived hyphae play an important role as initial binding agents (Jastrow, 1996). According to the conceptual scheme proposed by Six et al. (2000), macroaggregate turnover is greatly reduced under NT promoting the formation of C-enriched microaggregates within macroaggregates. Moreover, the SOC sequestered within these microaggregates remains protected from microbial attack resulting in longer residence time (Blanco-Canqui and Lal, 2004). Compared to macroaggregates, the biochemical structure of the SOC that stabilizes microaggregates tends to be highly processed and recalcitrant (Elliott, 1986). Also, soil biological activity under NT is increased (Madejon et al., 2009) promoting the production of organic binding by-products that stabilize soil aggregates.

When NT is maintained over time, soil aggregate stability is enhanced (Beare et al., 1994b) leading to the increase of total SOC (West and Post, 2002). In Florida, Ochoa et al., (2009) studied a NT chronosequence of 0, 6, 10 and 15 years under NT in commercial plots. They observed a relationship between the increase in surface soil water-stable macroaggregates and the hydrolysable organic carbon with longer years under NT. Thus, they concluded that continuous NT is beneficial for SOC buildup in soil macroaggregates.

In the Mediterranean semiarid agroecosystems, intensive tillage practices have led to the loss of soil structure and soil degradation (Álvaro-Fuentes et al., 2007). Recently, conservation tillage systems (e.g., reduced tillage or NT) have been increasingly adopted in these areas due to its agricultural and environmental benefits (Kassam et al., 2009). In these semiarid systems, several studies have investigated the impacts of

adoption of continuous NT on soil aggregation and physical C stabilization (e.g., Álvaro-Fuentes et al., 2009; Plaza-Bonilla et al., 2010). Nevertheless, the vast majority of these studies have been based on time-point comparisons (Staley et al., 1988). As a result, there is a lack of information about the continuous maintenance of NT on soil aggregation and SOC protection. Consequently, the objective of this experiment was to study the temporal dynamics of soil aggregation and SOC protection after the conversion of CT to NT in a rainfed Mediterranean agroecosystem. In order to achieve this objective we established a NT chronosequence 20 years ago in a representative Mediterranean dryland agroecosystems located in northeast Spain. We hypothesized that the maintenance of NT results in greater SOC protection within C-enriched water-stable macroaggregates.

2. Materials and Methods

2.1. Experimental site

A NT chronosequence experiment located in the semiarid Ebro river valley, NE Spain (41º48' N, 1º07` E, 330 m), was established 20 years ago in a previously intensive-tilled field of 7500 m^2. Mean annual precipitation, mean air temperature and mean annual evapotranspiration in the area are 430 mm, 13.8 ºC and 855 mm, respectively. The soil was classified as Typic Xerofluvent (Soil Survey Staff, 1994), with the following properties in the Ap horizon (0-28 cm) at the start of the experiment: pH (H_2O, 1:2.5): 8.5; electrical conductivity (1:5): 0.15 dS m^{-1}; $CaCO_3$ eq. (%): 40; Water retention (kg kg^{-1}): 0.16 and 0.05 at -33 and -1500 kPa, respectively; sand (2000-50 µm), silt (50-2 µm) and clay (<2 µm) content: 475, 417 and 118 g kg^{-1}, respectively. The edaphoclimatic conditions of the experiment could be considered as representative of the most part of cropping systems located in the dryland Mediterranean areas. In 1990, 1999, 2006 and 2009 successive portions of 1500 m^2 of the intensive-tilled field (i.e., 7500 m^2) were transformed to NT. Thus, in 2010, a surface of 1500 m^2 remained under CT and 6000 m^2 under NT with different years: 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years. In all five chronosequence phases the cropping system consisted in winter cereals rotation. Fertilization was based on pig slurry homogeneously applied for the whole experimental area in a dose of 50 kg N ha^{-1} yr^{-1} depending of the slurry composition. The CT treatment consisted of one pass of a moldboard plough to 25 cm depth immediately followed by one or two passes with a cultivator to 15 cm, both in September. The NT treatments consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. Planting was performed with a direct drilling disk machine set to 2-4 cm in November. Prior to the set up of the experiment, the historical management of the field was based on conventional intensive tillage with moldboard ploughing and pig slurry additions, similar to the management applied to the CT phase of the chronosequence. Neither slope nor differences in soil characteristics in the whole experimental area were found. The treatments were arranged in a randomized design with replicated plots. More details about the experimental design are given in Álvaro-Fuentes (201X).

2.2. Soil sampling and analyses

Soil sampling was performed in July 2010, right after crop harvest. Each phase of the chronosequence was divided in three areas. In each area, a composite sample was collected from three samples randomly selected. Soil samples were obtained using a flat spade in four soil layers from 0 to 30 cm depth (0-5, 5-10, 10-20 and 20-30 cm) and were stored in crush-resistant airtight containers. Once in the laboratory, soil was sieved with a 8 mm-sieve and air-dried at room temperature. For each sample, dry soil aggregate and water-stable aggregate distributions were obtained. Water-stable aggregate size separation was performed in a 100 g 8-mm sieved soil sub-sample according to a modified wet sieving method adapted from (Elliott, 1986). The method is extensively described in a previous work (Plaza-Bonilla et al., 2010). Four water-stable aggregate fractions were obtained: (i) large macroaggregates (2 – 8 mm), (ii) small macroaggregates (0.250 – 2 mm), (iii) microaggregates (0.053 – 0.250 mm) and (iv) silt-plus clay-sized particles (< 0.053 mm). All water-stable aggregate fractions were oven-dried at 50 ºC (48 h) in aluminum trays and weighed. Sand content of the aggregate classes (> 0.053 mm) was determined dispersing 5 g of a subsample in a sodium hexametaphosphate solution (5 g L^{-1}) using a reciprocal shaker. Sand correction was performed in each aggregate-size class because sand was not considered part of those aggregates (Elliott et al., 1991). The dry aggregate size distribution was conducted placing 100 g of air-dried sub-sample (8 mm sieved) on an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain) with the same sieves used for the water-stable aggregate size distribution. A sieving time of 1 min and the lowest power program of the machine were used.

SOC concentrations from the bulk soil and from each water-stable aggregate size-class were determined using the wet oxidation of the Walkley-Black method described by Nelson and Sommers (1996). In some treatments the amount of large macroaggregates (2-8 mm) was not enough to determine SOC concentration. Consequently, large (2-8 mm) and small (0.250-2 mm) macroaggregates were mixed and SOC determined as macroaggregate-C. The method was modified to increase the digestion of SOC. The modification consisted in extensive heating of the sample during the digestion, boiling the sample and the extraction solution at 150 ºC for 30 minutes (Mebius, 1960).

In each chronosequence phase, the stratification ratio (SR) was calculated dividing the SOC concentration in the 0-5 cm soil depth by those in the 5-10 cm, 10-20 cm and 20-

30 cm soil layers (Franzluebbers, 2002). Regression analyses were performed between the SR of SOC and the number of years under NT to assess the changes of this ratio over time and between SOC concentration and the proportion of water-stable aggregates fractions.

The data were analyzed using the SAS statistical software (SAS institute, 1990). To compare the effects of tillage treatments and soil depths, analysis of variance (ANOVA) for a randomized design was performed using the procedure general linear model. When significant, differences among treatments and depths were identified at the 0.05 probability level of significance using Duncan's test.

3. Results

3.1. No-tillage maintenance effects on soil organic carbon concentration

In the 0-5 cm soil depth, total SOC concentration was significantly greater in the NT-11 and NT-20 phases compared with the NT-4, NT-1 and CT phases. Furthermore, SOC concentration in the NT-4 phase was significantly greater than in the NT-1 and CT phases. However, below 5 cm soil depth no significant differences were found among treatments (Table 7).

The stratification of SOC on soil surface increased with the time under NT (Table 7 and Fig. 6). In the 0-5 and 20-30 cm soil layers, SOC concentration values ranged between 8.6 and 12.1, 8.5 and 17.3, 7.1 and 24.0 and 6.5 and 24.0 g C kg^{-1} dry soil in the NT-1, NT-4, NT-11 and NT-20 phases, respectively (Table 7). In the NT-11 and NT-20 phases, SOC concentration among soil depths was significantly different in the next order: 0-5 > 5-10 > 10-20 and 20-30 cm depth. However, in the NT-4 phase differences were only found between 0-5 cm and the rest of the analyzed depths. Moreover, in the NT-1 phase, total SOC concentration did not show any stratification trend with depth (Table 7). The regressions between SOC stratification ratios and the number of years under NT showed significant logarithmic relationships (Fig. 6). The SR for 0-5:20-30 cm varied between 1.2 and 4.1 for the NT-1 and NT-20 treatments, respectively. When the number of years under NT increased, changes in the SR for the 0-5:5-10 and the 0-5:10-20 depths were minimal. However, for the 0-5:20-30 depth, the regression showed an increase in the SR over the 20-yr period (Fig. 6).

Table 7 Total soil organic carbon (SOC) concentration in the 0-30 cm soil depth in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Soil depth (cm)	SOC (g kg^{-1})				
	CT	NT-1	NT-4	NT-11	NT-20
0 – 5	11.9 (0.3)‡ cA*¶	10.5 (0.5) cAB	17.3 (1.7) bA	24.0 (1.2) aA	24.0 (0.6) aA
5 – 10	11.7 (0.4) AB	12.1 (2.1) A	13.0 (1.2) B	15.0 (3.5) B	14.1 (1.6) B
10 – 20	10.3 (1.9) AB	11.0 (1.1) AB	9.3 (0.9) B	8.8 (0.8) C	9.0 (1.3) C
20 – 30	9.9 (0.4) B	8.6 (1.7) B	8.5 (2.1) B	7.1 (0.9) C	6.5 (2.3) C

* Within each depth values are significantly different between chronosequence phases at P<0.05

¶ Within each chronosequence phase, different letters indicate significant differences between depths at P<0.05.

‡ Values in parenthesis are the standard errors of the mean.

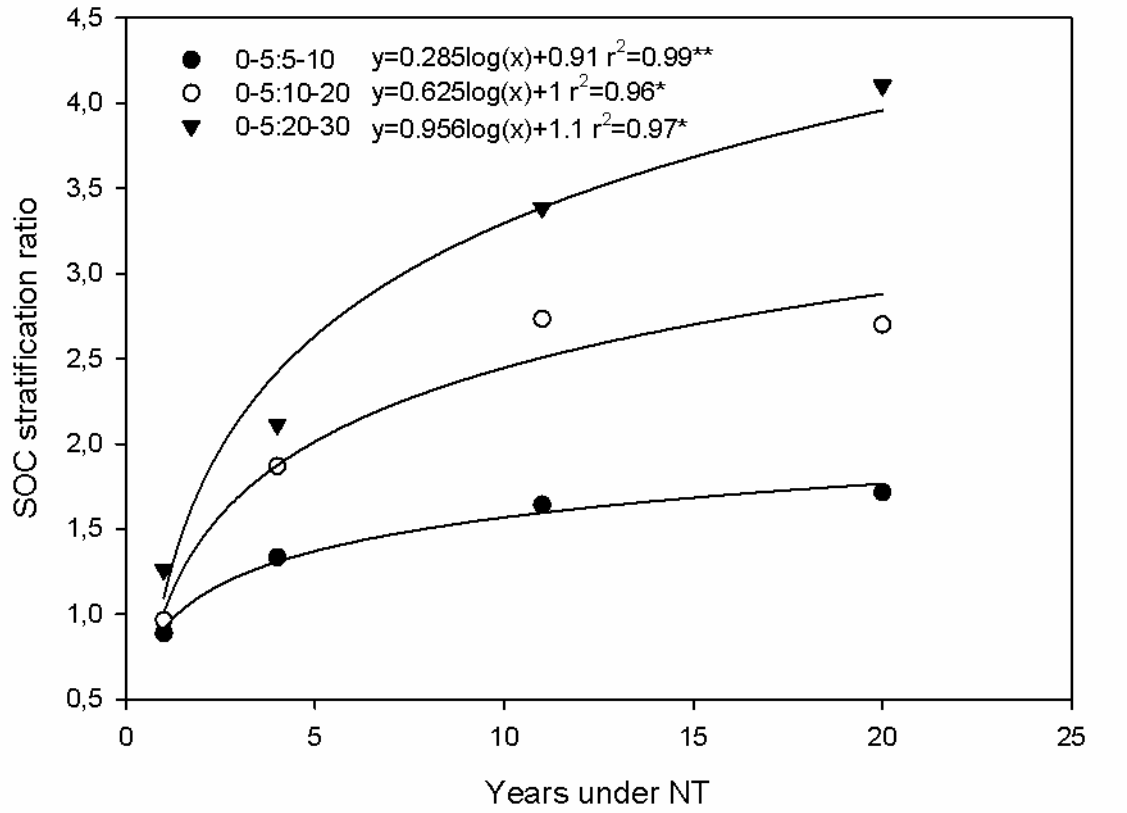

Figure 6 Regression analysis between soil organic carbon (SOC) stratification ratio and the number of years under no-tillage (NT) for three ratio depths (0-5:5-10 cm; 0-5: 10-20 cm; 0-5:20-30 cm). * Regression significant at P<0.05; **Regression significant at P<0.01.

3.2. No-tillage maintenance effects on dry and water-stable aggregate-size classes

Differences between treatments on dry macroaggregates were only found in the 10-20 and 20-30 cm soil depths (Fig. 7). In the 10-20 cm depth, the NT-4, NT-11 and NT-20 chronosequence phases showed greater proportion of large dry-sieved macroaggregates when compared with the NT-1 and CT phases, but this fact was compensated with a lower proportion of small dry-sieved macroaggregates (Fig. 7). In the 20-30 cm depth, greater large dry-sieved macroaggregates were found when NT was maintained over time (Fig. 7). Interestingly, between depths, greater dry-sieved small macroaggregates content was found in the 10-20 and 20-30 cm than in the 0-5 and 5-10 cm soil depths in the CT and NT-1 phases (Fig. 7).

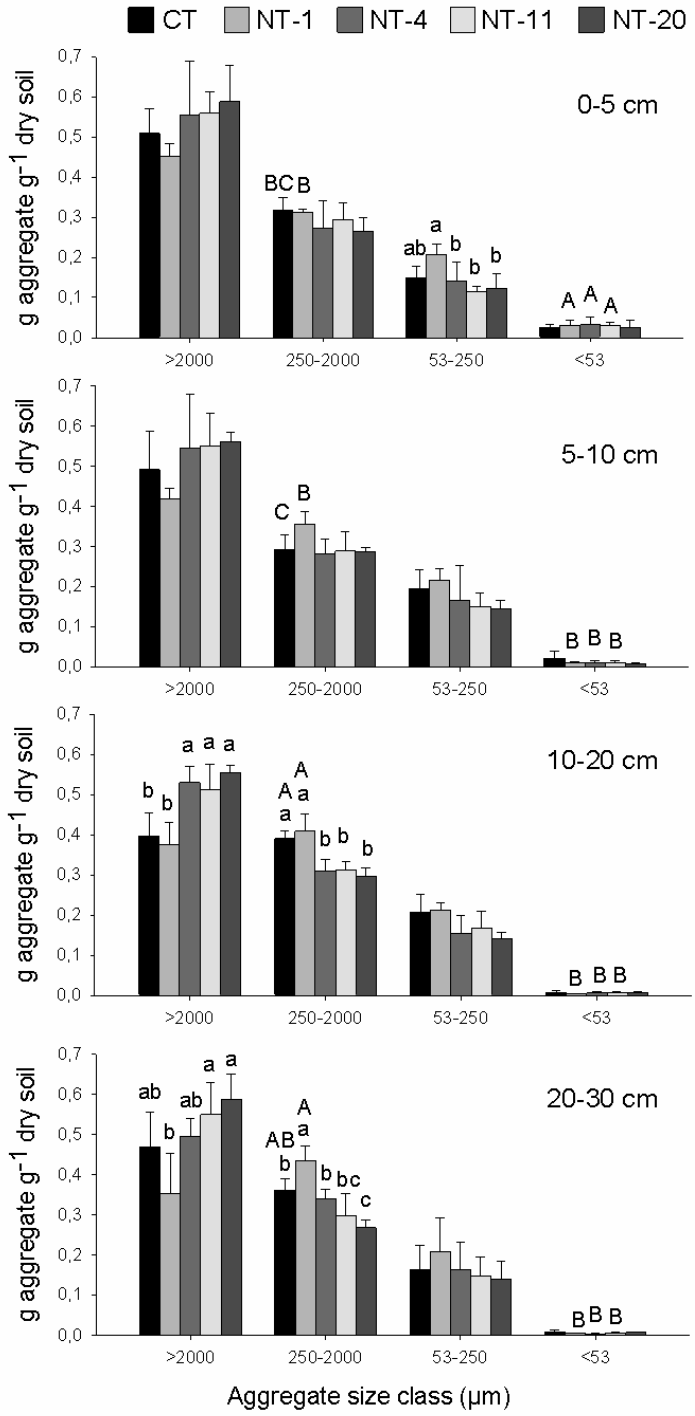

Figure 7 Dry aggregate size distribution at the 0-5, 5-10, 10-20 and 20-30 cm soil depths in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years under NT. Error bars represent standard errors. Within the same soil depth and aggregate fraction, different lowercase letters indicate significant differences between years under no-tillage at $P<0.05$. For the same year treatment and aggregate fraction, different uppercase letters indicate significant differences between depths at $P<0.05$.

In the 0-5 cm soil depth, water-stable macroaggregates ranged between 0.01 and 0.32 g aggregate g^{-1} dry soil (Fig. 8). Differences in water-stable aggregates between treatments were only found in the 0-5 and 5-10 cm depths (Fig. 8). In the 0-5 cm soil depth, greater proportion of large water-stable macroaggregates was found in the NT-11 and NT-20 phases compared with the other three phases (i.e., CT, NT-1 and NT-4). A similar trend was observed in the small macroaggregates, with greater proportion in the NT-4, NT-11 and NT-20 phases compared with the NT-1 and CT phases. In the 0-5 cm soil depth, a significant decrease in the proportion of water-stable microaggregates was observed when increasing the number of years under NT with the greatest proportion of water-stable microaggregates in the CT treatment (Fig. 8).

In the 5-10 cm soil depth, significant differences in large water-stable macroaggregates were found between the NT-20 phase and the NT-1 and CT phases. Furthermore, for this soil depth, the proportion of water-stable microaggregates also significantly differed between the CT phase and the NT-11 and NT-20 phases. Significant linear relationships were found between SOC concentration and the water-stable aggregate fractions for the different chronosequence treatments studied (Table 8). Large macroaggregates were positively related with SOC for the CT, NT-4, NT-11 and NT-20 treatments, whereas small macroaggregates were only related with SOC in the NT-4, NT-11 and NT-20 treatments. For the microaggregates fraction, significant linear relationships were found for the CT, NT-1 and NT-20 treatments. However, in the last case (i.e. NT-20) this relationship was negative. Finally, the silt-plus clay-sized particles were negatively related with SOC for all the chronosequence phases studied (Table 8).

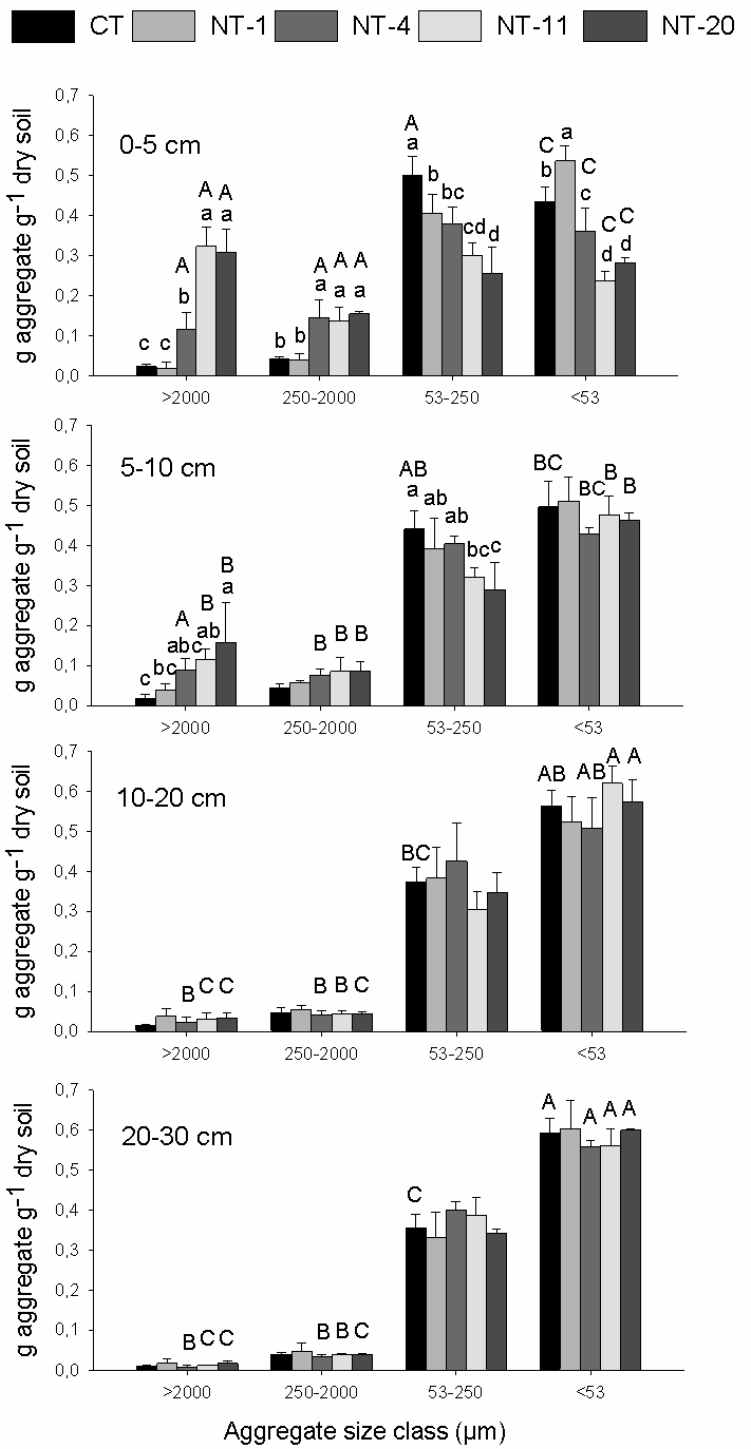

Figure 8 Water-stable aggregate size distribution at the 0-5, 5-10, 10-20 and 20-30 cm soil depths in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years under NT. Error bars represent standard errors. Within the same soil depth and aggregate fraction, different lowercase letters indicate significant differences between years under no-tillage at $P<0.05$. For the same year treatment and aggregate fraction, different uppercase letters indicate significant differences between depths at $P<0.05$.

Table 8 R^2 coefficients of the linear relationships between soil organic carbon (SOC) concentration and the proportion of water-stable large macroaggregates (2 – 8 mm), small macroaggregates (0.250 – 2 mm), microaggregates (0.053 – 0.250 mm) and silt-plus clay-sized particles (< 0.053 mm) for the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Water-stable aggregate fraction (mm)	CT	NT-1	NT-4	NT-11	NT-20
2-8	0.33*	n.s.	0.67**	0.92***	0.83***
0.250-2	n.s.	n.s.	0.79***	0.91***	0.90***
0.050-0.250	0.46*	0.49*	n.s.	n.s.	-0.35*
<0.050	-0.55**	-0.49*	-0.75***	-0.90***	-0.96***

n.s.: no significant; *P<0.05; **P<0.01; *** P<0.001

3.3. No-tillage maintenance effects on C concentration in the water-stable aggregate fractions

In the 0-5 cm soil layer, C concentration of the water-stable macroaggregates excluding their sand content was similar among chronosequence phases with values ranging from 18.6 to 30.7 g kg^{-1} (Table 9). However, in the 10-20 and the 20-30 cm soil layers, significant differences were found between phases with the greatest C concentration in the CT and NT-1 phases. Likewise, C concentration in microaggregates (0.053-0.250 mm) including their sand content and silt-plus clay-sized particles (< 0.053 mm) was significantly greater in the NT-4, NT-11 and NT-20 phases compared with the CT and NT-1 phases in the 0-5 cm soil layer (Table 9). Differences in macroaggregate-C between soil depths were found in all NT chronosequence phases when their sand content was taken into account. However, for the microaggregate-C (sand-proportion included) and the C associated to the silt-plus clay-sized particles, differences between soil depths were only found in some phases (i.e., the NT-4, NT-11 and NT-20 phases for microaggregates and the NT-11 and NT-20 phases for the silt-plus clay-sized particles). In both cases, the C concentration decreased with increasing soil depths (Table 9).

Table 9 Soil organic carbon (SOC) concentration in different water-stable aggregate classes in the 0-30 cm soil depth in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Soil depth (cm)	Water-stable Aggregate classes (mm)	SOC (g kg^{-1})				
		CT	NT-1	NT-4	NT-11	NT-20
0-5	0.250 – > 2	24.9 (1.4)‡ A¶	18.6 (5.3)	25.8 (9.3) A	29.5 (2.0) A	30.7 (1.7) A
	0.053 – 0.250	10.2 (1.4) b*	10.1 (2.3) b	16.4 (5.6) aA	18.1 (2.0) aA	19.5 (0.8) aA
	< 0.053	10.3 (2.3) b	8.8b (4.5)	19.5 (7.5) a	19.9 (2.7) aA	13.4 (1.7) abA
5-10	0.250 – > 2	23.5 (1.3) AB	22.1 (4.6)	19.5 (6.7) AB	22.7 (7.4) B	27.0 (9.5) A
	0.053 – 0.250	10.2 (0.9)	10.9 (2.7)	11.4 (2.8) AB	12.3 (2.2) B	13.1 (0.7) B
	< 0.053	10.0 (1.1)	9.3 (4.1)	11.9 (3.9)	11.8 (1.5) B	9.0 (3.3) B
10-20	0.250 – > 2	24.7 (2.2) aA	21.2 (6.6) ab	13.1 (6.7) bcB	10.4 (2.1) cC	14.7 (2.2) bcB
	0.053 – 0.250	10.7 (1.3)	10.1 (2.3)	8.2 (1.6) B	8.1 (1.7) C	8.8 (0.9) C
	< 0.053	6.0 (2.8)	8.3 (1.1)	9.7 (3.6)	8.9 (2.7) BC	7.4 (1.2) B
20-30	0.250 – > 2	18.6 (5.5) aB	13.8 (5.7) ab	8.8 (4.5) bB	6.8 (1.3) bC	10.1 (0.2) bBC
	0.053 – 0.250	9.9 (0.5)	9.2 (1.9)	7.5 (2.5) B	6.7 (0.6) C	6.9 (1.3) D
	< 0.053	7.9 (1.3)	6.5 (2.2)	9.9 (2.6)	7.7 (2.3) BC	7.5 (0.9) B

* For a given depth and water-stable aggregate fraction, different lowercase letters indicate significant differences between chronosequence phases at $P<0.05$

¶ For a given chronosequence phase and water-stable aggregate fraction, different uppercase letters indicate significant differences between depths at $P<0.05$.

‡ Values in parenthesis are the standard errors of the mean.

In the 0-5, 10-20 and 20-30 cm soil layers, C concentration (including their sand content) of soil macroaggregates was significantly different between chronosequence phases with the greatest macroaggregate-C concentration in the CT phase (Table 10). On the contrary, in the 0-5 and 5-10 cm soil layers, greater sand-free C concentration of microaggregates, was observed in the NT-11 and NT-20 phases compared to the NT-4, NT-1 and CT phases (Table 10). Differences in sand-free C concentration between depths were found in the three aggregate-size classes and in some chronosequence phases (Table 10). For instance, for the macroaggregates differences between soil layers were found in the NT-1, NT-4, NT-11 and NT-20 phases. In general, the sand-free C concentration decreased with increasing soil depth (Table 10).

Table 10 Sand-free soil organic carbon (SOC sand-free) concentration in different water-stable aggregate classes in the 0-30 cm soil depth in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Soil depth (cm)	Water-stable Aggregate classes (mm)	SOC (g kg^{-1}) sand-free				
		CT	NT-1	NT-4	NT-11	NT-20
0-5	0.250 – > 2	64.3 (7.3)‡ a*	54.8 (6.8) abA¶	46.0 (8.5) bA	49.5 (4.0) bA	50.9 (3.9) bA
	0.053 – 0.250	16.2 (2.2) c	18.3 (0.4) c	26.2 (4.8) bA	33.9 (4.5) aA	36.7 (2.6) aA
	< 0.053	10.3 (2.3) b	8.8 (4.5) b	19.5 (7.5) a	19.9 (2.7) aA	13.4 (1.7) abA
5-10	0.250 – > 2	62.9 (10.2)	52.7 (9.4) A	37.9 (5.6) AB	42.9 (11.0) A	49.2 (14.7) A
	0.053 – 0.250	17.7 (2.1) b	19.3 (1.8) b	19.2 (2.2) bB	24.7 (4.9) abB	28.0 (5.5) aB
	< 0.053	10.0 (1.1)	9.3 (4.1)	11.9 (3.9)	11.8 (1.5) B	9.0 (3.3) B
10-20	0.250 – > 2	63.5 (10.5) a	49.6 (5.7) bAB	33.7 (9.5) cAB	29.6 (0.7) cB	36.7 (6.1) bcAB
	0.053 – 0.250	19.0 (1.7)	18.1 (2.5)	14.0 (1.8) C	17.6 (3.4) C	16.0 (1.0) C
	< 0.053	6.0 (2.8)	8.3 (1.1)	9.7 (3.6)	8.9 (2.7) BC	7.4 (1.2) B
20-30	0.250 – > 2	51.2 (16.9) a	37 (9.1) abB	26.3 (6.3) bB	23.5 (4.7) bB	26.5 (3.8) bB
	0.053 – 0.250	17.6 (0.5) a	17.7 (1.9) a	12.3 (2.4) bC	12.3 (0.6) bC	13.9 (3.9) abC
	< 0.053	7.9 (1.3)	6.5 (2.2)	9.9 (2.6)	7.7 (2.3) BC	7.5 (0.9) B

* For a given depth and water-stable aggregate fraction, different lowercase letters indicate significant differences between chronosequence phases at $P<0.05$

¶ For a given chronosequence phase and water-stable aggregate fraction, different uppercase letters indicate significant differences between depths at $P<0.05$.

‡ Values in parenthesis are the standard errors of the mean

4. Discussion

The maintenance of no-tillage (NT) over time increased total SOC concentration. However, differences between chronosequence phases were only observed in soil surface. Those differences were positively related to the proportion of water-stable macroaggregates. The linear relationship established between SOC and water-stable large and small macroaggregates improved when the number of years under NT increased. Whereas the relationship between SOC concentration and the silt-plus clay-sized particles was negative for all the NT phases with an increasing R^2 value when increasing the number of years under NT. The relationships commented demonstrated the importance of the soil water-stable macroaggregates on the physical protection of SOC when increasing the number of years under NT. Thus, for example, in the 0-5 cm depth the maintenance of NT during 4 and 11 years (i.e., NT-4 and NT-11 phases) promoted 6-fold and 17-fold increase of water-stable large macroaggregates, respectively, compared to the NT-1 phase.

After NT adoption, several authors have reported increases in the proportion of soil water-stable macroaggregates together with gains in SOC concentration (Álvaro-Fuentes et al., 2008; Beare et al., 1994a). In an experiment with contrasting tillage systems and different number of years since the implementation of NT, Plaza-Bonilla et al., (2010) found greater differences in SOC levels and water-stable macroaggregates between CT and NT treatments when NT was maintained longer time. Adoption of NT promotes soil microbial activity in soil surface (Madejon et al., 2009; Staley et al., 1988) leading to greater production of organic binding by-products when decomposing fresh organic inputs (Abiven et al., 2009; Golchin et al., 1995; Golchin et al., 1994). These organic by-products play an important role in macroaggregate formation and stability, according to the hierarchy concept proposed by Tisdall and Oades, (1982). However, in our experiment, despite water-stable macroaggregates in soil surface (0-10 cm) increased significantly with the number of years under NT, differences in aggregate-C were only found in the microaggregate fraction. Under NT, the reduction in soil disturbance leads to the protection of SOC within macroaggregates. In particular, C within macroaggregates is stabilized in the form of microaggregate-sized particulate organic matter, enhancing the formation of C-enriched microaggregates (Denef et al., 2001). In the same area of this study, Álvaro-Fuentes et al., (2009) found greater microaggregate-C within macroaggregates in NT when compared to CT in three tillage

experiments. Six et al., (2000) stated that slower macroaggregate turnover and subsequent formation and liberation of C-enriched microaggregates occluded within macroaggregates could explain the greater SOC stocks usually found under NT. Our results corroborate this theory, with increasing proportions of stable macroaggregates and greater C concentration within microaggregates when the number of years under NT increased.

SOC stratification with depth increased with the number of years under NT. Franzluebbers, (2002) suggested that the stratification ratio (i.e. the proportion of SOC at the soil surface in relation to the SOC in deeper soil layers) could be a better indicator of soil quality than total SOC alone. In Mediterranean conditions, higher SR's under NT than under CT have been reported (López-Fando et al. 2007; Lopez-Garrido et al., 2011). In similar Central Spanish conditions, Hernanz et al. (2009) reported an increase in the SR over time in a NT system throughout a 20-year experiment. In our study, the SR in the 0-5:20-30 depths increased according to the years under NT with starting values of 1.2 in NT-1 up to 4.1 in the NT-20 phase. In a subtropical climate, Sa and Lal, (2009) also observed an increase in the stratification ratio of SOC when increasing the number of years under NT. Under NT crop residues are placed on the top of soil surface where their decomposition is reduced (Paustian et al., 1997). The stratification of SOC was closely related with the decrease with depth in the proportion of water-stable macroaggregates in the phases with more years under NT (i.e. NT-11, NT-20). Significant differences were found between depths in the sand-free C concentration in the macroaggregates of the NT-4, NT-11 and NT-20 cases. In the microaggregate fraction, differences in C concentration between soil depths were only found in the NT-4, NT-11 and NT-20 phases. Similarly, in the silt-plus clay-sized fraction, these differences were only found in the NT-11 and NT-20 phases. According to the data obtained, it could be hypothesized that the stratification of C concentration under NT is dependant on the size of soil aggregates. Consequently, C concentration in the greatest fractions (i.e. macroaggregates) showed faster stratification compared with the finest fractions (i.e. silt-plus clay-sized particles). In soil surface in the CT and NT-1 phases, the differences between the proportions of the water-stable and the dry-sieved macroaggregates were greater than in the NT-11 and NT-20 phases. On the contrary, in deeper soil, differences in the proportions of dry-sieved and water-stable macroaggregates were significant for all the chronosequence phases. Thus, it could be

assumed that in soil surface macroaggregates were more stable when the number of years under NT increased. The small proportion of water-stable macroaggregates located at deeper soil layers (i.e. 10-20 and 20-30 cm) in the CT and NT-1 phases, could be an explanation to the absence of differences in SOC concentration between chronosequence phases.

Different results were obtained in C concentration of the different aggregate fractions when this C concentration within aggregates was not corrected for sand content. Similar trend was observed by Plaza-Bonilla et al., (2010) who hypothesized that the redistribution of sand particles and/or the erosion of the silt and clay particles under inversion CT could explain the greater C concentration in soil macroaggregates under CT or the NT phase with only one year since the implementation (i.e. NT-1) when corrected for sand content.

5. Conclusions

Our results show that the maintenance of NT over time enhanced SOC concentration in soil surface reaching its maximum value after 11 years. Both the proportion of water-stable macroaggregates and the C concentration of microaggregates in soil surface increased according to the increase of the years under NT. Thus, the greater proportion of water-stable macroaggregates and the greater C-concentration within microaggregates were the main mechanisms of SOC protection in the NT chronosequence. A significant logarithmic stratification with depth over the NT chronosequence was observed in SOC concentration, which was related with the stratification of water-stable macroaggregates in soil depth. In these Mediterranean semiarid agroecosystems, the increase in the proportion of stable macroaggregates and the enrichment of C concentration of microaggregates are the main mechanisms of SOC protection when NT is maintained over time.

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Capítulo 3

Soil aggregate stability as affected by fertilization type under semiarid no-tillage conditions

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Soil aggregate stability as affected by fertilization type under semiarid no-tillage conditions

Abstract

Agricultural management practices play an important role in soil organic carbon (SOC) protection within soil aggregates. However, there is a lack of information on the effects of nitrogen (N) fertilization on C protection within aggregates under no-tillage (NT) systems. The effects of organic fertilization (with pig slurry and poultry manure) and mineral N fertilization on soil aggregation and physical C protection dynamics under NT soils were investigated. Two experiments were established in a semiarid area of northeast Spain. In the organic fertilization experiment, treatment with pig slurry at two N rates (100 and 200 kg N ha^{-1}), poultry manure (100 kg N ha^{-1}) and a control (0 kg N ha^{-1}) treatment were compared. In the mineral fertilization experiment, increasing rates of N fertilizer (0, 40, 80, 120 and 160 kg N ha^{-1}) were compared. Water-stable macroaggregates (>0.250 mm) and their C concentration, the distribution of dry-sieved aggregates, total SOC and microbial biomass C (MBC) were quantified in the soil surface in two cropping seasons. Organic fertilizers slightly increased the proportion of water-stable macroaggregates but caused no differences in MBC, SOC or water-stable macroaggregate C concentration. In the mineral N fertilization experiment, similar water-stable macroaggregate, water-stable macroaggregate C and SOC concentrations were observed among N fertilizer doses. Overall differences in water-stable macroaggregates between sampling dates were greater than differences between fertilization treatments. Our study demonstrates that, in the short-term, the use of organic or mineral N fertilizers hardly improves the stability of the macroaggregates and their C protective capacity when NT is performed. This finding could be related to the limitations imposed by water in the Mediterranean areas and the buffering effect of long-term NT adoption on soil aggregate stability and C protection.

1. Introduction

In Mediterranean agroecosystems, NT adoption increases crop water availability, resulting in greater biomass production (Cantero-Martínez et al., 2003; Cantero-Martinez et al., 2007). Moreover, in these systems it has been demonstrated that the use and maintenance of NT over time improves soil aggregation and C protection (Martin Lammerding et al., 2011; Plaza-Bonilla et al., 2010; Plaza-Bonilla et al., 2013). However, there is a lack of information about the effects of fertilization on soil C protection within aggregates when NT is performed. Soil structure is a key factor for soil maintenance and for the physical and biological processes that it involves. Soil structure mediates a number of soil properties such as crop nutrient availability, crop residue decomposition, soil erosion and, crop productivity (Bronick and Lal, 2005). In Mediterranean dryland areas there is a severe risk of soil erosion, so the maintenance of a suitable soil structure is essential in order to maintain soil quality (Álvaro-Fuentes et al., 2008a). One main indicator of soil structure is aggregate stability (Six et al., 2000). Soil aggregation is controlled by the quantity and quality of SOC, soil biota, ionic bridging, clay and silt content, and the presence of carbonates and gypsum (Amézketa, 1999).

SOC has a predominant effect on soil structure because its quantity and quality can be modified by agricultural management practices (Abiven et al., 2009; Whalen and Chang, 2002). Soil aggregation is a dynamic process, so it is expected to vary temporally (Chan et al., 1994). Temporal aggregate dynamics is influenced by climatic conditions, agricultural management practices (e.g., tillage, N fertilization and cropping system), crop growth and the decomposition kinetics of the organic residues applied to the soil (Álvaro-Fuentes et al., 2008b). Several studies have reported an improvement in soil physical properties after the long-term application of organic fertilizers (Abiven et al., 2009; Aoyama et al., 1999b; Celik et al., 2004; Wortmann and Shapiro, 2008) because of the increase in SOC content (Aoyama et al., 1999a; Paustian et al., 1992; Triberti et al., 2008). However, other studies have reported that manure application could reduce aggregate size, mainly due to the dispersive agents such as monovalent cations in the manure (Whalen and Chang, 2002). The increase in SOC caused by the application of organic fertilizers is a direct result of the manure composition and an indirect result of the increased crop growth and crop residue in response to the nutrient supply (Whalen and Chang, 2002). Aoyama et al. (1999a) proposed that organic fertilizers increase the pool of particulate organic matter, promoting the formation of

soil macroaggregates in the short-term, whereas in the long-term the organic residues could be transformed to mineral-associated C, contributing to aggregate stabilization. Moreover, the application of organic residues to the soil can increase the stability of soil aggregates because of the physical and chemical action of the molecules contained in the organic products and/or the increase in the hydrophobicity of soil aggregates (Abiven et al., 2009). Some authors have reported an increase in SOC when synthetic fertilizers are applied to the soil (Álvaro-Fuentes et al., 2012; Halvorson et al., 1999). Therefore, an increase in soil structural stability following the application of mineral N could be expected (Sainju et al., 2003). In contrast, greater SOC mineralization was observed when N fertilizers were used (Khan et al., 2007), reducing aggregate stability (Le Guillou et al., 2011). Therefore, it is unclear how mineral fertilizers can affect both soil aggregate stability and the physical protection of SOC.

Animal waste from a large livestock industry is a common crop fertilizer source used in Mediterranean Spain, (http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database, European Commission. 2012. Eurostat, accessed 1 Nov. 2012). Organic fertilizers include a broad range of organic materials with different characteristics and therefore different decomposition kinetics (Thuries et al., 2002). For example, because of its high ammonia content, pig slurry tends to show a similar behavior to mineral fertilizers in soils (Sánchez-Martin et al., 2010), whereas N mineralization in solid organic fertilizers such as poultry manure tends to be more time-persistent (Thuries et al., 2002). In Mediterranean dryland livestock production is typically combined with crop production, resulting in a variable availability of organic materials from diverse animal sources for crop fertilization. Investigating the effects of organic products on physical C protection within soil aggregates is therefore essential in those areas.

The main objective of our experiment was to study the effects of organic and mineral N fertilization on aggregate stability and carbon content under no-tillage. Our hypotheses were: (i) the use of organic fertilizers results in an increase in the proportion of water-stable macroaggregates; (ii) soil macroaggregate stability increases when more recalcitrant products (e.g., poultry manure) are used; and (iii) SOC can be expected to increase when mineral N fertilizers are applied, with a concomitant increase in water-stable aggregates.

2. Materials and Methods

2.1. Experimental sites

The study was performed in two NT experimental fields with different fertilization management established in 2007 and 2008 in northeast Spain. The site with organic fertilization (41º43'N, 1º27'E, Conill, Spain) and the site with mineral fertilization (41º42'N, 1º30'E, Sant Martí Sesgueioles, Spain) were 5 km apart. The main objective of both experimental fields was to study crop response to the application of different doses of mineral N and types and doses of the most common organic fertilizers in the area (i.e. pig slurry and poultry manure) in order to better advise the farmers in the area. Central Catalonia, where the two experimental fields were set up, is an area of intensive livestock production (swine and poultry) that generates substantial amounts of organic waste. Farmers therefore have a varied range of mineral and organic products with which to fertilize crops. N rates were chosen according to the usual fertilization management in the area and previous fertilization experiments performed in the area (Angás et al., 2006; Morell at el., 2011c).

The two sites have identical climatic conditions, with mean annual precipitation and potential evapotranspiration of 500 and 960 mm, respectively. Selected soil properties for both experiments are detailed in Table 11. Prior to the establishment of the experiments, both fields had been under NT for the previous 10 years with organic and mineral N fertilization at Conill and Sant Martí Sesgueioles, respectively. During that time, the typical pig slurry application rate in Conill was about 20 m^3 ha^{-1}, which represents about 100 kg N ha^{-1}.

Table 11 Site and general soil characteristics in the 0-30 cm soil depth of the two experimental sites.

Site and soil characteristics	Organic fertilization	Mineral fertilization
Year of establishment	2008	2007
Latitude	41º 43' N	41º 42' N
Longitude	1º 27' E	1º 30' E
Elevation (m)	748	625
pH (H_2O, 1,2.5)	7.8	7.9
$EC_{1,5}$ (dS/m)	0.39	0.30
$CaCO_3$ eq. (%)	32	24
Particle Size Distribution (%)		
Sand (2000–50 µm)	13.1	23.5
Silt (50–2 µm)	47.2	41.1
Clay (<2 µm)	39.7	35.4

Both experiments consisted of a randomized complete-block design with three replications. Plot size was 50 x 12 m in the organic N fertilization experiment and 50 x 6 m in the mineral N fertilization experiment.

2.2. Mineral N fertilization experiment

In October 2007, five mineral N doses were established: 0, 40, 80, 120 and 160 kg N ha^{-1}. One-third of the dose was applied before the seeding of the crop with ammonium sulphate (26% N). The other two-thirds were applied as top dressing at the tillering stage of the crop with ammonium nitrate (33% N). The cropping system consisted of an NT barley (*Hordeum vulgare L.)* monocropping. Planting was performed in October with a disc direct-drilling machine after the application of a total herbicide (1.5 L 36% glyphosate per hectare). Potassium fertilization was applied according to the results of soil analyses with KCl, as in the organic fertilization experiment. The crop was harvested by the end of June with a commercial medium-sized harvester. The straw residue was chopped and spread over the soil.

2.3. Organic fertilization experiment

In October 2008, four organic treatments were established: a control treatment without application, two treatments with two rates of pig slurry, 100 and 200 kg N ha^{-1} (PS100 and PS200, respectively) and one treatment with the application of 100 kg N ha^{-1} of poultry manure (PM100). All the organic fertilizers were obtained from commercial farms near the experiment and their main characteristics are presented in Table 12. PS100 and PM100 were applied before the seeding of the crop, whereas PS200 was split into half the dose before seeding and the other half at crop tillering, as is common in the area. Fertilizers were surface-applied using commercial machinery previously calibrated to apply the precise dose. Pig slurry was conventionally surface-spread via a vacuum tanker fitted with a splashplate (Fig. 9). Dry poultry manure was applied with a rear-discharge, box-type spreader equipped with beaters that broadcast the manure over a width of 12 m. Both types of machinery are commonly used in the area by farmers.

The cropping system consisted in an NT rotation of wheat (*Triticum aestivum L.*) and barley. Planting was performed in October with a direct-drilling disk machine to a depth of 3 cm, after the application of a non-selective herbicide (2 L 36% glyphosate per hectare). No mineral N was applied during the experiment, but in order to meet the crop's potassium requirement, KCl was applied before seeding according to the results

of soil analyses. The crop was harvested by the end of June with a commercial medium-sized harvester. The straw residue was chopped and spread over the soil.

Table 12 Composition of the pig slurry (PS) and the poultry manure (PM) applied in the organic fertilization experiment (values are expressed as g 100 g^{-1} of dry matter).

Organic fertilizers characteristics	PS				PM	
	Pre-seeding 2009/10	Tillering 2009/10	Pre-seeding 2010/11	Tillering 2010/11	Pre-seeding 2009	Pre-seeding 2010
Dry matter	4.3	4.3	3.6	3.1	55.6	58.1
C	-	42.4	-	-	-	39.1
N kjeldahl	3.2	3.0	3.5	4.4	6.1	3.9
Ammonium-N	6.8	9.2	10.2	10.0	0.8	1.5
P	2.5	2.2	2.2	3.0	1.4	1.5
K	3.8	5.4	5.6	5.4	3.2	2.9

Figure 9 Pig slurry application in the organic fertilization experiment at the tillering stage.

2.4. Soil sampling and analyses

During the 2009-2010 and the 2010-2011 cropping seasons, soil surface (0–5 cm) was sampled on seven different dates corresponding to different crop developmental stages: tillering and post-application of the top-dressing fertilization on 19 March 2010; flowering on 12 May 2010; post-harvest on 19 July 2010; post-summer fallow and post-

application of pre-seeding fertilization on 5 November 2010; tillering and post-application of top-dressing fertilization on 29 March 2011; flowering on 3 May 2011; and post-harvest on 25 July 2011. In each plot, two composite samples were obtained from two representative areas 10 m apart. Undisturbed soil samples were collected with a flat spade (Fig. 10). Each soil sample was stored in a crush-resistant airtight container. From each composite sample, SWC was measured gravimetrically by drying soil subsamples at 105ºC until constant weight. Once in the laboratory, the undisturbed samples were passed gently through an 8-mm sieve and air-dried at room temperature. For each sample, water-stable macroaggregate (>0.250 mm) separation and dry soil aggregate distribution were performed. Water-stable macroaggregate (>0.250 mm) size separation was performed according to a modified wet sieving method adapted from Elliott (1986). Briefly, a 100-g air-dried soil sub-sample was placed on the top of a 0.250-mm sieve and submerged for 5 min in deionized water at room temperature. The sample was manually sieved 50 times for 2 min. The water-stable macroaggregates obtained were oven-dried at 50ºC for 24 h and weighed. Sand content of the macroaggregates (>0.050 mm) was determined by dispersing a 5-g sub-sample in sodium hexametaphosphate solution using a reciprocal shaker. Sand-corrected macroaggregates (g g^{-1} dry soil) were expressed as:

Sand-corrected macroaggregates = Water-stable macroaggregate weight/ [1- (sand $\text{proportion}_{\text{macroaggregates}}$)]

Figure 10 Taking surface soil samples (0-5 cm) with a flat spade at the tillering stage of the crop

The dry aggregate size distribution was conducted by placing 100 g of air-dried soil sub-sample (8-mm-sieved) on an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain) with a series of three sieves (2, 0.250 and 0.050 mm) in order to obtain four aggregate fractions: (i) large macroaggregates (2–8 mm); (ii) small macroaggregates (0.250–2 mm); (iii) microaggregates (0.050–0.250 mm); and (iv) silt- plus clay-sized particles (<0.050 mm). A sieving time of 1 min and the lowest-power program of the machine were used. The mean weight diameter (MWD) (Youker and Mc Guiness, 1957) was used to express the dry soil aggregate distribution.

The organic C concentration of the bulk soil (SOC) and the water-stable macroaggregates (water-stable macroaggregate C) were determined using the wet oxidation method of Walkley-Black described by Nelson and Sommers (1996). The method was modified to increase the digestion of SOC. The modification consisted in boiling the sample and the extraction solution at 150ºC for 30 minutes (Mebius, 1960).

The microbial biomass C (MBC) in bulk soil (0–5 cm) was measured in the organic fertilization experiment during the last three sampling dates (March, May and July 2011) in order to elucidate possible effects on the stability of the macroaggregates. The analyses were performed according to the chloroform-fumigation and direct extraction method of Vance et al. (1987). The extracts were analyzed for organic C using a Shimadzu TOC-VCSH analyzer. The MBC extraction coefficient applied was 0.38 (Sparling and Zhu, 1993; Vance et al., 1987).

The data were analyzed using the SAS statistical software (SAS institute, 1990). To compare the effects of fertilizer treatments and sampling date, a repeated measures analysis of variance was performed for each site. When significant, differences among treatments were identified at the 0.05 probability level of significance using an LSD test. In the mineral N fertilization experiment the different rates of N were analyzed as continuous variables. In order to determine the relationships between some variables, linear regression analyses were performed with Sigmaplot 11 (Systat Software, Inc., 2008).

3. Results

Rainfall events and mean soil and air temperature during the whole experimental period are shown in Fig. 11. Soil temperature was below zero (ºC) for two weeks prior to the first sampling event during the tillering stage of the crop (March 2010) and one month before the March 2011 sampling. Moreover, a snowfall occurred two weeks before the March 2010 sampling. Soil and air temperature increased during the subsequent months, reaching their maximum in the post-harvest sampling events (July 2010 and July 2011). Rainfalls of 14, 5 and 18 mm occurred the week before the March 2010, May 2010 and May 2011 sampling dates, respectively. On the July 2010, November 2010, March 2011 and July 2011 sampling dates no rainfalls occurred.

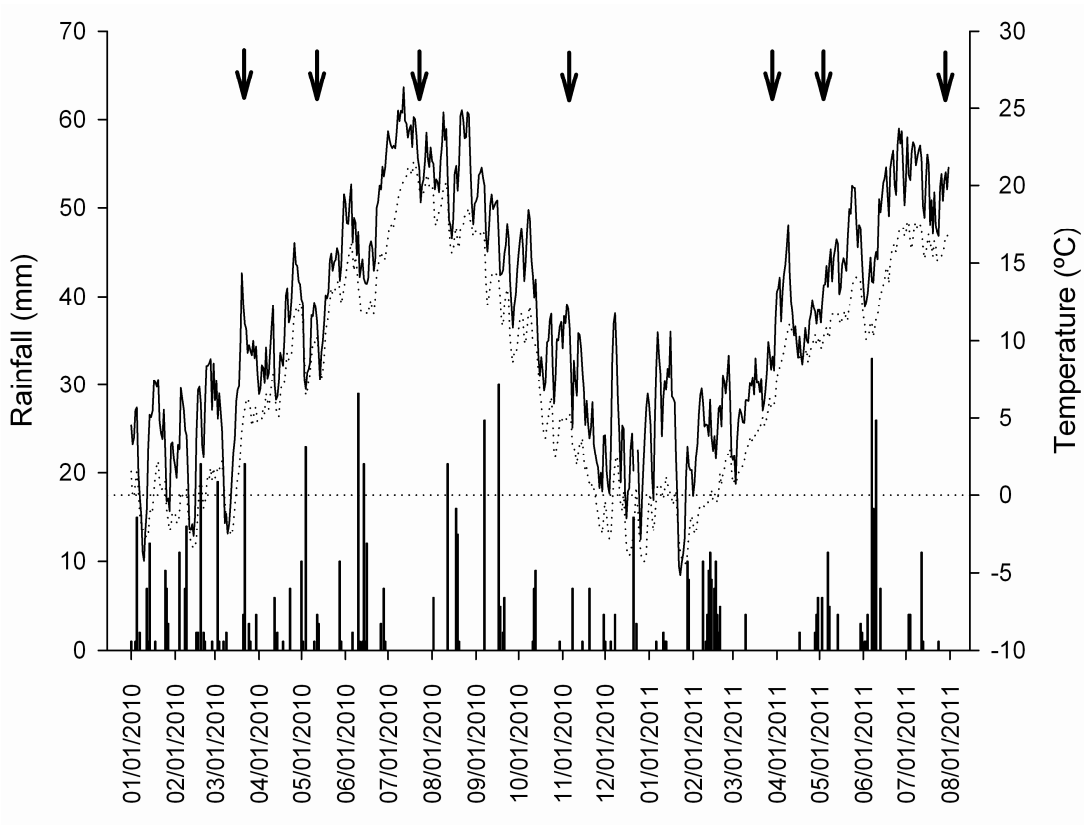

Figure 11 Soil temperature (dotted line), air temperature (continuous line) and rainfall events (columns) during the experimental period. Small vertical arrows indicate soil sampling events.

3.1. Mineral N fertilization effects on soil aggregation dynamics and C protection

In the mineral N fertilization experiment, water-stable macroaggregates ranged between 0.12 and 0.28 g g^{-1} dry soil (Fig. 12). No differences in soil water-stable macroaggregates were found between mineral N fertilization doses (Table 13). However, when sampling dates were compared, significant differences arose (Fig. 12).

The lowest proportion of water-stable macroaggregates was found in March 2010 (tillering and post-application of top-dressing fertilization) and the highest proportion in July 2010 (post-harvest).

The MWD of the dry-sieved aggregates ranged between 2.90 and 3.79 mm (Fig. 12), with significant differences between N doses and sampling dates (Table 13 and Fig. 12). The MWD increased significantly with the increase in the dose of N applied (data not shown). In turn, the highest values of MWD were found on the first two sampling dates (March and May, 2010), while the lowest values were found in July 2010 and July 2011 (Fig. 12).

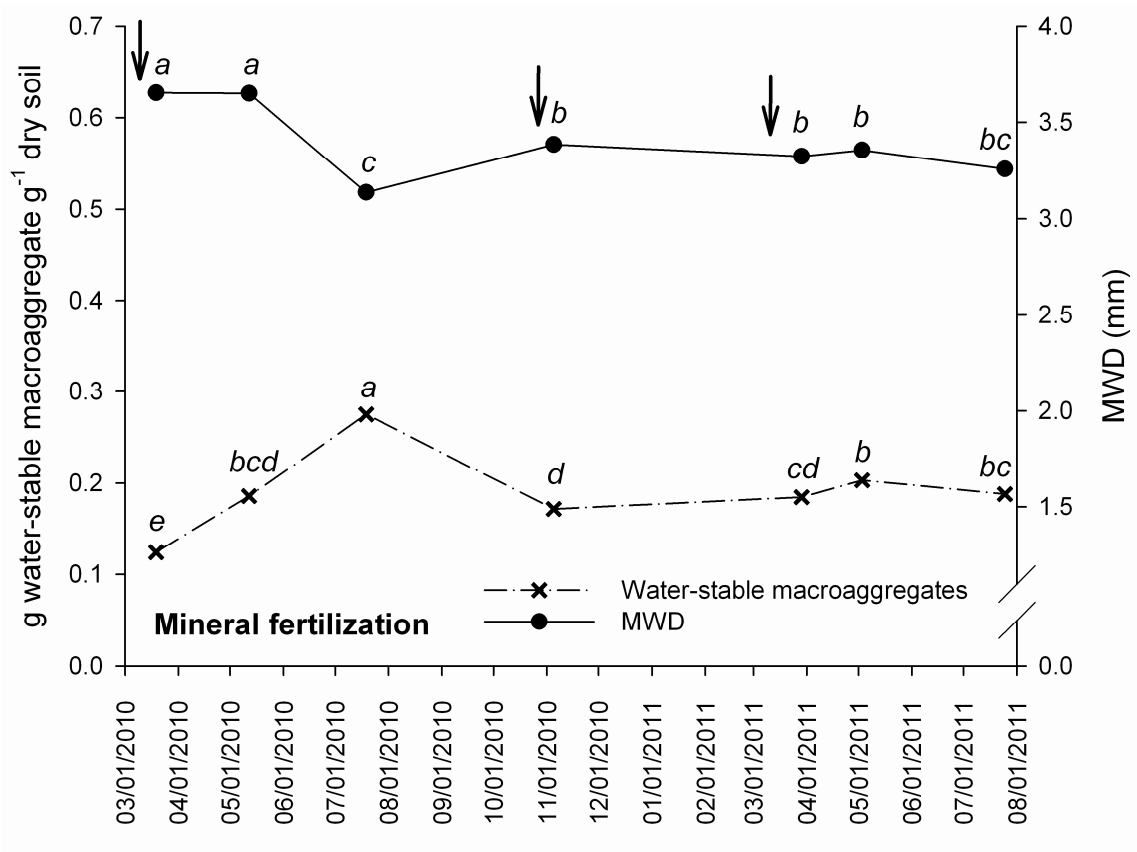

Figure 12 Proportion of water-stable macroaggregates (> 0.250 mm) and mean weight diameter of the dry-sieved aggregates (MWD) in soil surface (0–5 cm depth) of the mineral fertilization experiment as affected by sampling date. Values are the means of the five mineral N fertilizer rates compared (0, 40, 80, 120 and 160 kg N ha^{-1}). For each variable, different lowercase letters indicate significant differences between sampling dates at $P<0.05$. Vertical arrows indicate the application of fertilizers.

Over the whole experimental period, total SOC concentration ranged between 16.6 and 25.7 g kg^{-1} dry soil (data not shown). No effects of N doses or the interaction between N doses and sampling dates on SOC or macroaggregate C were observed (Table 13). However, significant differences between sampling dates were found in SOC and macroaggregate C (Table 13). Both SOC and macroaggregate C concentration were highest in July 2011 (Table 14).

Table 13 ANOVA *P*-values showing significant differences in the proportion of water-stable macroaggregates (>0.250 mm), the mean weight diameter of the dry-sieved aggregates (MWD), the soil organic carbon of the bulk soil (SOC) and the water-stable macroaggregates C as affected by organic and mineral N fertilization treatments, sampling dates and their interaction.

Variables	Source of variation	Organic fertilization	Mineral N fertilization
Water-stable macroaggregates	N Treatment	*	ns
	Date	***	***
	Date*N treatment	ns	ns
MWD	N Treatment	**	***
	Date	***	***
	Date*N treatment	ns	ns
SOC	N Treatment	ns	ns
	Date	ns	***
	Date*N treatment	ns	ns
Macroaggregate-C	N Treatment	ns	ns
	Date	**	***
	Date*N treatment	ns	ns

ns: non-significant; **P*<0.05; ***P*<0.01; *** *P*<0.001

Table 14 Soil organic carbon concentration (SOC) and water-stable macroaggregate C in soil surface (0–5 cm depth) in the organic and mineral fertilization experiments as affected by sampling date.

Variables	Sampling date	Organic fertilization	Mineral fertilization
SOC ($g\ kg^{-1}$ dry soil)	March 2010	46.9 (7.9)†	20.4 (1.6) c‡
	May 2010	47.7 (5.6)	21.2 (1.4) bc
	July 2010	49.6 (3.4)	18.7 (3.1) d
	November 2010	50.7 (3.3)	21.5 (1.4) bc
	March 2011	53.8 (2.5)	22.1 (1.9) ab
	May 2011	48.4 (6.0)	22.6 (3.4) ab
	July 2011	49.2 (2.9)	23.1 (1.4) a
Water-stable macroaggregate-C ($g\ kg^{-1}$ dry soil)	March 2010	93.6 (13.3) abc	44.6 (3.8) c
	May 2010	100.4 (22.9) a	48.3 (3.4) b
	July 2010	86.9 (14.8) bcd	41.0 (2.8) d
	November 2010	85.3 (13.7) cd	45.1 (4.7) c
	March 2011	80.5 (8.9) d	44.3 (3.5) c
	May 2011	85.5 (8.8) bcd	44.0 (6.5) c
	July 2011	94.1 (12.6) ab	53.6 (4.8) a

†Values in parenthesis are the standard errors of the mean.

‡Within each experiment (i.e. organic and mineral fertilization) and variable, different letters indicate significant differences between sampling dates at $P<0.05$.

3.2. Organic fertilization effects on soil aggregation dynamics and C protection

The proportion of water-stable macroaggregates ranged between 0.27 and 0.50 $g\ g^{-1}$ dry soil for the control treatment (i.e. 0 kg N ha^{-1}) in March 2010 and for the PS100 treatment in July 2010, respectively (data not shown). Significant differences in water-stable macroaggregates were found between organic fertilization treatments. When organic fertilizers were applied (PM100, PS100 and PS200), a greater proportion of water-stable macroaggregates was observed than in the control treatment (Table 15). Also, significant differences were found between sampling dates (Table 13), with the highest values in July 2010 and March 2011 (Fig. 13). However, no significant interaction was found between organic fertilization treatments and sampling dates. Also, significant differences were found between N fertilization treatments and sampling dates in MWD (Table 13). The highest MWD was observed in the PS200 and PM100 treatments (Table 15), whereas the lowest was observed in the control treatment (Table 15). When sampling dates were compared, the highest MWD was observed during the first three sampling events (March, May and July 2010) (Fig. 13).

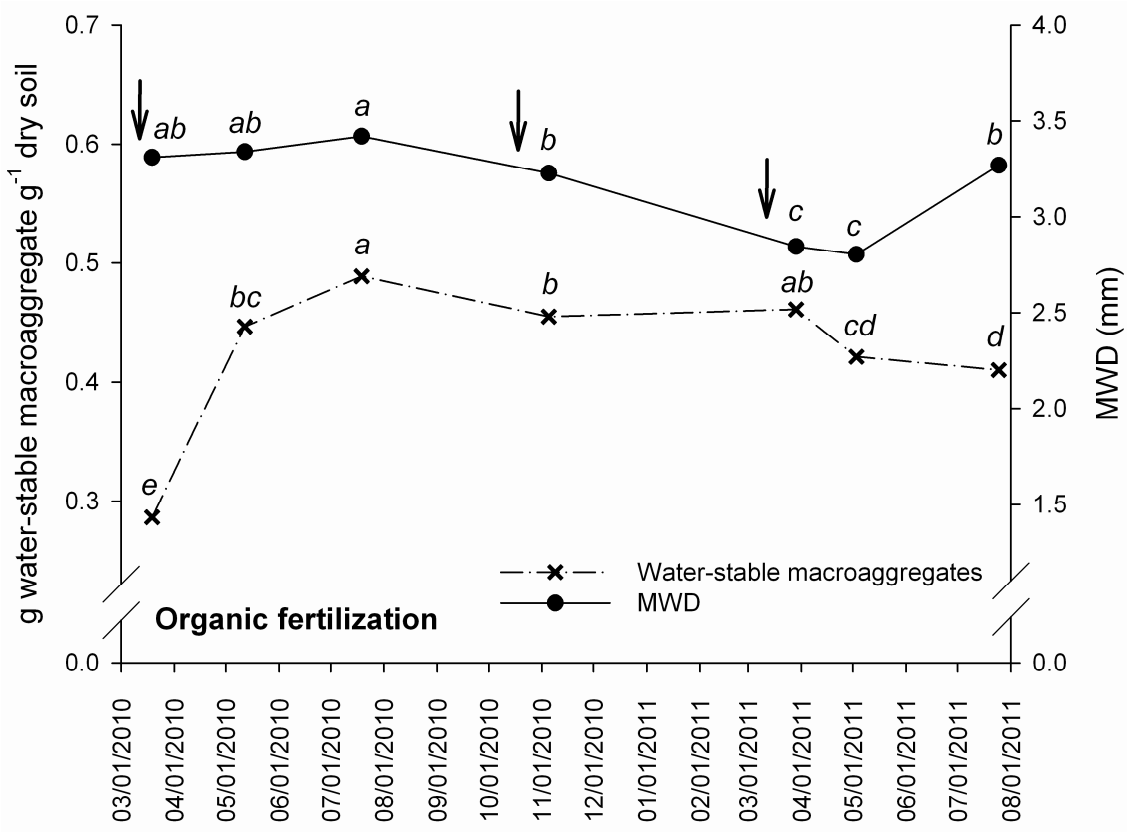

Figure 13 Proportion of water-stable macroaggregates (>0.250 mm) and mean weight diameter of the dry-sieved aggregates (MWD) in soil surface (0–5 cm depth) of the organic fertilization experiment as affected by sampling date. Values are the means of the four organic fertilization treatments compared (a control treatment, pig slurry at 100 and 200 kg N ha^{-1} and poultry manure at 100 kg N ha^{-1}). For each variable, different lowercase letters indicate significant differences between sampling dates at $P<0.05$. Vertical arrows indicate the application of fertilizers.

No significant differences were found between organic fertilization treatments, sampling dates or their interaction on SOC (Table 13). However, the sampling date significantly affected the macroaggregate C concentration. Water-stable macroaggregate C ranged between 78.0 and 105.0 for the PS200 treatment in March 2011 and for the PM100 treatment in May 2010, respectively (data not shown). No differences in MBC were found between organic fertilization treatments (Table 16). However, in the PS100 treatment, differences between sampling dates were found, with greater MBC in March 2011 and May 2011 than in July 2011 (Table 16).

Table 15 Proportion of water-stable macroaggregates (>0.250 mm) and mean weight diameter of the dry-sieved aggregates (MWD) in soil surface (0–5 cm depth) as affected by organic fertilization treatments with PS100 and PS200 (pig slurry 100 and 200 kg N ha^{-1}, respectively) and PM100 (poultry manure 100 kg N ha^{-1}). Values are the means of the seven sampling events.

Treatment	Water-stable macroaggregates	MWD
	g g^{-1} dry soil	mm
Control 0 kg N ha^{-1}	0.39 b†	3.02 c
PM100	0.43 a	3.23 ab
PS100	0.43 a	3.15 bc
PS200	0.44 a	3.30 a

†Within the same variable, different lowercase letters indicate significant differences between treatments at $P<0.05$.

Table 16 Soil microbial biomass C (MBC) in soil surface (0–5 cm depth) as affected by organic fertilization with PS100 and PS200 (pig slurry 100 and 200 kg N ha^{-1}, respectively) and PM100 (poultry manure 100 kg N ha^{-1}) in March 2011, May 2011 and July 2011.

Variable	Sampling date	Control 0 kg N ha^{-1}	PM100	PS100	PS200
MBC (mg C kg^{-1})	March 2011	810.6 (93.3)†	1229.8 (533.0)	1032.2 (145.8) a‡	1135.9 (179.3)
	May 2011	899.9 (172.9)	959.7 (250.9)	1082.4 (297.3) a	1372.4 (38.3)
	July 2011	590.6 (114.6)	579.2 (116.9)	654.6 (135.3) b	975.7 (218.6)

†Values in parenthesis are the standard errors of the mean.

‡Within each fertilization treatment, different letters indicate significant differences between sampling dates at $P<0.05$.

4. Discussion

The addition of organic fertilizers slightly increased the proportion of soil-surface water-stable macroaggregates and the MWD of the dry-sieved aggregates (Table 15). On the other hand, the application of increasing doses of mineral N fertilizer only affected the MWD of the dry-sieved aggregates (Table 13). This observation suggests that unstable aggregates are formed when N mineral fertilizers are applied. Consequently, in our experiment, N fertilization (both organic and mineral) had a minor effect on soil surface aggregation. As stated in the Materials and Methods section, soil management prior to the establishment of both experiments consisted of NT. In both experiments, the use of NT for more than 10 years could have resulted in the development of soil macroaggregates with high stability, in turn diminishing the possibility of any subsequent effect due to the application of fertilizers. Also, because both experiments have been conducted for only a few years, it could be hypothesized that effects of greater magnitude may occur after the long-term use of fertilizers. Under NT soil macroaggregates are more stable, resulting in greater protection of SOC (Álvaro-Fuentes et al., 2008b; Plaza-Bonilla et al., 2010). Aoyama et al. (1999b) concluded that manure application can provide a C-protective mechanism in annually tilled cropping systems similar to that provided by NT. In a soil management and N source experiment, Mikha and Rice (2004) observed greater water-stable large macroaggregates with the application of manure combined with NT adoption. However, they found no significant interaction between soil management and N source. In our organic fertilization experiment, the historical fertilization management prior to the establishment of the experimental field consisted of pig slurry. This organic addition for more than 10 years could have led to the high initial levels of surface SOC measured in the experiment (between 46.9 and 53.8 g kg^{-1} dry soil) (Table 14). The initial high SOC could have contributed to the low differences in water aggregate stability found between organic fertilization treatments. In the organic fertilization experiment, our results show a small significant linear relationship between the proportion of water-stable macroaggregates and the total SOC (data not shown). However, the R^2 values obtained are considerably lower than those reported by other authors (e.g., Chaney and Swift, 1984) indicating that macroaggregates could be C-saturated. Fortun et al. (1989) concluded that the impact of addition of organic products to the soil is greater in cases in which the initial concentration of soil C is low. Likewise, Stewart et al. (2008)

demonstrated that soils that are near to C-saturation are less efficient than C-depleted ones when storing added C.

As stated above, no effects of mineral N fertilization on water-stable macroaggregates were found (Table 13). This lack of response has also been reported by other authors (e.g., Aoyama et al., 1999a; Celik et al., 2004; N'Dayegamiye, 2009). Moreover, the application of N fertilizer affected neither SOC concentration nor water-stable macroaggregate C concentration (Table 13). Other authors have reported a lack of response of SOC or macroaggregate C to N mineral fertilization (Aoyama et al., 1999b; Mikha and Rice, 2004). In rainfed Mediterranean agroecosystems there is limited response to N application, mainly because of the restricted soil water availability for crop growth (Cantero-Martínez et al., 1995; Cantero-Martínez et al., 2003). In these systems, N fertilization has little impact on C inputs returned to the soil (crop residues and roots) (Morell et al., 2011a, Morell et al., 2011c) and, as a result, its effects on SOC tend to appear in the long term (Alvaro-Fuentes et al., 2012; Morell et al., 2011b). Although differences in biomass production between N fertilization doses have been observed in the experiment (data not shown), the small number of years since its implementation could have influenced the absence of differences in SOC. Other authors have reported a negligible or slight soil C sequestration under rainfed Mediterranean conditions when using mineral fertilizers (López-Bellido et al., 2010; Triberti et al., 2008).

We hypothesized that the application of recalcitrant organic residues (e.g., poultry manure) would be followed by a more constant stability of macroaggregates when compared with more labile organic products such as pig slurry. It has been reported that the C composition of pig slurry is more labile than that of solid organic fertilizers (Gigliotti et al., 2002; Bol et al., 2003). Thus, fast mineralization of the most labile parts of the C applied with the pig slurry could increase the water stability of the aggregates. Contrary to our hypothesis, no significant interaction was found between the N treatment and the date of sampling, demonstrating the absence of different temporal responses in macroaggregate stability when organic residues of different composition are applied.

According to the repeated-measures analyses of variance performed, the effect of the sampling date on water-stable macroaggregates, MWD and macroaggregate C concentration was significant in both experiments because of the high seasonal variation in these variables (Table 13). SOC was only significantly affected by the sampling date

in the mineral N fertilization experiment. However, as stated above, no significant interaction between sampling date and fertilization treatment was found for any of the variables measured. In both experiments, the lowest proportion of water-stable macroaggregates was observed on the first sampling date (March 2010) (Figs. 12 and 13). As stated in the Results section, two weeks before the first sampling date a snowfall accompanied by low temperatures occurred. It is known that rapid rewetting of the soil and freeze-thaw cycles cause swelling (Kemper et al., 1985; Dagesse, 2011) that can be followed by slaking and macroaggregate disruption (Denef et al., 2001). These factors could have reduced the stability of macroaggregates during that period. The great differences between sampling dates in water-stable macroaggregates in both experiments contrasts with the slight or null effect of the N treatments (in the organic and mineral N fertilization experiments, respectively) on that variable. Some authors have reported seasonal variations in aggregate stability greater than the differences between the treatments that they were comparing (Alderfer, 1950; Chan et al., 1994; Perfect et al., 1990). For example, a negative correlation between the SWC and the soil aggregate stability was reported by Angers (1992), Chan et al. (1994) and Yang and Wander (1998). However, unlike earlier studies, our experiment did not show a meaningful relation between macroaggregate stability and SWC. In the mineral N fertilization experiment, the greatest proportion of water-stable macroaggregates was found on the sampling date of July 2010. This fact contrasts with the SOC and water-stable macroaggregate C concentration on that sampling date, which showed their lowest values (Table 14). Perfect et al. (1990) studied the impact of soil moisture, roots and microbial biomass on temporal fluctuations in soil structural stability. They concluded that there was a dominant influence of soil water content on the stability of the soil surface. Water content at the time of sampling is clearly related to the forces of soil cohesion. Kemper and Rosenau (1984) found that soil cohesion decreased with an increase in soil water content. Also, Blanco-Moure et al. (2012) pointed out the deleterious effect of rains after long dry periods in the Mediterranean areas and demonstrated that slaking could be the dominant disaggregation process during these events.

In the Mediterranean cropping systems, the rainfall distribution pattern is bimodal, with the greatest peaks of rainfall in September-October and, to a lesser extent, in April-May (Austin et al., 1998). In these systems, during the spring months winter cereal crops are in the flowering stage of high water consumption. Thus, in spring 2010 and 2011, lower

SWC in the spring months could contribute to the greater proportion of water-stable macroaggregates found during these periods. Moreover, in the flowering period, root biomass could be considered at its maximum (Lampurlanés et al., 2001; Morell et al., 2011a), also enhancing the stability of soil macroaggregates (Jastrow et al., 1998). Jastrow and Miller (1997) also pointed out the role of root exudates in the stabilization of soil aggregates during their decomposition.

In a similar Mediterranean area and using a similar experiment to ours, Álvaro-Fuentes et al. (2007) studied the effects of soil tillage and cropping system on aggregate dynamics. They also found lower aggregate stability values in winter than in summer. However, they concluded that MBC was the main factor affecting water stability of aggregates. In our study, the relationship found between MBC and water-stable macroaggregates was low (data not shown). Perfect et al. (1990) also concluded that microbial biomass played a secondary role in controlling water aggregate stability during time one.

5. Conclusions

The application of organic fertilizers to the soil increased the proportion of water-stable macroaggregates. However, poultry manure did not provide greater macroaggregate stability than pig slurry and, in general, the application of none of these products increased the protection of C within aggregates or the total SOC concentration of the bulk soil. The use of increasing doses of mineral N fertilizer did not increase the stability of macroaggregates. The seasonal changes in soil macroaggregates stability had more impact than fertilization treatments (organic and mineral N). Our study demonstrates that, in the short-term, the use of organic or mineral N fertilizers hardly improves the stability of the macroaggregates and their C-protective capacity when NT is performed. This fact could be related to the limitations imposed by water in the Mediterranean areas and the buffering effect of long-term NT adoption on soil aggregate stability and C protection.

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Capítulo 4

Soil management effects on greenhouse gases production at the macroaggregate scale

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Soil management effects on greenhouse gases production at the macroaggregate scale

Abstract

Agricultural management practices play an important role in greenhouse gases (GHG) emissions due to their impact on the soil microenvironment. In this study, two experiments were performed to investigate the influence of tillage and N fertilization on GHG production at the macroaggregate scale. In the first experiment, soil macroaggregates collected from a field experiment comparing various soil management systems (CT, conventional tillage; NT, no-tillage) and N fertilization types (a control treatment without N and mineral N and organic N with pig slurry treatments both at 150 kg N ha^{-1}) were incubated for 35 days. Methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) production was quantified at regular time intervals by gas chromatography. In the second experiment, the effects of fertilization type and soil moisture on the relative importance of nitrification and denitrification processes in N_2O emission from soil macroaggregates were quantified. Nitrate ammonium, macroaggregate-C concentration, macroaggregate water-stability, microbial biomass-C and N (MBC and MBN, respectively) and water-soluble C (WSC) were determined. While NT macroaggregates showed methanotrophic activity, CT macroaggregates acted as net CH_4 producers. However, no significant differences were found between tillage systems on the fluxes and cumulative emissions of CO_2 and N_2O. Greatest cumulative CO_2 emissions, macroaggregate-C concentration and WSC were found in the organic N fertilization treatment and the lowest in the control treatment. Moreover, a tillage and N fertilization interactive effect was found in macroaggregate CO_2 production: while the different types of N fertilizers had no effects on the emission of CO_2 in the NT macroaggregates, a greater CO_2 production in the CT macroaggregates was observed for the organic fertilization treatment compared with the mineral and control treatments. The highest N_2O losses due to nitrification were found in the mineral N treatment while denitrification was the main factor affecting N_2O losses in the organic N treatment. Our results suggest that agricultural management practices such as tillage and N fertilization regulate GHG production in macroaggregates through changes in the proportion of C and N substrates and in microbial activity.

1. Introduction

The production and consumption of soil greenhouse gases (GHG) is mediated by several microbial processes (Conrad, 1996). For instance, soil carbon dioxide (CO_2) emissions are the result of microbial heterotrophic respiration while methane (CH_4) is normally oxidized by methanotrophic bacteria in aerobic soils (Goulding et al. 1995). Furthermore, soil nitrous oxide (N_2O) production is the result of nitrification and denitrification processes (Blackmer et al., 1980; Firestone et al., 1980; Poth and Focht, 1985). Those microbial processes are regulated by the physical protective capacity of aggregates that limit decomposition of organic C and N compounds (Elliott, 1986). Soil aggregates not only protect C and N, but they also regulate both the structure and the activity of the soil microbial community (Gupta and Germida, 1988; Miller et al., 2009). The intra-aggregate distribution of pores plays a major role in microbial access to oxygen, substrates and water. As Young and Ritz (2000) pointed out, soil structure regulates oxygen diffusion to habitat sites, depending on the connectivity and tortuosity of pore pathways. The aggregate architecture also controls the distribution of water films within soil matrix, affecting microbial microhabitats. Thus, the diffusion of oxygen to the center of aggregates will depend on the spatial arrangement of water films (Young and Ritz, 2000). The last factors affect the importance of denitrification and respiration activities and demonstrate the role played by soil aggregates regulating them (Beare et al., 1994; Estavillo et al., 2002). Moreover, due to their physical protective capacity, soil aggregates also regulate the microbial accessibility to substrates.

In a recent experiment, Lenka and Lal (2013) have suggested that the aggregate hierarchy theory of Tisdall and Oades (1982) could be extended to describe the effect of soil aggregation on GHG emission from soil. That theory postulates that the nature of the organic binding agents (transient, temporary and persistent) regulates different hierarchical stages of aggregation. Microaggregates are formed by the joining of primary particles and silt-sized aggregates and persistent organic binding agents, while these microaggregates are bound together into macroaggregates by temporary and transient organic binding agents. These organic materials are protected by the heterogeneity of the soil microenvironment which limits the access of decomposers and their enzymes (Schmidt et al., 2011; Ananyeva et al., 2013).

The agricultural practices play an important role in GHG emissions due to their effects on the soil microenvironment. Tillage breaks soil aggregates leading to enhanced

organic matter decomposition (Álvaro-Fuentes et al., 2008; Beare et al., 1994) and reduced C and N concentration (Plaza-Bonilla et al., 2010). Contrarily, the use and maintenance of no-tillage (NT) increases the stability of soil macroaggregates (Plaza-Bonilla et al, 2013b), a fact that could lead to a reduction in heterotrophic respiration due to a greater substrate protection, thus limiting the emissions of CO_2. Likewise, CH_4 production is also affected by tillage management. For instance, in a wheat-fallow rotation, Kessavalou et al. (1998) reported higher CH_4 uptake rates under NT when compared with a plough treatment. Also, Hütsch (1998a) reported 4.5-11 times greater CH_4 oxidation rates under NT than under conventional tillage (CT). Ball et al. (1999) hypothesized that the reduction in CH_4 oxidation usually found when tillage is performed could be due to the disturbance of the methanotrophic microbes by tillage, the changes in gas diffusivity or a long-term damage to methanotrophs due to disruption of soil structure. Tillage also has an impact on N_2O emissions. Estavillo et al. (2002), studying the effects of ploughing a permanent pasture on the emissions of this gas, observed an increase in both soil organic N mineralization and N_2O production rates from nitrification and denitrification processes after the breakage of soil aggregates by tillage.

Nitrogen fertilization has a strong impact on soil aggregation and C and N protection. The application of organic fertilizers such as pig slurry enhances the proportion of easily-decomposable C fractions (Morvan and Nicolardot, 2009) that could act as substrates for the denitrification process and the concomitant soil N_2O emissions to the atmosphere (Burford and Bremnner, 1975). Sexstone et al. (1985) quantified the diffusion of oxygen within soil aggregates establishing a relationship between their size and their potential to act as denitrifying microsites within soil. Nitrogen fertilization also plays a major role in methane oxidation. Different authors (Hütsch et al. 1993; Mosier et al. 1991; Steudler et al. 1989), working with incubated soil cores from agricultural, grassland and forest experiments, observed a decrease in CH_4 uptake when applying inorganic N to soil. Contrarily, recent findings suggest that ammonium-based fertilizers could stimulate the activity of methanotrophs (Bodelier and Landbroek, 2004).

In recent years, different experiments have been performed to analyze the effects of aggregate size on CO_2, CH_4 and N_2O production (Diba et al., 2011; Drury et al., 2004; Kimura et al., 2012). However, inconsistent results have been observed in the literature

due to the simultaneous diverse microbial processes that soil aggregates can hold (Sey et al., 2008). For instance, Parkin (1987) related the spatial heterogeneity in the N_2O emissions usually observed in most experiments with the presence of particulate organic matter within soil aggregates. Those studies demonstrate that different aggregate attributes such as size or C fractions within them regulate GHG production processes. However, few experiments have studied the effects of agricultural management practices on soil GHG production at the aggregate scale.

Thus, the objectives of this study were: (i) to analyze the effect of the use of different types of tillage and N fertilization on the production of GHG by soil macroaggregates and, (ii) to quantify the relative importance of the nitrification and denitrification processes on the macroaggregate emissions of N_2O depending on the type of fertilizer used. We hypothesized that (i) CT macroaggregates would emit a greater amount of GHG due to their lower protection of the organic C and N compounds when compared to NT macroaggregates and (ii) the application of pig slurry and mineral N would result in different rates of GHG production provided by soil macroaggregates.

2. Materials and Methods

Soil samples were collected from an experimental field established in 2010 in Senés de Alcubierre, NE Spain (41º 54' 12'' N, 0º 30' 15'' W), in an area with a temperate continental Mediterranean climate. This field experiment has a randomized block design with three replications comparing different tillage systems and N fertilization treatments. Two tillage systems (CT, conventional tillage with disk ploughing and NT, no-tillage) and two types of N fertilizers (mineral N with ammonium nitrate and ammonium sulphate and organic N with pig slurry), with three N doses (0, 75 and 150 kg N ha^{-1}), were compared. Each year, in the CT treatment, tillage is performed right before the seeding of barley (*Hordeum vulgare L.*) with one pass of a disk plough to 20 cm depth in October, after the application of organic and mineral fertilizers. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. Mineral N fertilizer was manually applied. The treatment with 150 kg N ha^{-1} was split into two applications: half of the dose before tillage as ammonium sulphate (21% N) and the other half at the beginning of tillering, in February, as ammonium nitrate (33.5% N). For the 75 kg N ha^{-1} treatment the entire dose was applied at tillering as ammonium nitrate. Equally, in the treatments with organic fertilization, the 75 kg N ha^{-1} rate was applied entirely at tillering and the 150 kg N ha^{-1} one was split into two applications, one half before tillage and the other half at tillering. The organic fertilization treatment consisted of the application of pig (*Sus scrofa*) slurry from a commercial farm in the area. The slurry was conventionally surface-spread using a commercial vacuum tanker fitted with a splashplate. The machinery was previously calibrated to apply the precise dose after analyzing the pig slurry. The main edaphoclimatic characteristics of the experimental site are listed in Table 17. Prior to the establishment of the experiment the field was conventionally tilled and fertilized with mineral N for four decades. In 2008, the field was under no-tillage. The cropping system is a continuous barley monoculture.

Table 17 General characteristics of the experimental site. Soil properties were measured in the Ap horizon (0-30 cm depth) at the beginning of the experiment.

General site characteristics	
Elevation (masl)	395
Mean air temperature (ºC)	13.4
Annual precipitation (mm)	327
Annual ETo (mm)	1197
Soil classification†	Typic calcixerept
pH (H_2O, 1:2.5)	8.0
Organic C (g kg^{-1})	15.6
Organic N (g kg^{-1})	1.4
EC 1:5 (dS m^{-1})	1.0
CaCO3 eq. (%)	28.9
Particle size distribution (%)	
Sand (2000-50 µm)	6.2
Silt (50-2 µm)	63.3
Clay (<2 µm)	30.5

†According to the USDA classification (Soil Survey Staff, 1994).

2.1 Experiment 1: GHG production from soil macroaggregates under different tillage and N fertilization treatments.

Soil samples were obtained from both tillage treatments (CT and NT) and the lower (0 kg N ha^{-1}, Control) and the higher (150 kg mineral N ha^{-1}, Mineral, and 150 kg organic N ha^{-} with pig slurry, Organic) fertilization treatments of the field experiment. Soil sampling was performed in March 2012 during the late tillering stage of the crop, three weeks after the top-dressing application of fertilizers. In each plot (i.e., tillage and N fertilization treatments), soil samples were collected from four areas that correspond to the four replications. From each sampling area, a composite sample of approximately 500 g was taken from the 0-5 cm soil depth using a flat spade and outside the wheel tracks areas. Afterwards, the samples were stored in crush-resistant airtight plastic containers for 3-4 hours. Once in the laboratory, the samples were gently passed through an 8mm sieve and air-dried at room temperature. Soil macroaggregates (0.250-8 mm) were obtained placing the 8-mm soil sieved sample on the top of a 0.250 mm sieve in an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain). A sieving time of 1 min and the lowest power program of the device were used to avoid macroaggregate breakage. The dry-sieved macroaggregates (0.250-8 mm) obtained were stored in aluminium trays taking care to avoid any breakage until further analyses.

Samples of 40-g each of dry-sieved macroaggregates (0.250-8 mm) were placed in 500 ml Mason jars (Fig. 14). Four jars were built for each tillage and N fertilization combination. A stainless steel fitting turned to accommodate two silicon-Teflon septa

was inserted in the lid of each jar to ensure air tightness. A volume of 12.8 ml of distilled water was added to each macroaggregate sample using a micropipette in order to avoid the breakage of the macroaggregates when adding the water, and also to obtain a gravimetric moisture content of about 32%. This value corresponds to the field capacity of the bulk soil of our experiment according to Saxton and Rawls (2006). All the jars were covered with a layer of parafilm which was pinpricked to ensure air exchange and avoid sample desiccation during the incubation process. The weight of the jars with the wet macroaggregate samples was recorded and then every 48 hours to check for water evaporation. Distilled water was added when needed. Air samples were withdrawn at 0_(0), 4_(0.17), 12_(0.5), 24_(1), 48_(2), 72_(3), 192_(8), 384_(16), 504_(21), 672_(28) and 840_(35) hours_(days) after the beginning of the incubation process. The parafilm layer of each jar was removed 15 min prior to each gas sampling. Then all the lids were tightly closed and a 15 ml headspace gas sample was withdrawn with the use of a gas-tight syringe, pumping twice before the extraction to ensure a total mixing of the gas in the jar (0 min sampling). Afterwards, 15 ml of ambient air were injected in the jars to compensate for the volume previously withdrawn. A second gas sampling was performed 60 min later. The gas samples obtained were injected in 12 ml Exetainer borosilicate glass vials (model 038W, Labco, High Wycombe, UK) until their analysis. Once the samplings were made (i.e., after 60 min) the lids were opened and the jars were covered with a parafilm layer until the next sampling event. Also, the jars were covered during the incubation to avoid light exposure. As explained in the next section, the difference in GHG concentration between 0 and 60 min samplings was used to calculate the GHG fluxes.

Figure 14 Soil macroaggregates (0.250-8 mm) incubation in 500 mL jars.

2.1.1 Gas and soil analysis

The gas samples were analyzed with an Agilent 7890A gas chromatography system equipped with an electron capture detector (ECD) and a flame ionization detector (FID) plus methanizer, and three automated valves to obtain the three gases of interest (i.e., CH_4, CO_2 and N_2O) for each gas sample injection (Fig. 15). A HP-Plot Q column (30 m long, 0.32 mm of section and 20 µm) was used along with a 15 m long pre-column of the same material. The injector and the oven temperature were set to 50ºC. The temperature of the FID and ECD detectors was set to 250ºC and 300ºC, respectively. The methanizer temperature was set to 375ºC. For the FID detector, H_2 was used as a carrier gas and N_2 as a make-up gas at 35 and 25 ml min^{-1}, respectively. In the case of the ECD detector, 5% methane in Argon was used as a make-up gas at 30 ml min^{-1}. The volume of sample injected was 1 ml. The system was calibrated using analytical grade standards (Carburos Metálicos, Barcelona, Spain).

Figure 15 Gas chromatography system for the quantification of soil GHG in air samples.

Soil CH_4, CO_2 and N_2O production in the jar headspace was calculated according to Holland et al. (1999). Gas concentrations (ppm) obtained with the chromatography system were converted to mass units with the ideal gas equation:

$$C_m = (C_v \times M \times P)/(R \times T)$$

where C_m is the mass/volume concentration (e.g., mg CO_2-C m^{-3} incubation jar headspace), C_v is the volume/volume concentration (ppm of each GHG obtained with the chromatography system), M is the molecular weight of each GHG (e.g., 12 g CO_2-C

mol^{-1} or 28 g N_2O-N mol^{-1}), P is atmospheric pressure, R is the universal gas constant and T is the incubation temperature (298 K). C_m was multiplied by the headspace volume of the incubation jars (5 x 10^{-4} m^{-3}) to obtain the mass of CH_4-C, CO_2-C or N_2O-N accumulated during the incubation. Thus, the mass of GHG produced (e.g., mg CH_4-C kg^{-1} macroaggregates h^{-1}) is calculated as follows:

$$f = ((C_1-C_0)/(m \times t)) \times 1000$$

where f is the mass of gas produced per unit of time, C_1 and C_0 are the mass of C or N produced at the end and at the beginning of two consecutive samplings, respectively, m is the mass of air-dried macroaggregates in each jar (0.04 kg) and t is the incubation period (1 h). Finally, the cumulative production of CH_4-C, CO_2-C and N_2O-N was calculated using the trapezoid rule by linear interpolation between two consecutive samplings.

Additionally, the initial mineral N (i.e., nitrate and ammonia), the C concentration, and the proportion of water-stable macroaggregates were quantified for each experimental unit. Once the incubation was finished, the microbial biomass-C and microbial biomass-N (MBC and MBN, respectively), the nitrate and ammonia content, the water-soluble C (WSC) and the C concentration of each 40 g macroaggregates sample were also quantified. Soil nitrate (NO_3^-) and ammonium (NH_4^+) were determined extracting 10 g of macroaggregates with 80 ml of 1 M KCl and using a continuous flow autoanalizer (Seal Autoanalyzer 3). The macroaggregate-C concentration was quantified by the wet oxidation method of Walkley-Black described by Nelson and Sommers (1996), with a modification to increase the digestion of soil organic carbon (SOC), which consisted in boiling the sample and the extraction solution at 150ºC for 30 min (Mebius, 1960). The proportion of water-stable macroaggregates and their sand content were determined following a modification of the method of Elliott (1986) as described in Plaza-Bonilla et al. (2013a). The microbial biomass-C and microbial biomass-N were determined with the chloroform-fumigation and direct extraction method of Vance et al. (1987) (Fig. 16). The extracts were analyzed for organic C and N with a multi C/N TOC-TNB analyzer 3100 (Analytik Jena, Jena, Germany). The extraction coefficient applied for both C and N was 0.38 (Sparling and Zhu, 1993; Vance et al., 1987). The WSC was extracted by shaking 10 g of macroaggregates in 40 ml of distilled water with 0.5 g potassium sulphate in a centrifuge tube for 30 min, centrifuging for 5 min at 5000 rpm and filtering

all supernatant solution through a Whatman no.42 filter. The organic C in the filtrate was determined by the same device used for the MBC-MBN determination.

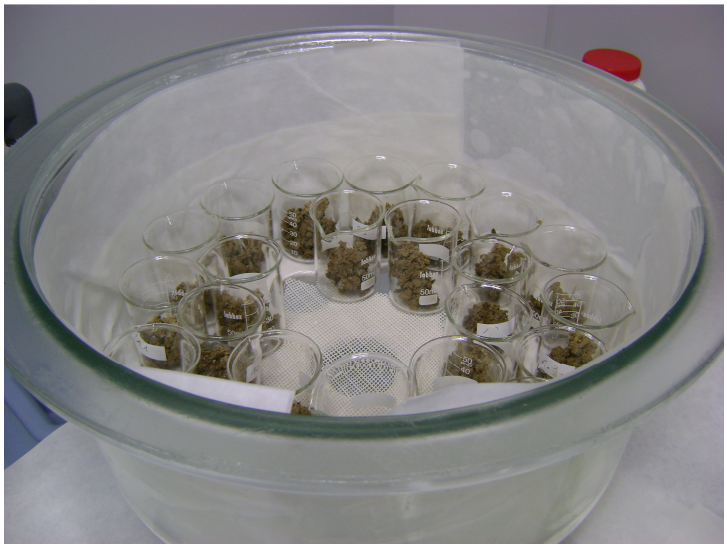

Figure 16 Determination of the microbial biomass carbon (MBC) after the incubation of soil macroaggregates

2.1.2 Data analysis

Cumulative GHG data were log-transformed and analyzed using the SAS statistical software (SAS Institute Inc., 1990). To compare the effects of tillage, fertilizer treatments and sampling time on cumulative GHG emissions, a repeated measures analysis of variance for a bifactorial design was performed for each gas. When significant, differences among treatments were identified at the 0.05 probability level of significance using an LSD test. For each sampling time, the linear relationship between CO_2 and N_2O production in the CT and NT macroaggregates was determined with the statistical package JMP 10 (SAS Institute Inc, 2012). To analyse the relationship between CO_2 production, proportion of water-stable macroaggregates and their C concentration, a stepwise regression was performed using the statistical package JMP 10 (SAS Institute Inc, 2012).

2.2 Experiment 2: Relative importance of nitrification and denitrification in N_2O production from soil macroaggregates under different N fertilization types.

Soil samples from the 0-5 cm soil depth were collected in the same fertilization treatments as in Experiment 1 (i.e., 0 and 150 kg N ha^{-1} as mineral N and pig slurry), only under NT. However, for this experiment soil sampling was performed on

December 2012, three weeks after the pre-seeding fertilization of the crop. In each plot (i.e., N treatment), six areas that would correspond to the six replications of the experiment were defined.

From each area, a 500 g composite soil sample was taken from the 0-5 cm soil depth using a flat spade, taking care to avoid the wheel track areas. Dry-sieved macroaggregates fractionation was analogous to Experiment 1. The experimental set up consisted of three N fertilization types (0 kg N ha^{-1}, mineral N at 150 kg ha^{-1} and organic N with pig slurry at 150 kg ha^{-1}), two soil moisture treatments (15% and 30 % gravimetric water content) and three levels of acetylene (0%, 0.01% and 5%, v v^{-1}). Each combination of the three factors was repeated six times according to the experimental replications. Therefore, the total number of observations was 108. To achieve this number of observations, the dry-sieved macroaggregates from each experimental replication was divided in six subsamples of 40 g that were placed in Mason jars. These six subsamples were divided in two groups. In the first group, three subsamples were moistened with distilled water to 15% gravimetric water content. The other three subsamples were moistened to 30% gravimetric water content. The lids of the jars were closed and a 15 ml headspace gas sample was taken for every jar (0 min sampling). Afterwards, for each soil moisture treatment three acetylene (C_2H_2) treatments were applied: 0%, 0.01% and 5% (v v^{-1}) corresponding to partial pressures of 0 Pa, 10 Pa and 5000 Pa, respectively) following the method proposed by Klemedtsson et al. (1988) to differentiate the relative contribution of the nitrification and denitrification processes in N_2O emissions. Different drawbacks of the method have been reported in the literature. Among them, Baggs (2008) enumerates (i) a possible underestimation of denitrification by preventing the supply of nitrifier-NO_3^-, mainly in aquatic systems (Groffman et al., 2006), (ii) acetylene could be used as a C-substrate for denitrification, and (iii) a limited diffusion of acetylene into fine textured soils. However, acetylene-based methods still have a role in systems with high NO_3^- concentrations (Groffman et al., 2006), such as the agricultural soil of our experiment, and are useful for comparative purposes between different treatments (Estavillo et al., 2002). According to each treatment, different volumes of ambient air were injected to equilibrate the pressure into the jars. The jars with the macroaggregates were incubated at 25ºC for 24 hours. After that, another 15 ml gas sample was withdrawn to calculate the accumulation of N_2O in the 24 hours period for each jar. Air gas samples were

stored and analyzed following the same methodology as in Experiment 1. It was assumed that the N_2O measured in the treatment without acetylene (i.e., 0% C_2H_2,) corresponded to the N_2O produced by the nitrification and denitrification processes. In turn, the N_2O measured in the treatment with 0.01% C_2H_2 corresponded only to that produced during the denitrification process (Davidson et al., 1986) and, finally, the N_2O measured in the treatment with a C_2H_2 concentration of 5% corresponded to the N_2O produced due to a complete denitrification (Yoshinari et al., 1977). The production of N_2O by the nitrification process was calculated from the difference between the N_2O measured in the 0% and the 0.01% C_2H_2 treatments, while the production of N_2O by the denitrification process corresponded to the amount of N_2O measured in the 0.01% C_2H_2 treatment, and complete denitrification (i.e., N_2O that would be reduced to N_2) was calculated as the difference between the N_2O measured in the 5% and the 0.01% C_2H_2 treatments. The gas samples were analyzed with an Agilent 7890A gas chromatography system equipped with an ECD detector with the same parameters as in Experiment 1. Moreover, the mineral N content as nitrate and ammonium and the WSC were also determined prior to the incubation following the methodology described above.

2.2.1Data analysis

The N_2O production data were transformed using the Box-Cox procedure and analyzed using the SAS statistical software (SAS Institute Inc., 1990). To compare the effects of fertilizer treatments and soil moisture on N_2O production an analysis of variance was performed. When significant, differences among treatments were identified at the 0.1 probability level of significance using an LSD test. Furthermore, the linear relationship between WSC and N_2O production was determined with the statistical package JMP 10 (SAS Institute Inc., 2012)

3. Results

3.1 Experiment 1: GHG production from soil macroaggregates under different tillage and N fertilization treatments.

Tillage significantly affected the fluxes of CH_4 produced by soil macroaggregates. As an average of all the samplings performed during the incubation period, macroaggregates of the CT treatment acted as emitters of CH_4 while those under NT acted as a CH_4 sink (Table 18). Also, significant differences on cumulative CH_4 fluxes were observed between CT and NT (Fig. 17a). According to the data, the methanotrophic activity in the NT treatment began after the first 72 hours of macroaggregate incubation (Fig. 17a). In contrast to CH_4, no significant differences were found between tillage systems on neither the fluxes nor the cumulative emissions of CO_2 and N_2O (Table 18, Fig. 17b and c).

Nitrogen fertilization treatments did not affect the fluxes of CH_4 and N_2O (Table 18). Also, cumulative emissions of CH_4 and N_2O did not differ between N fertilization treatments (Fig. 18a and 18c). CO_2 followed a different trend, with greater average fluxes in the organic treatment (1669.4 µg CO_2-C kg macroaggregates^{-1} h^{-1}) when compared with the control (1217.5 µg CO_2-C kg macroaggregates^{-1} h^{-1}) and the mineral (1199.4 µg CO_2-C kg macroaggregates^{-1} h^{-1}) treatments (Table 18). Also, cumulative CO_2 emissions were the greatest under the organic fertilization treatment in the first 48 hours of the incubation, without differences between the control and mineral treatments (Fig. 18b). When the incubation was finished (i.e., after 840 hours), the organic treatment presented a greater cumulative CO_2 emission when compared with the mineral treatment, while the control presented intermediate values (Fig. 18b).

The interaction between tillage and N fertilization significantly affected the fluxes of CO_2 (Table 18). The different N fertilization treatments did not show different CO_2 fluxes for the NT macroaggregates, whereas the CT macroaggregates under organic fertilization emitted greater amount of CO_2 (1824.5 µg CO_2-C kg macroaggregates^{-1} h^{-1}) compared with the control (1021.4 µg CO_2-C kg macroaggregates^{-1} h^{-1}) and mineral (1155.1 µg CO_2-C kg macroaggregates^{-1} h^{-1}) fertilization treatments (Table 18).

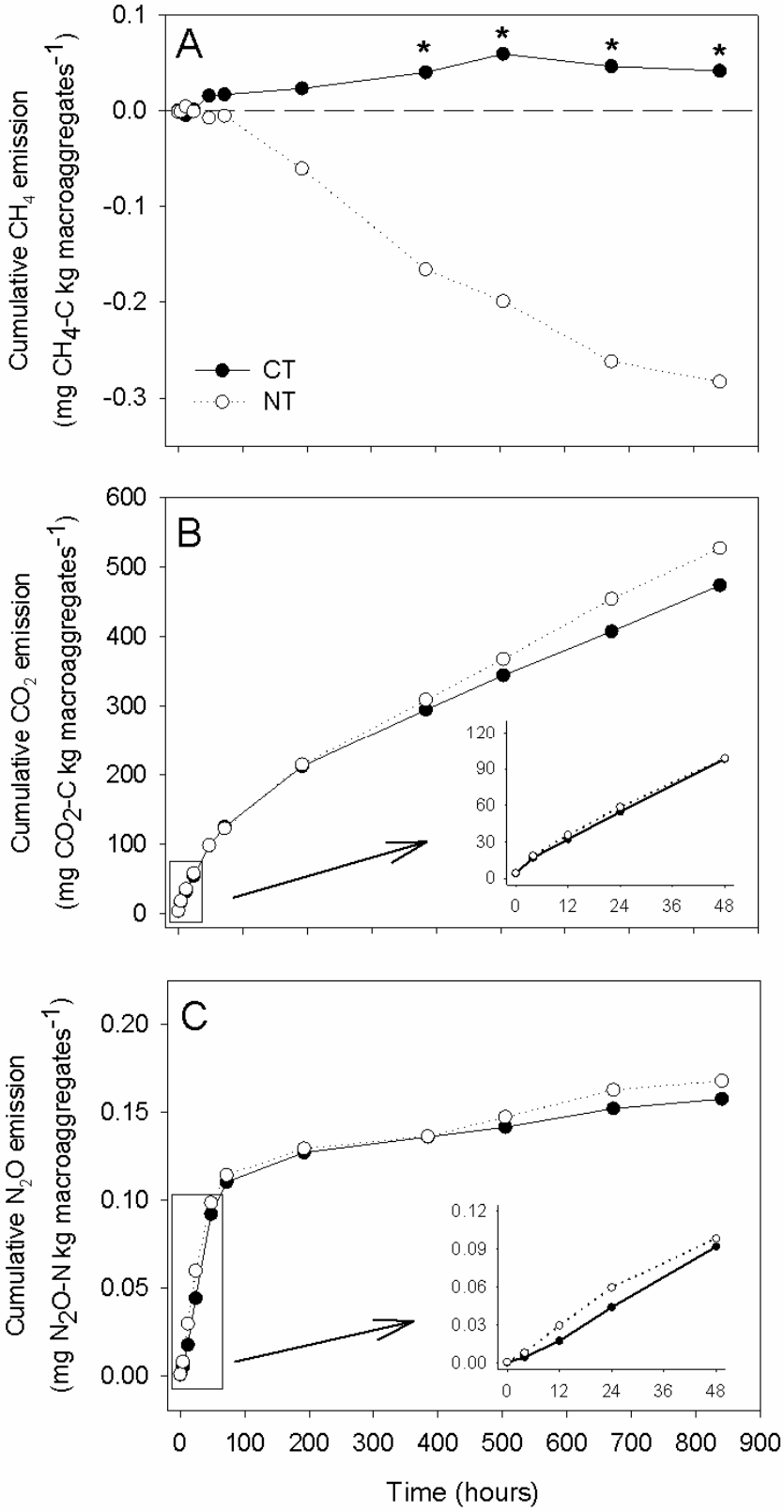

Figure 17 Cumulative CH_4 (A), CO_2 (B) and N_2O (C) production from dry-sieved macroaggregates (0.250-8 mm) as affected by tillage (CT, conventional tillage; NT, no-tillage). * For each sampling time, values are significantly different at $P<0.05$.

Table 18 Analysis of variance of the fluxes of CH_4, CO_2 and N_2O from dry-sieved macroaggregates (µg CH_4-C, CO_2-C and N_2O-N kg^{-1} macroaggregates h^{-1}, respectively) as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization treatments (0, control; mineral N at 150 kg N ha^{-1} and organic N with pig slurry at 150 kg N ha^{-1}), sampling time, and their interactions. Values are the means of all samplings (0, 4, 12, 24, 48, 72, 192, 384, 504, 672 and 840 hours after the beginning of the incubation).

Effects	CH_4 fluxes	CO_2 fluxes	N_2O fluxes
Tillage (T)	*	n.s.	n.s.
CT	0.073 a¶	1333.67	0.754
NT	-0.207 b	1406.19	0.919
N fertilization (N)	n.s.	*	n.s.
Control	-0.175	1217.45 b	0.556
Mineral	0.074	1199.36 b	0.866
Organic	-0.096	1669.39 a	1.042
Sampling time (t)	**	***	***
T x N	n.s.	*	n.s.
CT-Control	0.046	1021.40 d	0.458
CT-Mineral	0.214	1155.13 dc	0.676
CT-Organic	-0.040	1824.48 a	1.127
NT-Control	-0.470	1478.84 ab	0.686
NT-Mineral	-0.066	1243.58 bcd	1.056
NT-Organic	-0.153	1514.30 abc	0.956
T x t	***	n.s.	n.s.
N x t	n.s.	*	n.s.
N x T x t	n.s.	n.s.	n.s.

n.s.: not significant; *P<0.05; **P<0.01; *** P<0.001; ¶ For each gas and treatment, different letters indicate significant differences between treatments at P<0.05.

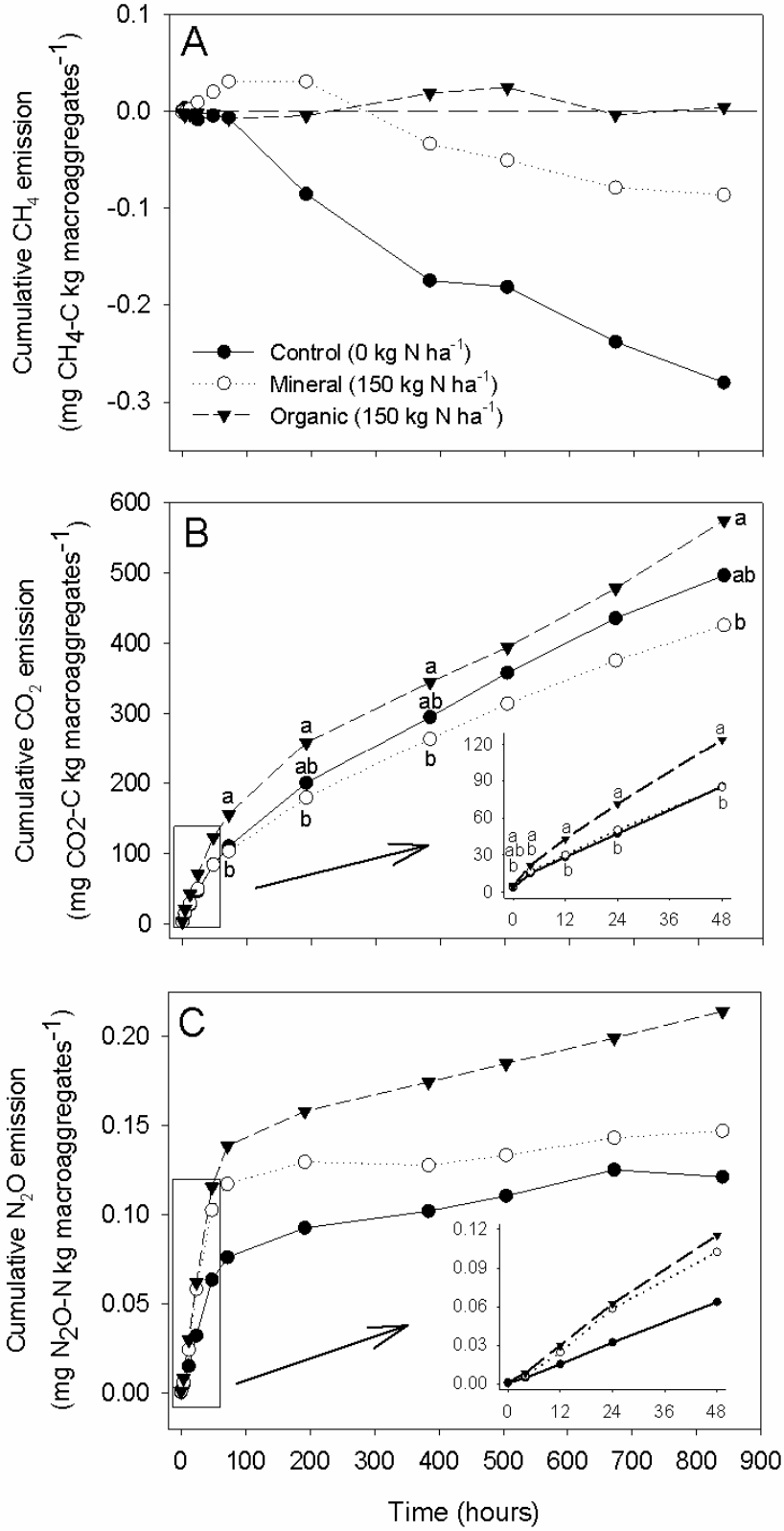

Figure 18 Cumulative CH_4 (A), CO_2 (B) and N_2O (C) production from dry-sieved macroaggregates (0.250-8 mm) as affected by N fertilization (0, control; mineral N at 150 kg N ha^{-1} and organic N with pig slurry at 150 kg N ha^{-1}). For each sampling time different letters indicate significant differences between N fertilization treatments at $P<0.05$.

No differences between tillage systems were found in the organic carbon (OC) concentration of dry-sieved macroaggregates before or after 840 hour incubation (Table 19). However, different results arose when analyzing the OC concentration in the soil macroaggregates under different N fertilization treatments. In this case, greater macroaggregate-C concentration was found in the organic fertilization treatment both before and after the incubation period when compared with the control and mineral treatments (Table 19). Nevertheless, the decrease in the OC concentration during the incubation was not statistically different between N fertilization treatments. Significant differences between tillage and N fertilization treatments were found on the initial NO_3^- concentration of the macroaggregates (Table 19). A greater initial NO_3^- concentration was found in the CT treatment than in the NT treatment, with 110.4 and 92.6 mg NO_3^--N kg^{-1} dry-sieved macroaggregates, respectively. In the case of N fertilization, the mineral treatment showed the greatest initial NO_3^- concentration while the control presented the smallest one and the organic treatment intermediate values. After the incubation period (i.e., 840 hours) the mineral and organic fertilization treatments showed greater NO_3^- concentration when compared with the control. Also, a significant interaction between tillage and N fertilization was found on this variable. Significant differences between tillage and N fertilization treatments were also found on the NO_3^- concentration variation (0 vs. 840 hours). In this case, the NT-control treatment presented the greatest increase in the NO_3^- concentration in the macroaggregates, followed by the CT-control treatment (Table 19). Differences between N fertilization treatments were also found on the initial NH_4^+-N concentration and its variation during the incubation process. The organic treatment presented the greatest values, followed by the mineral and the control treatments (Table 19). The reduction of the NH_4^+-N concentration during the incubation period was higher in the fertilized treatments (about 87% and 93% reduction in the NH_4^+ concentration in the mineral and the organic treatments, respectively) compared with the control (about 32% reduction) (Table 19). Furthermore, no differences between treatments were found on the MBC content. However, a greater MBN content was found in the organic treatment compared with the mineral and the control treatments (Table 19). The WSC content after the incubation process was significantly affected by both tillage and N fertilization treatments. Thus, a greater WSC content was found under NT than under CT and in the organic N treatment compared with the mineral and control ones (Table 19).

Table 19 Organic C concentration (OC, g kg^{-1}) and mineral N content (nitrate, NO_3^-, and ammonium, NH_4^+, in mg kg^{-1}) of dry-sieved macroaggregates (0.250-8 mm) before (0 hours) and after (840 hours) incubation, microbial biomass C and N (MBC and MBN, respectively; mg C or N kg^{-1}) and water-soluble C (WSC; mg C kg^{-1}) after the incubation (840 hours), and % of variation of C, nitrate and ammonium during the incubation as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization treatments (0, control; mineral N at 150 kg N ha^{-1}; and organic N with pig slurry at 150 kg N ha^{-1}), and their interactions.

Treatment	0 hours			840 hours						% variation 840-0 hours		
	OC	NO_3^-	NH_4^+	OC	NO_3^-	NH_4^+	MBC	MBN	WSC	OC	NO_3^-	NH_4^+
CT	6.07 (0.8)	110.4 (83.1) a¶	20.1 (15.1)	5.78 (0.8)	147.9 (72.7)	1.7 (0.7)	865.8 (232.4)	228.2 (115.6)	197.2 (43.1) b	-6.41 (3.7)	59.0 (68.9) b	-79.0 (28.9) b
NT	6.16 (0.9)	92.6 (56.0) b	13.3 (12.4)	5.72 (0.8)	141.9 (58.3)	2.0 (0.4)	954.4 (236.9)	236.5 (142.5)	234.9 (71.9) a	-7.78 (5.6)	100.8 (92.5) a	-62.3 (35.9) a
Control	5.54 (0.4) b	24.1 (4.5) c	3.0 (0.7) c	5.21 (0.6) b	87.3 (56.9) b	2.0 (0.8)	893.0 (278.4)	177.0 (70.98) b	199.5 (16.7) b	-6.07 (5.1)	182.2 (58.8) a	-32.5 (30.6) a
Mineral	5.94 (0.9) b	180.0 (43.4) a	19.0 (11.5) b	5.46 (0.5) b	183.6 (66.3) a	1.8 (0.5)	977.9 (213.1)	182.6 (96.5) b	174.3 (33.3) b	-7.61 (5.3)	7.1 (37.9) c	-86.8 (10.4) b
Organic	6.87 (0.5) a	100.4 (16.4) b	28.1 (11.7) a	6.60 (0.6) a	163.6 (12.3) a	1.7 (0.4)	859.4 (219.9)	337.5 (140.1) a	274.3 (68.5) a	-8.05 (3.8)	65.9 (22.9) b	-92.7 (4.4) b
CT-Control	5.51 (0.2)	26.0 (5.5) d	3.4 (0.8)	5.19 (0.2)	104.3 (82.2)	1.9 (1.3)	688.3 (81.3)	123.2 (44.7)	191.7 (19.0)	-5.87 (3.3)	132.2 (30.2) b	-49.2 (35.7)
CT-Mineral	5.62 (0.3)	214.4 (28.7) a	19.5 (4.2)	5.29 (0.1)	179.5 (92.4)	1.3 (0.2)	1023.2 (272.6)	208.9 (50.9)	156.5 (18.4)	-5.61 (4.7)	-13.8 (45.8) e	-93.0 (1.1)
CT-Organic	7.08 (0.4)	90.9 (8.3) c	37.4 (6.4)	6.86 (0.5)	159.9 (6.1)	1.9 (0.2)	885.9 (203.6)	352.6 (92.6)	243.4 (31.7)	-9.08 (2.2)	77.0 (16.6) c	-94.8 (0.8)
NT-Control	5.56 (0.6)	22.2 (2.6) d	2.5 (0.3)	5.23 (0.8)	70.4 (5.8)	2.1 (0.1)	1097.8 (249.9)	230.8 (45.1)	207.3 (11.3)	-6.27 (7.0)	219.7 (43.9) a	-15.7 (13.0)
NT-Mineral	6.27 (1.2)	145.7 (20.7) b	18.6 (17.0)	5.62 (0.8)	187.8 (40.7)	2.3 (0.1)	932.5 (161.6)	156.2 (131.5)	192.1 (37.5)	-9.61 (5.6)	28.1 (10.1) de	-80.5 (12.1)
NT-Organic	6.66 (0.6)	109.9 (17.8) c	18.7 (6.5)	6.34 (0.6)	167.4 (16.7)	1.6 (0.5)	832.9 (263.6)	322.5 (191.4)	305.2 (86.0)	-7.36 (5.0)	54.7 (24.8) cd	-90.5 (5.6)

¶ For each variable, different letters indicate significant differences between treatments at $P<0.05$. Values between parentheses are the standard deviations of the mean.

A highly significant polynomial relationship (R^2= 0.72; P<0.001) was observed between the initial NH_4^+ concentration in the macroaggregates and the cumulative N_2O-N emission during the first 48 hours of incubation (Fig. 19). Furthermore, significant linear relationships were observed between CO_2 and N_2O production in six samplings in CT and in three samplings in NT (Table 20).

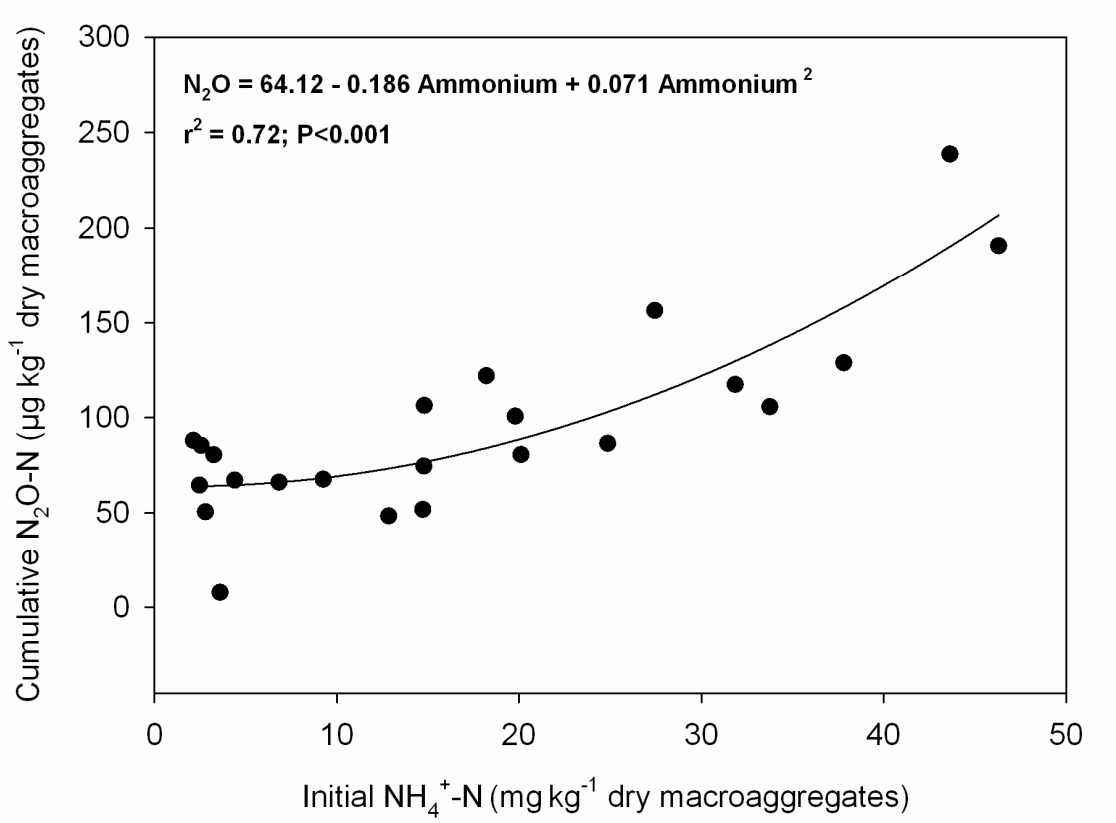

Figure 19 Regression analysis between the initial concentration of NH_4^+-N and the cumulative N_2O-N emissions after 48 hours of incubation of dry-sieved macroaggregates (0.250-8 mm).

Table 20 R^2 coefficients of the linear relationships between carbon dioxide (CO_2) and nitrous oxide (N_2O) production in conventional tillage (CT) and no-tillage (NT) macroaggregates at different times of the incubation period.

Tillage system	Sampling (hours)										
	0	4	12	24	48	72	192	384	504	672	840
CT	n.s.	0.71***	0.56**	0.61**	0.76***	0.33*	0.40*	n.s.	n.s.	n.s.	n.s.
NT	n.s.	0.72***	n.s.	n.s.	n.s.	n.s.	0.62**	n.s.	n.s.	n.s.	0.78***

n.s.: not significant; *P<0.05; **P<0.01; ***P<0.001

At the end of the incubation period, a greater proportion of water-stable macroaggregates was quantified under NT compared with CT (Fig. 20). Moreover, a significant interaction (P<0.05) between tillage and N fertilization was found on the water-stability of macroaggregates. While under NT no differences between fertilization treatments were observed in the proportion of water-stable macroaggregates, under CT a greater proportion of water-stable aggregates was found in the organic treatment when compared with the mineral treatment, with intermediate values in the control (Fig. 20).

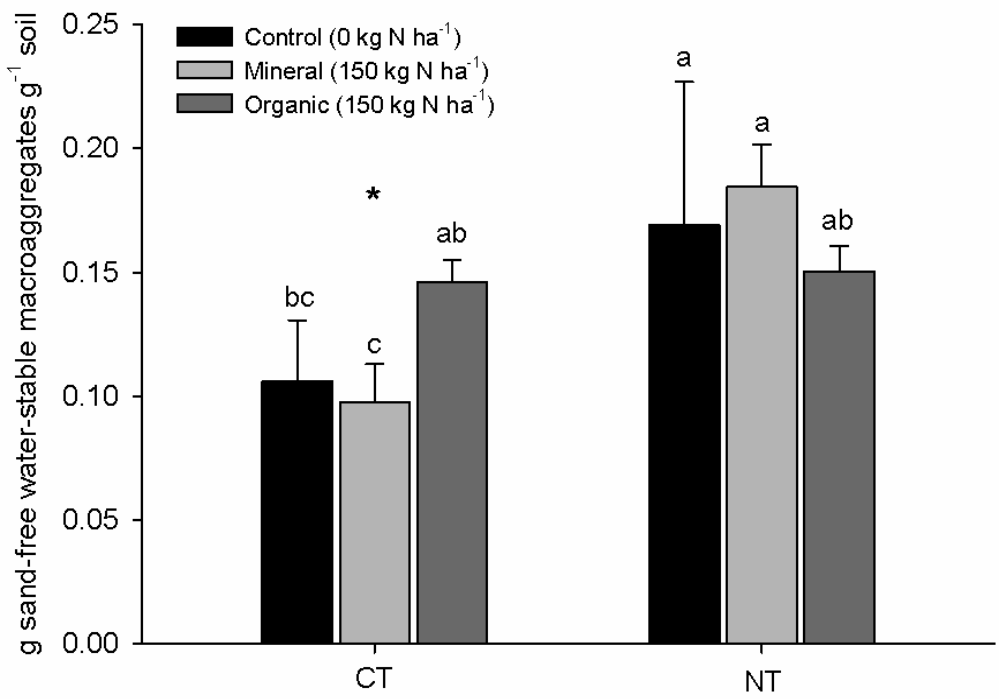

Figure 20 Proportion of sand-free water-stable macroaggregates (0.250-8 mm) as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization treatments (control without fertilization; mineral at 150 kg N ha^{-1}; organic at 150 kg N ha^{-1}) at the end of the incubation period (after 840 hours). Different letters indicate significant differences between tillage and fertilization treatments at *P*<0.05. * Indicate significant differences between tillage treatments at *P*<0.05.

3.2 Experiment 2: Relative importance of nitrification and denitrification on N_2O production from soil macroaggregates under different N fertilization types.

No differences between N fertilization treatments were found on NH_4^+ concentration of the macroaggregates before the incubation (Table 21). In contrast, before the incubation process, the fertilized treatments (mineral and organic) presented a greater NO_3^- concentration in the macroaggregates when compared with the control (Table 21). Moreover, significant differences between N fertilization treatments were found on the WSC concentration with greater values in the organic fertilization treatment when compared with the mineral and the control ones (Table 21).

Table 21 Mineral N content (nitrate, NO_3^-, and ammonium, NH_4^+, in mg kg^{-1}) and water-soluble C (WSC; mg C kg^{-1}) of dry-sieved macroaggregates (0.250-8 mm) before incubation, as affected by N fertilization treatments (0, control; mineral N at 150 kg N ha^{-1}, and organic N with pig slurry at 150 kg N ha^{-1}).

Treatments	NH_4^+	NO_3^-	WSC
Control	1.88	20.19 b¶	90.08 b
Mineral	2.13	85.22 a	92.45 b
Organic	2.58	88.96 a	114.47 a

¶ For each variable, different letters indicate significant differences between N fertilization treatments at $P<0.05$.

At the 15% moisture level, the incubation of the macroaggregates resulted in 173, 254 and 139 mg N_2O-N kg^{-1} h^{-1} for the control, mineral and organic treatments, respectively (Table 22). At the 30% moisture level, N losses reached 4751, 5552 and 4922 mg N_2O-N kg^{-1} h^{-1} for the control, mineral and organic treatments, respectively (Table 22). Both N fertilization and soil moisture content significantly affected the amount of N_2O-N produced due to the nitrification and denitrification processes (Table 22). The production of N_2O due to the nitrification process was greater in the mineral treatment when compared with the control, with intermediate values in the organic treatment. Different results were obtained in the production of N_2O due to the denitrification process. In this case, the organic treatment showed greater values than the control, while intermediate values were found in the mineral treatment (Table 22). Nevertheless, the production of N_2 due to a complete denitrification process was only affected by soil moisture, with the greatest values in the 30% moisture treatment when compared with the 15% moisture treatment (Table 22). Moreover, the production of N_2O due to nitrification and denitrification processes was 4.3 and 7.3 times greater in the 30% than

in the 15% moisture treatment, respectively (Table 22). No significant relationship was found between WSC and the amount of N_2O produced during the denitrification process (data not shown).

Table 22 Analysis of variance of the production of N_2O (mg N_2O-N kg^{-1} macroaggregates h^{-1}) from nitrification and denitrification processes and N_2 from denitrification process of dry-sieved macroaggregates as affected by N fertilization treatments (0, control; mineral N at 150 kg N ha^{-1}, and organic N with pig slurry at 150 kg N ha^{-1}), soil moisture (15 and 30% gravimetric water content), and their interactions.

Effects	Nitrification-N_2O	Denitrification-N_2O	Denitrification-N_2
N fertilization (N)	*	*	n.s.
Control	122.51 b¶	180.06b	1949.91
Mineral	455.25 a	303.12 ab	1436.76
Organic	247.90 ab	381.07 a	1752.38
Soil moisture (SM)	***	***	***
15%	115.06 b	69.68 b	5.32 b
30%	500.84 a	506.49 a	4120.01 a
NxSM	n.s.	n.s.	n.s.
Control – 15%	87.15	56.94	29.33
Control – 30%	193.22	303.18	4254.60
Mineral – 15%	159.37	73.39	21.0
Mineral – 30%	751.14	532.85	4268.27
Organic – 15%	60.13	78.70	0
Organic – 30 %	341.78	683.44	3896.46

n.s.: not significant; *P<0.1; **P<0.01; *** P<0.001; ¶ For each process, different letters indicate significant differences between N fertilization or moisture treatments at P<0.1.

4. Discussion

4.1 Effects of tillage and N fertilization on GHG production from soil macroaggregates

CH_4 was the only greenhouse gas produced by the macroaggregates that presented significant differences between tillage treatments. In the CT treatment macroaggregates acted as CH_4 producers, whereas macroaggregates of the NT treatment oxidized CH_4 mainly from the first 72 hours until the end of the incubation. Methanotrophic activity is reduced by anoxic conditions. In our experiment, an equal amount of water was added to the macroaggregates of both tillage treatments to bring them to the field capacity of undisturbed soil in our field experiment. Therefore, it could be hypothesized that differences in the intra-aggregate pore architecture and connectivity could have maintained a higher amount of aerobic microsites within the NT macroaggregates, thus facilitating the oxidation of CH_4. This hypothesis is in line with the findings of Kravchenko et al. (2013) who studied the effects of tillage on the intra-aggregate porosity of macroaggregates and observed higher intra-aggregate porosity >100 µm in NT macroaggregates when compared with CT macroaggregates. Another hypothesis could be the influence of the different types of tillage on the diversity of microorganisms within macroaggregates, which could have maintained a greater amount of methanotrophs in the NT treatment.

According to our results, no differences between tillage systems were found on the amount of macroaggregate-C mineralized as CO_2. That result could be related to the absence of differences in macroaggregate-C concentration prior and after the incubation. Different results were obtained by Fernández et al. (2010) when using soil of a long-term (14-yr) experiment. These authors observed higher production of CO_2 by NT macroaggregates when compared with CT macroaggregates and related this finding to the higher amount of organic C in the NT macroaggregates. Thus, in our experiment, the similar macroaggregate-C concentration found in the CT and NT treatments would have influenced the lack of differences in CO_2 production by macroaggregates.

Similarly to CO_2 production, no differences between tillage treatments were found on the fluxes and cumulative emissions of N_2O by soil macroaggregates. Although a higher initial NO_3^- concentration susceptible of being denitrified and a greater reduction in the concentration of NH_4^+ during the incubation were found under CT, no greater MBN content was found in this treatment when compared with NT. For that reason, the

hypothesis of a greater N immobilization under CT was not supported by our data. Another hypothesis could be a greater or complete denitrification under CT, in which the mineral N would be emitted as N_2. It is known that the $N_2/(N_2 + N_2O)$ ratio increases with decreasing O_2 concentration (Tiedje, 1988). That hypothesis would be in line with the smaller intra-aggregate porosity above referred and the related higher anaerobic conditions in CT macroaggregates.

Wrage et al. (2001) suggested that a greater soil organic matter content and better aggregate structure could facilitate O_2 diffusion, thus reducing the production of N_2O in NT soils. In our experiment, we found a greater water-stability of macroaggregates under the NT treatment when compared with the CT treatment as it has also been observed in other studies in the Mediterranean area (Álvaro-Fuentes et al., 2009; Plaza-Bonilla 2010, 2013b). However, the greater macroaggregate water-stability under NT was not followed by a lower production of N_2O as suggested by Wrage et al. (2001). Extrapolating our results to a structured soil, the greater macroaggregate water-stability found under NT could imply a more interconnected porous space in the soil matrix. This could lead to a greater aeration and reduced N_2O emissions in NT when compared with CT.

Macroaggregate CO_2 emissions were influenced by fertilization type: when the incubation was finished (i.e., after 840 hours), the macroaggregate CO_2 losses under the organic fertilization treatment were higher than under the mineral fertilization treatment. Moreover, the interaction between tillage and N fertilization types also affected the CO_2 produced by macroaggregates. Thus, under CT the CO_2 emissions were greater with the use of organic fertilizer compared with either the use of mineral fertilizers or in absence of fertilization. The application of pig slurry usually enhances the amount of readily decomposable C compounds in the soil (Arcara et al., 1999; Sánchez-Martín et al., 2008; Yang et al., 2003). This fact was observed in our experiment, in which a higher WSC content was measured in the macroaggregates of the pig slurry treatment. A similar trend was observed in macroaggregate water-stability. In this case, unlike the CT treatment, the NT treatment did not show an interaction with the type of fertilizer on macroaggregate water-stability. Contrarily, the application of organic fertilizer under CT led to greater proportion of water-stable macroaggregates than the control treatment. These findings suggest that the use of NT buffers the effects of the application of

organic fertilizers on the increase of macroaggregate stability (Plaza-Bonilla et al., 2013a).

We found significant linear relationships between the cumulative CO_2 production and (i) the macroaggregate-C concentration (R^2: 0.21; *P*: 0.016) and (ii) the proportion of water-stable macroaggregates (R^2: 0.19; *P*: 0.021). However, when both variables (i.e., macroaggregate-C concentration and proportion of water-stable macroaggregates) were included in a stepwise procedure in order to analyze their relationship with CO_2 production, no statistical significance was found. This finding suggests that the relationship found between CO_2 production and macroaggregate stability was due to a greater C concentration in those macroaggregates that are more water-stable resulting in a greater production of CO_2.

In contrast to tillage, the different N fertilization treatments had no significant effects on CH_4 emission. However, a trend (not significant) to CH_4 uptake could be observed in Figure 18a in the control treatment and near zero emissions in the organic treatment. Ammonium has been reported to be a competitive inhibitor of CH_4 oxidation (Whittenbury et al., 1970). Interestingly, the uptake of CH_4 that we observed in the NT and control treatments began after the first 72 hours of incubation and coincided with the reduction in the rate of N_2O emissions. Hütsch (1998b) pointed out that CH_4 metabolism only begins when the nitrification process is almost completed. That conclusion would explain the time-lapse that we found until the CH_4 uptake began in the NT and control treatments.

In our experiment, fertilization type did not lead to differences in the N_2O produced by soil macroaggregates. However, a trend to lower emissions under the control treatment and higher emissions under the organic fertilization with pig slurry was observed (Fig. 18c). It is already known that the denitrification process is intensified under the presence of easily decomposable C fractions such as WSC (Arcara et al., 1999). Thus, the application of organic wastes, such as animal manure, usually enhances N_2O emissions when compared with inorganic fertilizers (Heller et al., 2010) due to their easily decomposable C content and sufficient mineral N to activate the population of denitrifiers in soil (Johnson et al., 2007; Sánchez-Martín et al., 2008). The significant relationship that we found between CO_2 and N_2O emissions shows that that organic C decomposition acted as an electron donor for denitrifier bacteria (Kimura et al., 2012).

Besides, our results show the relationship between the initial ammonium concentration in soil macroaggregates and their N_2O production. That relationship could be explained by the role played by the NH_4^+ ion in the nitrification and denitrification processes. Ammonium oxidation is the first step in the nitrification process that produces NO_3^-, which in turn is the most important ion involved in the denitrification process.

4.2 N_2O production by soil macroaggregates as affected by the type of N fertilization

At 15% soil moisture, the nitrification process was the predominant N_2O producer, while at 30% soil moisture the denitrification process emitted nine times more N (as the sum of N_2O and N_2) than nitrification. These results agree with the conceptual model developed by Bouwman (1998) about N_2O emissions fractionation from nitrification and denitrification processes as a function of water-filled pore space. Although in small amounts, the denitrification process lead to N_2O production in the macroaggregates incubated at 15% soil moisture content. This could be related with the presence of anaerobic microsites within the macroaggregates. Sexstone et al. (1985) quantified oxygen profiles in wet aggregates and found anaerobic centers in all the aggregates that denitrified. However, aerobic denitrification could have also occurred. As in other studies (Bandibas et al., 1994; Diba et al., 2011; Liu et al., 2007), we observed an important increase of the N_2O evolved when doubling soil moisture. This finding demonstrates the role played by the absence of oxygen as electron acceptor on nitrification and denitrification processes (Bouwman, 1998).

The combination of higher concentration of NO_3^- and greater WSC in the organic N treatment would explain the greater amount of N_2O evolved due to the denitrification process when compared to the control treatment (Burford and Bremner, 1975; Mulvaney et al., 1997). Contrarily, the greater N_2O loss from nitrification in the mineral N treatment cannot be explained by a higher NH_4^+ concentration before the incubation. One hypothesis to explain this finding would be a greater organic N mineralization during the incubation that could have increased the amount of mineral N susceptible of being nitrified. Different authors have observed a greater mineralization in N fertilized soils compared with soils without N fertilization (Hatch et al., 2000; Zhang et al., 2012). Another hypothesis could be a more efficient nitrification process in the control treatment in comparison with the mineral treatment that would explain the lower N_2O emissions found in the control. Nemergut et al. (2008) and Ramirez et al. (2012) found changes in soil microbial community when using repeated application of mineral

fertilizer when compared to unfertilized soils. Thus, it could be hypothesized that the mineral fertilizer applications in our field experiment could have led to changes in the microbial community structure with higher nitrification efficiency in the unfertilized treatment. Furthermore, our data shows a similar N_2O emission in the mineral and the organic fertilization treatments. This suggests that readily decomposable C was not a limiting factor to denitrification in the mineral treatment. The lack of differences between fertilization treatments on macroaggregate N_2O production after the first 24 hours of incubation in Experiment 1 corroborates this hypothesis.

5. Conclusions

Tillage and N fertilization treatments affected the production of GHG at the soil macroaggregate scale due to changes in C and N substrates within macroaggregates. Moreover, the different methanogenic and methanotrophic activities found in the tillage treatments suggest changes in the microbial diversity within soil macroaggregates when either conventional tillage or no-tillage are used. Easily decomposable C compounds associated with the organic fertilization together with the presence of nitrate stimulated the denitrifying activity. The use of mineral and organic fertilizers leads to differences in the relative importance of the nitrification and the denitrification processes in the production of N_2O by soil macroaggregates: while N_2O losses due to the nitrification process were preponderant in the mineral fertilization treatment, denitrification N_2O losses had a higher importance under organic fertilization due to a higher presence of C-labile compounds. A significant effect of the interaction between tillage and N fertilization treatments on CO_2 production, with higher emissions under CT when applying organic fertilizers and no differences between types of fertilizers on CO_2 emissions under NT, demonstrated the capacity of NT aggregates to protect C. Our study shows that tillage and N fertilization and their interaction play a major role in GHG production from soil macroaggregates due to their impact on the soil mineral and organic substrates that regulate the microbial activity.

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Capítulo 5

Soil C fluxes, biomass-C inputs and soil carbon stocks as affected by tillage and N fertilization in dryland conditions

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Abstract

In Mediterranean areas the quantification of yield-scaled CO_2 and CH_4 emissions over a range of management practices is needed in order to identify their efficiency in terms of gas emitted per unit of mass produced. Moreover, there is a lack of knowledge about the impact of the interaction of tillage with different types and rates of N fertilizers on the fluxes of CH_4. In this study, CO_2 and CH_4 fluxes were measured in a long-term experiment and a short-term field experiment, which were established in 1996 and 2010, respectively. In the long-term experiment, two types of tillage (NT, no-tillage, and CT, conventional intensive tillage) and three N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared. In the short-term experiment, two tillage systems (NT and CT), three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of fertilizer products (mineral N and organic N with pig slurry) were compared. The emissions of CO_2 and CH_4 were measured with non-steady-state chambers during two (long-term experiment) and three (short-term experiment) cropping seasons. For each chamber and sampling, water-filled pore space and soil temperature were also determined. Above- and below-ground C inputs, soil organic carbon (SOC) stocks and grain yield were quantified for the three cropping seasons studied in the short-term experiment. Finally, for each experiment and treatment, the ratio between the CO_2 equivalents emitted (as CH_4 and CO_2) and grain yield was calculated. In both experiments, the soil acted as a CH_4 sink for most of the experimental period, except during pig slurry applications when sharp peaks of CH_4 and CO_2 fluxes were observed under NT. In both experiments, the NT treatment presented a greater mean CO_2 flux compared with CT. In the long-term experiment CH_4 oxidation was greater under NT than under CT, whereas contrary results were found in the short-term experiment. The fertilization treatments also affected CO_2 emissions in the short-term experiment, with the greatest fluxes measured when 75 and 150 kg organic N ha^{-1} as pig slurry were applied to the soil. Despite the larger CO_2 emissions under NT, the emission of CO_2 equivalents per kg of grain produced under NT was five and two times less than under CT in the long- and short-term experiments, respectively. In addition, while the lowest amount of CO_2 emitted per kilogram of grain was observed in the NT treatments with 75 kg mineral N ha^{-1} and with 150 kg organic N ha^{-1} (0.47 kg CO_2-equivalent kg $grain^{-1}$), the greatest value was observed in the CT treatment with 75 kg

mineral N ha^{-1} (1.64 kg CO_2-equivalent kg $grain^{-1}$), thus suggesting the importance of the analysis of the interaction between tillage and N fertilization. No differences between tillage and fertilization treatments were observed in SOC stocks after three years of contrasting management practices. In conclusion, our results demonstrate that the interaction between tillage and N fertilization practices plays a major role on the emissions of CH_4 and CO_2 and stress the importance of identifying the best combination of those practices in order to assure the highest environmental and productive sustainability of Mediterranean cropping systems.

1. Introduction

The agricultural sector is responsible for the 12-15% of the global anthropogenic emissions of greenhouse gases (GHG) (Smith et al. 2007). However, a large mitigation potential could be achieved by improving agricultural practices through a proper choice of crop and land management systems. Moreover, optimized agronomic practices can also increase the soil C sink, a process that was proposed in the Kyoto Protocol (United Nations, 1998) as a climate change mitigation measure (Smith, 2004). As a consequence, there is a strong need to quantify GHG emissions associated with different agricultural management systems to identify those practices with the smallest emission footprints while maintaining the environmental and economic sustainability of the agricultural activity.

Carbon dioxide (CO_2) and methane (CH_4) are two of the most important GHG due to their global warming potential (GWP) and long residence time in the atmosphere (IPCC 1995). Soil emission of gaseous C-compounds as CO_2 and CH_4 from agricultural systems represents a loss of soil organic carbon (SOC) that directly affects the fertility and sustainability of soils. Soil CO_2 emissions are the result of mineralization and respiration processes. However, only the respiration process represents a net C loss from the soil to the atmosphere due to its heterotrophic nature. The CO_2 produced by microbial activity diffuses through the soil pore system, being transported to the soil surface due to concentration gradients (Risk et al. 2008). Likewise, agricultural soils can also act as net emitters or oxidizers of CH_4 depending on the relative importance of the methanotrophy and methanogenesis processes (Hütsch, 2001). The methanotrophic process involves the microbial oxidation of CH_4 in aerobic conditions while the methanogenesis process entails the anaerobic digestion of soil organic matter (Le Mer and Roger, 2001). Although the last processes can occur simultaneously in arable ecosystems, upland soils usually act as net CH_4 oxidizers (Conrad, 1995).

In field crops, tillage and N fertilization are management practices that imply a significant investment by farmers. Consequently, these two practices have a greater potential for optimization and play a major role in the mechanisms that drive the production, transport and consumption of GHG in soils. During tillage operations, soil structure is greatly disturbed and the CO_2 contained in the soil pore system is lost due to a process known as degassing (Reicosky et al. 1997). Besides that physical release of gas, tillage also affects soil microbial activity due to changes in substrate availability

and micro-environmental conditions. For instance, tillage buries crop residues, thus increasing the contact between C-rich substrates and soil particles where greater soil moisture and nutrients are available (Balesdent et al. 2000; Paustian et al. 1997). On the other hand, tillage also accelerates the breakdown of soil aggregates, thus liberating the organic carbon protected within them and increasing its availability to soil microorganisms (Beare et al. 1994). Tillage can also influence the processes that regulate the emission and/or consumption of CH_4 due to its impact on soil water regime, soil structure and microbial diversity. For instance, Ball et al. (1999) hypothesized that no-tillage (NT) increases the oxidation of CH_4 due to the absence of soil disturbance, a greater gas diffusivity, and the reduction of damage to CH_4 oxidizers compared with conventional tillage (CT). In field experiments, a greater (Ball et al. 1999; Kessavalou et al. 1998) or equal (Alluvione et al. 2009; Piva et al. 2012; Sainju et al. 2012) CH_4 oxidative capacity under NT when compared with CT has been found. Other authors have reported an interaction between tillage and N fertilization treatments, with greater or lower CH_4 oxidation under NT depending on the type of fertilizer (Venterea et al. 2005).

Nitrogen fertilizer application affects the soil C stock and GHG emission. In a semiarid area of NE Spain, Morell et al. (2011) measured an increase in the amount of C sequestered in the soil after 15 years of mineral N fertilizer application due to a greater crop residue production. These authors also measured greater CO_2 emissions when applying mineral N in wet years, without differences between fertilized and unfertilized treatments during dry years. The type of fertilizer applied also has a large influence on soil CO_2 emissions (Ding et al. 2007). In a dryland area of Central Spain, Meijide et al. (2010) carried out an experiment comparing the effects of different fertilizers on CO_2 emissions during one barley growing season These authors did not find differences on CO_2 emissions when applying pig slurry, composted organic waste or urea to the soil. Different studies have shown that the addition of N reduces the uptake of atmospheric CH_4 by soil (Hütsch et al. 1993). This finding has been mainly related to a direct inhibition of CH_4 oxidation by the presence of ammonium in the soil (Conrad, 1996; Whittenbury et al. 1970). Also, the changes in soil gas diffusivity and the promotion of anaerobic microsites due to the enhancement of soil respiration when applying organic slurries can reduce the oxidation of CH_4 and increase its production (Meijide et al. 2010).

The main characteristic of rainfed Mediterranean agroecosystems is the scarcity of water available for crop growth (Cantero-Martínez et al. 2007), a limiting factor that also affects the crop response to N fertilization (Cantero-Martínez et al. 2003; Ryan et al. 2009). In those areas, the use of reduced tillage or no-tillage techniques has been pointed out as a promising strategy to increase the amount of SOC due to the increase in C inputs under NT in consonance with a higher soil water conservation and a greater physical protection of carbon within soil aggregates compared with CT (Cantero-Martínez et al. 2007; Álvaro-Fuentes et al. 2008a). Also, in Mediterranean Spain the application of animal waste to agricultural soils is a common practice, due to the intensive animal production in this area (Yagüe and Quílez, 2013).

Prior to the present study, Morell et al. (2011) studied in the same semiarid area the effect of different types of tillage and rates of mineral N on the emissions of CO_2. In turn, Meijide et al. (2010) quantified the emissions of both CO_2 and CH_4 under different types of organic and mineral fertilization. However, to date, no studies have been conducted in the Mediterranean area to investigate the impact of different tillage, N rates and fertilizer types on the emissions of CH_4 and CO_2 including their effect in the yield-scaled emissions of those gases.

As a consequence, our objective was to quantify the interactive effects of tillage and N fertilization type and rate on the emission of CH_4 and CO_2 and on biomass-C inputs and SOC stocks as well, in order to identify and implement greater environmentally sustainable practices while maintaining crop yields. We hypothesized that the interaction between tillage and N fertilization practices would affect crop performance and soil microbial activity and, consequently, the GHG emission and soil C storage in Mediterranean rainfed cropping systems.

2. Material and Methods

2.1 Sites and treatments description

2.1.1 Long-term experiment

A tillage and mineral N fertilization experiment was established in 1996 in Agramunt, NE Spain (41º48'36''N, 1º07'06''E, 330 masl). The climate in the area is Mediterranean temperate, with mean values of annual precipitation, annual air temperature and annual reference evapotranspiration (FAO Penman-Monteith methodology) of 430 mm, 13.8 ºC and 855 mm, respectively. The soil was classified as Typic Xerofluvent (Soil Survey Staff, 1975). Selected soil properties at the start of the experiment in the 0-30 cm layer were: pH (H_2O, 1:2.5): 8.5; electrical conductivity (1:5): 0.15 dS m^{-1}; organic C concentration: 7.6 g kg^{-1}; sand (2000-50 µm), silt (50-2 µm) and clay (<2 µm) content: 465, 417 and 118 g kg^{-1}, respectively. Two types of tillage (NT, no-tillage, and CT, conventional intensive tillage with moldboard plow) and three mineral N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared in a randomized block design with three replications. The medium N rate (60 kg N ha^{-1}) was chosen according to the productive potential in the area while the high N rate (120 kg N ha^{-1}) was chosen because it was the most common among farmers. Plot size was 50 m x 6 m. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. The CT treatment consisted of one pass of moldboard plow to 25 cm depth followed by one-two passes of a cultivator to 15 cm depth, both in September-October. Mineral N fertilizer was manually applied and split into two applications: one-third of the dose before seeding as ammonium sulphate (21% N) and the rest of the dose at the beginning of tillering, in February, as ammonium nitrate (33.5% N). The cropping system consisted of a barley (*Hordeum vulgare* L.*;* cv. Hispanic in the 1996-2010 period and cv. Cierzo in the 2010-2013 periods) monocropping, which is a traditional system in the area. Planting was performed in November with a direct drilling disk machine set to 2-4 cm depth and 17 cm between rows. Harvesting was carried out with a commercial medium-sized combine in June. The straw residue was chopped and spread over the soil by the same machine. The historical management of the field prior to the establishment of the experiment was based on conventional intensive tillage with moldboard plowing and winter grain cereals monoculture.

2.1.2 Short-term experiment

An experimental field was established in Senés de Alcubierre, NE Spain (41º 54' 12'' N, 0º 30' 15'' W, 395 masl) in 2010. Mean values of annual precipitation, annual air temperature and annual evapotranspiration (FAO Penman-Monteith methodology) in the area are 327 mm, 13.4 ºC and 1197 mm, respectively. The soil was classified as a Typic Calcixerept (Soil Survey Staff, 1975) (Fig. 21). Selected soil properties at the start of the experiment in the 0-30 cm depth were: pH (H_2O, 1:2.5): 8.0; electrical conductivity (1:5): 1.04 dS m^{-1}; organic C (g kg^{-1}): 15.6; organic N (g kg^{-1}): 1.4; sand (2000-50 µm), silt (50-2 µm) and clay (<2 µm) content: 62, 633 and 305 g kg^{-1}, respectively. The cropping system before and during the experiment consisted of a barley (cv. Meseta) monoculture. During the four years prior to the set-up of the experiment, soil management consisted of NT with mineral N fertilizer additions at rates of 75-100 kg ha^{-1}. Before that period two passes with a subsoiler or a chisel were used since the 1970's decade.

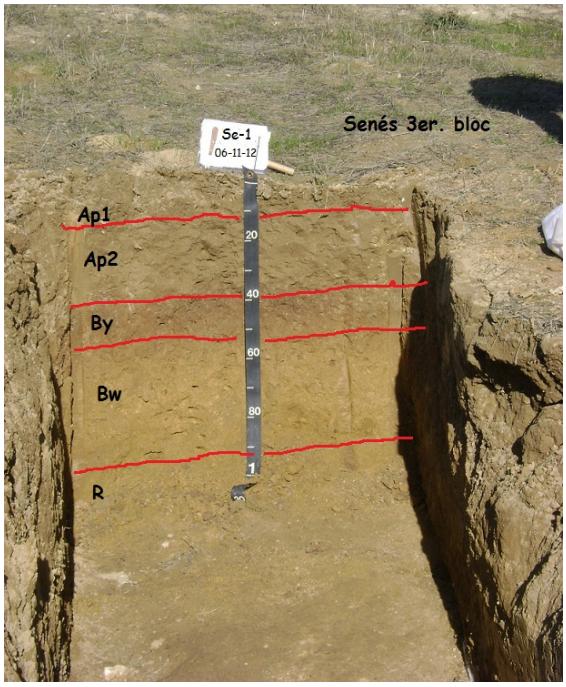

Figure 21 Soil profile of the short-term field experiment: the different soil horizons can be identified

Two tillage systems (CT, with two passes of chisel plowing, and NT), three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of fertilizer products (mineral N and organic N with pig slurry) were compared. The highest N rate (150 kg N ha) was chosen according to the most common dose applied by farmers in the area, while the

medium rate (75 kg N ha) was chosen to evaluate the possibility of reducing the N application rate without compromising crop yields. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) to control weeds before sowing. Mineral N fertilizer was manually applied. The treatment with 150 kg N ha^{-1} was split into two applications: half of the dose before tillage as ammonium sulphate (21% N) and the other half at the beginning of tillering, in February, as ammonium nitrate (33.5% N). For the 75 kg N ha^{-1} treatment the entire dose was applied at tillering as ammonium nitrate. Likewise, in the treatments with organic fertilization, the 75 kg N ha^{-1} rate was applied entirely at tillering and the 150 kg N ha^{-1} rate was split into two applications of 75 kg N ha^{-1} each, one before tillage and the other one at tillering. The organic fertilization treatment consisted in the application of pig slurry from a commercial farm of the area. The slurry was conventionally surface-spread using a commercial vacuum tanker fitted with a splashplate. The machinery was previously calibrated to apply the precise dose after analyzing the pig slurry composition (Fig. 22). Main characteristics of the pig slurry applied during the whole experimental period are shown in Table 23. Similarly to the long-term experiment, planting was performed in November with a disk direct drilling machine set to 2-4 cm depth and 17 cm between rows and harvesting was carried out with a commercial medium-sized combine in June. The straw residue was chopped and spread over the soil by the same machine. The experiment consisted of a randomized complete block design with three replications. Plot size was 40 x 12 m in the organic N fertilization treatment and 40 x 6 m in the mineral N fertilization treatment.

For both experiments, air temperature and rainfall observations were recorded on a daily basis using an automatic weather station located at each experimental site.

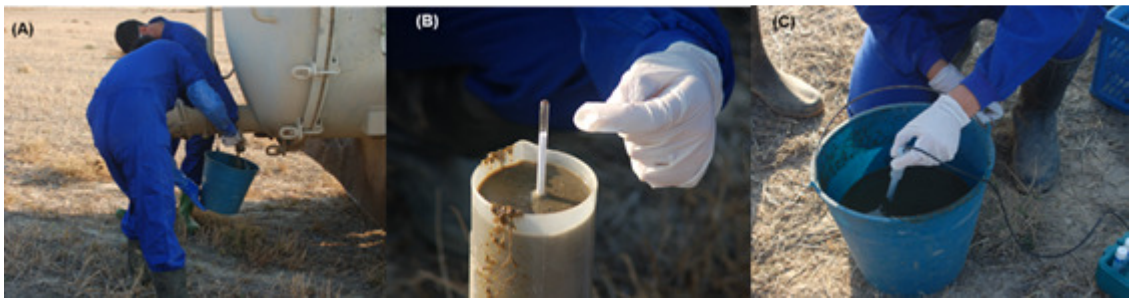

Figure 22 Taking pig slurry samples (A) for density (B) and electrical conductivity (C) quantification

Table 23 Composition of the pig slurry used in the organic fertilization treatment as pre-seeding and top-dressing applications in the short-term experiment during the three growing seasons studied (2010-2011, 2011-2012, 2012-2013) (values in g kg^{-1} dry weight).

Pig slurry characteristics	2010-2011		2011-2012		2012-2013	
	Pre-seeding	Top-dressing	Pre-seeding	Top-dressing	Pre-seeding	Top-dressing
Dry matter	45.0	94.0	19.0	19.5	56.0	138.0
Organic C*	412.5	nd	337.0	392.5	391.5	396.0
Kjeldahl N*	34.2	23.6	29.8	34.2	24.2	23.6
Ammonium N	44.5	33.0	104.9	125.5	36.4	42.5
P	18.7	19.3	16.9	17.1	16.8	18.7
K	22.6	18.2	77.4	81.45	27.9	26.5

* Values of the dry residue

nd: not determined

2.2 Gas sampling and analyses

Both CO_2 and CH_4 emissions were measured every two or three weeks with the non-steady-state chamber methodology (Hutchinson and Mosier, 1981), except during the fertilizer applications when a more intensive sampling was performed. In these particular moments, gas measurements were made the day prior to the application and 4 and 72 hours after the application. The measurement period covered three cropping seasons (2010-2011, 2011-2012 and 2012-2013) in the short-term experiment and two cropping seasons (2010-2011 and 2011-2012) in the long-term experiment. Samplings were also performed during the summer fallow period (June-November) in order to quantify the entire year emissions (Fig. 23). However, due to methodological constraints, the first gas samplings started in both experiments in February 2011 at the time of top-dressing fertilizer application.

At the beginning of the each experiment, two polyvinyl chloride rings (31.5 cm internal diameter) per plot were inserted 5 cm into the soil. The rings were only removed at the time of tillage, planting and harvesting operations, allowing a minimum lapse of 24 hours following ring rearrangement at the initial location before any gas sampling to avoid the concomitant effects of soil disturbance on gas emissions. Polyvinyl chloride chambers (20 cm height) were fitted into the rings when measurements were performed. Moreover, a polytetrafluoroethylene vent (10 cm long and 0.4 cm internal diameter) was installed on one side of the chambers in order to overcome possible changes in volume and pressure during the deployment of chambers and gas sampling. The chambers were covered with a reflective insulation fabric (model Aislatermic, Arelux, Zaragoza, Spain) that consisted of two reflective layers of aluminum film bonded to an internal layer of

polyethylene bubbles in order to diminish internal increases of temperature. A metal fitting was attached in the center of the top of the chamber and lined with two silicon-Teflon septa as a sampling port.

Soil gas samples (15 mL) were obtained with polypropylene syringes at 0, 30 and 60 minutes after closing the camber and injected into 12 mL Exetainer® borosilicate glass vials (model 038W, Labco, High Wycombe, UK). Each block of the experiment (i.e., 10 treatments) was sampled by one operator in order to reduce as much as possible the amount of time during the sampling process, thus avoiding temperature-induced biases (Rochette et al. 2012).

Figure 23 Soil gas sampling in the tillering (a), stem elongation (b) and pre-harvest (c) stages of the crop, and in the summer fallow (d).

Gas samples were analyzed with an Agilent 7890A gas chromatography system equipped with a flame ionization detector (FID) + methanizer and two valves in order to obtain the gases of interest (i.e., CH_4 and CO_2) for each gas injection. A HP-Plot Q column (30 m long, 0.32 mm of section and 20 µm) was used, with a pre-column 15 m long of the same characteristics. The injector and the oven temperatures were set to

50ºC. The temperatures of the FID detector and the methanizer were set to 250 and 375 ºC, respectively. For the detector, H_2 was used as a carrier gas and N_2 as a make-up gas at 35 and 25 mL min^{-1}, respectively. The volume of sample injected was 1 mL. The system was calibrated using ultra-high purity CH_4 and CO_2 standards (Carburos Metálicos, Barcelona, Spain). Emission rates were calculated taking into account the linear increase in the gas concentration within the chamber over the sampling time and correcting for the air temperature.

2.3 Biomass sampling and analyses

In the short-term experiment, crop above-ground biomass was measured right before the harvest in the three growing seasons studied by cutting the plants at the soil surface level along 0.5 m of the seeding line at three randomly selected locations per plot. The samples were dried at 65ºC during 48 h and weighed. Root biomass was measured at flowering in April 2012 and in May 2013 in the short-term experiment. For each plot, four soil cores (0-30 cm) were obtained, two in the seeding line and the other two within lines. Special care was taken in order to avoid wheel track locations. Each soil sample was dispersed with a 5% sodium hexametaphosphate solution in a reciprocal shaker during at least 30 minutes and then washed by hand with a low-pressure shower jet through a 0.5-mm sieve to recover the roots, following the methodology proposed by Böhm (1979). Once washed, the sieve was submerged in a tray filled with water in order to ease the skimming of the roots. Finally, the roots were oven-dried at 65ºC and weighed. Root biomass per unit of area was calculated dividing the weight of roots by the area sampled with the core. Afterwards, above- and belowground biomass samples were analyzed for C content by dry combustion. The above- and below-ground biomass C inputs were calculated by multiplying the weight of each fraction by its C content.

Grain yield of each treatment was measured in 2012 in the long-term experiment and in 2011, 2012 and 2013 in the short-term experiment by harvesting the plots with a commercial combine and weighing the grain. After determining the grain moisture content, grain yield was corrected to 10% moisture.

2.4 Soil sampling and analyses

Soil samples from the 0-5 cm soil layer were collected at every sampling date near each gas sampling chamber. Water-filled pore space (WFPS) was calculated as the quotient between soil volumetric water content and total porosity. The volumetric water content was calculated as the gravimetric water content times the soil bulk density. The

gravimetric water content was obtained by oven drying the soil samples at 105 ºC for the long-term experiment and at 50ºC for the short-term experiment until constant weight. In the short-term experiment, soil was dried at 50ºC in order to avoid the dehydration of the gypsum present in the soil at this experiment (Porta, 1998). Soil porosity was calculated as a function of soil bulk density assuming a particle density of 2.65 Mg m^{-3}. In turn, soil bulk density was determined using the cylinder method (Grossman and Reinsch, 2002). Moreover, at each gas sampling date, soil temperature was measured at 5 cm soil depth with a hand-held probe.

In the short-term experiment, a soil sampling was made at the end of the experiment (June 2013) to quantify SOC stocks. Two sampling areas per plot were selected and soil samples taken from the whole soil profile at five depths: 0-5, 5-10, 10-25, 25-50 and 50-75 cm. For the same depths, soil bulk density was determined using the cylinder method (Grossman and Reinsch, 2002). Once in the laboratory the samples were 2-mm sieved and then air-dried. The SOC concentration was determined using the dichromate wet oxidation method of Walkley and Black described by Nelson and Sommers (1996). During the oxidation extensive heating at 150ºC for 30 minutes was used in order to increase the digestion of SOC (Mebius, 1960). Finally, the SOC stock was calculated using the equivalent soil mass procedure proposed by Ellert and Bettany (1995).

2.5 Calculations and data analysis

For both experiments, the cumulative soil C losses due to the emissions of CO_2 and CH_4 during the whole experimental period were quantified on a mass basis (i.e., kg C ha^{-1}) by using the trapezoid rule. Also, for both experiments, a yield-scaled ratio between the C lost as CH_4 and CO_2 and the production of grain for each treatment was calculated in order to quantify the efficiency of each treatment in terms of kg of CO_2 equivalents emitted per kg of grain produced. In the long-term experiment, this ratio was calculated for the 2011-2012 growing season by integrating the emissions of CH_4 and CO_2 from the pre-seeding application of fertilizers until the harvest of the crop, taking into account that CH_4 has a GWP 25 times greater than CO_2 (Forster et al. 2007), and dividing that result by the amount of grain produced by each treatment in that season. The ratio was also calculated in the short-term experiment for the 2011-2012 and 2012-2013 growing seasons by integrating the emissions of CH_4 and CO_2 from the pre-seeding application of fertilizers in the 2011-2012 growing season until the harvest of the 2012-2013 season

and dividing that result by the sum of grain produced by each treatment in both cropping seasons.

For each site, data for WFPS and CO_2 and CH_4 fluxes were analyzed using the SAS statistical software (SAS institute, 1990) performing a repeated measures analysis of variance (ANOVA). Moreover, ANOVAs for the cumulative C-losses of the two gases, the above- and below-ground biomass-C inputs, the SOC stocks, and the yield-scaled ratios between the C-gases emitted and the grain produced were also performed. When significant, differences among treatments were identified at 0.05 probability level of significance using a Tukey test.

3. Results

3.1 Environmental conditions and soil water-filled pore space during the experiments

Rainfall and air temperature for the 2010-2011, 2011-2012 and 2012-2013 cropping seasons are presented in Figure 24. In both sites a large variation in precipitation was recorded during the three cropping seasons, as expected in our experimental Mediterranean conditions. As it can be seen in the figure, annual rainfall ranged from 211 to 530 mm and from 280 to 537 mm in the long-term and short-term experiments, respectively. In the long-term experiment (Fig. 24A), the 2010-2011 and 2011-2012 cropping seasons were characterized by a low precipitation, while the 2012-13 season registered greater rainfall than the 30-yr average for the area (430 mm). In the short-term experiment (Fig. 24B), the 2011-12 cropping season was drier than the average (327 mm), while the 2012-13 season recorded an exceptionally large amount of rainfall (537 mm), mainly in the autumn and spring months. Air temperature showed the highest values during the summer months (June-August) and the lowest during the winter months (December-February).

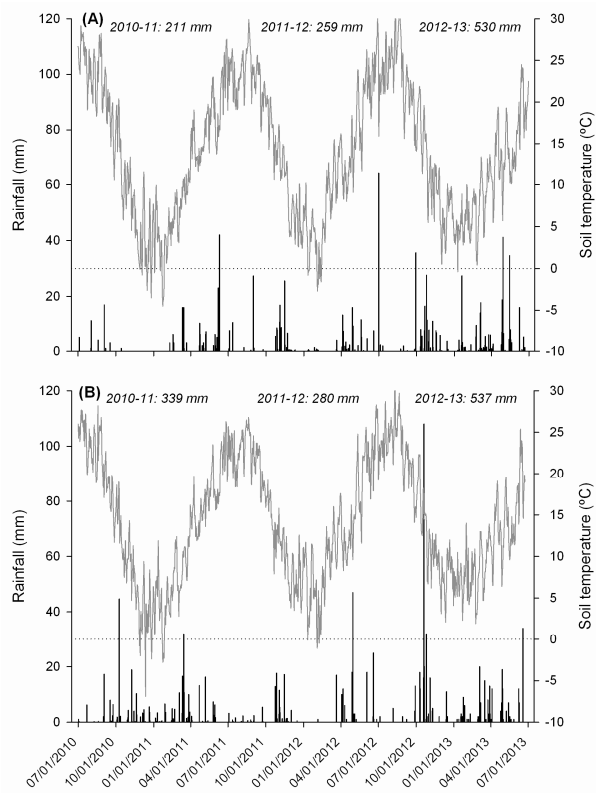

Figure 24 Air temperature (continuous line) and rainfall events (bars) in (A) the long-term experiment and (B) the short-term experiment.

Over the experimental period, soil temperature ranged from -1.3 to 29.1 ºC in the long-term experiment (Fig. 25A) and from 1.4 to 29.3 ºC in the short-term one (Fig. 25B). Also, for both experiments, soil temperature was below 15 ºC during the applications of fertilizers except for the pre-seeding application of the 2011-2012 cropping season in the short-term experiment when soil temperature reached 23.7 ºC (Fig. 25B). In both experiments, tillage significantly affected WFPS (Tables 24 and 25). In the long-term experiment, mean WFPS values were 19.8 and 44.1% for the CT and NT treatments, respectively (Table 24), while in the short-term experiment, the same treatments presented a mean WFPS of 18.5 and 32.0%, respectively (Table 25). Contrarily, neither the nitrogen treatments nor the interaction between tillage and nitrogen showed significant differences on WFPS (Table 24).

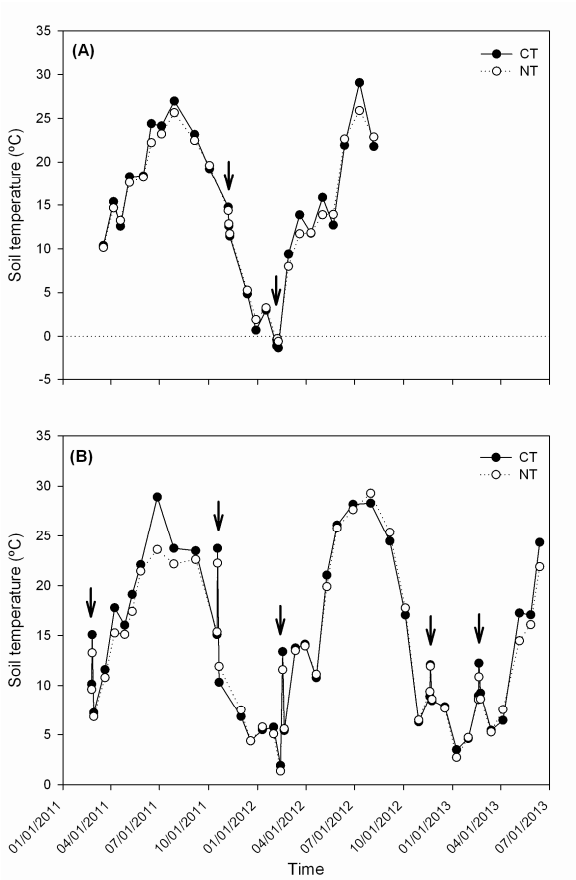

Figure 25 Soil temperature as affected by tillage (CT, conventional tillage; NT, no-tillage) in (A) the long-term experiment and (B) the short-term experiment. Vertical arrows indicate fertilizer applications.

Table 24 Analysis of variance of water-filled pore space (WPFS) (%), fluxes of CH_4 and CO_2 (mg CH_4-C m^{-2} d^{-1} and mg CO_2-C m^{-2} d^{-1}, respectively), cumulative C losses for both gases during the whole experimental period (kg C ha^{-1}), 2011-2012 grain yield (kg ha^{-1} at 10% moisture) and ratio between the CH_4 and CO_2 losses, expressed in CO_2 equivalents, and the production of grain in the 2011-2012 growing season as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization (0, control; 60, mineral N at 60 kg N ha^{-1}; 120, mineral N at 120 kg N ha^{-1}), date of sampling and their interactions in the long-term field experiment. Values of gas fluxes and WFPS are the means of all samplings.

Effects	Long-term experiment						
	WFPS	Gas fluxes		Cumulative C losses		2011-12 Grain yield	kg CO_2 eq. kg $grain^{-1}$
		CH_4	CO_2	CH_4	CO_2		
Tillage	*<0.001*	*0.009*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
CT	19.84 b¶	-0.249 a	516.29 b	-1.065 a	2610.57 b	245.8 b	4.64 a
NT	44.08 a	-0.424 b	779.33 a	-2.396 b	3984.85 a	1554.3 a	0.91 b
Nitrogen	*0.191*	*0.068*	*0.739*	*0.061*	*0.748*	*<0.001*	*0.072*
0	32.07	-0.443	617.00	-2.249	3139.09	719.6 c	3.51
60	31.55	-0.341	667.89	-1.700	3338.43	940.7 b	2.90
120	32.27	-0.230	663.21	-1.242	3415.62	1039.8 a	1.92
Tillage x Nitrogen	*0.55*	*0.435*	*0.875*	*0.427*	*0.922*	*<0.001*	*0.094*
CT – 0	19.32	-0.390	462.21	-1.747	2370.03	178.4 c	6.00
CT – 60	22.21	-0.183	551.47	-0.726	2716.57	226.8 c	4.97
CT – 120	17.99	-0.174	535.63	-0.726	2745.12	332.1 c	2.95
NT – 0	44.82	-0.495	768.85	-2.751	3908.16	1260.8 b	1.01
NT – 60	40.89	-0.494	779.89	-2.676	3960.29	1654.5 a	0.83
NT – 120	46.54	-0.285	789.19	-1.760	4086.11	1747.5 a	0.89
Date	*<0.001*	*0.011*	*<0.001*				
Tillage x Date	*<0.001*	*0.488*	*<0.001*				
Nitrogen x Date	*0.995*	*0.846*	*0.844*				
Tillage x Nitrogen x Date	*0.021*	*0.324*	*0.529*				

¶ For each variable, different letters indicate significant differences between treatments at $P<0.05$.

3.2 Tillage and N fertilization effects on CH_4 emissions.

In the long-term experiment, although greater oxidation of CH_4 was observed under NT (2.4 kg C-CH_4 ha^{-1}) when compared with CT (1.1 kg C-CH_4 ha^{-1}) (Table 24), no differences between tillage treatments were found on the dynamics of this gas over time (data not shown). Contrary results were found in the short-term experiment, where greater mean CH_4 oxidation was found under CT (2.7 kg C-CH_4 ha^{-1}) compared with NT (1.2 kg C-CH_4 ha^{-1}) (Table 25). Moreover, in this experiment, the temporal dynamics of the CH_4 fluxes was affected by tillage, with higher emission peaks of CH_4 under NT compared with CT during two of the five fertilizer applications in the period covered by the experiment (Fig. 26A). Also, for both CT and NT, a net emission of CH_4 from the soil to the atmosphere occurred during the coldest months (December-February) (Fig. 26A).

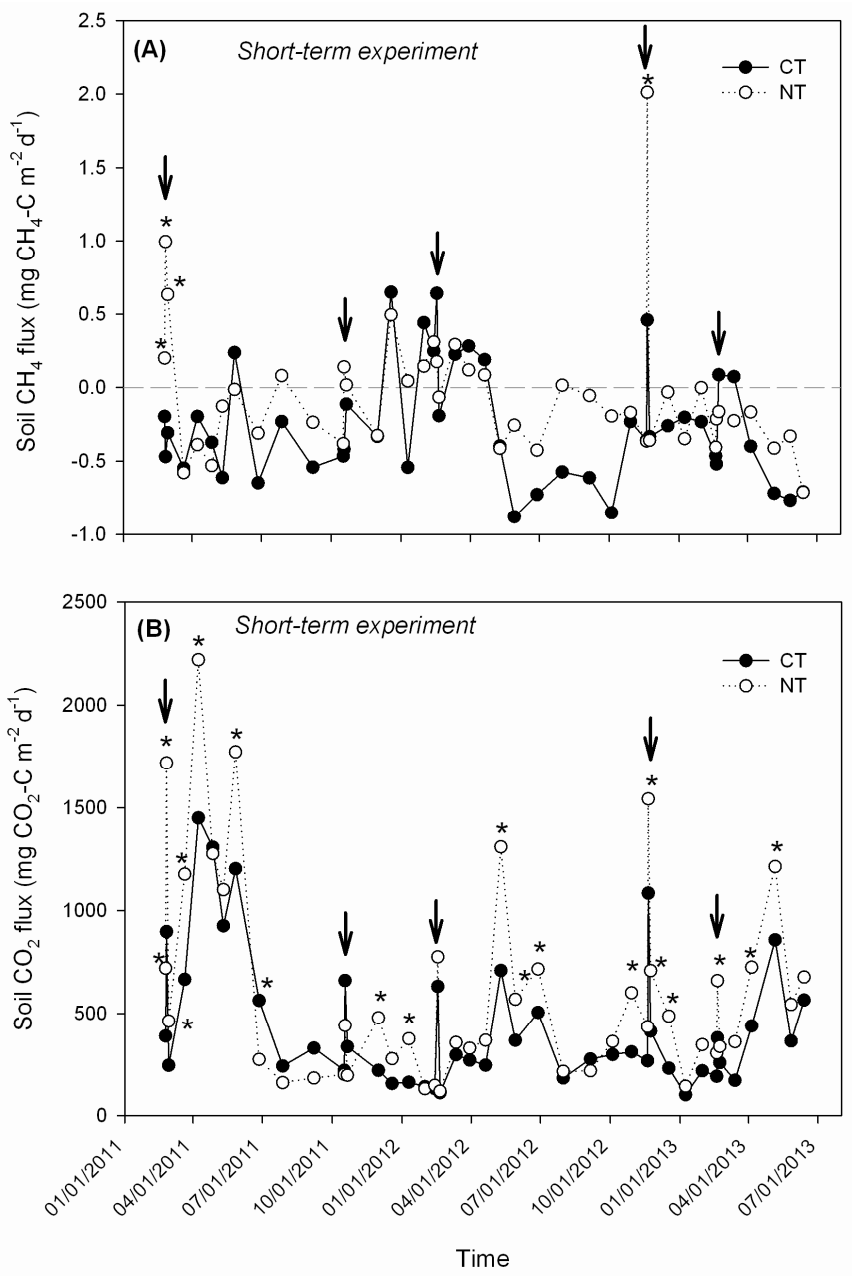

Figure 26 Soil CH_4 (A) and CO_2 (B) emissions in the short-term experiment as affected by tillage (CT, conventional tillage; NT, no-tillage). *Indicates significant differences between tillage treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

No significant effects of mineral N rate on the dynamics of CH_4 fluxes were found in the long-term experiment. However, a trend to lower CH_4 oxidation when increasing the rate of mineral N applied to the soil can be observed in Table 24. In contrast, in the short-term experiment, although no differences between fertilization treatments were noted in the mean values of CH_4 fluxes, N fertilization affected the dynamics of CH_4 fluxes, with significant differences at six dates, four of them coincident with fertilizer

applications (Table 25, Fig. 27A). Moreover, a significant (r^2: 0.27; P<0.001) logarithmic relationship was found between CH_4 fluxes and soil temperature (Fig. 28). Contrarily, no significant relationship was found between CH_4 fluxes and soil gravimetric moisture content (data not shown).

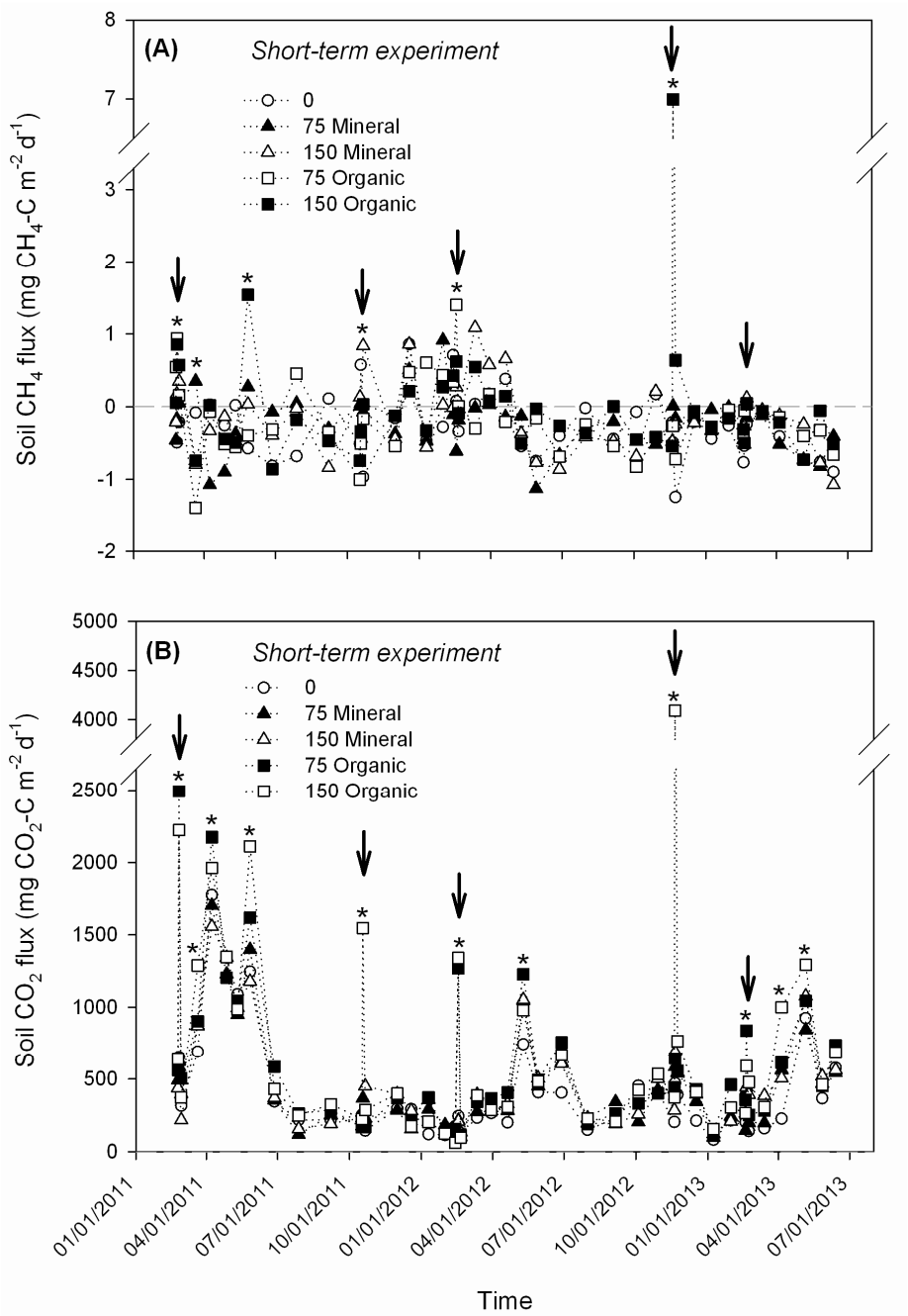

Figure 27 Soil CH_4 (A) and CO_2 (B) emissions in the short-term experiment as affected by nitrogen fertilization (0, control; 75 Mineral, 75 kg N ha^{-1} of mineral N; 150 Mineral, 150 kg N ha^{-1} of mineral N; 75 Organic, 75 kg N ha^{-1} as pig slurry; 150 Organic, 150 kg N ha^{-1} as pig slurry). * Indicates significant differences between fertilization treatments for each date at P<0.05. Vertical arrows indicate fertilizer applications.

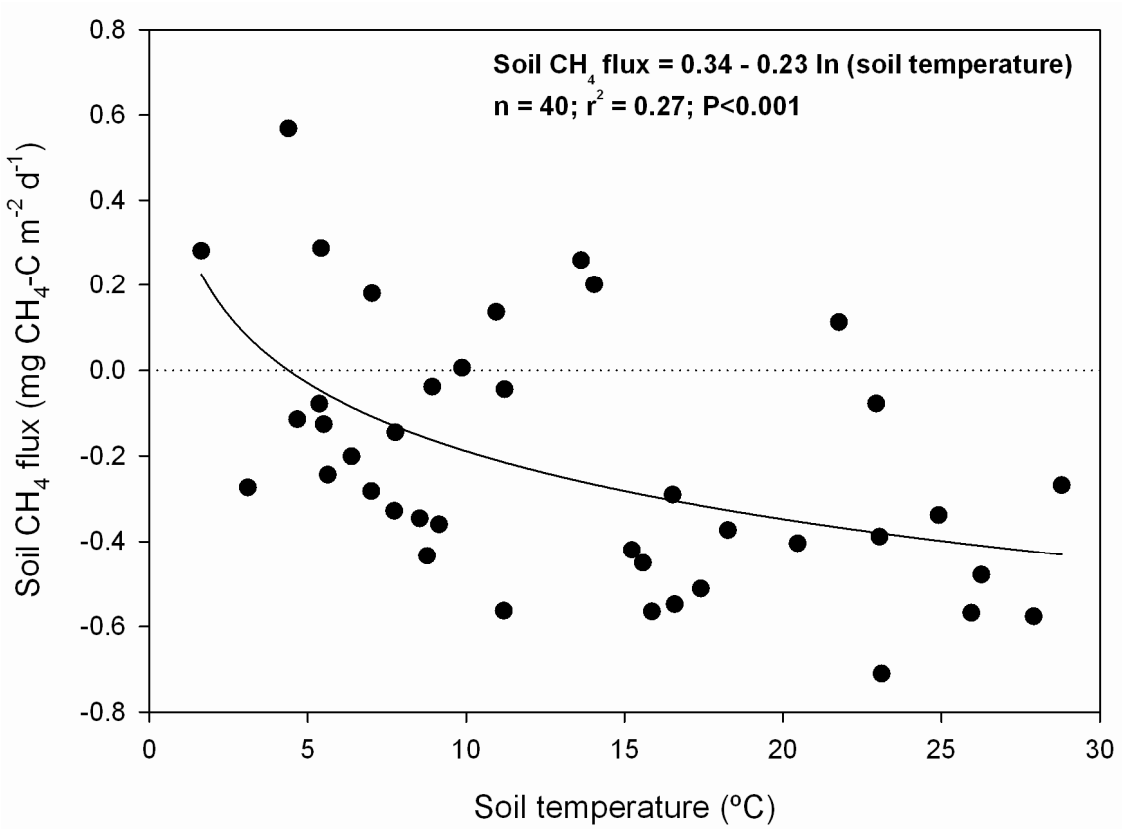

Figure 28 Regression analysis between soil temperature and CH_4 flux. Each point represents the average of all treatments for each sampling date. Data from samplings performed three hours after each fertilizer application are excluded in order to avoid the effect of N on CH_4 oxidation.

Table 25 Analysis of variance of water-filled pore space (WPFS) (%), fluxes of CH_4 and CO_2 (mg CH_4-C m^{-2} d^{-1} and mg CO_2-C m^{-2} d^{-1}, respectively), cumulative C losses for both gases during the whole experimental period (kg C ha^{-1}), 2011-2012 plus 2012-2013 grain yield (kg ha^{-1} at 10% moisture) and the ratio between the loss of CH_4 and CO_2 in CO_2 equivalents and the production of grain (sum of the 2011-2012 and 2012-2013 growing seasons) as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N as pig slurry at 75 kg N ha^{-1} and 150 Org, organic N as pig slurry at 150 kg N ha^{-1}), date of sampling and their interactions in the short-term field experiment. Values of gas fluxes and WFPS are the means of all samplings.

Effects	Short-term experiment						
	WFPS	Gas fluxes		Cumulative Closses		2011-13 grain yield	kg CO_2 eq. kg $grain^{-1}$
		CH_4	CO_2	CH_4	CO_2		
Tillage	*<0.001*	*0.013*	*<0.001*	*0.006*	*<0.001*	*<0.001*	*<0.001*
CT	18.47 b¶	-0.281 b	455.99 b	-2.690 b	3312.67 b	2262.8 b	1.07 a
NT	32.01 a	-0.062 a	627.08 a	-1.161 a	4480.39 a	5692.3 a	0.51 b
Nitrogen	*0.732*	*0.082*	*<0.001*	*0.7*	*<0.001*	*<0.001*	*<0.001*
0	24.29	-0.287	425.21 c	-2.436	3226.56 c	2358.6 d	1.00 a
75 Min	24.54	-0.250	461.29 c	-2.073	3574.64 bc	3651.1 c	1.05 a
150 Min	26.11	-0.191	486.52 c	-1.827	3802.07 bc	3885.2 c	0.72 ab
75 Org	25.78	-0.176	607.85 b	-2.055	4293.78 ab	4657.4 b	0.62 b
150 Org	25.47	0.051	727.55 a	-1.238	4585.60 a	5335.4 a	0.55 b
Tillage x Nitrogen	*0.057*	*0.567*	*0.384*	*0.378*	*0.426*	*<0.001*	*<0.001*
CT – 0	16.35	-0.419	365.68	-3.512	2819.18	992.3 f	1.42 ab
CT – 75 Min	16.16	-0.369	385.82	-2.903	2899.34	1308.3 ef	1.64 a
CT – 150 Min	22.11	-0.173	402.77	-1.914	3130.57	2225.7 de	0.89 bc
CT – 75 Org	18.50	-0.366	480.46	-3.654	3497.46	2755.4 cd	0.75 c
CT- 150 Org	19.22	-0.079	643.77	-1.468	4216.79	4032.6 b	0.63 c
NT – 0	32.23	-0.154	484.51	-1.360	3633.94	3725.0 bc	0.57 c
NT – 75 Min	32.92	-0.136	535.02	-1.243	4249.95	5993.9 a	0.47 c
NT – 150 Min	30.11	-0.208	569.61	-1.739	4473.56	5544.8 a	0.55 c
NT – 75 Org	33.06	0.010	732.80	-0.456	5090.09	6559.5 a	0.49 c
NT- 150 Org	31.72	0.179	810.37	-1.008	4954.40	6638.3 a	0.47 c
Date	*<0.001*	*<0.001*	*<0.001*				
Tillage x Date	*<0.001*	*0.039*	*<0.001*				
Nitrogen x Date	*<0.001*	*<0.001*	*<0.001*				
Tillage x Nitrogen x Date	*0.034*	*<0.001*	*0.019*				

¶ For each variable, different letters indicate significant differences between treatments at $P<0.05$.

3.3 Tillage and N fertilization effects on CO_2 emissions

Tillage significantly affected the average CO_2 emissions for the entire period studied, with a greater mean CO_2 flux under NT than under CT in both experiments (Tables 24 and 25). In the long-term experiment, soil CO_2 fluxes ranged between 91.5 and 1872.2 mg CO_2-C m^{-2} d^{-1} increasing in the summer months (June-September) when compared with winter months (December-March) (Fig. 29). In this experiment, greater CO_2 fluxes were observed under NT compared with CT in most of the sampling dates (Fig. 29). In the short-term experiment, a trend to higher CO_2 emissions during the fast growing period of the crop (February-May) was observed for both tillage treatments (Fig. 26B). As in the long-term experiment, significant differences between tillage treatments for most of the sampling dates were found with greater values under NT than under CT (Fig. 26B).

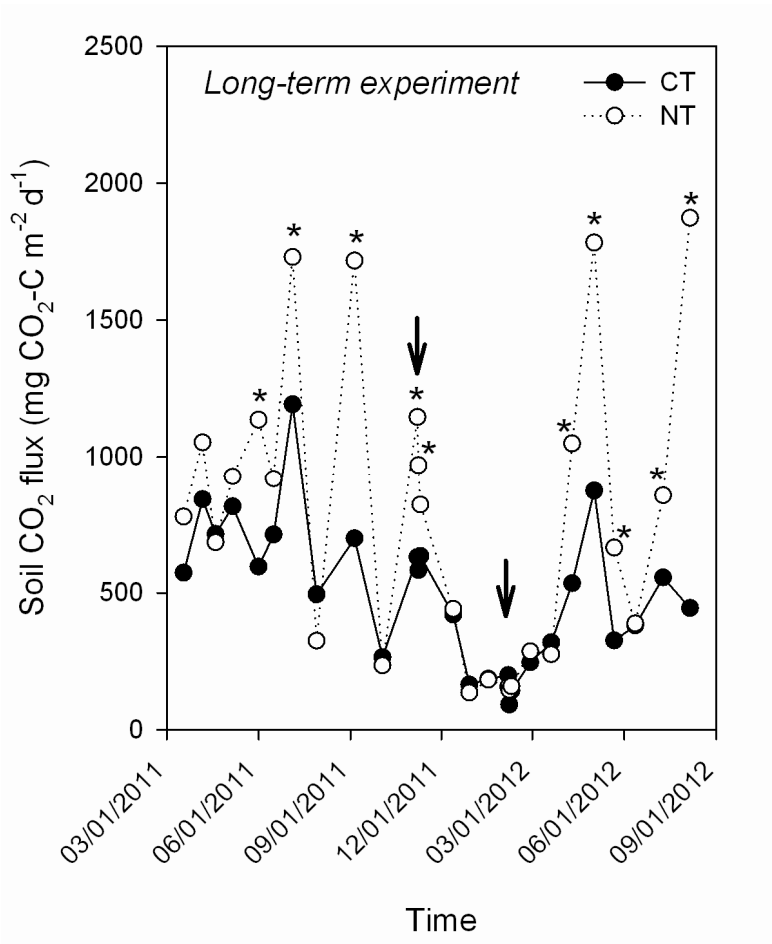

Figure 29 Soil CO_2 emissions in the long-term experiment as affected by tillage (CT, conventional tillage; NT, no-tillage). * Indicates significant differences between tillage treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

In the long-term experiment, the mineral N rates applied did not affect soil CO_2 emissions (Table 24). Contrarily, fertilization treatments affected CO_2 emissions in the short-term experiment (Fig. 27B). In this case, the application of organic N fertilizers resulted in short-lasted peaks of CO_2. The average CO_2 values also showed differences among fertilization treatments, with the greatest value in the 150 kg N ha^{-1} organic fertilizer treatment (Table 25). In addition, during the fast development period of the crop (February-May), significant differences were also found between N fertilization treatments (Fig. 27B).

3.4 Cumulative C losses, grain yield and yield-scaled CH_4 and CO_2 emissions.

In the long-term experiment, and taking into account the whole period of gas measurements, the soil absorbed 1.07 and 2.40 kg CH_4-C ha^{-1} and emitted 2610.6 and 3984.9 kg CO_2-C ha^{-1} in the CT and NT treatments, respectively, with significant differences between them (Table 24). On the contrary, no significant differences were found between N fertilization treatments or the interaction between tillage and N fertilization on the absorption/emission of CH_4 and CO_2. Although no significant, a trend to a lower consumption of CH_4 by the soil when increasing the N rate was quantified in that experiment (Table 24). Unlike the long-term experiment, during the whole gas measurement period in the short-term experiment, the absorption of CH_4-C by the soil amounted for 2.69 and 1.16 kg CH_4-C ha^{-1} under CT and NT, respectively, being these values significantly different (Table 25). Significant differences between tillage and N fertilization treatments were also found on cumulative CO_2-C losses. In the case of tillage, CT emitted 3312.7 kg CO_2-C ha^{-1}, while NT emitted 4480.4 kg CO_2-C ha^{-1} (Table 25). In the case of N fertilization, the losses of C as CO_2 ranged from 3226.6 kg CO_2-C ha^{-1} for the control treatment to 4585.6 kg CO_2-C ha^{-1} for the 150 kg organic N ha^{-1} treatment (Table 25).

Greater grain production was observed in both experiments under NT. In the long-term one, grain yield in the 2011-12 growing season was 246 and 1554 kg ha^{-1} for the CT and NT treatments, respectively (Table 24). In the short-term experiment, grain yield, expressed as the sum of the 2011-12 and 2012-13 growing seasons, reached 2263 and 5692 kg ha^{-1} for the CT and NT treatments, respectively (Table 25). The application of increasing rates of N significantly increased grain yield in the long-term experiment, with 720, 941 and 1040 kg grain ha^{-1} for the 0, 60 and 120 kg N ha^{-1} treatments, respectively (Table 24). In turn, in the short-term experiment, the greatest yields were

obtained when adding 75 and 150 kg ha^{-1} of organic N as pig slurry (4657 and 5335 kg grain ha^{-1}), compared with the same rates of mineral N fertilizer (3651 and 3885 kg grain ha^{-1}) (Table 25).

As it has been explained in the Materials and Methods section, the quotient between the amount of CO_2 equivalents emitted as CH_4 and CO_2 and the production of grain was calculated for each treatment. In the long-term experiment, the NT treatment emitted five times less CO_2 equivalents per kg of grain than the CT treatment (Table 24). Also, in that experiment, the use of increasing rates of mineral N fertilizer did not show statistical differences between treatments in the CO_2 equivalents emitted per kg of grain, although a trend to a higher efficiency (i.e., less emissions of CO_2 per unit of grain produced) was observed when increasing the amount of N fertilizer applied.

In contrast to the long-term experiment, in the short-term experiment, significant differences between tillage, fertilization and their interaction in the equivalents of CO_2 emitted per unit of grain produced were observed (Table 25). In this short-term experiment, the lowest ratio between the CO_2 produced per each kilogram of grain was observed in the NT treatment with either 75 kg mineral N ha^{-1}or 150 kg organic N ha^{-1} (0.47 kg CO_2 equivalents kg $grain^{-1}$), while the highest ratio corresponded to CT with 75 kg mineral N ha^{-1} (1.64 kg CO_2 equivalents kg $grain^{-1}$) (Table 25). Following the same trend found in the long-term experiment, in the short-term experiment the NT treatment presented two times less emission of CO_2 equivalents per kg of grain produced than the CT treatment. Furthermore, the organic fertilizer treatments (75 and 150 kg organic N ha^{-1}) presented lower ratios than the control and the 75 kg mineral N ha^{-1} treatments, while the application of 150 kg mineral N ha^{-1} led to intermediate values (Table 25).

3.5 Tillage and N fertilization effects on soil C inputs and stocks in the short-term experiment.

In the short-term experiment, tillage and N fertilization treatments significantly affected the above-ground C inputs (crop residues), while no differences between treatments were found in the below-ground C inputs (root biomass) (Table 26). As an average of all treatments, the above-ground C inputs accounted for the 86.5% of the total C inputs to the soil while the below-ground C inputs only accounted for the13.5%. For the three growing seasons studied (2010-2011, 2011-2012 and 2012-2013), the CT and NT

treatments led to mean values of above-ground C inputs of 97 and 155 g C m^{-2}, respectively (Table 26). These values imply that the above-ground C inputs are 60% greater under NT than under CT. On average, the application of 150 kg organic N ha^{-1} led to the greatest amount of above-ground C inputs (169 g C m^{-2}) and the control treatment to the lowest (93 g C m^{-2}) (Table 26). After three years of contrasting treatments, no differences between tillage and fertilization treatments were observed in SOC stocks (Table 27). Mean SOC stock for the whole sole profile (0-75 cm) expressed on an equivalent soil mass basis was 98.7 and 95.8 Mg C ha^{-1} in the CT and NT treatments, respectively.

Table 26 Analysis of variance of above- and below-ground C inputs (g C m^{-2}) as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N as pig slurry at 75 kg N ha^{-1} and 150 Org, organic N as pig slurry at 150 kg N ha^{-1}), growing season and their interactions in the short-term field experiment.

Effects	Short-term experiment						
	Above-ground C inputs				Below-ground C inputs		
	2010-11	*2011-12*	*2012-13*	*Mean*	*2011-12*	*2012-13*	*Mean*
Tillage		*<0.001*				*0.191*	
CT	100.79	53.56	179.70	96.90 b	12.87	23.98	17.81
NT	176.01	105.28	233.14	154.93 a	20.46	23.28	21.51
Nitrogen		*<0.001*				*0.086*	
0	81.75 c	59.60	169.31 b	92.56 c	16.02	20.13	17.67
75 Min	184.31 ab	90.23	164.56 b	132.33 b	15.80	12.00	14.28
150 Min	125.45 abc	92.29	224.51 ab	133.64 b	16.53	30.41	21.16
75 Org	106.58 bc	48.93	202.69 ab	101.78 bc	19.81	35.61	26.99
150 Org	193.90 a	106.05	271.02 a	169.25 a	15.16	19.89	27.60
Tillage x Nitrogen		*<0.001*				*0.630*	
CT – 0	58.67 c	62.91 abc	151.82 b	84.08 d	15.50	11.34	13.84
CT – 75 Min	115.43 bc	20.83 c	147.44 b	76.13 d	7.16	9.57	8.12
CT – 150 Min	83.72 c	91.86 abc	200.26 b	116.93 cd	12.20	32.60	20.36
CT – 75 Org	92.42 c	26.31 c	212.88 b	89.48 cd	16.24	40.20	28.22
CT- 150 Org	153.71 abc	65.87 abc	186.12 b	117.89 cd	13.26	20.04	16.65
NT – 0	104.83 bc	56.28 abc	186.81 b	101.05 cd	16.54	28.92	21.49
NT – 75 Min	253.19 a	159.63 a	181.68 b	188.53 ab	24.45	14.43	20.44
NT – 150 Min	167.18 abc	92.72 abc	248.77 ab	150.35 bc	20.87	26.03	22.16
NT – 75 Org	120.75 abc	71.54 abc	192.51 b	114.09 cd	23.37	28.73	25.51
NT- 150 Org	234.10 ab	146.22 ab	355.92 a	220.62 a	17.05	19.65	18.09
Growing season (GS)		*<0.001*				0.073	
Tillage x GS		*0.455*				*0.323*	
Nitrogen x GS		*<0.001*				*0.349*	
Tillage x Nitrogen x GS		*0.033*				*0.447*	

¶ For each variable, different letters indicate significant differences between treatments at $P<0.05$.

Table 27 Soil organic carbon stock expressed on equivalent mass basis (SOC_{esm}) as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N as pig slurry at 75 kg N ha^{-1} and 150 Org, organic N as pig slurry at 150 kg N ha^{-1}) in the short-term field experiment.

Soil depth	SOC_{esm} stock (Mg C ha^{-1})											
(cm)	CT						NT					
	0	75 Min	150 Min	75 Org	150 Org	Mean	0	75 Min	150 Min	75 Org	150 Org	Mean
0-10	17.9 (2.4)*	16.9 (2.5)	17.2 (2.5)	21.1 (3.6)	19.3 (4.1)	18.5 (3.1)	21.2 (1.7)	19.7 (6.0)	19.5 (3.3)	21.3 (8.1)	20.9 (6.0)	20.5 (4.7)
10-75	78.4 (10.6)	70.5 (17.2)	83.1 (13.1)	88.7 (5.1)	80.3 (10.7)	80.2 (11.9)	61.2 (17.7)	80.9 (19.0)	71.9 (20.6)	76.5 (7.8)	85.7 (1.1)	75.3 (15.5)
0-75	96.2 (12.9)	87.4 (18.0)	100.2 (14.5)	109.8 (8.7)	99.7 (7.7)	98.7 (13.3)	82.5 (17.0)	100.6 (24.4)	91.4 (23.9)	97.8 (15.9)	106.6 (6.7)	95.8 (18.0)

* Values in parentheses are the standard deviations of the mean.

4. Discussion

4.1 Tillage effects

In both the long-term and short-term field experiments, the soil acted as a net sink of CH_4, in line with the data obtained by Meijide et al. (2010) in a comparison of fertilizer types conducted in a dryland area of Central Spain. However, in both experiments we obtained contrasting results between tillage systems, with greater CH_4 oxidation under NT in the long-term experiment and lower in the short-term one. Different authors have suggested that CH_4 oxidation can be reduced by tillage due to either its effects on gas diffusivity or long-term damage to the methanotrophic community (Ball et al. 1999; Hütsch, 2001). As a consequence, we could hypothesize that the number of years under NT can influence the methanotrophic capacity of a soil. In a NT chronosequence performed in a dryland area similar to that in the present study, Plaza-Bonilla et al. (2013) found an improvement of soil structure when the number of years under NT increased. Thus, the greater metanotrophic activity found under NT in the long-term experiment would be related to a better soil structure that could counterbalance the higher WFPS found under this system. Contrarily, the greater CH_4 oxidation found under CT in the short-term experiment would be explained by its short duration and the possible lack of differences between tillage treatments in soil porous architecture or methanotrophic communities. Other possible explanation for those contrasting results between experiments could be the effect of soil texture, which was coarser in the long-term experiment. Dörr et al. (1993) studied the effects of soil texture on CH_4 uptake by soil and observed that gas permeability of coarse-textured soils was one order of magnitude higher than that of fine-textured ones. Soil texture also determines the size of structural units such as soil aggregates and concomitant hot spots of microbial activity (Ball, 2013). In the NT chronosequence above referred, Plaza-Bonilla et al. (2013) found an increase in the proportion of soil macroaggregates (>0.250 mm) when the number of years under NT increased. That finding could be related to an improvement in gas permeability when NT is maintained over time, which would explain the higher CH_4 oxidation under NT in the long-term experiment compared with CT and the divergent results in the short-term experiment.

Different authors have observed important effects of soil moisture content on CH_4 emissions (Czepiel et al. 1995; Khalil and Baggs, 2005), with a secondary role of soil temperature (Smith et al. 2003). Contrarily, we did not find any relationship between

CH_4 fluxes and WFPS but observed that CH_4 fluxes were related to soil temperature, with a greater absorption of CH_4 at high temperatures. These contrasting results could indicate that the low soil water content found during most of the experimental period did not represent a limitation for the methanotrophic activity in our field experiments.

In both experiments, higher CO_2 fluxes and also cumulative CO_2-C losses were observed under NT compared with CT. Soil CO_2 emissions are the result of two processes. First, the autotrophic respiration of plant roots, which does not represent a net loss of C from the soil and, second, the heterotrophic respiration of decomposer microorganisms that use SOC as a source of energy for their activity. As a consequence, in rainfed cereal cropping systems of Mediterranean areas, it is usual to find greater CO_2 emissions during the active growth period of the crop (February-May period) (Álvaro-Fuentes et al. 2008b; Morell et al. 2011). In this period, about 30% - 50% of the total CO_2 flux could be accounted for by root respiration (Rochette et al. 1999).

In the literature, NT has often been claimed as a soil management system that reduces the emission of CO_2 from soils to the atmosphere compared with CT (Kessavalou et al. 1998). However, some authors have found that, as compared with more humid regions, in dryland Mediterranean agroecosystems, the use of NT implies greater or equal CO_2 emissions when compared with CT, mainly in wet years (Álvaro-Fuentes et al. 2008b; Morell et al. 2011). The greater CO_2 emissions found under NT could be due to the enhancement of soil respiration and mineralization processes. In Mediterranean areas, NT usually increases the production of biomass (Cantero-Martínez et al. 2003), a fact that is reflected by the greater C inputs to the soil that we have found under this tillage system. The higher biomass production under NT in these areas represents an input of C that can be either slowly incorporated into the soil or metabolized by the microorganisms, thus affecting CO_2emissions. In addition, the higher soil water content under NT could have enhanced microbial activity. In line with this hypothesis, in the Mediterranean area, Madejón et al. (2009) and Álvaro-Fuentes et al. (2013) found greater microbial biomass C and enzymatic activities under NT than under CT.

Although in the short-term experiment the above-ground C inputs to the soil were 60% greater under NT than under CT this did not lead to differences in SOC stocks between tillage treatments. After three years of experiment, the lack of differences in SOC stocks between tillage treatments could be explained by the higher CO_2 emissions found under NT compared with CT and the low number of years of tillage systems comparison. The

SOC stock is the result of a balance between C inputs and C outputs. Thus, though greater C inputs were found under NT, greater C losses as CO_2 were also measured under this treatment. Six et al. (2004) suggested that the C sequestration capacity of a soil when using NT is only realized in the long-term. Álvaro-Fuentes et al. (in press) studied the effect of the maintenance of NT on the accumulation of C in the soil and observed that the maximum annual SOC sequestration occurred after 5 years of NT adoption. However, in that study, the management prior to the adoption of NT was a CT system based on moldboard plowing, which can be considered a high-intensity type of tillage. In our case, prior to the establishment of the short-term experiment, the area had been managed with NT for four years. Then, when the experiment started, the CT treatment was based on two passes of chisel. That management implies a lower intensity of tillage when compared with the use of a moldboard plow and, as a consequence, we expect differences between tillage treatments in SOC accumulation in a longer period of time than in the study of Álvaro-Fuentes et al. (in press).

Even though we quantified greater CO_2 emissions from the soil to the atmosphere under NT in both experiments, our results showed five and two times lower yield-scaled CO_2 equivalents under NT than under CT in the long-term and short-term experiments, respectively. These findings demonstrate the necessity of a holistic evaluation of the GWP of each agricultural management practice, taking into account its associated grain production. In 2006, Mosier et al. introduced the concept of greenhouse gas intensity, relating GWP to crop yield. Later, Van Groenigen et al. (2010) pointed out the need to link agronomic productivity and environmental sustainability and postulated that expressing GHG emissions as a function of land area is not helpful and may be counterproductive, and suggested that GHG emissions should be assessed as a function of crop yield. Although the latter authors referred to the effect of nitrogen application on N_2O emissions, our results demonstrate that the relation between GWP and yield is also applicable to other GHG (i.e., CH_4 and CO_2) and agricultural management practices such as soil tillage.

4.2 Nitrogen type and rate effects

According to our results, the application of pig slurry to the soil led to peaks of CH_4 and CO_2 emissions while the application of mineral fertilizers did not show punctual increases in the emission of these gases. The instantaneous (i.e., after three hours) increase in the emission of CH_4 after the application of pig slurry implied a change in

the role of soil, from CH_4-oxidizer to emitter. This change in the dynamics of CH_4 fluxes could be the result of different processes. First, as an average of all applications, the pig slurry used in our experiment contained about 94% water by weight. Thus, each addition of pig slurry to the soil represented an input of about 3 mm of water. Although this is a relatively small amount, it could have produced anaerobic conditions in some soil microsites, especially in the most superficial soil layer, thus changing them from a methanotrophic activity to a methanogenic one. Also, due to the liquid nature of the organic manure, the NH_4^+ present in the pig slurry could have infiltrated into the soil matrix much faster compared with the application of mineral fertilizer. It is known that the application of NH_4^+ to the soil has an inhibitory effect for the methanotrophic communities as a result of competitive inhibition of methane monooxygenase, the enzyme responsible for CH_4 oxidation (Dunfield and Knowles, 1995; Le Mer and Roger, 2001). Chadwick et al. (2000) also pointed out the importance of (i) the volatilization of the CH_4 dissolved in the slurry and (ii) the microbial degradation of the short-chained volatile fatty acids present in animal manures, as other mechanisms that can produce peaks of CH_4 when applying pig slurry to the soil. In agreement with our results, other researchers have reported a reduction of CH_4 uptake when N fertilizers are used (Powlson et al. 1997; Sainju et al. 2012).

As explained above, pig slurry also produced peaks of CO_2 in the subsequent hours following the application. In consonance with our results, Sánchez-Martín et al. (2008) also observed a higher emission of CO_2 after applying organic fertilizers to the soil when compared to urea. These authors suggested that the increase of microbial activity as a result of the incorporation of easily decomposable C compounds to the soil could explain the greater emission of CO_2 when manures are used. Rochette et al. (2000) studied the mechanisms controlling the dynamics of CO_2 emission following the application of pig slurry in a long-term experiment. Similarly to our results, they observed a peak of CO_2 during the first two days after the application. In their experiment, after the phase of rapid decomposition of the labile fraction of the slurry C, a linear and slower phase of CO_2 loss occurred. When they analyzed the soil immediately after the application of the slurry, they found an increase of soil microbial biomass and extractable C that would explain the increase in the emissions of CO_2. Thus, this finding could explain the dynamics in the emissions of CO_2 that we found in

the short-term experiment, with important bursts of CO_2 right after the application of pig slurry.

We did not find significant differences in the cumulative losses of C as CO_2 when applying increasing rates of mineral N in the long-term and short-term experimental fields. On the contrary, the application of pig slurry in the short-term experiment led to higher CO_2 fluxes compared to the mineral fertilizer. Similar results were found by Jarecki et al. (2008) who compared the effects of N mineral fertilizer and pig slurry on GHG emissions. Moreover, in the short-term experiment, although greater C inputs to the soil were found under organic fertilization than under the mineral one, no differences in SOC stocks were found between both fertilizer types. Plaza et al. (2004) studied the effects of the application of increasing rates of pig slurry (i.e., from 30 to 150 m^3 ha^{-1} y^{-1}) to the soil in a semiarid area of Spain. They did not observe differences in SOC between pig slurry rates and suggested that this result could be attributed to the small amount of organic C and the relatively large N content of that manure, which could lead to microbial oxidation of native soil organic C. Thus, our findings on higher CO_2 emissions and C inputs when using pig slurry and the lack of differences in SOC stocks when compared to the control or the mineral treatments could be explained by an enhanced mineralization of the C contained in the pig slurry. Moreover, although no significant, the higher input of C as below-ground biomass that we found when applying pig slurry would also suggest a greater root respiration under this treatment (Morell et al. 2012).

The application of pig slurry reduced the CO_2 equivalents per unit of grain produced when compared to the mineral treatments, thus showing a higher environmental sustainability in terms of CO_2 and CH_4 production when this organic fertilizer is used. However, we did not find differences in that ratio between N rates regardless the type of N fertilizer applied.

4.3 Interactive effects of tillage and nitrogen

The importance of the interactive effects of tillage and nitrogen fertilization arises when studying the ratio between the CO_2 equivalents emitted and the mass of grain produced. Under NT, the application of different rates of mineral or organic N fertilizer did not reduce significantly that ratio. On the contrary, when CT was used, the application of pig slurry lowered significantly the ratio when compared to the control treatment or the application of 75 kg mineral N ha^{-1}. Our data indicate that the use of NT combined with

the application of medium rates of mineral or organic N fertilizers, can greatly reduce the amount of CO_2 and CH_4 emitted per kg of grain in Mediterranean dryland agroecosystems.

5. Conclusions

The results of this study showed that tillage and N fertilization and their interaction affected the emissions of CH_4 and CO_2. The NT treatment led to higher emissions of CO_2 to the atmosphere compared to CT. However, although in general the soil acted as a CH_4 sink, contrasting results were obtained in the two experimental fields. Thus, whereas in the long-term experiment greater CH_4 oxidation was observed under NT compared with CT, in the short-term experiment, CH_4 oxidation was much lower under NT. The application of pig slurry led to immediate peaks of CH_4 and CO_2 emission fluxes and also enhanced the C lost as CO_2 during all the experimental period. Contrarily, there were no significant differences in the cumulative losses of C as CO_2 when applying increasing rates of mineral N in both the long-term and short-term experimental fields. Compared with CT, the use of NT reduced 5 and 2 times the ratio between the CO_{2eq} and the production of grain in the long-term and short-term field experiments, respectively. Moreover, the use of pig slurry also reduced the ratio when compared to the mineral or the control treatments. Our study demonstrates that, in dryland Mediterranean agroecosystems, the combination of NT and medium rates of either mineral or organic N fertilization can be an appropriate management strategy in terms of CO_2 and CH_4 emissions and grain yield production.

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Capítulo 6

Tillage and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed Mediterranean area

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Tillage and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed Mediterranean area

Abstract

There is a strong need to identify the combination of tillage and N fertilization practices that reduce the amount of nitrous oxide (N_2O) emissions while maintaining crop productivity in dryland Mediterranean areas. We measured the fluxes of N_2O in two experimental fields with 3 and 15 years since their establishment. In the long-term experiment, two types of tillage (NT, no-tillage, and CT, conventional intensive tillage) and three mineral N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared. In the short-term experiment, the same tillage systems (CT and NT) and three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of fertilizers (mineral N and organic N with pig slurry) were compared. During two (long-term experiment) and three (short-term experiment) cropping seasons the emissions of N_2O were measured. Additionally, water-filled pore space and soil mineral N content as nitrate and ammonium were determined. Grain yields, N-biomass inputs and soil total nitrogen (STN) stocks were also quantified and the N_2O yield-scaled ratio as kg of CO_2 equivalents per kg of grain produced was calculated for each treatment. In both experiments tillage treatments significantly affected the dynamics of N_2O fluxes. Cumulative losses of N as N_2O were similar between tillage treatments in the long-term field experiment. Contrarily, although not significant, cumulative N losses were about 35% greater under NT than CT in the short-term experiment. While NT significantly increased the production of grain and the inputs of N to the soil as above-ground biomass in both experiments, CT emitted 0.362 and 0.104 kg CO_2 eq. kg $grain^{-1}$ in the long-term and the short-term experiment, respectively, clearly more than NT that emitted 0.033 and 0.056 kg CO_2 eq. kg $grain^{-1}$, respectively. Nitrogen fertilization rates did not affect the average N_2O fluxes or the total N losses during the period of gas measurement in the long-term experiment. Contrarily, in the short-term experiment, a higher emission of N_2O when increasing the amount of N applied to the soil was quantified for both mineral and organic fertilizers. In this experiment, the losses of N as N_2O were: 700, 911, 1804, 1007 and 1283 g N ha^{-1} for the control, the 75 and 150 kg mineral N ha^{-1} and the 75 and 150 kg organic N ha^{-1} treatments, respectively. The use of pig slurry increased grain production when compared with the mineral N treatment, thus reducing the yield-scaled emissions of N_2O to a 44%. Our results showed that in rainfed Mediterranean agroecosystems, the use of NT and pig slurry are effective means of yield-scaled N_2O emissions reduction.

1. Introduction

Human activities impact the N cycle through the production and use of fertilizers and fossil fuel combustion (Galloway et al., 2004). Agricultural and natural N inputs to the biosphere from N fertilizers, animal manure, biological N_2 fixation and atmospheric N deposition increased from 155 to 345 Tg N yr^{-1} between 1900 and 2000 (Bouwman et al., 2013). That increase entails major losses of N from the agricultural systems such as nitrate leaching, erosion and gaseous emissions by denitrification. Among them, denitrification is the major terrestrial N removal process (Bouwman et al., 2013; Seitzinger et al., 2006).

The emission of N to the atmosphere as nitrous oxide (N_2O) has received recent attention due to its role as a powerful greenhouse gas (GHG) with a global warming potential (GWP) 298 times greater than the carbon dioxide (CO_2) (Forster et al., 2007) and its involvement in the depletion of the ozone (O_3) layer in the stratosphere that could result in harmful effects due to solar ultraviolet radiation (Crutzen, 1974). The transformation of N to N_2O has been related mainly to two biological processes, being the first process the loss of N as N_2O during the nitrification of NH_4^+ under aerobic conditions and the second the reduction of NO_3^- under anaerobic conditions. Both processes account for 70% of global N_2O emissions (Braker and Conrad, 2011). Although having a reduced relative importance in soils, other processes such as chemodenitrification, chemical decomposition of hydroxylamine, nitrifier-denitrification and coupled nitrification-denitrification may also be involved in the production of N_2O (Bremner, 1997; Butterbach-Bahl et al., 2013).

Several environmental factors influence the production of N_2O in soils. The presence of readily-available C fractions such as water-soluble organic C enhances the denitrification process by acting as a source of energy for denitrifying organisms (Burford and Bremner, 1975). Another important factor is the availability of oxygen in the soil, a parameter that is directly affected by the presence of water. In this line, Linn and Doran (1984) studied the regulatory effect of water-filled pore space (WFPS) on N_2O production in soils under different types of tillage. The last authors pointed out that a WFPS of 65% is the threshold above which denitrification is the main process producing N_2O, while below that value nitrification plays a major role. Denitrification also depends on temperature because of enzyme dependence on that parameter (Saad and Conrad, 1993) and on pH, since soil acidity restricts denitrification intensively

(Bandibas et al., 1994). Moreover, the supply of ammonium and nitrate also regulates the potential loss of N from soil as N_2O. As Smith et al. (1997) suggested, the more N is cycled through the system, the more is the amount converted to N-oxides. That process was detailed by Firestone and Davidson (1989) with their "hole-in-the-pipe" conceptual model. In this model, an increase in the flow that moves throughout the pipe (the whole N in the soil) results in an increase in the amount of N lost through the hole (losses such N-oxides). According to the last factors, it is easy to understand that any agricultural practice that implies changes in the soil N substrates or soil environmental conditions can lead to important variations in soil N_2O production.

Fertilization is a key process controlling soil N_2O fluxes. Bouwman et al. (2002) carried out a meta-analysis on 139 N_2O studies conducted in agricultural fields and observed an increase of N_2O emissions with increasing N application rates, mainly with application rates above 100 kg N ha^{-1}. Those results are also supported by the findings of Rees et al. (2013) who synthesized different European agricultural experiments. Earlier studies showed a greater amount of N_2O lost to the atmosphere when agricultural soils were manured than when mineral N fertilizers were used (Bouwman, 1990). However, other authors have obtained no differences between organic and mineral fertilizers (Meijide et al., 2009) or higher N_2O emissions when using mineral products (Meijide et al., 2007; Aguilera et al., 2013). Moreover, different results arise when separating between organic solid and liquid fertilizers (Aguilera et al., 2013). In their meta-analysis, the last authors found that only organic solid fertilizers led to significantly lower N_2O emissions than mineral fertilizers (Aguilera et al., 2013). Although in recent years several publications have covered the effect of fertilization on N_2O emission, much less attention has been paid to the interaction of different tillage and fertilization practices.

The use of conservation tillage practices has been claimed as a mechanism to reduce the CO_2 atmospheric pool by increasing the amount of organic carbon in the soil. In this line, several studies have shown the benefits in terms of soil organic carbon sequestration when using no-tillage (NT) over a broad range of edaphoclimatic conditions (Follett, 2001). However, different authors have also suggested that the benefits obtained with the use of NT could be counterbalanced by an increase in N_2O emissions due to the greater amount of water in the soil and soluble forms of C in non-tilled systems (Aulakh et al., 1984; Ball et al., 1999; Smith et al., 2001). Nevertheless, Six et al. (2004) suggested that the emissions of N_2O could be reduced when

maintaining NT over time. According to this last observation, van Kessel et al. (2013) conducted a meta-analysis on 239 direct comparisons between conventional tillage (CT) and NT and reduced tillage (RT) and pointed out that, on average, both NT and RT did not show greater N_2O emissions when compared with CT. Moreover, they found a significant reduction in these emissions in long-term experiments (> 10 yr) under NT and RT practices, mainly in dry climates. In the same way, Grandy et al. (2006), when analyzing contrasting types of tillage in a long-term experiment in Michigan, found no differences between NT and CT in N_2O emissions.

In the Mediterranean area the use of RT or NT systems is increasingly adopted due to its agricultural and environmental benefits (Kassam et al., 2012). For instance, a better crop performance under NT due to greater soil water availability has been reported (Cantero-Martínez et al., 2003; Giambalvo et al., 2012). In turn, in some areas of semiarid Spain, animal waste from a large livestock intensive farming sector is a relevant economic activity. The application of organic fertilizers as amendments are a valuable resource for low-fertility soils and could lead to the increase in the amount of soil organic C and N (Hernández et al., 2013). However, the interaction between the C input concomitant with the application of organic fertilizers and the greater amount of water stored in the soil usually found under NT in the Mediterranean agricultural systems could enhance the emission of N_2O to the atmosphere (Smith et al., 2001).

The objective of our study was to identify the optimum combination of tillage and N fertilization practices to reduce the amount of N_2O emitted from the soil to the atmosphere per unit of production in dryland Mediterranean areas. Our main hypotheses were that (i) due to the higher conservation of water under NT the emission of N_2O under this tillage system would be higher when compared with CT, (ii) although the greater emission under NT could be compensated by a greater yield, and (iii) in turn, the combination of organic fertilizers and NT would increase the N emitted as N_2O due to the presence of labile C in the composition of the organic materials.

2. Material and Methods

2.1 Experimental sites

The study was carried out in two experimental fields with different tillage and fertilization management established in 1996 (long-term experiment) and 2010 (short-term experiment) in northeastern Spain. Selected site characteristics and soil properties for both experiments are detailed in Table 28.

Table 28 General site and soil characteristics in the 0- to 30-cm soil depth at the beginning of the experiments at the two study sites.

Site and soil characteristics	Long-term experiment (Agramunt)	Short-term experiment (Senés de Alcubierre)
Year of establishment	1996	2010
Latitude	41º 48’ 36’’ N	41º 54’ 12’’ N
Longitude	1º 07’ 06’’ E	0º 30’ 15’’ W
Elevation, m	330	395
Annual precipitation, mm	430	327
Mean annual air temperature, ºC	13.8	13.4
Annual ETo, mm	855	1197
Soil classification†	Typic Calcixerept	Typic Xerofluvent
pH (H_2O, 1:2.5)	8.5	8.0
$EC_{1,5}$, dS m^{-1}	0.15	1.04
Organic C, g kg^{-1}	7.6	15.6
Organic N, g kg^{-1}	-	1.4
Particle size distribution, %		
Sand (2000-50 µm)	46.5	6.2
Silt (50-2 µm)	41.7	63.3
Clay (<2 µm)	11.8	30.5

† According to the USDA classification (Soil Survey Staff, 1994).

In the long-term experiment, two types of tillage (NT, no-tillage, and CT, conventional intensive tillage) and three N fertilization rates (0, 60 and 120 kg N ha^{-1}) were compared. The CT treatment consisted of one pass of moldboard plow to 25 cm depth followed by two passes of a cultivator to 15 cm depth, both in September-October. Nitrogen fertilizer was manually-applied and split into two applications: one-third of the rate before seeding as ammonium sulphate (21% N) and the rest at the beginning of tillering, in February, as ammonium nitrate (33.5% N). The cropping system consisted of continuous barley (*Hordeum vulgare L.,* cv. Hispanic from 1996 to 2010 and cv. Cierzo from 2010 to 2013). The historical management of the field prior to the establishment of the experiment was based on conventional intensive tillage with moldboard plowing and winter cereal monoculture.

In the short-term experiment, two tillage systems (CT with disk plow and NT), three N fertilization doses (0, 75 and 150 kg N ha^{-1}) and two types of N fertilizers (mineral N with ammonium sulphate and ammonium nitrate and organic N with pig slurry) were compared. The use of disk plow in the CT treatment is shown in Fig 30.

Figure 30 Conventional tillage with disk plow in the short-term field experiment.

In 2011, the CT treatment was carried out with two passes of chisel instead of disk plow due to the dry conditions of the soil. The treatment with 150 kg mineral N ha^{-1} was split into two manual applications, half of the dose before tillage as ammonium sulphate (21% N) and the other half at the beginning of tillering, in February, as ammonium nitrate (33.5% N),whereas the 75 kg N ha^{-1} treatment was applied entirely at tillering as ammonium nitrate. Similarly, in the pig slurry treatments, the 75 kg N ha^{-1} rate was applied entirely at tillering and the 150 kg N ha^{-1} one was split into two applications, one before tillage and the other one at tillering. The pig slurry was obtained from a commercial farm in the area and was conventionally surface-spread via a commercial vacuum tanker fitted with a splashplate, previously calibrated to apply the precise dose after analyzing the pig slurry composition. The cropping system before and during the experiment consisted of a barley (*Hordeum vulgare L.,* cv. Meseta) monoculture. Four years prior to the set-up of the experiment, soil management consisted of NT with mineral N fertilizer and application rates between 75 and 100 kg N ha^{-1}. Before that period passes of subsoiler and chisel were used since the 1970's decade.

For both experimental fields, the NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. Planting was performed in November with a disk direct drilling machine set to 2-4 cm and harvesting was carried out with a commercial medium-sized combine in June. The straw residue was chopped and spread over the soil (Fig. 31). Both experiments consisted of a randomized block design with three replications. Plot size in the long-term field experiment was 50 m x 6 m while in the short-term experiment plot size was 40 x 12 m in the organic N fertilization treatment and 40 x 6 m in the mineral N fertilization treatment. Also, for both experiments, air temperature and rainfall values were recorded hourly using an automated weather station located in each experimental area.

Figure 31 Barley harvest in the short-term field experiment with a commercial combine.

2.2 Soil N_2O emission quantification and analyses

The emission of N_2O from the soil to the atmosphere was measured with the non-steady-state chamber method (Hutchinson and Mosier, 1981). At the beginning of each experiment, two polyvinyl chloride rings (31.5 cm internal diameter) per plot were inserted into the soil to a depth of 5 cm. The rings were only removed at the time of tillage, planting and harvesting operations. Vented chambers of the same material and 20-cm height were fitted into the rings when the measurements were performed. The chambers were covered with a reflective insulation layer (model Aislatermic, Arelux, Zaragoza, Spain). A metal fitting was attached in the center of the top of the chamber and was lined with two silicon-Teflon septa as a sampling port.

Gas measurements were performed every two to three weeks. During the fertilizer applications more frequent samplings were performed (i.e., 24 h prior and 3 h and 72 h after fertilization). The sampling protocol consisted in one operator sampling each block of the experiment (Fig. 32). That procedure intended to reduce as much as possible the amount of time during the sampling process, thus avoiding temperature-induced biases (Rochette et al., 2012). Gas samples of 15 mL were obtained with polypropylene syringes at 0, 30 and 60 minutes after closing the chamber. Each sample was immediately injected into 12 mL Exetainer® borosilicate vials (model 038W, Labco, High Wycombe, UK). To quantify the amount of N_2O, gas samples were analyzed with an Agilent 7890A gas chromatography system equipped with an electrical conductivity detector (ECD) and an HP-Plot Q column (30 m long, 0.32 mm of section and 20 µm) with a pre-column 15 m long of the same characteristics. The injector and oven temperatures were set to 50ºC. The temperature of the ECD detector was set to 300 ºC, using a 5% methane in Argon gas mixture as a make-up gas at 30 mL min^{-1}. The system was calibrated using analytical grade standards (Carburos Metálicos, Barcelona, Spain). Soil N_2O emission rate was calculated taking into account the linear increase in the N_2O concentration within the chamber with time and correcting the values for the air temperature. The experiment covered three cropping seasons (2010-2011, 2011-2012 and 2012-2013) in the short-term experiment and two cropping seasons (2010-2011 and 2011-2012) in the long-term experiment. However, due to experimental constraints, gas samplings for both experiments began at the time of top-dressing application of fertilizers in February 2011.

2.3 Soil N-biomass input quantification

The quantification of the amount of N returned to the soil as above- and below-ground ground crop residues was performed in both experiments. Above-ground N biomass inputs were quantified in the 2011-2012 growing season in the long-term experiment and in the 2010-2011, 2011-2012 and 2012-2013 growing seasons in the short-term experiment by sampling plants along 0.5 m of the seeding line just before harvest, at three randomly selected locations per plot. Once in the laboratory the samples were dried at 65ºC during 48 h and threshed. Then all the plant but the grain (i.e., above-ground crop residues) was weighed. Root biomass was measured at flowering in April 2012 in the long-term and short-term experiments and in May 2013 in the short-term experiment. For each plot four soil cores (0-30 cm) were obtained, two in the seeding

line and the other two within lines. Each soil sample was dispersed with a 5% sodium hexametaphosphate solution in a reciprocal shaker during at least 30 minutes and then washed by hand with a low-pressure shower jet through a 0.5-mm sieve to recover the roots, following the methodology proposed by Böhm (1979). Once washed, the sieve was submerged in a tray filled with water in order to ease collection of floating roots. Finally, root biomass was oven-dried at 65ºC and weighed. Root biomass per unit of area was calculated dividing the weight of roots by the area sampled with the core. Afterwards, above- and belowground biomass samples were analyzed for N content by dry combustion. The above- and below-ground biomass N inputs were calculated by multiplying the weight of each fraction by its N concentration. Grain yield of each treatment was measured in 2012 in the long-term experiment and in 2011, 2012 and 2013 in the short-term experiment by harvesting the plots with a commercial combine and weighing the grain.

Figure 32 Gas sampling procedure in the short-term field experiment.

2.4 Soil sampling and analyses

Soil samples from the 0-5 cm soil layer were obtained at every sampling date near (<1 m far) each gas sampling chamber for the determination of the water-filled pore space (WFPS) and the ammonium (NH_4^+) and nitrate (NO_3^-) present in the soil. The WFPS was obtained as the quotient between soil volumetric water content and total porosity. The volumetric water content was calculated as the gravimetric water content times the soil bulk density. The gravimetric water content was obtained by oven drying the soil samples at 105 ºC for the long-term experiment and at 50ºC for the short-term experiment until constant weight. In the short-term experiment, soil was dried at 50ºC in order to avoid the dehydration of the gypsum present in the soil of this experiment (Porta, 1998). Soil porosity was calculated as a function of soil bulk density assuming a particle density of 2.65 Mg m^{-3}. In turn, soil bulk density was determined with the cylinder method. The soil NH_4^+ and NO_3^- contents were calculated by extracting 50 g of fresh soil with 100 mL of 1M KCl. The extracts were analyzed with a continuous flow autoanalizer (Seal Autoanalyzer 3, Seal Analytical, Norderstedt, Germany). Both ions were transformed to kg N ha^{-1} in a dry soil basis taking into account soil moisture and bulk density.

Also, soil total nitrogen (STN) stocks were calculated in June 2013 in the short-term experiment. To this end, soil samples were taken from five depths (0-5, 5-10, 10-25, 25-50 and 50-75 cm) at two selected areas per plot. For the same depths soil bulk density was determined using the cylinder method. Afterwards samples were air-dried and 0.5-mm sieved. The STN concentration was determined by dry combustion. Finally, the concentration values were transformed to STN stocks following the equivalent soil mass procedure (Ellert and Bettany, 1995).

2.5 Total N loss as N_2O, yield-scaled N_2O emission and statistical analysis

Total N loss as N_2O during the entire period of gas measurements was quantified with the trapezoid rule. In the long-term experiment, the yield-scaled N_2O ratio, expressed as kg of CO_2 equivalents emitted per kg of grain produced, was calculated for the 2011-2012 growing season by integrating the N_2O fluxes from the pre-seeding application of fertilizers until harvest, taking into account that the GWP for the N_2O is 298 times higher than that of CO_2 (Forster et al., 2007) and dividing that result by the amount of grain produced by each treatment in that cropping season. In the short-term experiment, the ratio was calculated for two growing seasons (2011-2012 and 2012-2013) by

integrating the emissions of N_2O from the pre-seeding application of fertilizers in the 2011-12 growing season until the harvest of the 2012-13 season and dividing that result by the sum of grain produced by each treatment in both cropping seasons.

Data for N_2O fluxes, soil ammonium and nitrate content and WFPS were analyzed using the JMP 10 statistical package (SAS Institute Inc, 2012) performing a repeated measures analysis of variance (ANOVA) with tillage, N fertilization, date of sampling and their interactions as sources of variation. When needed, a Box-Cox data transformation was used in order to normalize the data and the variances. Also, different ANOVA were performed for cumulative N loss, above- and below-ground biomass N inputs, STN stocks and yield-scaled N_2O ratio with tillage, N fertilization and their interaction as sources of variation. When significant, differences among treatments were identified at 0.05 probability level of significance using a Tukey test. The statistical package JMP 10 (SAS Institute Inc., 2012) was also used to test the presence of linear relationships between N_2O fluxes and soil ammonium, nitrate, WFPS and temperature at 0-5 cm soil depth.

3. Results

3.1 Weather conditions during the experimental period

Air temperature and precipitation in the long- and short-term experiments are presented in Table 29. Given the average annual precipitation at the long-term (430 mm) and the short-term (327 mm) experimental sites (Table 29), the 2010-2011 and 2011-2012 growing seasons could be considered drier than average in both experiments. In contrast, the 2012-2013 growing season registered a higher rainfall than the average in the short-term experiment, with a total of 537 mm (Table 29). Throughout the experimental period, the air temperature showed the typical pattern of the Mediterranean region with hot summers and cold winters.

Table 29 Mean monthly air temperature (T) and monthly precipitation (P) in the short-term and long-term field experiments during the 2010-2011, 2011- 2012 and 2012- 2013 growing seasons.

Month	Long-term experiment				Short-term experiment					
	2010-2011		2011-2012		2010-2011		2011-2012		2012-2013	
	T	P	T	P	T	P	T	P	T	P
July	25.4	5	22.6	19	24.7	7.4	22.1	6	23.5	2
August	23.2	16	24.9	29	22.9	0.5	23.9	4.6	25.6	7
September	18.5	21	22.0	2	17.7	37.3	20.6	8.9	20.2	18
October	12.9	1	16.5	22	12.1	57.5	14.5	32.4	15.5	212
November	6.2	0	10.9	82	6.9	38.8	10.2	45.4	10.1	27
December	3.2	0	5.7	2	2.9	21.4	7.1	4.9	7.7	16
January	2.7	0	4.3	4	3.6	19.4	6.9	0	7.1	27
February	5.3	12	3.6	2	6.8	13.3	5.5	1	6.8	6
March	8.7	36	11.3	5	8.9	90.5	12.0	17	10.0	85
April	14.3	20	11.8	62	14.5	16.2	11.7	105	12.4	66
May	18.2	27	18.3	19	17.8	35.5	18.3	8	13.4	18
June	20.6	73	23.8	12	20.6	0.7	23.0	47	19.1	53
Year	13.3	211	14.7	260	13.3	338.5	14.6	280.2	14.3	537

3.2 Tillage effects

3.2.1. WFPS, soil ammonium and soil nitrate content

In the long-term experiment, the WFPS only exceeded the value of 65% in two of all the samplings performed (Fig. 33A) whereas in the short-term experiment WFPS values were below that threshold during the whole experimental period (Fig. 33B). Significant differences between tillage treatments in WFPS were found in both experiments, being NT the treatment which presented the highest values in most samplings dates (Fig. 33A

and B). In both experiments, the mean soil ammonium values were low (<2 kg NH_4^+-N ha^{-1}) (Tables 30 and 31). However, as shown in Figs. 34A and 34C, the application of fertilizers led to a fast and short-lived increase in soil ammonium content, reaching values of 100 and 129 kg NH_4^+-N ha^{-1} in the long-term (Fig. 34A) and short-term (Fig. 34C) experiments, respectively. Significant differences between tillage treatments in soil ammonium content were only found in the long-term experiment (Fig. 34A). In this experiment, soil nitrate content in the soil surface (0-5 cm) was affected by tillage, with higher mean values under CT (56.4 kg NO_3^--N ha^{-1}) when compared with NT (36.1 kg NO_3^--N ha^{-1}) (Table 30). In the long-term experiment, although relatively low values were observed during the first year, a greater accumulation of nitrate in the soil was observed under CT compared with NT during the last months studied (February-August 2012) (Fig. 34B). In the case of the short-term experiment (Fig. 34D), tillage treatments presented a similar nitrate content in the soil. However, in some sampling events significant differences between treatments also arose. Also, and as a general trend, a higher amount of nitrate in the soil was observed in the short-term experiment (Fig. 34D) when compared with the long-term one (Fig. 34B).

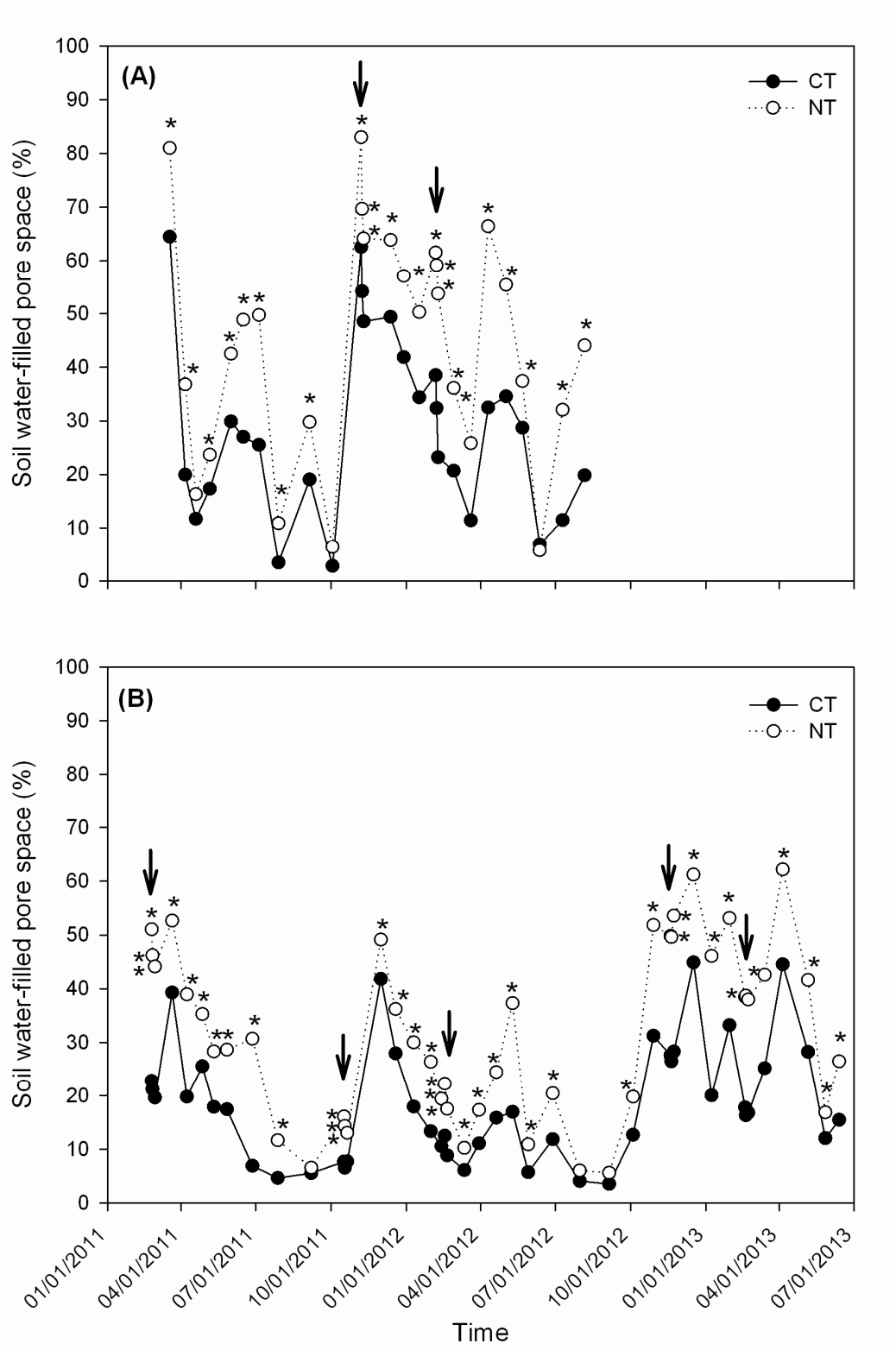

Figure 33 Water-filled pore space as affected by tillage (CT, conventional tillage; NT, no-tillage) in (A) the long-term experiment and (B) the short-term experiment. * Indicates significant differences between tillage treatments for each date at $P<0.05$. Vertical arrows indicate fertilizer applications.

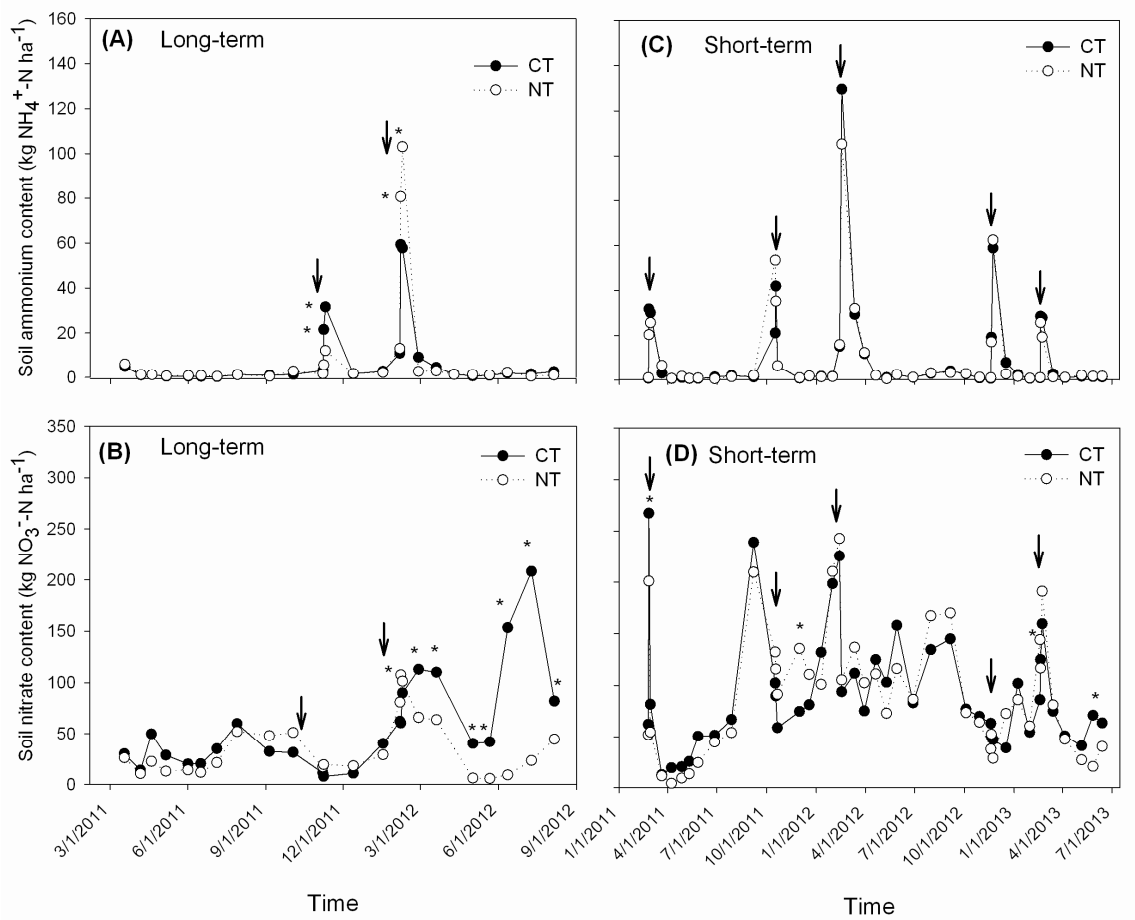

Figure 34 Soil ammonium (A) and nitrate (B) content in the long-term experiment and ammonium (C) and nitrate (D) content in the short-term experiment in the 0-5 cm soil layer as affected by tillage (CT, conventional tillage; NT, no-tillage). * Indicates significant differences between tillage treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

3.2.2. Soil N_2O emission, N-biomass inputs and yield-scaled N_2O emission

In the long-term experiment, the average N_2O flux throughout the entire experimental period was 0.137 and 0.141 mg N_2O-N m^{-2} d^{-1} for the CT and NT treatments, respectively, without significant differences between them (Table 30). Contrarily, in the short-term experiment, a greater average N_2O flux was observed under NT (0.205 mg N_2O-N m^{-2} d^{-1}) than under CT (0.139 mg N_2O-N m^{-2} d^{-1}). In both experiments, tillage treatments significantly affected the temporal dynamics of N_2O fluxes (Fig. 35). In the long-term experiment, N_2O fluxes ranged from -0.09 to1.14 mg N_2O-N m^{-2} d^{-1} while in the short-term experiment the fluxes varied from -0.05 to 0.91 mg N_2O-N m^{-2} d^{-1} (Fig. 35). Tillage treatments presented significant differences for six sampling dates in the long-term experiment in which, half of the dates, CT presented the highest values and in the other half NT (Fig. 35). Differently, in the short-term experiment, significant

differences were found only in four sampling dates, being always NT the highest N_2O emitter. When integrating all the sampling period neither the long-term experiment nor the short-term experiment showed significant differences between tillage systems in cumulative N losses as N_2O (Tables 30 and 31).

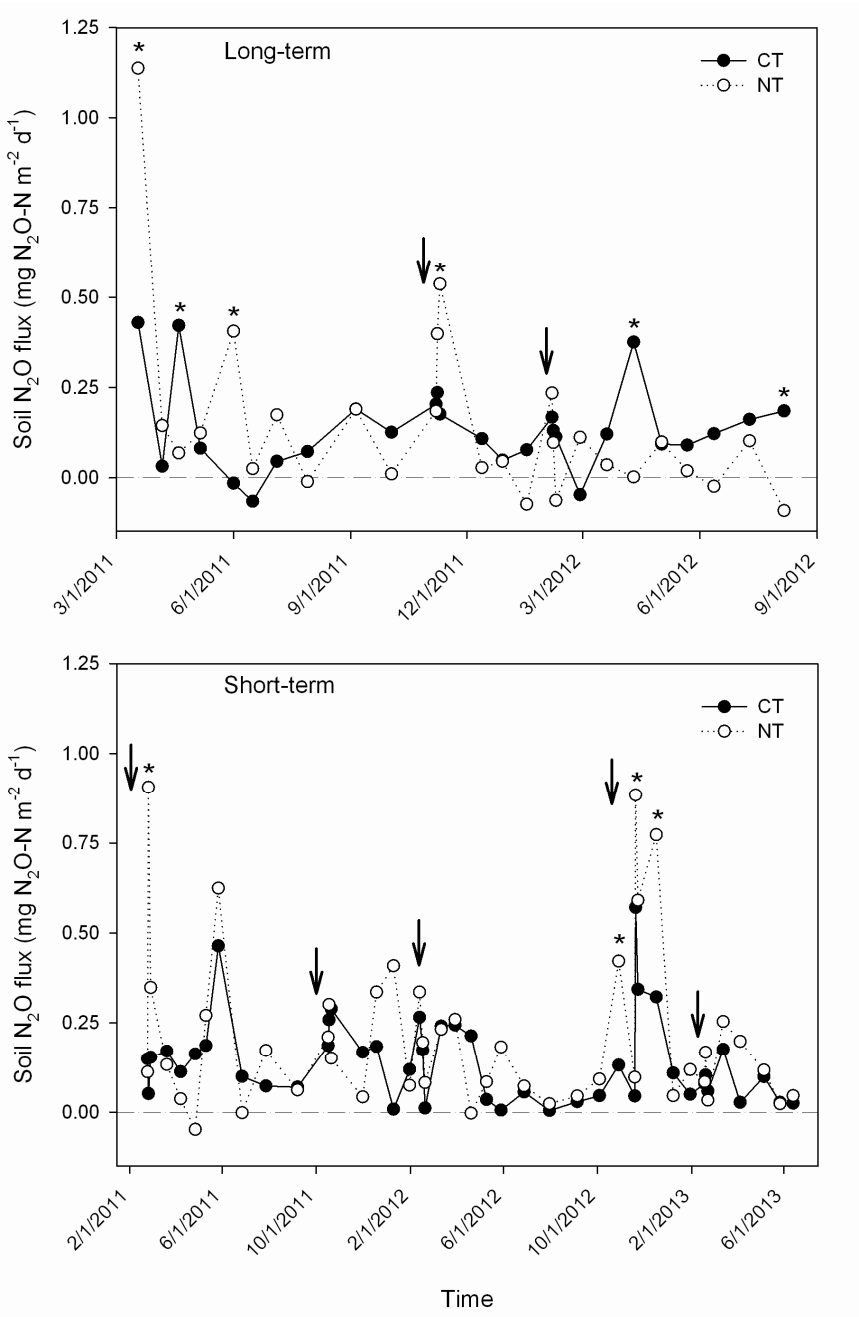

Figure 35 Soil N_2O fluxes in the long- and short-term experiments as affected by tillage (CT, conventional tillage; NT, no-tillage). * Indicates significant differences between tillage treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

Table 30 Analysis of variance of nitrate and ammonium content in the soil (0-5 cm) (kg NO_3^--N ha^{-1} and kg NH_4^+-N ha^{-1}, respectively), above- and below-ground N-biomass inputs, N_2O flux (mg N_2O-N m^{-2} d^{-1}), cumulative N-loss during the whole experimental period (g N ha^{-1}), 2011-12 grain yield (kg ha^{-1} at 10% moisture) and the ratio between the loss of N_2O in CO_2 equivalents and the grain yield of the 2011-12 growing season as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization treatments (0, 60 and 120 kg mineral N ha^{-1}), date of sampling (growing season in the case of N-biomass inputs) and their interactions in the long-term field experiment.

Effects	Long-term experiment							
	Soil nitrate (0-5 cm)	Soil ammonium (0-5 cm)	N-biomass input (g N m^{-2})		N_2O flux	Cumulative N-loss	2011-2012 Grain yield	kg CO_2 eq kg $grain^{-1}$
			Above-ground	Below-ground				
Tillage	*0.004*	*0.476*	*<0.001*	*0.167*	*0.817*	*0.591*	*<0.001*	*<0.001*
CT	56.40 a¶	8.59	2.11 b	1.21	0.137	616	245.8 b	0.362 a
NT	36.07 b	9.59	6.97 a	1.49	0.141	549	1554.3 a	0.033 b
Nitrogen	*<0.001*	*<0.001*	*0.047*	*0.098*	*0.094*	*0.191*	*<0.001*	*0.164*
0	19.11 c	3.44 b	3.33 b	1.16	0.113	473	719.6 c	0.095
60	50.02 b	10.69 a	4.84 ab	1.67	0.122	532	940.7 b	0.290
120	69.82 a	13.15 a	5.46 a	1.21	0.181	742	1039.8 a	0.208
Tillage x Nitrogen	*0.163*	*0.275*	*0.452*	*0.461*	*0.290*	*0.425*	*<0.001*	*0.155*
CT – 0	21.68	3.49	1.88	1.14	0.100	442	178.4 c	0.170
CT – 60	60.07	8.70	2.22	1.58	0.153	681	226.8 c	0.563
CT – 120	87.46	13.57	2.24	0.90	0.158	724	332.1 c	0.354
NT – 0	16.53	3.39	4.78	1.18	0.126	503	1260.8 b	0.019
NT- 60	39.98	12.69	7.45	1.76	0.093	383	1654.5 a	0.018
NT – 120	51.93	12.72	8.69	1.52	0.205	759	1747.5 a	0.062
Date	*<0.001*	*<0.001*			*<0.001*			
Tillage x Date	*<0.001*	*<0.001*			*<0.001*			
Nitrogen x Date	*<0.001*	*<0.001*			*<0.001*			
Tillage x Nitrogen x Date	*<0.001*	*0.340*			*0.121*			

¶ For a given variable, different lower-case letters indicate significant differences between treatments at $P<0.05$.

Table 31 Analysis of variance of nitrate and ammonium content in the soil (0-5 cm) (kg NO_3^--N ha^{-1} and kg NH_4^+-N ha^{-1}, respectively), above- and below-ground N-biomass inputs, N_2O flux (mg N_2O-N m^{-2} d^{-1}), cumulative N-loss during the whole experimental period (g N ha^{-1}), 2011-2012 plus 2012-2013 grain yield (kg ha^{-1} at 10% moisture) and the ratio between the loss of N_2O in CO_2 equivalents and the production of grain of the 2011-2012 plus 2012-13 growing seasons as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization treatments (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N with pig slurry at 75 kg N ha^{-1} and 150 Org, organic N with pig slurry at 150 kg N ha^{-1}), date of sampling (growing season in the case of N-biomass inputs) and their interactions in the short-term field experiment.

Effects	Short-term experiment							
	Soil nitrate (0-5 cm)	Soil ammonium (0-5 cm)	N-biomass input (g N m^{-2})		N_2O flux	Cumulative N-loss	2011-13 Grain yield	kg CO_2 eq kg $grain^{-1}$
			Above-ground	Below-ground				
Tillage	*0.820*	*0.577*	*<0.001*	*0.225*	*0.011*	*0.069*	*<0.001*	*0.004*
CT	98.18	11.43	3.50 b	1.18	0.139 b	971	2262.8 b	0.104 a
NT	96.01	10.86	5.09 a	1.43	0.205 a	1311	5692.3 a	0.056 b
Nitrogen	*<0.001*	*<0.001*	*<0.001*	*0.058*	*<0.001*	*0.004*	*<0.001*	*0.009*
0	64.78 bc¶	2.86 c	3.13 c	1.05	0.102 c	700 b	2358.6 d	0.075 ab
75 Min	106.53 ab	8.69 b	4.51 ab	1.00	0.132 bc	911 b	3651.1 c	0.096 ab
150 Min	148.29 a	15.95a	4.86 ab	1.52	0.271 a	1804 a	3885.2 c	0.131 a
75 Org	59.11 c	12.29 ab	3.34 bc	1.79	0.143 bc	1007 ab	4657.4 b	0.048 b
150 Org	106.78 ab	15.88 a	5.64 a	1.13	0.213 ab	1283 ab	5335.4 a	0.051 b
Tillage x Nitrogen	*0.482*	*0.298*	*<0.001*	*0.547*	*0.164*	*0.726*	*<0.001*	*0.136*
CT – 0	57.02	3.11	3.30 cd	0.89	0.088	624	992.3 f	0.099 ab
CT – 75 Min	116.54	8.40	2.58 d	0.53	0.098	647	1308.3 ef	0.144 ab
CT – 150 Min	153.67	17.54	4.60 bcd	1.35	0.279	1847	2225.7 de	0.179 a
CT – 75 Org	68.29	10.79	3.02 cd	1.88	0.106	736	2755.4 cd	0.052 b
CT- 150 Org	95.40	17.30	4.00 bcd	1.14	0.125	1001	4032.6 b	0.048 b
NT – 0	72.54	2.62	2.96 cd	1.21	0.117	776	3725.0 bc	0.051 b
NT – 75 Min	96.52	8.97	6.44 ab	1.46	0.166	1175	5993.9 a	0.048 b
NT – 150 Min	142.91	14.35	5.12 abc	1.68	0.264	1761	5544.8 a	0.083 ab
NT – 75 Org	49.92	13.79	3.65 cd	1.68	0.178	1278	6559.5 a	0.043 b
NT- 150 Org	118.16	14.46	7.28 a	1.12	0.299	1565	6638.3 a	0.055 b
Date	*<0.001*	*<0.001*	*<0.001*	*0.093*	*<0.001*			
Tillage x Date	*<0.001*	*0.512*	*0.483*	*0.209*	*0.002*			
Nitrogen x Date	*<0.001*	*<0.001*	*0.074*	*0.325*	*<0.001*			
Tillage x Nitrogen x Date	*0.717*	*0.732*	*0.006*	*0.443*	*0.003*			

¶ For a given variable, different lower-case letters indicate significant differences between treatments at $P<0.05$.

In both the long-term and short-term experiments, the use of NT significantly increased the production of grain (Tables 30 and 31). In the long-term experiment, grain yield was 246 and 1554 kg ha^{-1} in the 2011-2012 growing season for the CT and NT treatments, respectively. In the short-term experiment, the sum of grain yield for the 2011-2012 and 2012-2013 growing seasons accounted for 2263 and 5692 kg grain ha^{-1} under CT and NT, respectively (Table 31). Moreover, in the long-term experiment, NT showed greater N inputs as above-ground biomass when compared with CT, with 6.97 and 2.11 g N m^{-2}, respectively (Table 30). In the short-term experiment, the above-ground biomass N input average of the three growing seasons studied (2010-2011, 2011-2012 and 2012-2013) was 3.5 and 5.1 g m^{-2} for the CT and the NT treatments, respectively, with significant differences between them (Table 31). However, the below-ground N input was not significantly different between tillage treatments in any of the experiments (Tables 30 and 31).

When cumulative N-N_2O losses were related to grain yield, in both experiments NT significantly reduced yield-scaled N_2O emissions compared with CT. In the long-term experiment, the CT treatment emitted 0.362 kg CO_2 eq. kg^{-1} grain while the NT treatment emitted 0.033 kg CO_2 eq. kg^{-1} grain (Table 30). In the case of the short-term experiment, CT and NT averaged 0.104 and 0.056 kg of CO_2 eq. emitted per kg of grain, respectively (Table 31).

3.3 Nitrogen fertilizer type and rate effects

3.3.1. Soil ammonium and nitrate content

As it can be seen in Fig. 36A, in the long-term experiment, the application of 60 and 120 kg N ha^{-1} was accompanied by an increase in soil ammonium compared with the control treatment. However, during the rest of the experimental period soil ammonium content remained low and no differences between N fertilization rates were observed (Fig. 36A). On the other hand, the increasing N rates in the long-term experiment (0, 60 and 120 kg N ha^{-1}) were accompanied by increasing amounts of nitrate in the soil surface (0-5 cm): 19.1, 50.0 and 69.8 kg NO_3^--N ha^{-1}, respectively (Table 30). Differences between N fertilization rates were found in the dynamics of soil nitrate content (Fig. 36B), with the highest values under the 120 kg N ha^{-1} treatment in most of the sampling dates and the lowest in the control treatment. Likewise, in the short-term experiment the application of fertilizers was accompanied by large increases in the amount of ammonium in the soil

(Table 31 and Fig. 36C). Significant differences between N fertilization treatments in soil ammonium content were quantified during those dates, with greater values when 150 kg N ha^{-1} from mineral or organic sources were applied to the soil in four of the five fertilizer applications covered by the experiment. Similarly, significant differences were found between N fertilization treatments for the nitrate content at the soil surface layer (0-5 cm), with the greater values under the treatment with 150 kg mineral N ha^{-1} (Fig. 36D).

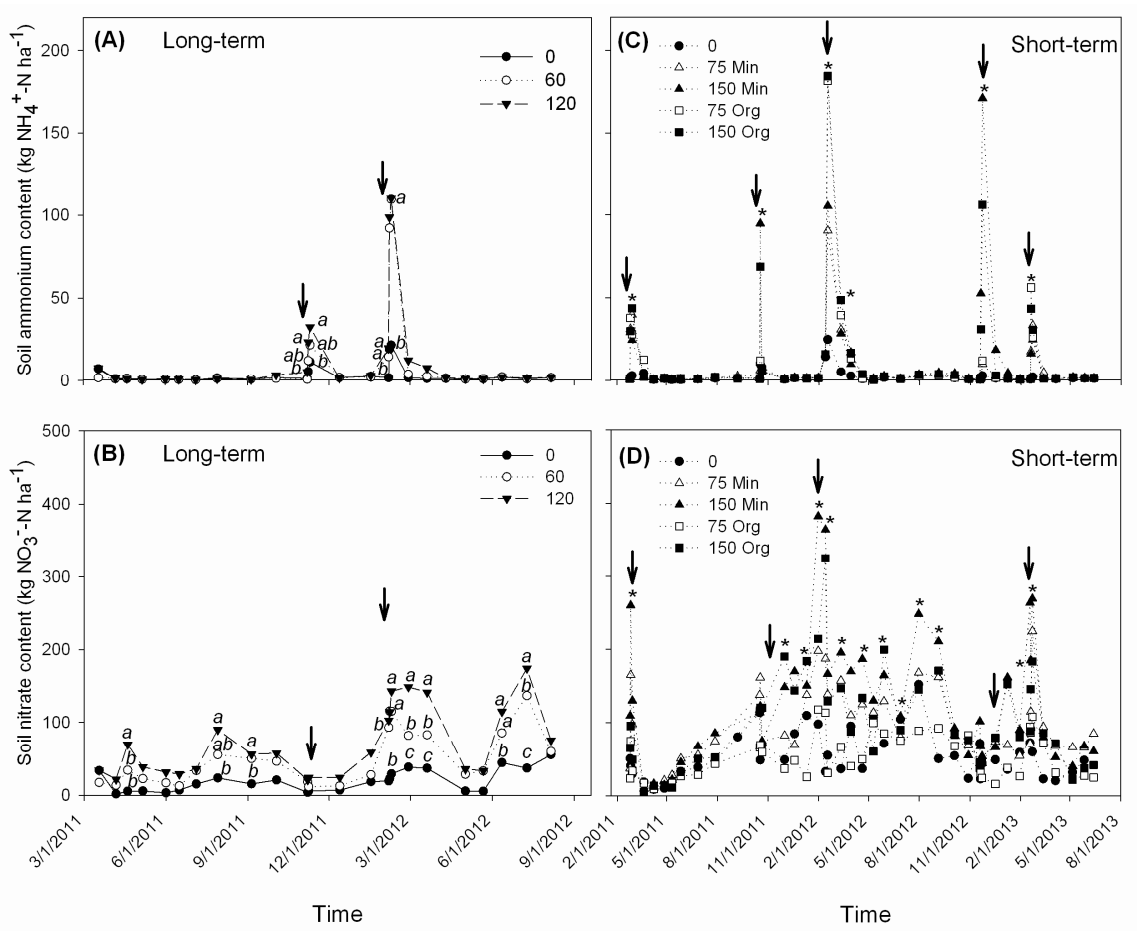

Figure 36 Soil ammonium (A) and nitrate (B) content in the long-term experiment and ammonium (C) and nitrate (D) content in the short-term experiment in the 0-5 cm soil layer as affected by N fertilization treatment (long-term experiment: 0, 60 and 120 kg mineral N ha^{-1}; short-term experiment: 0, control; 75 Min, 75 kg mineral N ha^{-1}; 150 Min, 150 kg mineral N ha^{-1}; 75 Org, 75 kg organic N ha^{-1} with pig slurry; 150 Org, 150 kg organic N ha^{-1} with pig slurry). * and different lower-case letters indicate significant differences between N fertilization treatments for each date and experiment at P<0.05. Vertical arrows indicate fertilizer applications.

3.3.2. Soil N_2O emission, N-biomass inputs and yield-scaled N_2O emission

The average N_2O fluxes quantified during the gas measurement period in the long-term experiment were 0.113, 0.122 and 0.181 mg N_2O-N m^{-2} d^{-1} for the 0, 60 and 120 kg mineral N ha^{-1} N rates, respectively, without significant differences between treatments (Table 30). Contrarily, in the short-term experiment significant differences between N fertilization treatments arose (Table 31). In this experiment, the lowest fluxes were observed in the control treatment (0.102 mg N_2O-N m^{-2} d^{-1}) and the highest when 150 kg mineral N ha^{-1} were applied (0.271 mg N_2O-N m^{-2} d^{-1}), with a higher emission of N_2O when increasing the amount of N applied to the soil for both mineral and organic fertilizers. However, no differences were found between N types (mineral or organic) on the average N_2O flux for a given N rate (Table 31). The temporal dynamics in the emission of N_2O from the soil to the atmosphere as affected by fertilization rates and types is shown in Fig. 37. In the long-term experiment, significant differences between N rates were observed in four sampling dates (Fig. 37). Likewise, in the short-term experiment, differences between treatments were observed in four sampling dates, three of them just after the application of fertilizers to the soil, in which the highest fluxes were registered in the treatments with applications of 150 kg N ha^{-1} (Fig. 37). Furthermore, for both experiments low negative fluxes were also found in different sampling dates.

The increasing rates of mineral N fertilizer (0, 60 and 120 kg N ha^{-1}) significantly increased the production of grain in the long-term experiment (720, 941 and 1040 kg grain ha^{-1}, respectively) (Table 30). In contrast, in the short-term experiment only the highest rate of mineral N (150 kg N ha^{-1}) showed greater grain yield that the control (0 kg N ha^{-1}) and the medium rate (75 kg N ha^{-1}) (Table 31). However, the use of pig slurry as a fertilizer increased the grain production compared with the mineral fertilizer and also showed a significant increase in yield (sum of the grain produced in the 2011-2012 and 2012-2013 growing seasons) when increasing the rate of slurry applied (4657 and 5335 kg grain ha^{-1} for the application of 75 and 150 kg organic N ha^{-1}, respectively) (Table 31). The above-ground N input to the soil in the long-term experiment was 3.33, 4.84 and 5.46 g N m^{-2} for the 0, 60 and 120 kg N ha^{-1} treatments, respectively, with significant differences between them (Table 30). In turn, the same parameter in the short-term experiment reached 3.1, 4.5, 4.9, 3.3 and 5.6 g N m^{-2} for the control, the 75 and 150 kg mineral N ha^{-1} and the 75 and 150 kg organic N ha^{-1} treatments, respectively,

with significant differences between them (Table 31). The input of N to the soil due to below-ground biomass did not show differences between N fertilization practices neither in the long-term experiment nor in the short-term experiment (Tables 30 and 30). Differences between N fertilization treatments in yield-scaled emissions of N_2O were only found in the short-term experiment. The ratio increased when the mineral N rate increased (0.075, 0.096 and 0.131 kg CO_2 eq. kg^{-1} grain for the 0, 75 and 150 kg mineral N ha^{-1}, respectively). Furthermore, the application of pig slurry showed the lowest ratio (for both 75 and 150 kg N ha^{-1} rates) (Table 31).

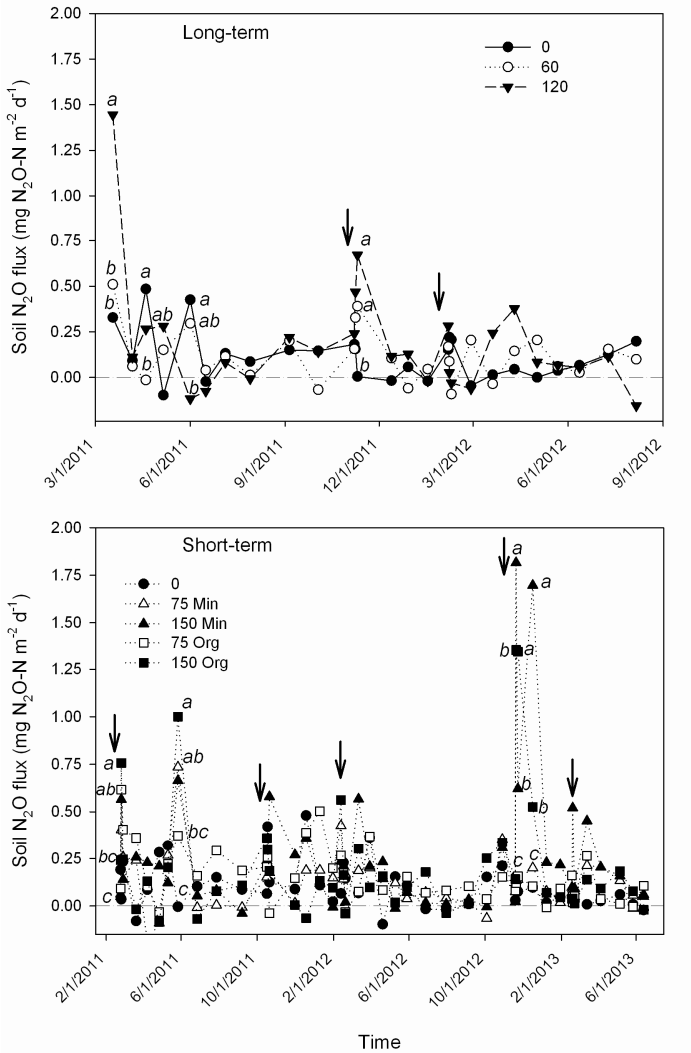

Figure 37 Soil N_2O fluxes in the long- and short-term experiments as affected by N fertilization treatment (long-term experiment: 0, 60 and 120 kg mineral N ha^{-1}; short-term experiment: 0, control; 75 Min, 75 kg mineral N ha^{-1}; 150 Min, 150 kg mineral N ha^{-1}; 75 Org, 75 kg organic N ha^{-1} with pig slurry; 150 Org, 150 kg organic N ha^{-1} with pig slurry). Different lower-case letters indicate significant differences between N fertilization treatments for each date and experiment at $P<0.05$. Vertical arrows indicate fertilizer applications.

3.4 Tillage and nitrogen interaction

In the short-term experiment, the interaction between tillage and N fertilization interaction was significant for N_2O fluxes, above-ground N inputs and grain yield (Table 31). In the case of the long-term field experiment, the interaction between tillage and N fertilization rates was only significant for soil surface NO_3^- and grain yield (Table 30). In the long-term experiment, no differences in grain yield were found between N rates under CT. However, under NT grater grain yield was observed when 60 and 120 kg N ha^{-1} were applied in comparison with the control treatment (Table 30). Contrarily, in the short-term experiment, in NT the application of mineral and organic N at different rates did not lead to significant differences in grain yield but in CT, for a given N rate, the use of pig slurry increased the production of grain when compared with the use of mineral N (Table 31). In the case of the above-ground N inputs to the soil as crop residues, in the short-term experiment, CT did not show differences between N fertilization treatments. However, in NT greater above-ground N inputs were quantified when applying 75 and 150 kg mineral N ha^{-1} and 150 kg N ha^{-1} of pig slurry when compared to the control treatment (Table 31).

3.5. Soil total N (STN) stocks

In the short-term experiment, STN stocks for the whole soil profile (0-75 cm) ranged from 5.2 and 8.9 for NT without N (control treatment) and CT with 75 kg organic N ha^{-1}, respectively (Fig. 38). However, after three years of contrasting treatments no differences between combinations of tillage and N fertilization were observed in the stocks neither in the soil surface (0-10 cm) nor in the entire soil profile (0-75 cm) (Fig. 38).

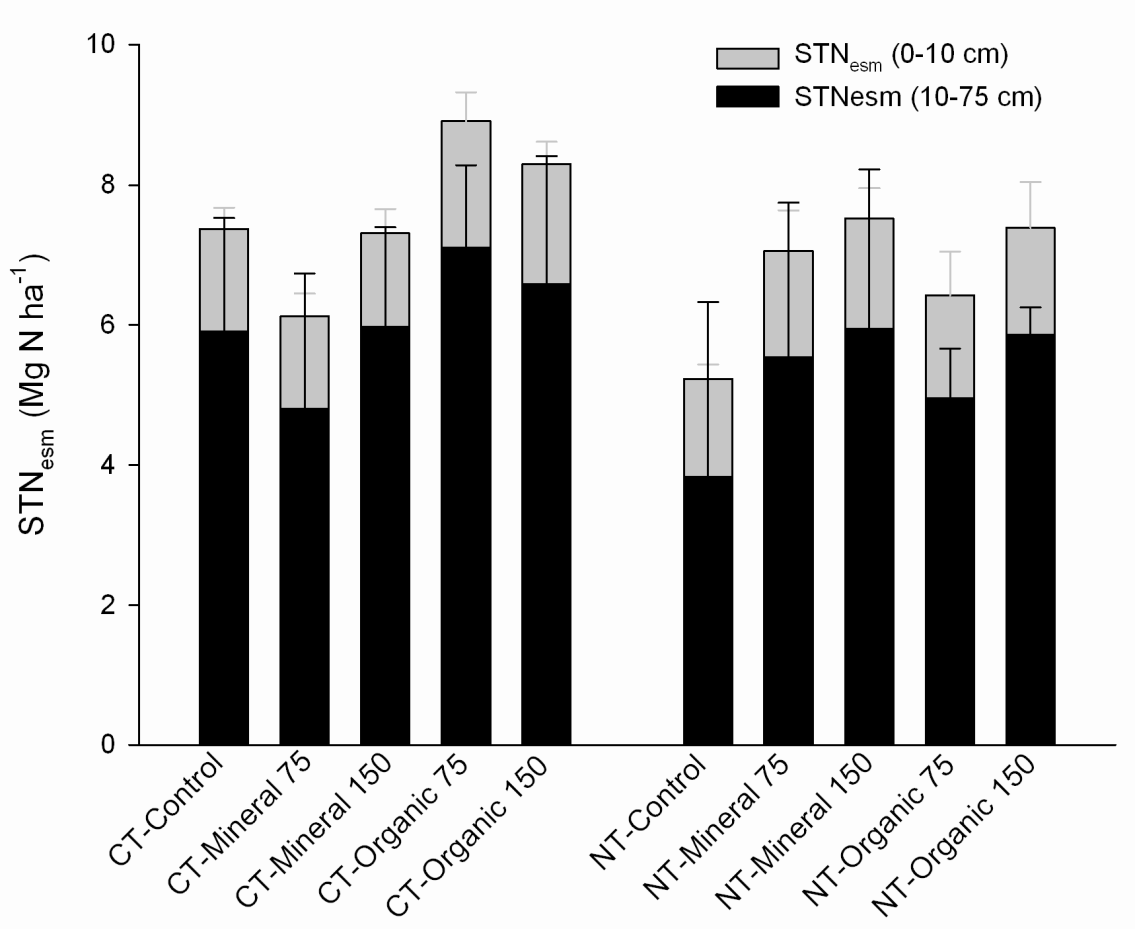

Figure 38 Soil total nitrogen stock in equivalent soil mass (STN_{esm}) for the 0-10 and 10-75 cm depths as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization treatments (0, control; 75 Min, mineral N at 75 kg N ha^{-1}; 150 Min, mineral N at 150 kg N ha^{-1}; 75 Org, organic N with pig slurry at 75 kg N ha^{-1} and 150 Org, organic N with pig slurry at 150 kg N ha^{-1}) in the short-term field experiment. Black and grey vertical bars indicate standard errors for STN_{esm} 10-75 cm and the STN_{esm} 0-10 cm, respectively.

4. Discussion

4.1 N_2O fluxes range and production mechanisms

In our study, most of the N_2O fluxes measured were within the range found in other similar studies performed in Mediterranean conditions (Meijide et al., 2009; Aguilera et al., 2013). In addition, WFPS values were below 65%. According to the findings of Linn and Doran (1984), this result would suggest that nitrification was the main process producing N_2O and that denitrification was restricted to anaerobic microsites due to the O_2 inhibitory effect on this process (Sexstone et al., 1985). In a fertilizer study conducted in rainfed Spain, Meijide et al. (2009) pointed out that denitrification was the N_2O producing process for only a few days during their experiment, on the basis of their denitrification estimates using the acetylene technique. Moreover, in our study, the low fluxes observed were accompanied by several episodes of negative values of N_2O. Soil consumption of N_2O has been measured under various edaphoclimatic conditions (Chapuis-Lardy et al., 2007). Although this process is usually related to the complete denitrification of N_2O to N_2 as a consequence of low soil mineral N and high water contents, recently its occurrence has also been reported in dry, oxic soils (Wu et al., 2013; Rees et al., 2013). Aerobic denitrification is assumed to be one of the main processes for N_2O consumption in dry soils with high O_2 concentration (Bateman and Baggs, 2005). On the other hand, no significant linear relationships were observed between the fluxes of N_2O and the different variables measured in any of the two experimental fields (data not shown).

4.2 Tillage effects

Different studies have shown greater emission of N_2O under NT than under CT (Skiba et al., 2002; Ball et al., 1999). This finding is usually related to a higher level of aeration and higher gas diffusivity when the soil is tilled. However, other authors have suggested lower N_2O emissions when NT is practiced for a long-term (> 10 years) (Omonode et al., 2011; Van Kessel et al., 2013) or in well aerated soils (Rochette, 2008). In our study, tillage significantly affected the average emissions of N_2O in the short-term experiment, with greater values under NT than under CT. In contrast, in the long-term experiment, the mean N_2O fluxes were the same for both tillage treatments. The improvement of soil structure under long-term NT could explain the different N_2O emission pattern observed between the short-term and the long-term experiments. This fact would be related to the increase in soil organic matter content under NT in the long-term experiment that would

have led to a reduction of anaerobic microsites and improved soil gas diffusivity (van Kessel et al., 2013). According to this hypothesis, in the same experimental site we have observed an increase in the proportion of soil water-stable macroaggregates when NT is maintained over time (Plaza-Bonilla et al., 2013). An improvement in soil structure could have resulted in greater soil gas diffusivity under NT, thus reducing the possibilities for denitrification and N_2O emissions. Moreover, the lower amount of nitrate found in NT compared with CT in the long-term experiment could also have impacted N_2O production and fluxes due to a lower amount of N available for denitrification.

The use of NT greatly improved the yield-scaled N_2O emissions compared with CT regardless the number of years of the experiment. In rainfed Mediterranean agrosystems, the occurrence of terminal drought during the grain filling period usually leads to a reduction in potential yield (Loss and Siddique, 1994; González et al., 2007). However, in these conditions, the use of NT increases the amount of water stored in the soil leading to a greater production of above-ground biomass and grain yield, mainly in dry years (Cantero-Martínez et al., 2003). This fact is also demonstrated in our results since above-ground N inputs and grain yield were higher in NT compared with CT, thus reducing the emissions of N_2O as CO_2 equivalents per kg of grain produced. These results are in line with those of Van Groenigen et al. (2010), who concluded that N uptake must be maximized in order to minimize N_2O emissions while maintaining or increasing crop yield.

4.3 Nitrogen type and rate effects

The application of increasing rates of mineral N fertilizer led to higher emissions of N_2O, although those emissions were only significantly different in the short-term experiment. Different authors reported a positive relationship between N_2O emissions and soil mineral N content and N application rates (Bouwman, 1996; Bouwman et al., 2002; Halvorson et al., 2008). In turn, in other studies it has been observed that when the threshold for satisfying crop N needs is exceeded, N_2O emissions increase dramatically (Snyder et al., 2009). In the area of our experiments, Angás et al. (2006) observed higher nitrogen use efficiency at lower N rates on a tillage and N application rate study. Their results would explain the greater N_2O-N losses we found when increasing the amount of mineral N applied to the soil. However, although the application of mineral N led to an increase of grain yield, this increase was not enough to counterbalance the increase in

N_2O emissions resulting in the lack of differences between mineral N rates on yield-scaled N_2O emissions.

As it has been explained in the Results section, no differences between fertilizer types in N_2O fluxes or N lost to the atmosphere were observed for a given N rate applied to the soil. In our study, we used mineral N and pig slurry, because these are the most common fertilizing materials used in the area. It is known that pig slurry tends to show a similar behavior to mineral fertilizers due to its high NH_4^+ content (Sánchez-Martín et al., 2010), which can be rapidly nitrified in well aerated soils. Some studies have suggested, however, that liquid manure can activate the denitrifying soil microbial community due to the readily oxidizable C and sufficient mineralizable N they provide (Johnson et al., 2007). But in dryland agrosystems the addition of C compounds through pig slurry could mainly affect NO fluxes whereas in irrigated or more humid systems they could merely stimulate N_2O emissions (Meijide et al., 2009).

The use of 75 and 150 kg N ha^{-1} as pig slurry led to a grain yield 28% and 37% higher than the equivalent rate of mineral N, respectively. On the contrary, the lack of differences in above-ground N inputs between both types of fertilizers could be explained by a better allocation of resources for grain production when using pig slurry. The increase in grain yield with pig slurry led to a 2 to 2.5 times reduction in the yield-scaled N_2O emissions. Our results agree with those of Hernández et al. (2013) who compared the response of barley crop to the application of mineral fertilizer and different rates of pig slurry in a rainfed area of Spain and found a positive effect of the pig slurry on grain yield. Similarly to Thomsen and Sørensen (2006) they also found an enhanced crop N uptake when using pig slurry compared with mineral fertilizer.

4.4 Tillage and nitrogen interaction effects

In the long-term experiment, the interaction between tillage and mineral N fertilization rates affected significantly the production of grain. Under NT the application of 60 kg N ha^{-1} was followed by an increase in grain production, whereas under CT there were no differences in grain yield. In semiarid Mediterranean agroecosystems, water is the most limiting factor for crop production and the response to N application depends on soil water availability (Cantero-Martínez et al., 1995). Consequently, tillage systems that maintain a higher amount of water in the soil, such as NT, usually lead to a better crop response to N application (Angás et al., 2006; Morell et al., 2011). In the short-term

experiment, a significant interaction between tillage and N fertilization was found. In this case, whereas the different types and rates of N did not produce a significant increase in crop yield under NT, the application of pig slurry under CT significantly improved grain production compared with mineral N fertilization. Maltas et al. (2013) compared the production of different crops when using mineral and organic fertilizers in a 12-yr field experiment under a reduced tillage management similar to that in our long-term experiment. They observed a significantly higher grain yield when pig slurry was applied and suggested that this positive effect of slurry could be due to a more diversified mineral fertilization provided by animal manures.

5. Conclusions

Our results demonstrate that under rainfed Mediterranean conditions tillage significantly affected the loss of N as N_2O emitted to the atmosphere, with higher N_2O emissions under NT in the short-term (<4 years). However, when NT was used in the long-term (>10 years), N_2O fluxes were lower than under CT. The application of N fertilizers increased N_2O emissions mainly when high rates of mineral N were used and particularly immediately after the application of fertilizers. Despite the use of mineral or organic fertilizer was not accompanied by differences in N_2O fluxes, a significant increase in grain yield using pig slurry resulted in lower yield-scaled N_2O emissions. Likewise, in the two field experiments reported in this study, the use of NT significantly reduced the yield-scaled emissions of N_2O due to better crop performance. Therefore, we can conclude that in rainfed Mediterranean agroecosystems, the use of NT and pig slurry as N fertilizer is an efficient management practice for reducing the amount of N_2O emitted per kg of grain produced.

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Discusión general

Efecto del tipo de laboreo sobre la emisión de gases de efecto invernadero y la protección del carbono en agregados del suelo

El principal objetivo de esta tesis doctoral fue identificar aquellas prácticas de manejo que permiten disminuir las emisiones de gases de efecto invernadero en sistemas agrícolas extensivos de secano mediterráneo sin menoscabar la productividad y calidad del suelo de éstos. Para abordar este objetivo general, se cuantificaron las emisiones de gases de efecto invernadero (i.e., CO_2, CH_4 y N_2O) del suelo a la atmósfera, la producción del cultivo y la agregación del suelo bajo diferentes prácticas de laboreo y de fertilización nitrogenada.

En general, los sistemas de laboreo impactaron en la dinámica de los gases estudiados. No obstante, la respuesta fue diferente en función de los años transcurridos desde el cambio de manejo. Así, mientras que en el experimento de corta duración (<4 años) bajo siembra directa se generó una mayor pérdida de N en forma de N_2O y una menor oxidación del CH_4, en el experimento de largo plazo (>10 años) la emisión de N_2O fue similar entre ambos tipos de laboreo y, a su vez, la siembra directa pasó a oxidar una mayor cantidad de CH_4 en comparación con el laboreo intensivo. En esta línea, en condiciones de secano, van Kessel et al. (2013) concluyeron que la siembra directa debe mantenerse en el tiempo con el fin de resultar una estrategia efectiva para la mitigación de las emisiones de N_2O. Estas diferentes relaciones encontradas entre la emisión de gases, el sistema de laboreo y los años desde la adopción de la técnica podrían explicarse por los cambios en la arquitectura de los poros del suelo acontecidos al utilizar la siembra directa de forma ininterrumpida a lo largo del tiempo. De esta manera, los resultados obtenidos en la crono-secuencia de siembra directa mostraron un incremento en la proporción de macroagregados estables al agua al aumentar el número de años bajo siembra directa. Esta mayor proporción de macroagregados estables relacionada con una mayor cantidad de años bajo siembra directa daría lugar a poros del suelo de mayor tamaño reduciéndose, así, la probabilidad de encontrar sitios anaeróbicos en la estructura del suelo en donde puedan producirse procesos de desnitrificación y/o de inhibición de la oxidación del CH_4. Nuestros resultados corroborarían la hipótesis presentada por Govaerts et al. (2009) y basada en que la mayor proporción de macroagregados estables y la mayor aireación del suelo en sistemas de siembra directa inhibiría la desnitrificación y estimularía la oxidación del metano.

No obstante, al contrario de lo observado con los gases CH_4 y N_2O, la utilización de la siembra directa generó unas mayores emisiones de CO_2 en comparación con los sistemas de laboreo intensivo. Esta mayor emisión de CO_2 puede explicarse por diferencias en respiración radicular y/o en la actividad microbiana del suelo. Para el primer caso, en un estudio llevado a cabo en el mismo campo de larga duración se encontraron diferencias en respiración radicular debido a diferencias en producción de biomasa radicular entre sistemas de laboreo (Morell et al., 2012). Por tanto, la mayor emisión de CO_2 en suelos de siembra directa podría estar relacionada con la mayor biomasa radicular medida en esta Tesis al utilizar éste sistema de manejo. En cuanto a la hipótesis basada en una mayor actividad microbiana, la mayor cantidad de agua en el suelo y de C orgánico susceptible de ser mineralizado encontrado en los sistemas de siembra directa pudo favorecer la mayor actividad de los microorganismos. Tal y como se ha comentado anteriormente, la siembra directa generó una mayor cantidad de macroagregados estables en el suelo, fomentando la formación de microagregados dentro de estos macroagregados y, a su vez, secuestrando C orgánico en estos microagregados del suelo. En la literatura se ha señalado que este C orgánico secuestrado en el interior de estos microagregados del suelo son los que mayormente contribuyen al secuestro de C orgánico en el tiempo por parte de los sistemas de siembra directa (Six et al., 2000).

La mayor conservación del agua en sistemas de siembra directa ha sido ampliamente documentada en los sistemas extensivos de secano mediterráneo (Cantero-Martínez et al. 2003, Morell et al., 2011). Este incremento de agua pudo acelerar la descomposición de la materia orgánica del suelo debido al impacto de la humedad en la actividad de los microorganismos (Almagro et al., 2009). Sin embargo, además del impacto en las pérdidas de C del suelo, el mayor contenido de agua en los sistemas de siembra directa repercutió también en una mayor producción de biomasa en estos sistemas y, por tanto, en un mayor rendimiento de cosecha respecto a los sistemas de laboreo intensivo. En esta Tesis ha quedado comprobado que esta mayor producción de los sistemas de siembra directa en condiciones de secano mediterráneo ha generado unas menores emisiones de CO_2 equivalentes por cada kg de grano producido. Así, bajo laboreo intensivo las emisiones de gases de efecto invernadero fueron 2.1 y 5.3 veces mayores en comparación con la siembra directa en los experimentos de corto y largo plazo, respectivamente. Por tanto, en los agroecosistemas estudiados, la siembra directa resulta

un sistema de manejo de suelo más sostenible en términos ambientales y productivos que los sistemas de laboreo intensivo.

Efecto la dosis y el tipo de fertilización nitrogenada sobre la emisión de gases de efecto invernadero y la protección del carbono en agregados del suelo

En las condiciones de secano mediterráneo en las que se planteó esta Tesis, el impacto de la aplicación de fertilizante mineral en la emisión de N_2O del suelo a la atmósfera fue variable en función de la antigüedad del experimento. Así, de los dos experimentos considerados, en el de largo plazo (>10 años), la aplicación de diferentes dosis de nitrógeno mineral no tuvo ningún efecto sobre la emisión de N_2O. En cambio, en el experimento de corto plazo (<4 años) se obtuvieron unas mayores emisiones de este gas al incrementar la dosis de N mineral aplicado. El incremento de emisiones de N_2O al incrementar las dosis de N mineral aplicado está en sintonía con lo observado en estudios similares (Bouwman, 1996; Halvorson et al., 2008). Sin embargo, la falta de respuesta observada en el experimento de largo plazo representa un resultado sorprendente y que necesita ser estudiado en futuras investigaciones. Esta falta de respuesta al incremento de dosis de fertilizante nitrogenado mineral también se observó para las emisiones de CO_2 y de CH_4 y en ambos ensayos utilizados. Hay que tener en cuenta que en el estudio de agregación y de acumulación física del C del suelo, no se encontraron diferencias ni en la proporción de macroagregados estables ni en el C acumulado en estos macroagregados al incrementar las dosis aportadas de N mineral. Por tanto, en los secanos mediterráneos estudiados, el impacto de la aplicación de N mineral en la dinámica del C del suelo es mínimo. Sin embargo, el tipo de fertilizante nitrogenado utilizado (mineral y orgánico en base a purín porcino) sí tuvo efecto en la emisión de CO_2 del suelo con unas mayores emisiones de ese gas de efecto invernadero con las aplicaciones de purín. A su vez, la aplicación de este tipo de fertilizante orgánico también generó unas mayores emisiones de CO_2 provenientes de los macroagregados en condiciones controladas de laboratorio. Estas mayores emisiones en macroagregados en los que se ha aplicado purín en comparación a los que se ha aplicado fertilizante mineral se explicaría por el aporte de formas de carbono orgánico fácilmente degradables al aplicar este fertilizante orgánico (Arcara et al., 1999). En cambio, se ha observado como el efecto del fertilizante orgánico sobre la protección física del C depende de los niveles de carbono orgánico total del suelo. En el experimento realizado, los efectos sobre la protección del C en los macroagregados al aplicar fertilizantes orgánicos en suelos

manejados con siembra directa fueron limitados debido al elevado nivel inicial de carbono de ese suelo. Así, solo se observó un pequeño aumento en la estabilidad al agua de los macroagregados al aplicar purín porcino y gallinaza en comparación con el control sin fertilizar. El anterior resultado sugirió que en los suelos en los que se utiliza la siembra directa, el elevado nivel de estabilidad de los agregados que se consigue tiene un efecto tampón sobre subsiguientes mejoras debidas a la aplicación de fertilizantes orgánicos. De forma similar, en una comparación de sistemas de manejo del suelo y tipos de fertilización, Mikha y Rice (2004) no observaron ninguna mejoría en la estabilidad de los agregados con el uso combinado de la siembra directa y la aplicación fertilizantes orgánicos en comparación con la siembra directa sin fertilizar.

Sin embargo, el tipo de fertilizante no provocó diferencias en las emisiones de CH_4 y N_2O del suelo a la atmosfera para una misma dosis de N en la aplicación de fertilizante mineral o de purín de cerdo. Esos resultados contrastan con los de otros estudios en los que se ha observado un aumento de la desnitrificación debido al aporte de carbono orgánico fácilmente degradable al aplicar purín (Arcara et al., 1999). Esas diferencias podrían venir explicadas por los bajos niveles de humedad del suelo y por tanto de espacio poroso lleno de agua en nuestro experimento, que provocarían que la emisión de N_2O fuese mayormente debida al proceso de nitrificación (Linn y Doran, 1984).

Por último, es importante destacar el impacto que tuvieron los diferentes tratamientos de fertilización nitrogenada en el rendimiento y en las emisiones asociadas. En promedio, la aplicación de purín incremento un 33% el rendimiento de grano de cebada respecto la aplicación de fertilizante mineral. Este mayor rendimiento redujo entre un 41% y un 72% las emisiones equivalentes de CO_2 por kg de grano producido en comparación con los tratamientos en los que se aplicó fertilizante mineral. Por tanto, la aplicación de subproductos orgánicos ganaderos en estos sistemas de secano es una estrategia de fertilización prometedora no sólo por el impacto en los rendimientos de los cultivos sino también por las emisiones de gases de efecto invernadero asociadas a ésta.

Finalmente, a partir de los resultados obtenidos en esta Tesis se puede concluir que para los agroecosistemas extensivos de secano mediterráneo una estrategia de manejo basada en el empleo de siembra directa junto con un aporte de dosis medias de N en forma de purín porcino optimiza ambiental y productivamente el sistema debido a que minimiza las emisiones de gases de efecto invernadero manteniendo la productividad del cultivo.

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Conclusiones generales

Incidencia e importancia del sistema de laboreo

1. En sistemas de secano mediterráneo, la utilización de la siembra directa generó una menor emisión de CO_2 equivalente por kg de grano producido en comparación con el laboreo intensivo.

2. A pesar de las menores emisiones de gases de efecto invernadero por kg de grano producido, la siembra directa provocó unas mayores emisiones de N_2O y una menor oxidación del CH_4 durante los primeros años desde su establecimiento. Sin embargo, a largo plazo (>10 años) las diferencias en la emisión de N_2O entre ambos sistemas de manejo de suelo desaparecieron e, incluso, se invirtieron en el caso del CH_4. Por lo tanto, a partir de los resultados obtenidos en esta Tesis, queda demostrado que la siembra directa es un sistema de manejo de suelo que debe ser mantenido a lo largo del tiempo con el fin de resultar una estrategia efectiva para mitigar las emisiones de N_2O y CH_4 en condiciones de secano mediterráneo.

3. A pesar de que las emisiones de CO_2 fueron mayores en la siembra directa que en el laboreo intensivo, el mantenimiento de la siembra directa a lo largo del tiempo llevó a unos mayores niveles de C orgánico del suelo debido a la estabilización física del C orgánico en microagregados formados en el interior de macroagregados estables del suelo.

4. Las diferencias encontradas en agregación del suelo entre sistemas de laboreo afectaron a la emisión de gases de efecto invernadero y, en particular, de CH_4. Así, mientras que en los macroagregados estables de siembra directa se cuantificaron procesos de oxidación de CH_4, en los de laboreo intensivo el principal proceso observado fue la emisión de CH_4.

Efecto del tipo y dosis de fertilización nitrogenada

5. Los resultados de esta Tesis indican que en sistemas extensivos de secano mediterráneo la aplicación de dosis crecientes de fertilizante nitrogenado (mineral y purín porcino) provocan un aumento de la pérdida de N del suelo en forma de N_2O hacia la atmósfera. Sin embargo, para una misma dosis de nitrógeno aplicado, el tipo de fertilizante no dio lugar a diferencias en las emisiones de N_2O del suelo a la atmósfera.

6. En el sistema de secano estudiado, la aplicación de purín porcino generó picos puntuales en la emisión de CO_2 y CH_4 justo después de la fertilización. Además, cuando los resultados se promediaron a lo largo de toda la campaña, la aplicación del fertilizante orgánico resultó en un incremento en las emisiones de CO_2 en comparación con la aplicación de fertilizante mineral.

7. A pesar de las mayores emisiones de CO_2 del suelo a la atmósfera, la aplicación de purín porcino disminuyó las emisiones de CO_2 equivalente por kg de grano producido en comparación con la fertilización mineral.

8. En sistemas de siembra directa, la aplicación de fertilizantes orgánicos generó cambios en el secuestro de C orgánico en comparación con la aplicación de fertilizantes minerales. Además, en condiciones controladas de temperatura y humedad del suelo, la aplicación de purín porcino también provocó un aumento en la emisión de CO_2 de los macroagregados.

Interacción entre el sistema de laboreo y la fertilización nitrogenada

9. A partir de los resultados obtenidos en esta Tesis se puede concluir que en los sistemas de secano mediterráneos el uso de la siembra directa, junto con el aporte de dosis medias de purín porcino, es una estrategia de manejo óptima ya que minimiza las emisiones de gases de efecto invernadero y mantiene la productividad del cultivo. A su vez, ambas prácticas mejoran la estabilidad de los agregados, maximizando la cantidad de carbono orgánico secuestrado y mejorando el estado estructural del suelo.

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