Universidad Politécnica de Madrid
Escuela Técnica Superior de Ingeniería Agronómica,
Alimentaria y de Biosistemas

**GREENHOUSE GAS EMISSIONS IN
CONSERVATION AND CONVENTIONAL
AGRICULTURE SYSTEMS UNDER RAINFED
MEDITERRANEAN AGRO-ECOSYSTEM**

TESIS DOCTORAL

Ángela Téllez del Río

Ingeniero Agrónomo

Madrid, 2017

Universidad Politécnica de Madrid

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Alimentaria y de Biosistemas

Departamento de Química y Tecnología de Alimentos

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Madrid, 2017

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EL SECRETARIO

A mi familia y a Víctor,

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RESUMEN

Resumen

Durante las últimas décadas, las prácticas de Agricultura de Conservación han ido progresivamente en aumento en agroecosistemas de secano semiárido de la zona Mediterránea debido a sus beneficiosos efectos en los suelos agrícolas, como por ejemplo: incremento de la materia orgánica, mejora de la calidad y fertilidad, y aumento del secuestro del Carbono orgánico del suelo (SOC). Sin embargo, el efecto de estas prácticas agrícolas en las emisiones de gases de efecto invernadero (GEI), particularmente en la emisión de óxido nítrico (N_2O), no es consistente y existe, todavía hoy, una gran controversia en este tema con resultados bastante contradictorios. Además, dada la creciente preocupación por la contaminación medioambiental y la productividad agrícola, un mejor entendimiento del manejo de estas prácticas agrícolas es necesario para poder hacer frente a dos retos en los próximos años: incrementar la producción de alimentos para satisfacer la creciente demanda asociada al aumento de la población mundial y reducir la contaminación medioambiental. El principal objetivo de esta tesis es: *“Evaluar el impacto de las prácticas de agricultura de conservación de largo duración (las cuales incluyen siembra directa, mínimo laboreo y rotación de cultivos) en las emisiones de GEI (N_2O y metano (CH_4)) en agrosistemas de secano con bajo aporte de nitrógeno (N) en condiciones de clima Mediterráneo semiárido, con el fin de poder determinar la combinación más óptima en términos agronómicos y medioambientales.*

Para lograr este objetivo, se establecieron varios ensayos de campo en condiciones de clima Mediterráneo semiárido (Comunidad de Madrid, centro de España), que incluían una rotación de cultivos de secano (barbecho-cereales –leguminosa) en tres sistemas de laboreo: siembra directa, mínimo laboreo y laboreo tradicional, durante varias campañas. Las emisiones de GEI (principalmente N_2O , pero también metano, CH_4), parámetros de suelo (por ejemplo, C orgánico disuelto o N mineral) y los rendimientos fueron evaluados. Además, la abundancia de desnitrificadores mediante técnicas moleculares de PCR (codificados por los genes *nirK* y *nirS*) y la capacidad de desnitrificación fueron cuantificados en un momento puntual (primavera). Finalmente, los datos generados durante estas campañas se usaron para calibrar y validar el modelo “Decomposition and Denitrification” (DNDC) en los sistemas de siembra directa y laboreo tradicional en la rotación trigo-veza en condiciones de clima Mediterráneo semiárido.

En esta tesis, las emisiones de N_2O fueron bajas debido al ajuste de la fertilización nitrogenada a las condiciones del suelo. El impacto de la rotación de cultivos en las emisiones de N_2O se atribuyó principalmente a los siguientes factores: ratios de aporte de N, tipo y manejo de residuos procedentes del cultivo anterior en la rotación de cultivos, y de la interacción del sistema de laboreo con los residuos. El trigo rotacional redujo las pérdidas de

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N_2O e incrementó la oxidación de CH_4 y el rendimiento en grano comparado con el trigo monocultivo, y por tanto, mostró una reducción en las emisiones de N_2O escaladas al rendimiento. De este modo, las rotaciones de cultivo que incluyen leguminosas, se pueden considerar como una estrategia óptima para disminuir las emisiones de GEI (N_2O y CH_4) e incrementar el rendimiento de cultivo, junto con los beneficios agronómicos asociados a la rotación de cultivos, como por ejemplo: reducción de las malas hierbas y el uso de pesticidas, mejora de la calidad del suelo y reducción del uso de fertilizantes minerales N debido a la fijación de N por las leguminosas.

En general, el efecto de los sistemas de laboreo de larga duración en las emisiones de N_2O fue similar entre siembra directa, mínimo laboreo y laboreo convencional en la rotación de secano (barbecho-cereal-leguminosa) bajo condiciones de clima Mediterráneo semiárido. Teniendo en cuenta esa falta de efecto del laboreo en las emisiones de N_2O , fue necesario considerar otras fuentes o sumidero de emisiones de GEI para poder identificar prácticas de mitigación de estos GEI.

La oxidación de CH_4 no se vio afectada por las prácticas de laboreo de larga duración en este agrosistema de secano de la cuenca Mediterránea semiárida. En general, los sistemas de laboreo mostraron un poder de calentamiento global asociado a las emisiones de GEI positivo, lo que demostró que a pesar de las bajas emisiones de N_2O , el consumo de CH_4 no compensó las pérdidas de N_2O . Así, la evaluación del poder de calentamiento global asociado a las emisiones de GEI durante varias campañas en diferentes cultivos no demostró un claro efecto sobre la mitigación de los sistemas de laboreo en las emisiones de GEI. Sin embargo, si consideramos otros factores, tales como el almacenamiento de C orgánico del suelo (secuestro de C orgánico del suelo) y el poder de calentamiento global neto, es decir, considerando las emisiones de CO_2 -equivalentes derivadas de los insumos y operaciones agrícolas, las diferencias entre laboreos aparecen. La siembra directa incrementó el almacenamiento de C orgánico del suelo y redujo el poder de calentamiento global neto comparado con los otros 2 sistemas de laboreo, ya que los CO_2 -equivalentes derivados de los insumos y operaciones agrícolas fueron menores en siembra directa que en mínimo laboreo y laboreo convencional, debido a una reducción en el número de operaciones agrícolas. Por lo tanto, la combinación de siembra directa y rotación de cultivos, que incluya cereal y leguminosa, son prácticas recomendables para mitigar las emisiones de CO_2 -equivalentes sin afectar a los rendimientos comparado con los sistemas de mínimo laboreo y laboreo tradicional en agrosistemas de

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secano semiárido. Además, la siembra directa reduce las emisiones de N_2O escaladas al rendimiento en años secos (con precipitaciones inferiores a 200 mm).

Los sistemas de laboreo afectaron a la capacidad de desnitrificación ($\text{N}_2\text{O}+\text{N}_2$) y a la abundancia del gen *nirK* para el caso del cultivo de veza, a diferencia del barbecho y el trigo rotacional, durante el momento puntual analizado (primavera). Sin embargo, una mayor densidad del gen *nirK* junto con condiciones favorables para la desnitrificación se observó en el barbecho en comparación con el trigo rotacional, lo que explicaría las mayores emisiones de N_2O observadas en las parcelas de barbecho durante ese periodo crítico evaluado en comparación con el trigo rotacional.

En general, en este agro-ecosistema de secano semiárido de la cuenca Mediterránea, la producción agrícola de los cultivos (trigo y veza en rotación y trigo en monocultivo) no se vio afectada por los sistemas de laboreo estudiados. Las emisiones escaladas al rendimiento fueron bajas ($< 7 \text{ g N}_2\text{O-N kg}^{-1} \text{ N absorbido}$) en todos los sistemas de laboreo, debido principalmente a las bajas emisiones de N_2O medidas.

La calibración y validación del modelo “DNDC” en los sistemas de laboreo siembra directa y laboreo convencional mostró un buen ajuste entre datos observados y simulados en el contenido de agua del suelo y los rendimientos de los cultivos de veza y trigo en rotación. Sin embargo, el modelo DNDC en general sobreestimó los flujos de N_2O diarios en todos los tratamientos evaluados (siembra directa y laboreo tradicional en trigo y veza en rotación), produciendo desviaciones en las emisiones acumuladas de N_2O entre datos observados y simulados en los dos sistemas de laboreo. Estos resultados se pueden considerar como un primer paso hacia el cumplimiento de la metodología del TIER 3, con el fin de mejorar el inventario nacional de emisiones GEI en agro-ecosistemas de secano semiárido en clima Mediterráneo.

ABSTRACT

Abstract

Conservation agriculture practices have widespread in the last decades in semiarid Mediterranean agroecosystems because of their beneficial effects on the increase in soil organic matter, improve soil quality and fertility, and enhance soil organic carbon (SOC) sequestration. However, the impact of these agricultural management practices on greenhouse gas (GHG) emissions, particularly nitrous oxide emissions (N_2O), is not consistent and controversy on this matter remains today. Given increasing concerns about the environmental pollution and agronomic productivity, a better understanding of these agricultural management practices is required to face two challenges in the coming future: increase the food production to meet the increase in world population and reduce the environmental pollution. Thus, the main objective of this thesis was: *“To evaluate the impact of long-term conservation agricultural practices (which involved no tillage (NT) and minimum tillage (MT) and crop rotation) versus conventional agricultural practices (CT and monoculture) on GHG emissions (N_2O and CH_4) in low N input systems under rainfed semiarid Mediterranean agro-ecosystem in order to find the best optimal combination between environmental and agronomic perspective”*.

To achieve this objective, several field experiments were performed under semiarid Mediterranean conditions (Comunidad de Madrid, Centre of Spain), involving rainfed cereal-legume crop rotation and wheat in monoculture in three long-term tillage systems: no tillage (NT), minimum tillage (MT) and conventional tillage (CT), during several campaigns with contrasting climatic conditions. Greenhouse gas emissions (N_2O and CH_4), soil parameters (e.g. dissolved organic C and mineral N) and crop yields were quantified. Additionally, denitrification capacity and denitrifier abundance (targeted by nirK and nirS genes) by molecular PCR approach were measured at a critical time (spring). Finally, field-experiment dataset collected during these campaigns were used for the calibration and validation of the Decomposition and Denitrification (DNDC) model in NT and CT systems for wheat-vetch rotation under rainfed Mediterranean semiarid conditions.

Nitrous oxide fluxes measured in this thesis were low by adjusting fertilizer N rate to soil N conditions. The impact of crop rotation on N_2O emissions is attributed mainly to the following factors: N fertilization rates, type and management of crop residue from the previous crop in rotation and the interaction effect of tillage with the crop residues. In this sense, rotational wheat reduced N_2O losses and increased CH_4 oxidation and grain yield compared to wheat in monoculture and, in turn, produced an abatement of YSNE. Thus, the crop rotations including legumes could be considered as a good strategy for decreasing GHG emissions (N_2O

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and CH₄) while increasing crop yield, together with the agronomic benefits associated to crop rotation (i.e.: reduction of weed population and use of pesticides, improvement of soil quality, decrease the use in N fertilizer through increasing N input by legumes N fixation).

Overall, the impact of long-term tillage systems on N₂O emissions was similar between NT/MT and CT in a fallow-cereal-legume rotation under rainfed conditions. Given the lack of effect of tillage systems on N₂O emissions, considering other GHG sink or sources are necessary to identify mitigation practices.

Methane uptake was not affected by long-term tillage practices in rainfed Mediterranean semiarid agro-ecosystem.. Tillage systems (NT, MT and CT) overall showed a positive GHG-GWP emission, revealing that in spite of low measured N₂O emissions, CH₄ uptake did not offset N₂O losses. Thus, the evaluation of GHG-GWP during several campaigns in different crops did not show a clear effect about the mitigation of tillage systems on global GHG emission. However, if we considered other factors such as: ΔSOC content and net-GWP (CO₂-eq emissions), differences arised between tillage systems. No tillage systems increased the ΔSOC content and reduced net GWP compared to MT and CT, because of CO₂-eq emissions for farm inputs and operations in NT system were lowest than MT/CT and less important due to a reduction in the number of operations. Therefore, the combination of NT systems and crop rotations, including cereal-legume crops, can be a good strategy to reduce CO₂-eq emissions without yield penalties compared to MT and CT systems in rainfed semiarid agro-ecosystems. In addition to, NT can mitigate YSNE in dry years (rainfall < 200 mm).

Tillage systems affected to denitrification capacity (N₂O+N₂) and *nirK* gene abundance in vetch crop, as opposed to fallow and wheat, at a crucial time (spring). However, greater *nirK* density together with favourable conditions for denitrification process was observed in fallow than in rotational wheat plots, which may explain the higher N₂O fluxes measured in fallow than in wheat at that time (spring).

Overall, crop yields (rotational wheat, wheat in monoculture and vetch) were not affected by tillage systems under semiarid Mediterranean conditions. Low YSNE (< 7 g N₂O-N kg⁻¹ N uptake) was found for all tillage systems in this rainfed Mediterranean agro-ecosystem, mainly due to the low measured N₂O emissions.

Calibration and validation of DNDC model for NT and CT systems showed a fair agreement in soil water content and crop yields for wheat and vetch in rainfed Mediterranean agro-ecosystems. But the DNDC model overall overestimated the daily N₂O fluxes for all

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treatments (WNT, WCT, VNT and VCT), producing deviations between model and measured cumulative N₂O emissions in NT and CT systems. These results can be considered as a potential step towards Tier 3 approach in order to improve the national GHG emissions inventory in rainfed semiarid Mediterranean agro-ecosystems.

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CHAPTER 1

General introduction

1. GHG emissions and agricultural systems

The intensification of agricultural systems over the past 60 years together with the increase in use of N fertilizers allowed to the world population to increase from 3 to 7 billion due to the beneficial impacts on crop yields (Erisman et al., 2008). These economic and population growth together with other anthropogenic drivers have led to an increase in the atmospheric concentration of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) and point to the evidence for human influence on the climate systems (IPCC, 2014). Total anthropogenic greenhouse gas emissions (GHG) have continued to increase over 1970 to 2010, particularly between 2000 and 2010, despite a growing number of climate change mitigation policies. Anthropogenic GHG emissions in 2010 have reached approximately 49 ± 4.5 Gt CO₂-eq yr⁻¹ (IPCC, 2014).

The agricultural sector is globally one of the main drivers of environmental pollution and contributes up to 10-12% of total anthropogenic GHGs (Stocker et al., 2013), mainly through the release of N₂O and CH₄. For example in Spain, agriculture accounts for 10% of GHGs emissions (MAGRAMA, 2012). An important source of these anthropogenic N₂O emissions is produced by the use of synthetic N-fertilizer, which represents approximately 56-70% of total global N₂O emissions (Butterbach-Bahl et al., 2013). Unfortunately, as a result of the population growth, which could reach 9.8 billion by 2050 (UN, 2017), and the necessary increase in N fertilizers use to meet the growing demand for food and feed, anthropogenic N₂O emissions are expected to double by 2050 (Eric and David, 2014). This negative tendency in N₂O emission needs to be changed and therefore, based on the recent Paris Agreement, the EU Roadmap recommend a reduction in European agricultural emissions of 36-37% for 2030, and 42-49% for 2050 (Domingo et al., 2014). Taken into account that N₂O is the most important driver of stratospheric ozone depletion (Ravishankara et al., 2009), its reduction is also beneficial to mitigate this effect.

To achieve this target, maintaining or even boosting crop production, is necessary to evaluate, quantify and identify the best mitigation strategies of GHG emissions within the agricultural sector, such as: improved agronomic practices, tillage, nutrient use, cropland and residue management, restoration of organic soils as a Carbon sinks and restoration of degraded lands (Smith et al., 2007).

Nitrous oxide emissions results mainly from two microbial soil processes: nitrification and denitrification, and a lesser extent from nitrifier denitrification and coupled nitrification-denitrification (Fig. 1.1). The interaction of controlling factors for microbial production and consumption of N₂O emissions is complex because depends on the links to biotic (e.g. inter- and

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intra-species competition, food webs, plant–microbe interaction) and abiotic (e.g. soil climate, physics and chemistry) factors (Butterbach-Bahl et al., 2013) and therefore, it is important to know these main microbial soil processes of N-cycle:

- Autotrophic nitrification: is the aerobic oxidation of (NH_4^+) to nitrate (NO_3^-) by NH_3 -oxidizing bacteria or Archaea, which has been suggested as the dominant process in calcareous low-C content soils (Aguilera et al., 2013a) and it is limited at low O_2 availability and high water filled-pore-space (WFPS) values (Firestone and Davidson, 1989).

Fig.1.1. Biotic and abiotic processes of nitrous oxide (N_2O). Processes potentially leading to N_2O formation and consumption, involved N compounds, their reaction pathways as well as their oxidation states are shown. According to current knowledge, anaerobic ammonia oxidation does not contribute to N_2O formation or consumption. By contrast, N_2O may at least serve as a substrate for biological dinitrogen fixation. Processes predominantly requiring anaerobic (or micro-aerobic) conditions are underlined by grey illuminated segments (Butterbach-Bahl et al., 2013).

- Heterotrophic denitrification: is the stepwise reduction of NO_3^- to N_2 by phylogenetically heterogeneous microorganisms, most of them belong to various subclasses of Bacteria, and a lesser extent to some Archaea and fungi (Philippot and Hallin, 2005). Denitrification process happens at limited O_2 supply when NO_3^- or NO_2^-

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are used as alternative terminal electron acceptors (Fig. 1.1). Owing to the high degree of taxonomic diversity among denitrifiers and to its role in the N-cycle, the denitrifying community is considered as a useful model for investigating the value of microbial biodiversity in ecosystem functioning (Philippot and Hallin, 2005). Seven enzymes catalyze the 4 reductive steps of the denitrification pathway (Zumft 1997; Philippot 2002) (Fig. 1.2):

- i. Reduction of soluble NO_3^- to NO_2^- is catalyzed either by a membrane-bound (*nar*) or a periplasmic nitrate reductase (*nap*).
- ii. The reduction of soluble NO_2^- to gaseous NO is catalyzed by two nitrite reductases, *nirK* or *nirS*. The 2 proteins are different regarding structure and catalytic site, despite having identical functions. *NirS* contains a cytochrome cd1 active site (*Cd-nir*), whereas *nirK* is a member of the multi-copper oxidase metalloprotein family (*Cu-nir*).
- iii. NO is further reduced to N_2O , this reduction is catalyzed by the nitric oxide reductases, two-component type (*nor*) or single-component type (*qNor*), encoded by genes *norB* and *norZ*, respectively.
- i. The final step in denitrification is the conversion of N_2O to N_2 , catalyzed by the enzyme nitrous oxide reductase (*nos*). This step effectively closes the overall nitrogen cycle as N_2 gas may again be converted to soluble nitrogen oxides through the action of N-fixing and nitrifying organisms.

Fig.1.2. Denitrification pathway, in which it is described the enzymes and the encoded genes for each step (Philippot and Hallin, 2005).

Denitrification process is the main contributor to N_2O losses at WFPS ranging from 60 to 90%, or even at WFPS < 40% when crop residues are not removed from soil (Li et al., 2016). Denitrification is limited when organic carbon (C) availability is low. Soil conditions are important to determine not only total denitrification rates, but also the end products of this process, i.e. the $\text{N}_2\text{O}/\text{N}_2$ ratio, which decreases at high pH (Baggs et al., 2010), high DOC/ NO_3^- -ratio (Abalos et al., 2013; Benckiser et al., 2015) and at WFPS >80% (Friedl et al., 2016). Much larger quantities of the innocuous N_2 are emitted globally in comparison to those of N_2O (particularly when conditions are favorable for complete denitrification, e.g. grasslands), affecting the N use efficiency (NUE) in agro-ecosystems.

The NO_3^- produced by nitrification can be also used by denitrifiers when favorable conditions for both processes occur in soil neighboring microhabitats (Wrage et al., 2001), and this is known as coupled nitrification-denitrification (Fig. 1.1). Another process resulting in N_2O production is the nitrifier denitrification, which is the oxidation of NH_3 to NO_2^- followed by the reduction of NO_2^- to N_2O or N_2 . This sequence of reactions is carried out only by NH_3 -oxidizers,

and could be a significant contributor to N₂O losses when moisture conditions/organic matter content are sub-optimal for denitrification (Kool et al., 2011) (Fig. 1.1).

Methane emissions are other environmental threat due to its radiative properties and long residence time in the atmosphere resulting in a global warming potential 28 times higher greater than CO₂ on a weight basis (Myhre et al., 2013). In addition to, CH₄ also plays an important role in stratospheric ozone depletion (IPCC, 2007). Major sources of anthropogenic methane (CH₄) emissions within the agricultural sector are from rice production under flooded conditions, stored manure and enteric fermentation by ruminant livestock (Snyder et al. 2009), and agricultural sector accounts for about 50% of CH₄ emissions (IPCC, 2007). The production of CH₄ is attributed to methanogenesis under anaerobic conditions, especially when soil is inundated such as in rice paddies, is carried out by methanogenic bacteria during the anaerobic digestion of organic matter (Le Mer and Roger, 2001). Methane is also oxidized by methanotrophic bacteria in aerobic soils (Hütsch, 2001). Therefore, soils can be net source or sink for CH₄ depending on the land use and management practices (Baggs et al., 2006; Vallejo et al., 2014). Usually, undisturbed soils act as a net CH₄ sink, but a dramatic decrease on the CH₄ oxidation rates is experienced when soils are converted to agriculture, which effect has been mainly related to the soil disturbance and to the ammonium-based N fertilization (Hütsch, 1998; Le Mer and Roger, 2001; Baggs and Blum, 2004).

In spite of these environmental pressures, the agricultural sector offer environmentally friendly strategies for mitigation of climate change, which underscored the necessity of change in today's agricultural practices (Smith, 2007; Rees et al., 2013a), in order to achieve a cleaner and sustainable agricultural production.

2. Conservation agriculture: benefits and problematic

Conservation agriculture (CA) practices involves mainly crop rotations and conservation tillage systems, i.e.: no tillage (NT) and minimum or reduced tillage (MT), and has been recognized as a sustainable alternative to the conventional practices (Verhulst et al., 2010). According to FAO 2011, conservation agriculture is based on three linked principles:

- 1) *Continuous minimum mechanical soil disturbance*: This consists in applying of low soil disturbance, such as no-tillage and direct seeding or it can be also carried out soil disturbance not more than 25% of the soil surface but not wider than 15 cm in bands.
- 2) *Permanent organic soil cover*: This means to mulch from crop residues, other organic mulch materials or living crops, including cover crops. The soil surface should be covered ideally by 100%, but never less than 30% of the soil surface, in order to increase soil organic matter content.

3) *Diversification of crop species grown in sequences and/or associations*: This refers to rotations and sequences of annual crops, mixed-, inter- or relay cropping, cover crops in perennial orchard or plantation crops, including legumes for their N effect as well as for their flowering in support of pollinator populations (Kassam et al., 2012).

Conservation agriculture practices have been boosted in soils of dry Mediterranean climate, such Spain, France and Portugal, during the last decades (Basch et al., 1995; Álvaro-Fuentes et al., 2008; Moreno et al., 2010). Because these areas are characterized by low organic matter status with poor soil aggregate structure, and are prone to degradation and desertification due to intensive tillage and overgrazing (Kassam et al., 2012). In fact, Spain is the leading countries in the adoption of CA in Europe, where CA is practised on about 650,000 ha, mainly in annual crops i.e.: wheat, barley, much less maize and sunflowers (Kassam et al., 2012). Adoption of conservation agriculture practices, especially combined with retention of crop residues, can build up soil organic matter, reduce CO₂ emissions and enhance soil organic C (SOC) sequestration (West and Marland, 2002; Plaza-Bonilla et al., 2015).

Compared to conventional systems, conservation agriculture practices, provides several benefits: maintain or increase yields due to the combination of improved soil porosity and soil moisture as well as nutrient availability; reduce production cost (i.e.: fuel, pesticides and fertilizer costs) and labour requirements; improve soil fertility; reduce soil erosion; and increase SOC stocks (Cantero-Martinez et al. 2007; Kassam et al., 2012; Alvaro-Fuentes et al., 2014). Conservation tillage increase C in soils because of the reduction in decomposition rates with physical protection of C within aggregates and favors a higher microaggregates formation within macroaggregate in soils (Six et al., 2000). This fact can lead a reduction of microbial heterotrophic respiration and as result decreasing CO₂ emissions (Plaza-Bonilla et al., 2013). In contrast, conventional tillage (CT) breaks microaggregate formation, and consequently the soil C is less protected from microbial decomposition, thus increasing the emission of CO₂ (Ogle et al., 2012). Conservation agriculture systems can also be an effective tool in suppressing weeds associated to a good residue management and crop rotations (Kassam et al., 2012), which also breaks insect pest and pathogen build-ups (Upadhyaya and Blackshaw, 2007). However, a higher weed, pest and disease pressure have been observed in conservation tillage systems compared to CT systems (Sans et al., 2011; Soane et al., 2012), which can lead to an increase in the use of agrochemicals and consequently, promoting pollution in soil and water bodies (Annet et al., 2014). Therefore, more long-term research is needed to overcome these possible setbacks in conservation tillage systems (Armengot et al., 2015), for example: through an integrated management of pests, disease and weeds (Kassam et al., 2012) in CA systems.

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Although many studies have reported potential benefits associated to conservation agriculture, its impact on GHG emissions is still uncertain (Abdalla et al., 2013). Agricultural management practices, such as conservation tillage (NT or MT) and crop rotation, can affect to the production and consumption of N_2O and CH_4 emissions from soils, directly by affecting N mineral availability and SOM, or indirectly by modifying the soil microclimate and cycling of C and N (Snyder et al., 2009).

The effect of NT and MT systems on N_2O emissions is highly variable, increases and decreases in N_2O emissions have been reported depending on: soil moisture, climatic conditions, soil characteristics, interaction of tillage with other agricultural practices, placement on N fertilizer, the duration and type of tillage practices (Van kessel et al., 2013). In this sense, a review of Six et al. (2004) reported an increase of N_2O emissions during the first 10 years after conversion from conventional tillage (CT) to NT, but thereafter N_2O emissions tended to reduce. However, that abatement in N_2O emissions after long-term NT was only significant in humid climates.

Recently, Van Kessel et al., (2013) published a meta-analysis in which NT/MT showed not significant overall effect on N_2O emissions, probably because the main responsible processes of N_2O production are regulated by opposing physical and chemical factors. But when data of this meta-analysis were segregated by climate and duration of experiment, differences were emerged. In dry climates, Van Kessel et al., (2013) observed that at short-term area-scaled N_2O emissions in NT/MT increased significantly compared to conventional tillage (CT), whereas at long-term (≥ 10 years) NT/MT mitigated significantly N_2O emissions (Fig.1.3). This fact highlights the importance of carrying out long-term field experiment in order to assess the effects of changes in physical, chemical and biological soil properties after several years of NT or MT on N_2O emissions. In Mediterranean areas, little effect has been described overall between tillage systems (Plaza-Bonilla et al., 2014a; Guardia et al., 2016a).

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Fig.1.3. Percent change in (a) area-scaled N₂O emissions, (b) crop yield, and (c) yield-scaled N₂O emissions in no-till (NT) and reduced tillage (RT) treatments compared with conventional tillage segregated by experiment duration and climate regime. Numbers in parentheses indicate the number of comparisons, followed by the number of studies from which the comparisons were derived. Error bars are 95% confidence intervals (CI). Significant effects of NT or RT are denoted by * (where error bars do not overlap zero). P-values are for differences in effect sizes between categories (Van kessel et al., 2013).

Other concern associated to conservation tillage (NT/MT) compared to CT is the decline in crop yield, often described across different climates zones in the literature review. This tendency for yield decline was overall caused by changes in disease pressure and N deficiency under NT/MT (Six et al., 2004; Ogle et al., 2012; Van kessel et al., 2013). In addition to, the current meta-analysis of Van Kessel et al. (2013) found a particularly high significant reduction of yield in dry climates, with an average loss of 12 % for short term and 9 % for long-term of NT/MT (Fig.1.3).

Crop rotations together with a good residue management improve soil quality and reduce inputs consumption (Kassam et al., 2012; Sanz-Cobena et al., 2017). The inclusion of fallow and/or legume in crop rotations may offer opportunities for mitigating direct and indirect N₂O emissions due to zero synthetic N input (Jensen et al., 2012; Jeuffroy et al., 2013a). Crop residues quality (high or low C:N ratio) and management (with or without incorporation into the soil) affect to N₂O emissions (Mahli and Lemke, 2007). Generally, higher N₂O emissions have been observed after incorporation of residues with a low C:N ratio (i.e. legumes), resulting in a rapid stimulation of microbial decomposition and increasing substrate for nitrification and denitrification (Baggs et al., 2003). Whereas, residues with high C:N ratio (i.e. cereal, maize) can promote soil N immobilization when are applied without additional source of N input, leading to a temporary reduction of N₂O emissions (Huang et al., 2004). In addition to, the effect of tillage and residue type on mineralization rates together with the application of N fertilizer can play an important role in N₂O emissions, contributing to explain N₂O fluxes due to the interaction between tillage and crop rotations (Guardia et al., 2016a). Therefore, further research is needed to acquire a better insight into all of those interactions and its effects on N₂O emissions.

Improving N use efficiency of the cropping system is a challenge in the conservation agriculture practices. In this sense, in rainfed semiarid regions, low N input systems (i.e. Integrated Soil Fertility Management, ISFM) are being promoted in order to match N input to the expected N uptake by crops (Kimani et al., 2003) and thus, optimize the use of available sources for crop growth. These ISFM systems involved the use of low rate of mineral N fertilizers and organic inputs (i.e.: crop residues, green manure, etc.), aiming to maximize

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agronomic efficiency (Vanlauwe et al., 2011). These strategies based on a better use of N can mitigate N₂O emissions and reduce the cost from over use of N fertilizers in semiarid regions, such as Spain (Guardia et al., 2016b).

According to IPCC Tier 1, the relationship between N input and N₂O emissions is linear, and recommend an emission factor (EF) default value around 1% (IPCC, 2006). In Mediterranean rainfed systems, the review of Aguilera et al. (2013a) reported that EF is one order of magnitude lower (0.08%) than IPCC Tier 1, due to low N₂O emissions and low N input. However, numerous studies have reported non-linear relationships between those two variables, which underscore the need of more specific IPCC Tier 2 approach for N₂O emissions based on N inputs. In this line, reporting yield scale N₂O emissions may be quite useful particularly for tillage practices which affect to yield and N₂O emissions (Mosier et al., 2006). Under dry climate conditions, Van kessel et al. (2013) described that yield-scaled N₂O increased significantly (57%) after short term adoption of NT/RT (<10 years), but decreased significantly (27%) after 10 years of NT/RT, compared to CT (Fig.1.3.). Therefore, further studies based on GHG emissions and crop productivity are necessary in order to find the best combination of agronomic productivity and environmental sustainability.

Conservation tillage systems (NT/MT) influence in the production and oxidation of CH₄ emissions due to the alteration of soil biochemical properties (Ussiri et al., 2009). Overall, most studies have described either no significant effect or a decrease in CH₄ emissions (Venterea et al., 2005; Abdalla et al., 2013) following the adoption of NT/ MT. Soils in NT/MT systems have a better porous structure that sometimes increases soil gas diffusivity and, in turn, promote high methanotrophic activity (Hutsch, 2001; Ball et al. 1997). In addition to, the application of inorganic N fertilizer and crop residues can reduce CH₄ oxidation in soils, due to the fact that the key enzymes in methanotrophs and chemo-autotrophic nitrifiers (CH₄ monooxygenase and NH₃ monooxygenase, respectively) are similar and that both are able to oxidize either CH₄ or NH₃ (Dunfield and Knowles, 1995; Hütsch et al. 1996). To date, relatively few studies have evaluated the effect of tillage systems and crop rotation on CH₄ emissions in rainfed semiarid Mediterranean conditions.

Agricultural conservation practices often affect fluxes of more than one gas, such as: N₂O, CH₄, SOC stocks and CO₂ emissions, sometimes in opposite ways. An adequate assessment of the effects of tillage and crop rotation on GHG balance require to estimate the global warming potential basis (CO₂-equivalent emissions), particularly due to the relatively low weight of N₂O losses in Mediterranean areas (Aguilera et al., 2015) in comparison to other climatic areas (Rees et al., 2013b), and the capacity of conservation tillage as a practice to mitigate GHG emissions through soil C sequestration. Moreover, the best way to evaluate if the

conservation tillage can increase C stocks and its relative contribution to the GWP is through long-term experiments. In this sense, Alvaro-Fuentes et al. (2014) observed that the maximum SOC sequestration rate may occur after 5 years of NT adoption.

3. Tillage systems and denitrifier abundance

The tillage practices exert an important effect on the transformation of microbial N, especially on the denitrification process (Robertson and Tiedje, 1987), due to shifts on pH and microbial community composition. No tillage systems can improve the soil habitat for microorganisms, because of increased C levels and physical properties of the soil (Martin-Lammerding et al., 2011), which affects to denitrification activity. Nitrite reductase, which catalyzes the reduction of soluble NO_2^- into NO encoded by *nirS* and *nirK* genes, is the key enzyme regulating soil denitrification (Zumft, 1997). Amplification of these key functional bacterial genes, using real-time quantitative PCR (qPCR), represents a potential method to evaluate denitrifier abundance and their influence on N_2O emissions in soils. To date, few studies have focused on quantitative analysis of bacterial community composition in relation to N turnover rates, specifically those related to N_2O emissions and how the agricultural practices, such as tillage, can have a significant effect on the abundance and activity of soil denitrifiers, and as consequence alter the N_2O fluxes from soils.

In this sense, Philippot et al. (2009) demonstrated a significant correlation between the distribution of N_2O -reducing bacteria and potential N_2O emissions that appeared to be driven by soil pH, whereas in another study (Henry et al., 2008), no relationship between $\text{N}_2\text{O}:\text{N}_2$ ratio and denitrifier community size or composition after addition of C compounds to soil was found. Baudoin et al. (2009) reported that the No tillage system simultaneously increased the size of the soil N pool and accelerated the N cycle, by stimulating the denitrifier community. These contrasting results highlight the need for further studies combining of molecular analysis of the microbial population with measurements of N_2O production and process rates. A better insight into the regulation of these processes may provide a key to better understanding of the variability of N_2O fluxes at the soil-atmosphere interface, which can be used to modify management practice to lower emission (Butterbach-Bahl et al., 2013).

4. Simulation of N_2O emission at field scale. DNDC model

Process-based models have been used extensively in agroecosystem research to help improve our understanding of soil N dynamics and cycling, influence of addition of N fertilizer and other crop management techniques (tillage practices, crop rotation, etc.) on yields of cropping systems (Grant et al., 2016) and environmental N losses. Process based model are able to simulate the interactions of soil-plant-atmospheric processes while accounting for the

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mass balance of nutrients and water (Fig. 1.4). In recent years, a mechanistic model such as DeNitrification and DeComposition (DNDC) model has become a valuable tool to simulate and predict N losses through trace GHG emissions in agroecosystems (Li et al., 1992; Li, 2000; Giltrap et al., 2010) as well as other interdependent factors such as soil C and crop productivity (Smith et al., 2013).

Fig.1.4. The two-component DNDC model with six submodels: soil climate, crop growth, decomposition, denitrification, nitrification and fermentation (Li, 2000).

To calibrate and evaluate the DNDC model in conservation agriculture systems compared to conventional systems can be a good approach to provide detailed information on the impact of conservation tillage versus CT on N₂O emissions and crop yield, estimate cultivar performance and optimize agricultural management practices under semiarid Mediterranean conditions in order to predict the impact of future climate change on these areas and develop adaptation strategies (Jeuffroy et al., 2013b; Wang et al., 2015).

CHAPTER 2

Objectives

Main objective

Conservation agricultural practices improve soil quality and increase the soil C sink. However, the impact of these practices on GHG emissions is still uncertain and highly dependent on duration of experiment, climate conditions, N fertilization placement and crop residues management. Our main hypothesis is that the long-term conservation agriculture practices with adjusted N-input rates can provide an optimum balance between GWP and crop yields in semiarid agro-ecosystems. Therefore, the main objective of this Thesis is:

“To evaluate the impact of long-term conservation agricultural practices (which involved NT/MT and crop rotation) versus conventional agricultural practices (CT and monoculture) on GHG emissions (N_2O and CH_4) in low N input systems under rainfed semiarid Mediterranean agro-ecosystem in order to find the best optimal combination between environmental and agronomic perspective”.

The research work has been divided in 4 experiments, which are presented in 4 different Chapters. Each Chapter has its own specific objective in order to reach the main objective of the thesis, as explained below.

Objective 1

“To assess the long-term effects of three tillage systems (conventional tillage (CT), minimum tillage (MT), and no tillage (NT)) on N_2O and CH_4 emissions in legume crop (vetch crop) preceded by wheat”.

To address this objective, conservation tillage practices versus conventional tillage practices were evaluated in a vetch crop under rainfed Mediterranean conditions in Chapter 3. In addition to, to gain a better understanding of the processes associated with tillage management practices on N_2O emissions, the abundance of denitrification bacterial genes (*nirK* and *nirS*), denitrification activity and some microbiological characteristics (Dissolved organic content (DOC) and Microbial biomass content (MBC)) for each tillage were also analyzed in spring season.

Objective 2

“To evaluate the interacting effect of three tillage systems (conventional tillage (CT), minimum tillage (MT), and no tillage (NT)) and two land-covers (fallow and wheat) on GHG (N_2O and CH_4) fluxes in a low N input system”.

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In a long-term field experiment, the impact of conservation tillage practices (NT/MT) versus conventional tillage practices (CT) and the crop residues management in two phases of crop rotation (fallow and wheat) were evaluated for one year under rainfed Mediterranean conditions in Chapter 4. Furthermore, denitrification capacity and denitrifier abundance (encoded by *nirK* and *nirS* genes) were measured in order to explore the tillage effect on microbial N transformations, in Chapter 4.

Objective 3

“To study the effect of three long-term tillage systems (CT, MT and NT) and two cropping systems (wheat in monoculture and wheat in a 4-year rotation with fallow as preceding crop) on N_2O and CH_4 emissions during two campaigns”.

In a long-term field experiment, conservation agriculture practices were compared to conventional agriculture practices for two years in Chapter 5. Yield-scaled emissions and net GWP (CO_2 -equivalent emissions) were calculated to understand the overall GHG balance (since these practices can affect the consumption of inputs and SOC stocks) and gain a complete overview of the agronomic and environmental suitability of these practices.

Objective 4

“To Calibrate and validate the DNDC model for predicting N_2O emissions, yield, soil N content (NO_3 and NH_4) in two contrasting tillage systems (No tillage (NT) and conventional system (CT)) and the crop rotation (fallow-wheat-vetch) under semiarid conditions”.

To address this objective, the field-experiment dataset obtained in the chapter 1, 2, 3 and the paper of Guardia et al. (2016) were used for testing the DNDC model in order to evaluate management options with regard N_2O losses, in Chapter 6.

CHAPTER 3

**Nitrous oxide and methane emissions from a vetch
cropping season are changed by long term tillage
practices in a Mediterranean agroecosystem**

1. Introduction

Nitrous oxide (N_2O) and methane (CH_4) emissions from agricultural soils account for 10-12% of global anthropogenic greenhouse gases (GHGs) (Stocker et al., 2013). Different microbial soil processes produce these gases in soils: nitrification and denitrification for N_2O (Firestone and Davidson, 1989); and methanogenesis and methanotrophy for CH_4 (Chan and Parkin, 2001a). These processes and consequently the total GHG emissions are conditioned not only by soil properties, such as aeration, moisture content or pH, but also by the inputs of C and N applied to the soils. Although crop residues and N fertilizer application are associated with an increase of GHG emissions from soils, especially for N_2O , it is possible to mitigate total emissions by adequate agricultural management practices adapted to each cropping system (Rees et al., 2013b).

Agricultural systems based on the adoption of conservation tillage (e.g. no tillage (NT) and minimum tillage (MT)) instead of conventional tillage (CT) are widespread because they generally promote positive effects, such as increases in soil organic C (SOC) and soil fertility and reductions in the external energy inputs and soil erosion (Soane et al., 2012). Tillage practices can also affect aeration, rates of N and C cycling and therefore, GHG emissions. To date, the effect of tillage on N_2O emissions is still uncertain (van Kessel et al., 2013). Several studies found that NT/MT can produce greater (Baggs et al., 2003), lower (Malhi et al., 2006; Yonemura et al., 2014) or similar (Grandy et al., 2006) N_2O emissions compared to CT. In many cases, this variability is associated with climate and residue management (Ball et al., 2014; Yonemura et al., 2014). Additionally, NT/MT usually retains greater soil moisture than those under CT, which may enhance denitrification, also affecting N_2O production. In this sense, recent studies of denitrifier gene abundance have been used to provide a better understanding of N_2O emissions due to denitrification in soils (Morales et al., 2010). Regarding CH_4 fluxes, tillage practices affect a range of biochemical properties and impact on the rates of CH_4 production and consumption. Thus, Liu et al. (2006) reported that NT can reduce CH_4 uptake compared to that of CT, whereas Jacinthe et al. (2014) showed that the maintenance and duration of NT increased CH_4 oxidation. On the other hand, legume-cereal rotations are promoted as sustainable cropping systems, both in conservation and conventional agriculture, because the legume crop provides an additional N supply for the cereal decreasing the requirements for application of N fertilizers (Pappa et al., 2012). Jensen et al. (2012) found that N_2O fluxes from legumes were not significantly different to background emissions and were also lower than comparable N-fertilized crops and pastures. However, uncertainties remain, and recent studies of N_2O emissions from legume crops have raised considerable doubt, observing a high variability between sites ($0.03\text{--}7.09 \text{ kg N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$) (Rochette and Janzen, 2005). Part

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of the uncertainty in N₂O emissions may be due to the effect of residues from previous crop on the dynamic of C and N along legume cropping period. Immobilization and mineralization of cereal residues (C/N >30) could be affected by N supplied through fixation and thus, impact on GHGs. Since soil moisture controlled processes producing GHGs, an additional variability in emissions depending on climatic conditions could be expected. To date, there is scarce information on GHG emissions in legume-cereal crop rotations under both conservation and conventional tillage, especially under semiarid conditions.

The objective of this study was to assess the long-term effects of three tillage systems (NT, MT and CT) on N₂O and CH₄ emissions under a vetch crop. We hypothesized that after 17 years of contrasting tillage practices we would find differences in N₂O and CH₄ emissions; with an expectation that NT/MT would increase abiotic parameters such as soil C and moisture content leading to enhanced denitrification rates, and N₂O and CH₄ emissions. To gain a better understanding of the underlying processes associated with tillage management practices on N₂O emissions, the abundance of denitrification bacterial genes (*nirK* and *nirS*), denitrification activity and some microbiological characteristics for each tillage were also analyzed in spring season. In addition, soil Cu content was measured because of the important role that it seems to play in the activity of nitrite reductase (Enwall et al., 2010) and N₂O reductase enzymes (Sullivan et al., 2013).

2. Material and methods

2.1. Site characteristics

The field experiment was carried out at “La Canaleja” Field Station (40° 32'N, 3° 20'W, 600 m), in Alcalá de Henares (Madrid, Spain). This long-term tillage experiment began in 1994 and consisted of NT, MT and CT. Since 1994, several (cereal-legume) crop rotations have been cultivated (Martín-Lammerding et al., 2011). From 2005, the annual crop rotation were fallow-wheat (*Triticum aestivum* L. var. Marius)-vetch (*Vicia sativa* L. var. Senda)-barley (*Hordeum vulgare* L. var. Kika), within each tillage system.

The soil is a sandy-loam Calcic Haploxeralf according to the USDA soil taxonomy system (Soil Survey Staff, 2010). Some of the main physicochemical properties of the top soil layer (0-15 cm), measured by conventional methods, were: sand, 50.8 %; silt, 37.7%; clay, 11.5%; CaCO₃, 41.6 g kg⁻¹; pH_{H2O}, 7.9; and EC, 121.3 µS cm⁻¹. Table 3.1 shows soil organic C (SOC), N (SN) and bulk density in each tillage system. The site has a semiarid Mediterranean climate with a dry summer and wet winter. The 10 year mean annual average temperature and rainfall for this area were 13.6°C and 412 mm.

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Table 3.1 Main soil properties: soil organic C (SOC) and soil N (SN) contents and bulk density (BD) for each tillage treatments, at 0-15cm depth.

Tillage system	SOC (g kg ⁻¹)	SN (g kg ⁻¹)	BD (Mg m ⁻³)
NT	7.76±2.4	0.75±0.2	1.46±0.03
MT	7.08±1.0	0.66±0.1	1.39±0.08
CT	6.24±1.3	0.67±0.1	1.41±0.12

Numbers represent mean value ± standard deviation.

2.2. Experimental design

The current study was conducted between November 2010 and October 2011. The experimental design was a split-plot randomized complete block with three replicates per treatment. Each block was divided into three main plots assigned to the three tillage systems (NT, MT and CT) and each of them was divided into four sub-plots (10 x 25 m) allocated to all phases of the crop rotation mentioned above. In this study, we quantified the effect of three tillage systems on GHG emissions for the vetch phase of the rotation. Tillage practices consisted of: CT (mouldboard plough, 20 cm depth) and MT (chisel plough, 15 cm depth) on 26th October 2010, followed by a cultivator pass for seedbed preparation. No tillage involved direct drilling and spraying with glyphosate (at a rate of 2 L ha⁻¹ of Sting Monsanto ®) for weed control. Under NT, crop residues were left on the soil surface. Vetch (120 kg seeds ha⁻¹) was sown on 8th November 2010 and harvested on 30th May 2011. Following local agricultural practices, fertilizer was not applied to the vetch plots. After vetch harvesting, all crop residues were chopped and left on the surface, independent of tillage system.

2.3. N₂O and CH₄ analysis

Fluxes of N₂O and CH₄ were measured using the closed chamber technique as described in detail by Sanz-Cobena et al. (2014). One chamber of 19.3 L (diameter 35.6 cm, height 19.3 cm) was placed in each sub-plot. The chambers were closed by fitting them into stainless steel rings which were inserted into the soil (at 5 cm depth) to minimize lateral diffusion of gases. Measurements were always made with vetch plants inside the chamber.

Gas samples (vol. 20mL) were collected by syringe at 0, 30 and 60 min after chamber closure and collected in evacuated gas chromatography vials. Samples were taken from chambers three times per week during the first and second week, then twice per week during the first month. Subsequently, sampling was carried every two weeks until the end of the crop

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period and was also taken two times per week during rainfall periods. Then, one sampling per month was taken after harvest.

Samples were analyzed by gas chromatography using a HP-6890 gas chromatograph equipped with a headspace autoanalyzer (HT3), both from Agilent Technologies (Barcelona, Spain). HP Plot-Q capillary columns transported gas samples to a ^{63}Ni electron-capture detector (Micro-ECD) to analyze N_2O concentrations and to a flame ionization detector (FID) to measure CH_4 . The temperatures of the injector, oven and detector were 50, 50 and 350°C , respectively.

The increases in N_2O and CH_4 concentrations within the chamber headspace were generally linear ($R^2 > 0.90$) during the sampling period (1h), as commonly observed for this type of chamber (Abalos et al., 2013). Emission rates of fluxes were estimated as described in MacKenzie et al. (1998). Cumulative N_2O -N and CH_4 -C emissions were calculated as proposed by Menéndez et al. (2006).

To facilitate the analysis, cumulative N_2O emissions were calculated in three relevant time periods based on vetch growth stages: I period (seedling development from November to December), II period which included early and full flowering stages and beginning of senescence (from January to mid- April) and III period which included pod setting and grain-filling to harvest (from the end of April to beginning of June). Additionally, the intercrop period (IV period) was measured from the end of June to October.

2.4. Soil analysis

In order to relate gas emissions to soil properties, soil samples were collected from each plot at 0-15 cm depth on every gas-sampling occasion. Three soil cores (2.5cm diameter and 15cm length) were randomly taken close to the ring from each plot, then mixed and homogenized in the laboratory. Soil dissolved organic-C (DOC), NH_4^+ -N and NO_3^- -N concentrations and water-filled pore space (WFPS) were determined as described in detail by Abalos et al. (2013). Rainfall data were obtained hourly from a meteorological station located at the field site. A temperature probe was inserted 15 cm into the soil to measure soil temperature in each tillage system, which stored hourly data on a data logger.

Under Mediterranean conditions, the beginning of spring is generally a favourable period for soil microbial activity, and specially denitrification because of the increase of soil temperature and rainfall events. For this purpose, six soil cores were randomly collected from each plot. Three of the six soil cores were mixed, homogenized for analysis of denitrification activity. The other three were sieved (2 mm), mixed, homogenized and stored at -80°C for

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microbiological analyses. Additional soil properties were analyzed in these soil cores: total copper (Cu) (using microwave oven digestion and AA spectrophotometry (Shuman and Hargrove, 1985)), microbial biomass C (MBC) content (by the chloroform fumigation-extraction method described by Vance et al., 1987) and DOC content.

Denitrification activity was measured to evaluate the effect of C availability on N₂O emissions using the acetylene block technique (Abalos et al., 2013). Three replicates for each tillage treatment were used. For this purpose, 30 g of fresh soil were placed in 250 mL glass bottles with 12.5 mL of a solution containing 42.9 mM KNO₃. Bottles were closed with air-tight lids equipped with septa and flushed with N₂ (10 minutes) to create anaerobic conditions. Then, bottles were divided into two groups (with and without 8% (v/v) of acetylene (C₂H₂)). These laboratory incubations were undertaken at 25°C and, after 0, 12 and 24 h, gas samples (10 mL) from each bottle were taken and stored in evacuated vials. N₂O concentrations were analyzed by gas chromatography as previously described. Different problems have been detected in the literature (Meijide et al., 2009) in relation to this technique, such as (i) the poor diffusion of C₂H₂ into the soil, (ii) the use of this compound by microorganisms as a C source for denitrification, (iii) the catalytic decomposition of NO in presence of C₂H₂ (Bollmann and Conrad, 1997) and (iv) the inhibition of nitrification at low concentrations (< 0.1%) (Muller et al., 1998). Nevertheless, this technique gives a qualitative estimate of denitrification activity and can be used to make comparisons between treatments (Estavillo et al., 2002).

Regarding the molecular biological analyses, DNA was extracted from three 250-mg aliquots of soil samples with the NucleoSpin Soil Kit (MACHEREY-NAGEL), according to the protocol of the manufacturer. The amount and quantity of DNA was assessed with a NanoDrop (ND-1000 Spectrophotometer) before PCR amplification and cloning. Soil DNA samples were used on standard PCR-amplifications with specific primers for *nirK* and *nirS* genes (Table 3.2). *NirK* and *nirS* amplicons were gel purified, cloned using the pGEM-T Easy cloning kit (Promega) and introduced into *E. coli* strain DG-1. Plasmid DNA was extracted using the Nucleospin plasmid miniprep kit (Marcherey-Nagel) and the concentration determined by NanoDrop. The presence of the *nirK* and *nirS* fragments was verified by gel electrophoresis after restriction enzyme digestions and by sequence analysis.

Quantitative PCR assays were performed to calculate *nirK* and *nirS* genes abundance. Standard curves were generated by plotting the threshold cycle obtained in quantitative PCR reactions performed with a 7300 Real Time PCR System (Applied Biosystems) from serial dilutions between 10¹⁰ and 10³ copies of *nirK* and *nirS* containing plasmids linearized with *SalI*. The amplification efficiency (E) was estimated by using the slope of the standard curve according to the equation:

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$$E = \left(10^{-\frac{1}{slope}} \right) - 1$$

Quantitative PCR assays were carried out on 25 µl assay mixtures containing 10 ng of soil total DNA, 1 µM of each primer (Table 3.2) and 10 µl of SYBR green PCR Master Mix (Applied Biosystems). Thermal cycling conditions for the *nirK*, *nirS* are shown in Table 3.2. Thermal cycling, florescent data collection and data analysis were carried out with the 7300 Real Time PCR System sequence detection system (Applied Biosystems). For each treatment, three replicate DNA extractions from each soil were assayed. Two independent quantitative PCR assays were performed with these samples for each gene. Threshold cycles obtained with *nirK* and *nirS* primers were referred to copy number using the standard curves.

Table 3.2 Quantitative PCR primers and thermal Cycling conditions used for quantification of different genes.

Primers	Sequence (5'-3')	Thermal conditions	Reference
<i>nirK</i>	ATYGGCGGVCA YGGCGA GCCTCGATCAGRTT RTGGTT	95°C, 15 min, 1cycle.	Henry et al. (2004)
		95°C for 15s, 63°C to 58 °C for 30s (-1°C by cycle), 72°C for 30s, 80°C for 15s, 6 cycles	
		95 °C for 15 s. 60 for 30s, 72 °C for 30s, 80 °C for 15s, 40 cycles.	
		95 °C for 15 s 60 to 95°C, 1 cycle.	
<i>nirS</i>	AACGGYSAAGGARACSGG GASTTCGGRTGSGTCTTSAYGAA	95°C, 15 min, 1cycle.	Throback, et al. (2004)
		95°C for 15s, 65°C to 60 °C for 30s (-1°C by cycle), 72°C for 30s, 80°C for 15s, 6 cycles	
		95 °C for 15 s. 60 for 30s, 72 °C for 30s, 80 °C for 15s, 40 cycles.	
		95 °C for 15 s 60 to 95°C, 1 cycle.	

2.5. Statistical analysis

Statistical analyses on the collected data were performed using Statgraphics Plus 5.1. The normality of the fluxes ($\text{N}_2\text{O-N}$ and $\text{CH}_4\text{-C}$) and soil parameters (NH_4^+ , NO_3^- , MBC and DOC content, denitrification activity and genes abundance) were verified using the Kolmogorov-Smirnov test. Where necessary, the data were log or square root transformed before analysis, such as *nirK* abundance, denitrification activity and $\text{CH}_4\text{-C}$ fluxes. Analyses of variance (two-way ANOVA, ($P < 0.05$), which included terms for each tillage system and block were performed for all variables over the experiment (except for rainfall). The Tukey test was used for multiple comparisons between means. For non-normally distributed data (mean soil NH_4^+ content), the Kruskal-Wallis test was used on non-transformed data to evaluate differences at $P < 0.05$. Linear regression analyses were carried out to determine relationships between gas fluxes and soil parameters.

3. Results

3.1. Environmental conditions, WFPS, DOC and mineral N

Mean soil temperatures ranged from 3.6 to 36.8°C, during the experimental period (one year). No significant differences between tillage systems ($P > 0.05$) were found in soil temperature at any sampling time (Fig. 3.1a). Total rainfall (from November 2010 to October 2011) was 512.4 mm for the experimental period, being higher than the previous 10-year mean (435.2 mm). Soil WFPS in the upper soil layer depended on rainfall events and tillage, and ranged from 76.1 to 32.2% for all tillage treatments (Fig. 3.1b), during the growth of vetch. No tillage had a significantly higher WFPS content ($P < 0.05$) than MT and CT on many sampling occasions.

Fig. 3.1 (a) Rainfall and soil temperature and (b) weekly soil water-filled pore space (WFPS) content in the 0-15 cm soil layer during the experimental period.

The DOC content of the top soil (0-15 cm) (Fig. 3.2a) was influenced by the tillage system, with values ranging from 11.3 to 61.8 mg C kg⁻¹; 8.1 to 45.4 mg C kg⁻¹ and 4.9 to 32.6 mg C kg⁻¹ for the NT, MT and CT, respectively. The NT treatment significantly increased ($P < 0.05$) DOC content in comparison to MT and CT, on many sampling dates. In fact, mean DOC content for NT (taking into account the whole crop period) were significantly higher (29.7% and 51.5%; $P < 0.05$) than those for MT and CT, respectively.

Fig. 3.2 (a) Soil dissolved organic carbon (DOC) content, (b) soil ammonium content and (c) soil nitrate content, under three tillage systems: no tillage (NT), minimum tillage (MT) and conventional tillage (CT), in the 0–15 cm soil layer during the experimental period. Vertical lines indicate standard errors. *Continuous arrow* indicates the date of tillage practices and *dotted arrow* indicates the date of seeding.

Soil NH_4^+ concentration (Fig. 3.2b) was below $4 \text{ mg NH}_4^+\text{-N kg}^{-1}$ in all treatments ($P > 0.05$) and varied between 0.2 to $3.5 \text{ mg NH}_4^+\text{-N kg}^{-1}$ in the three tillage systems. Soil NO_3^- content (Fig. 3.2c) increased in the three weeks after sowing in the three tillage treatments, with values above $16 \text{ mg NO}_3^-\text{-N kg}^{-1}$. Afterwards, soil NO_3^- content decreased in all plots with values below $6 \text{ mg NO}_3^-\text{-N kg}^{-1}$. In the last month of vetch crop (May), NO_3^- content increased in all treatments. Mean soil NH_4^+ and NO_3^- content was not significantly different ($P > 0.05$) among tillage practices during the experimental period.

3.2. Denitrification activity and genes abundance

Denitrification activity was affected by the tillage practices (Fig. 3.3a) in the spring season. Minimum tillage plots ($8.01 \text{ mg N kg}^{-1}\text{d}^{-1}$) showed significantly higher ($P < 0.05$) denitrification activity than NT ($5.94 \text{ mg N kg}^{-1}\text{d}^{-1}$) and CT ($4.82 \text{ mg N kg}^{-1}\text{d}^{-1}$).

The *nirK* copy numbers were higher than *nirS* copy numbers in the three tillage systems (Fig. 3.3b). The *nirS* abundance was below 1.3×10^3 gene copies g^{-1} dry soil in all tillage treatments (data not shown) indicating *nirS* gene abundance was below the detection limit as reported by Baudoin et al. (2009) under NT and CT soils. The *nirK* abundance was significantly higher for MT than NT and CT ($P < 0.05$), with values ranging from 2.4×10^5 to 7.1×10^7 copies g^{-1} dry soil.

Fig. 3.3 (a) Mean values for denitrification capacity to $\text{N}_2\text{O}+\text{N}_2$ and (b) Abundance of *nirK* genes in each tillage system: no tillage (NT), minimum tillage (MT) and conventional tillage (CT), under vetch crop. Vertical bars indicated standard errors. *Different lowercase letters* indicate significant differences between tillage treatments (LSD test at $P < 0.05$).

Several soil properties were also measured at the same sampling date alongside denitrification activity and genes abundance (Table 3.3). DOC content and MBC were significantly different ($P < 0.05$) among tillage systems, whereas Cu content was similar among them. No significant correlations were observed between denitrification activity and these soil properties, or between gene abundance and soil properties. There was a statistically significant correlation between *nirK* abundance and denitrification activity ($R^2 = 0.83$, $n = 9$, $P < 0.01$).

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Table 3.3 Selected soil properties: dissolved organic C (DOC), microbial biomass C (MBC) and total Cu, in each tillage system: no tillage (NT), minimum tillage (MT) and conventional tillage (CT) treatments, in spring season.

Treatment	DOC (mg C kg ⁻¹ soil)	MBC (mg C kg ⁻¹ soil)	Total Cu (mg Cu kg ⁻¹ soil)
NT	30.2±8.2a	304.2±27.1a	14.5±0.5a
MT	27.1±7.6a	186.2±23.9ab	14.7±0.5a
CT	11.2±4.4b	93.9±8.8b	13.8±0.4a

Numbers represent mean value ± standard error. Different letters within columns indicate significant differences between tillage treatments according to Tukey test ($P < 0.05$).

3.3. N₂O and CH₄ emissions

Throughout the experimental period, daily N₂O fluxes were consistently low, and ranged from -0.14 to 0.33 mg N₂O-N m⁻² d⁻¹ for NT, MT and CT (Fig. 3.4a). Higher N₂O fluxes in all treatments were observed just three weeks after tillage (one week after seeding) with values of 0.19, 0.18 and 0.14 mg N₂O-N m⁻² d⁻¹ for NT, MT and CT, respectively. Negative N₂O fluxes were observed in all tillage systems on some sampling occasions, during the experimental period. Conventional tillage showed the highest N₂O emission peaks ($P < 0.05$) (0.16 and 0.15 mg N₂O-N m⁻² d⁻¹) in December and April, during the growth period. However, NT and MT had the highest emission peaks (0.22 and 0.33 mg N₂O-N m⁻² d⁻¹ for NT and MT, respectively) at the end of growing season (maturity).

Total cumulative N₂O emissions (Fig. 3.4b and Table 3.4) were statistically greater ($P < 0.05$) for MT (0.12 kg N₂O-N ha⁻¹) than NT (0.05 kg N₂O-N ha⁻¹) and CT (0.05 kg N₂O-N ha⁻¹) during the experimental period (one year). Additionally, different N₂O flux patterns were observed among tillage systems during the growth period of vetch under semiarid environmental conditions (Fig. 3.4b and Table 3.4). Cumulative N₂O emissions for CT and MT increased following a similar trend during periods I and II until the last growing season, when MT increased roughly from April to mid- June (period III) and reached values greater than 10 mg N₂O-N m⁻². However, cumulative N₂O emissions for NT were negative in the II period and then increased quickly from the beginning of May to harvest. This increase of N₂O fluxes was similar to MT, during the last growing period (period III). As for the intercrop period (IV period), cumulative N₂O fluxes were quite low ($P > 0.05$), ranging from -0.1 to 0.3 mg N₂O-N m⁻² for all tillage treatments. No significant correlations ($P > 0.05$) were found between soil properties and N₂O fluxes.

Fig. 3.4 (a) Daily fluxes of N₂O and (b) Cumulative N₂O emissions for no tillage (NT), minimum tillage (MT) and conventional tillage (CT) treatments during the four measurement periods (I, II, III and IV period). Vertical lines indicate standard errors. Continuous arrow indicates the date of tillage practices and dotted arrow indicates the date of seeding.

Methane emissions ranged from -0.91 to 0.31 mg CH₄-C m⁻² d⁻¹; from -1.38 to 0.37 mg CH₄-C m⁻² d⁻¹ and from -0.80 to 1.51 mg CH₄-C m⁻² d⁻¹, for NT, MT and CT, respectively (data not shown). Cumulative CH₄ emissions (Table 3.4) were not significantly different among tillage practices, during the experimental period.

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Table 3.4 Cumulative N₂O-N over the different periods of field-experiment, and total cumulative N₂O-N and CH₄-C emissions in each tillage system.

Tillage treatment	Cumulative N ₂ O-N (g N ₂ O-N ha ⁻¹)				Total N ₂ O-N (g N ₂ O-N ha ⁻¹)	Total CH ₄ -C (g CH ₄ -C ha ⁻¹)
	I period	II period	III period	IV period		
NT	17.1±1.4 a	-39.5±2.1b	72.6±1.5a	0.9±0.1a	51.1±1.2 b	-137.4± 11.5a
MT	16.4±0.9a	26.3±1.7a	79.6±1.2a	1.6±0.1a	124.2± 2.2a	-473.1± 5.6a
CT	29.9±0.9a	25.8±0.7a	-0.9±0.6b	-1.1±0.1a	53.7±1.4 b	-231.2± 12.7a

Numbers represent mean value ± standard error. Different letters within columns indicate significant differences by applying the Tukey test at ($P < 0.05$)

4. Discussion

4.1. Effect of long-term tillage systems on N₂O emissions

The total N₂O losses measured over one year in a vetch crop under three tillage systems (< 0.16 kg N₂O ha⁻¹) were in the low range of emissions reported by Jensen et al. (2012) for legume crops (0.03-7.09 kg N₂O-N ha⁻¹). Other authors have also found similar values, such as Barton et al. (2013) for a lupin crop (0.038 kg N₂O-N ha⁻¹) and Sanz-Cobena et al. (2014) for a vetch crop (0.16 kg N₂O-N ha⁻¹) under semiarid conditions.

In this study, different patterns of N₂O emissions were observed among tillage treatments during the vetch crop. Four periods were identified (Fig. 3.4b, Table 3.4): a first period characterised by positive and similar N₂O fluxes and little relationship to the tillage practices; second period characterised by a sink for N₂O on many sampling dates and a clear dependence on the tillage, a third period with significant pulses of N₂O dependent on tillage and a fourth period considered as an intercrop period from the end of June to October.

During the 1st period coinciding with seedling development (Fig. 3.4b), N₂O peaked for all tillage systems as a consequence of some rainfall events which rewetted the dry soils, increasing WFPS. It is known that addition of water to a dry soil activates the microbial populations producing pulses of N₂O (Sanchez-Martin et al., 2010a). As aerobic processes (nitrification) generally control production of N₂O when rewetting dry soils (Davidson et al., 1993), possible differences in the capacity of soils for denitrification due to tillage were not significant at this stage. In this sense, DOC and WFPS in the upper soil surface during this period were higher for NT than for MT and CT, but N₂O fluxes were low and did not differ among treatments. Other rainfall events happened in November and December contributing to rewetting of deeper soils

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layers, leading to low pulses of N_2O in all treatments, probably associated with the nitrification process because of limiting WFPS observed in all tillage systems (mean WFPS values were: 53, 44 and 47%, in NT, MT and CT respectively). During this period, soils maintained a content of NO_3^- above 10 mg N kg^{-1} , which was very similar for all treatments (Fig. 3.2c). Available mineral N (from fertilizer and from mineralization) by the previous crop (wheat) was presumably the cause of this N source. At the end of this 1st period (December), rainfall events were recorded (38 mm) and were likely to have contributed to the reduction of soil NO_3^- contents ($< 5 \text{ mg kg}^{-1}$) as a result of leaching. Furthermore, the poor quality of the wheat residue (C/N ratio > 30), which was left on the soil from the previous crop in NT or incorporated into the soil for MT and CT, together with the low soil temperatures could have boosted immobilization of mineral N, contributing to lower soil NO_3^- content in all tillage systems, at the end of this period.

During the 2nd period, small or even negative fluxes predominated in the three tillage systems (Fig. 3.4b). The low available N mineral and WFPS content ($< 58\%$) may probably limit these N_2O fluxes under field conditions. Sink for N_2O has been described in other cropping systems by Abalos et al. (2013) and Merino et al. (2004), probably driven by denitrification processes, in which N_2O may be reduced to N_2 . In this period the low content of NO_3^- ($< 5 \text{ mg N kg}^{-1}$) observed in all treatments probably favoured the use of other electron acceptor sources by denitrifying bacteria, such as N_2O produced in soil through different processes or even atmospheric N_2O close to soil surface. Theoretically, the main N_2O consumption pathway could be denitrification. But Chapuis-Lardy et al. (2007) pointed out that the process responsible for N_2O uptake in dry soils are not very clear, and proposed nitrifier denitrification and aerobic denitrification, as other N_2O -reducing processes. No tillage plots in this period maintained the highest WFPS and DOC content, reducing gas diffusion and favouring better anaerobic conditions in the NT soil microsites, and thus gave more frequent negative fluxes than MT and CT (Fig. 3.4b). Cavigelli and Robertson (2000) also reported higher N_2O consumption in NT than in CT. In that study, the amount of the active N_2O reductase might be greater in the denitrifying community for NT than CT, which would be expected to result in a higher proportion of N_2 as a product of denitrification in NT soils. Although this enzyme activity has not been measured in our experiment, we speculate that it could have occurred mainly in NT plots. At the end of 2nd period (April), the decrease in WFPS ($< 50\%$), favouring aerobic conditions, together with the low content of soil NH_4^+ seem to indicate that nitrification might be also a significant source of N_2O (Li and Lang, 2014), especially in MT and CT systems.

For the 3rd period (Fig. 3.4b), which coincided with the last growing stage of vetch (pod-setting and grain filling), N_2O fluxes increased notably for NT and MT. The combination of

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changes in N requirement, which increased soil NO_3^- content (Fig. 3.2c), and favourable climatic factors such as rainfall events (142 mm in May) and an increase of soil temperature (Fig. 3.1) could have favoured denitrification, enhancing N_2O fluxes for several weeks in NT and MT. The increase in soil mineral N was as a consequence of the low plant N uptake and the release of mineral N from the decaying nodules and roots in the rhizosphere at this stage (Yang and Cai, 2005). Other studies reported N_2O emissions at the end of legume growth that were independent of tillage, and also associated the denitrification of sources such as N rhizodeposition by legume roots (Wichern et al., 2008) or mineralization of labile legume N released from nodulated roots (Rochette and Janzen, 2005). In the CT plots, the low DOC content may be the key factor limiting denitrification process, thus, reducing N_2O fluxes during this period. For the 4th period, N_2O fluxes (Fig. 3.4b, Table 3.4) were almost negligible in all tillage systems. The crop residues were left on the soil surface in all plots after harvest. This fact along with the lack of rainfall (< 60 mm) and high mean soil temperature (> 28°C) may have delayed the decomposition of residues as well as limited soil microbial processes controlling N_2O production or consumption (Aguilera et al., 2013a).

Although there are conflicting results in literature concerning the effects of tillage on N_2O emissions, our experiment confirmed that long-term tillage practices affected N_2O fluxes during a vetch crop (Table 3.4), through the changes in some soil properties, mainly DOC and WFPS contents during the cropping period and particularly, differences seen for MBC content, *nirK* abundance and denitrification activity during the 2nd period. In addition, significant differences in N_2O fluxes were found among tillage systems especially for the 2nd and 3rd period. In both periods, different conditions for nitrification and denitrification, which interacted with tillage, were responsible for soil N transformations and N_2O losses (Li and Lang, 2014). Barton et al. (2013) indicated that in legume-cereal rotation, nitrification rather than denitrification was the main source of N_2O under semiarid conditions in Australia. Although, the measurements taken in our experiment do not give enough information to clarify which pathway was responsible for this source of N_2O , we speculated that also denitrification could be an important source of N_2O , especially during 2nd and 3rd periods for NT, when WFPS were higher than 60% during 130-140 days compared to 45-50 and 85-90 days for MT and CT, respectively.

Tillage and crop rotation (including a legume crop) changed soil characteristics in this long-term experiment (1994-2011) with significant effects on organic matter distribution (Table 3.1) (Martín-Lammerding et al., 2011). This was important in contributing to a significantly higher DOC content in the NT treatment than CT on most sampling occasions. As labile organic C is an energy source for denitrifiers, higher denitrification activity would therefore be expected in the NT soil. However, results of this experiment did not confirm this hypothesis, at least for

the 2nd period, because denitrification activity (measured in March) was not correlated with DOC content at that time. Denitrification activity was only significantly correlated with *nirK* gene abundance, which followed the order: MT > NT > CT (Fig. 3.3b). This is also consistent with other authors (i.e. Baudoin et al., 2009), who found a positive correlation between *nirK* abundance and denitrification activity, suggesting that NT stimulated higher *nirK* and denitrification activity than CT. Additionally, soil Cu content also measured at that time were similar among tillage systems and no correlation with *nirK* abundance was found, whereas Enwall et al. (2010) pointed out a positive correlation between the *nirK* community structure and this micronutrient.

The abundance of *nirK* gene (Fig. 3.3b) measured in this study at this date varied within the range (9.7×10^4 to 4.8×10^8 copies g⁻¹ dry soil) reported previously by Baudoin et al. (2009) and Henry et al. (2004). Since relative denitrifier abundance may change over time and the detection of functional genes did not imply that the corresponding activity is present (Philippot and Hallin, 2005), the results of *nirK* gene abundance should be considered with caution and only as an indicator of microbial processes at a critical time (spring) for this agroecosystem.

Considering the sum of all periods (Table 3.4), the MT treatment enhanced total N₂O emissions in comparison to CT and NT, which gave similar emissions. The lower N₂O sink found in MT compared with NT, especially during 2nd period, was associated with lower WFPS contents on many sampling occasions and may explain these results. Additionally, we speculated that changes in the denitrifier population promoted by long-term tillage may have also led to different N₂O fluxes. From an environmental point of view, NT must be a recommended management practice (instead of MT) in order to mitigate N₂O emissions and specially to increase C sequestration (Table 3.1) in agricultural soils.

The patterns of N₂O fluxes for one year under a legume crop (Fig. 3.4b) discussed above would only be relevant if legumes were included in a crop rotation with a crop which uses N fixed (e.g. cereals), therefore reducing soil NO₃⁻. In the case of legumes monoculture, the results could be different because the soil NO₃⁻ content under legume monoculture may follow different patterns to those observed in cereal-legume rotations.

4.2. Tillage effect on methane emissions

In this study, a net uptake of CH₄ was found for all treatments (Table 3.4) as is common in rainfed agrosystems (Barton et al., 2013; Sanz-Cobena et al., 2014), and low rates of CH₄ emissions were observed compared to other CH₄ fluxes measured from other rainfed crops under Mediterranean conditions (Meijide et al., 2010). It is known that methanotrophic activity

is partially inhibited by NH_4^+ at the cellular level, due to competitive inhibition of the enzyme responsible for CH_4 oxidation (CH_4 monooxygenase) with the NH_3 monooxygenase (Dunfield and Knowles, 1995). For legume crops, as no N fertilizer was applied, this potential effect was not expected, particularly given that soil NH_4^+ content was always below 4 mg kg^{-1} . Even the last part of the vetch crop, when soil mineral N increased, a high CH_4 oxidation was observed for all treatments.

Although, no significant differences on total CH_4 fluxes were observed among tillage systems, these results followed similar trends as those reported by Omonode et al. (2007). Other studies (i.e. Ussiri et al., 2009; Jacinthe et al., 2014) have suggested the implementation and the duration of conservation tillage systems as strategy to decrease CH_4 emissions to the atmosphere by restoring the CH_4 sink strength of agricultural soils. This effect is attributed to the more suitable biological, chemical, and physical soil factors on methanotrophic bacteria activity (Hütsch, 1998).

5. Conclusions

Nitrous oxide emissions from the three tillage systems were low under a vetch crop in a Mediterranean rainfed soil. Long-term tillage and crop rotation affected soil properties (e.g. DOC, WFPS), which controlled N_2O emissions. Although, nitrification may have contributed to N_2O emissions in this agroecosystem during 1st and 2nd period, associated with the low content of soil WFPS and NH_4^+ , differences in N_2O losses among tillage systems may be mainly attributed to different conditions for denitrification, especially from January to June. The net emission of N_2O from January to April was characterised by negative fluxes associated with complete denitrification, which were promoted under NT plots. In this period, denitrification activity and *nirK* gene abundance were higher for MT than NT and CT. The higher WFPS and DOC in NT than in MT combined with a low soil NO_3^- content contributed to favourable conditions for total denitrification under NT compared to MT. At the end of the crop period, the favourable soil conditions for denitrification and the increase in soil NO_3^- probably as a consequence of the release of mineral N from the decaying nodules and roots in the rhizosphere produced higher fluxes from NT and MT than CT. Considering the whole experimental period, greater total N_2O emissions were observed in MT than NT and CT. Therefore, NT could be considered as a suitable alternative to MT to mitigate N_2O emissions within legume-cereal crop rotations under rainfed Mediterranean conditions. Additionally, a net uptake of CH_4 was observed for all tillage treatments as is common in rainfed agroecosystems. Further studies should focus on different microbial groups (i.e. denitrifier and nitrifier populations) and their contribution to production and consumption of N_2O and CH_4 fluxes in soils in order to target mitigation strategies and better understand how tillage systems affect these gases in legume-

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cereal rotation, under semiarid climate. As microbial population control production and consumption of GHG emissions, targeting microbial population can deep our understanding of the relationships between the microbial community ecology, N and C loss, and the agroecosystems' impact on climate change.

CHAPTER 4

**N₂O and CH₄ emissions from a fallow-wheat rotation
with low N input in conservation and conventional
tillage under a Mediterranean agroecosystem**

1. Introduction

Agricultural management practices affect the production of greenhouse gases (GHGs), especially nitrous oxide (N_2O) and methane (CH_4), which have global warming potentials 265 and 28 times higher (without inclusion of climate-carbon feedbacks), respectively, than carbon dioxide (CO_2) (Myhre et al., 2013). Agricultural soils emit approximately 10.3-12.8 Tg N_2O -N year⁻¹ (Butterbach-Bahl et al., 2013), as a result of two main biological processes, i.e. nitrification and denitrification. In the soils, CH_4 can be mainly consumed by methanotrophic bacteria or produced by methanogenic bacteria in aerobic and anaerobic soil conditions, respectively (Hütsch, 1998).

In Europe, Spain is the leading country in terms of the adoption of conservation agriculture (Kassam et al., 2012). Conservation agriculture practices include crop rotations and conservation tillage (no tillage (NT) and minimum tillage (MT)), which overall have the potential to increase organic matter in the topsoil and often reduce CO_2 emissions compared with conventional tillage (CT) (López-Bellido et al., 2010). However, the impact of tillage practices on N_2O emissions is highly variable depending on several biophysical parameters (Snyder et al., 2009), the degree of residue incorporation into the soil (Oorts et al., 2007), soil texture (Rochette, 2008), the duration of tillage practices and climatic variability (Six et al., 2004). Recently, Van-Kessel et al. (2013) has reported that long-term (>10 years) NT/MT practices reduced N_2O emissions in dry climates, because of better soil structure and higher aeration in NT/MT that can decrease anaerobic microsites in soil where denitrification can occur (Ussiri et al., 2009; Dendooven et al., 2012), supporting earlier findings (Six et al., 2004).

In order to understand the effect of tillage systems on N_2O emissions, the processes involved in N_2O emission, such as denitrification and nitrification need to be studied. It is known that tillage systems exert an important effect on the transformation of microbial N, especially on the denitrification process (Robertson and Tiedje, 1987). Nitrite reductase, which catalyses the reduction of soluble nitrite into nitric oxide, is the key enzyme regulating soil denitrification (Zumft, 1997). Two different types of nitrite reductases have been described, a cytochrome *cd1* enzyme and a Cu-containing enzyme, encoded by *nirS* and *nirK* genes. Amplification of these functional bacterial genes represents a potential method to evaluate denitrifier abundance and their influence on N_2O emissions in soils. To date, little is known about how denitrifier abundance changes due to agricultural management practices, such as tillage (Morales et al., 2010). Some studies (e.g. Attard et al., 2011) have shown that NT can promote higher denitrification rates compared with CT due to an increase in denitrifier abundance, which act as a source of N_2O . However, other studies have reported that

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denitrification activity can be regulated by environmental factors rather than denitrifier composition (Butterbach Bahl et al., 2013) and consequently denitrification activity and abundance are not necessarily tightly coupled (Zumft, 1997).

Over-application of N fertilizers is often the largest source of N₂O emissions. Therefore, strategies based on a better use of N are considered as a way of reducing N₂O emissions (Rees et al., 2013b). In regions where mineral fertilizers are not usually available, such as in Sub-Saharan countries, low N input systems (i.e. Integrated Soil Fertility Management system) are being promoted as an alternative to optimize the use of all suitable available resources for crop growth (Kimani et al., 2003). These systems are also being used in semiarid areas of developed countries, like Spain, where the expected crop yields are also low due to soil properties and climatic conditions. Low N input systems combine a low rate of mineral N fertilizers with crop residues to improve crop quality and agricultural sustainability. After the addition of cereal residues in the soils, a period of net N immobilization followed by a period of net N remineralization (Cayuela et al., 2009) generally occurs, which depends mainly on the quality of residue (i.e. C:N ratio) and the initial soil mineral N content (Baggs et al., 2000).

Agricultural practices often affect fluxes of more than one gas, through more than one mechanism, sometimes in opposite ways so that the net benefit depends on the combined effects of all gases (Snyder et al., 2009). In the case of tillage effects on CH₄ fluxes, conservation tillage generally enhances gas diffusivity due to a porous structure leading to a greater CH₄ oxidation (Ussiri et al., 2009). On the contrary, lower CH₄ uptake in NT compared to CT has been reported by Yonemura et al. (2014), when soil conditions in NT reduced gas diffusivity. The number of years under NT also affects soil aeration and as a consequence higher methanotrophic capacity of soils has been observed in long-term rather than in short-term experiments (Plaza-Bonilla et al., 2014b). However, application of crop residues and N fertilizer also affects CH₄ emissions in conservation agriculture systems. It is known that NH₄⁺ and urea fertilizer hamper CH₄ uptake due to interference of the enzyme activity responsible for CH₄ oxidation (CH₄ monooxygenase) with NH₃ monooxygenase (Dunfield and Knowles, 1995). Additionally, the management of crop residues due to tillage practices (i.e. incorporated or left on soil surface) can produce differences in CH₄ fluxes between tillage systems (Hütsch, 2001).

In low N input systems, a crop rotation with a fallow with incorporation of cereal crop residues is a useful option to maintain soil fertility and accumulate soil water (van Donk et al., 2010). In this fallow period, N₂O and CH₄ emissions can be affected by the fertilizer applied to the previous crop (as a residual effect), rainfall events (Sanchez-Martin et al., 2010b) and also by the mineralization of crop residues. However, will the fallow period have a positive or a negative effect with regards to GHG emissions in the long term? Although, the world

agricultural soil surface using low N inputs is increasing, information is scarce about GHG emissions from cereal crop rotations, especially in semiarid areas. To date, there have not been any studies assessing N₂O and CH₄ fluxes in a fallow-winter wheat crop rotation under conservation agriculture using low N input fertilizer systems.

The main objective of this study was to investigate the long-term impact of three tillage systems (NT, MT and CT) and land-covers (fallow/wheat) on GHG (N₂O and CH₄) fluxes in a low N input system under semiarid conditions. Our first hypothesis was that N₂O emissions in fallow would be lower than wheat subplots as a consequence of the absence of mineral N fertilizer and the presence of cereal crop residues, which would promote N immobilization leading to low N₂O emissions. The second hypothesis was that long-term NT and MT systems (>18 years) would reduce N₂O emissions compared with CT due to the improvement of soil structure and the decrease in anaerobic microsites, which would also affect CH₄ emissions.

2. Material and methods

2.1. Experimental site and design

The field experiment was located at “La Canaleja” Field Station (40° 32’N, 3° 20’W, 600 m), in northeast of Madrid (Spain). The long-term tillage experiment began in 1994. The tillage systems were: NT, MT and CT. Crop rotations always included legume and cereal crops (Martín-Lammerding et al., 2011). The annual crop rotation were fallow-wheat (*Triticum aestivum* L. var. Marius)-vetch (*Vicia sativa* L. var. Senda)-barley (*Hordeum vulgare* L. var. Kika), within each tillage system. An Integrated Soil Fertility Management system was applied from 2000.

The area has a mild Mediterranean climate with dry summers and wet winters. The 10-year mean annual temperature and rainfall for this area were 13.6 °C and 412 mm. The soil is a sandy-loam Calcic Haploxeralf (Soil Survey Staff, 2010). Some of the main physico-chemical properties of the top soil layer (0-15 cm), measured by conventional methods, were: sand, 50.8%; silt, 37.7%; clay, 11.5%; CaCO₃, 41.6 g kg⁻¹; pH_{H2O}, 7.9 and EC, 121.3 µS cm⁻¹. At the start of this study, soil organic C (SOC) was 7.76, 7.08 and 6.24 g kg⁻¹, and total soil N was 0.75, 0.66 and 0.67 g kg⁻¹, for NT, MT and CT respectively at 15 cm depth. Bulk density was 1.46, 1.39 and 1.41 Mg m⁻³, whereas soil porosity was 0.45, 0.48 and 0.47, for NT, MT and CT, respectively.

The current experiment was conducted for one year (from November 2010 to October 2011) and was designed on the basis of a three-replicated split-plot. Tillage was the main factor (plot) and land-cover was the secondary factor (subplot) in randomized complete block design.

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In this study, only fallow (F) and wheat (W) subplots were surveyed, resulting in eighteen subplots (10 m x 25 m). The tillage practices were applied in the autumn of 2010 (26th October 2010) for wheat and the beginning of February for fallow subplots; and consisted of: mouldboard plough at 20 cm depth (CT) and chisel plough at 15 cm depth (MT), followed by a cultivator pass for seedbed preparation. No tillage (NT) involved direct drilling and spraying with herbicides for weed control, leaving the crop residues on the soil surface. In the fallow subplots, the barley straw (C:N ratio of 112) was applied depending on the tillage system at a rate equal to 1,896 kg C ha⁻¹, corresponding to the average straw yield from the previous barley crop.

Wheat was sown on 8th November 2010 with 150 kg seeds ha⁻¹. Fertilizer was applied at seeding at a rate of 16 kg N ha⁻¹ as N-P-K (8-24-8) and at tillering (20th March 2011) at 40 kg N ha⁻¹ as NH₄NO₃. For the calculation of N rate, the expected N uptake by wheat and the soil available N (which was estimated through soil analysis) were taken into account. Finally, wheat was harvested on 16th of June. After wheat harvesting, all crop residues were chopped and left on the surface, independently of the tillage system.

2.2. Sampling and analysis of gases

Fluxes of N₂O, CH₄ and CO₂ were measured using the closed chamber technique as described by Sanz-Cobena et al. (2014), with a volume of 19.3 L (diameter 35.6 cm, height 19.3 cm). The chambers were placed in each subplot and closed (for 1 h) by fitting them into stainless steel rings which were inserted at the beginning of the study into the soil (at 5 cm depth) to minimize the lateral diffusion of gases and avoid the soil disturbance associated with the insertion of the chambers in the soil. The rings were only removed during management practices. Samples were always made with wheat plants inside the chamber. When the plants were higher than 19 cm, plastic intersections of 19 cm covered with Teflon were used between the ring and the chamber. Thermometers were placed inside three randomly selected chambers during the closure period of each measurement in order to correct the fluxes for temperature. Gas samples were taken from chambers three times per week during the first and second weeks after fertilizer applications, then twice per week during the following month, and subsequently every two weeks until the end of the crop period. Then, one sampling per month was taken after harvest. Gas samples from the fallow subplots were taken on the same dates as wheat subplots.

Gas samples (20 mL) were taken by syringe at 0, 30 and 60 min after chamber closure to test the linearity of gas accumulation and then stored in gas chromatography vials. Samples were analyzed by gas chromatography using a HP-6890 gas chromatograph equipped with a headspace autoanalyzer (HT3), both from Agilent Technologies (Barcelona, Spain). HP Plot-Q

capillary columns transported gas samples to a ^{63}Ni electron-capture detector (ECD) to analyze N_2O concentrations and to a flame ionization detector (FID) to measure CH_4 . The temperatures of the injector, oven and detector were 50, 50 and 350 °C, respectively.

Increases in GHG concentrations within the chamber headspace were generally linear ($R^2 > 0.90$) during the sampling period (1h), as commonly observed by this type of chamber (Sanz-Cobena et al., 2014). Emission rates of fluxes were estimated as the slope of the linear regression between concentration and time (after corrections for temperature and pressure) and from the ratio of chamber volume to soil surface area (Abalos et al., 2014). Cumulative N_2O -N and CH_4 -C emissions per subplot were estimated by linear interpolations between sampling dates.

Yield scaled N_2O emissions were calculated based on van Groenigen et al. (2010), considering total N uptake (i.e. by wheat grain and straw).

2. 3. Soil and crop analysis

To relate GHG emissions to soil properties, soil samples from 0-15 cm were collected from each subplot on every gas-sampling occasion. Three soil cores (2.5 cm diameter and 15 cm length) were taken close to the ring from each subplot, then mixed, and homogenized in the laboratory.

Soil dissolved organic carbon (DOC) and nitrate (NO_3^- -N) concentrations were analyzed by extracting 8 g of fresh soil with 50 mL of deionized water. An Orion 720A NO_3^- -N electrode (Thermo Fisher Scientific, Beverly, MA, USA) was used to analyze NO_3^- -N concentrations, while DOC content was determined using the permanganate oxidation method, described by BOE (1987). Soil ammonium (NH_4^+ -N) concentrations were determined by extracting 8 g of fresh soil with 50 mL of KCl (1 M) and measured with an UV-visible spectrophotometer (UV-1603, Shimadzu). The water filled pore space (WFPS) was calculated by dividing volumetric water content by total soil porosity. Moisture contents were determined by gravimetric analysis. Total soil porosity was calculated according to the relationship: soil porosity = $1 - (\text{soil bulk density}/2.65)$, assuming a particle density of 2.65 Mg m^{-3} . Rainfall and temperature data were obtained hourly from a meteorological station located at the field site. A temperature probe was inserted 15 cm into the soil to measure soil temperature in each tillage system.

In order to explore the tillage effect on microbial N transformations, some microbiological characteristics and denitrification capacity were measured from soil samples collected on 19th March 2011. The beginning of spring is a characteristic period of Mediterranean climate, which promotes microbial activity, especially denitrification owing to an

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increase in air temperature and rainfall events. For this purpose, six soil cores were randomly collected close to the ring from each plot. Three of the six soil cores were mixed and homogenized for analysis of denitrification capacity. Additional soil properties were analyzed in these soil cores: total copper (Cu) by using microwave oven digestion and AA spectrophotometry (Shuman and Hargrove, 1985) and microbial biomass C (MBC) content by the chloroform fumigation-extraction method described by Vance et al. (1987). Soil Cu content was measured because of the important role that seems to play in the activity of nitrite reductase (Enwall et al., 2010) and N₂O reductase enzymes (Sullivan et al., 2013). The other three were sieved (2 mm), mixed, homogenized and stored at -80°C for microbiological analyses.

Denitrification capacity was measured using the technique of acetylene blockage (Abalos et al., 2013) for assessing the effect of C availability on N₂O emissions. Three replicates for each treatment were used. To this end, 30 g of fresh soil were placed in 250 mL glass bottles with 12.5 mL of a solution containing 42.9 mM KNO₃. Bottles were closed with air-tight lids equipped with septa and flushed with N₂ (10 min) to create anaerobic conditions. Excess pressure and O₂ were also removed using a small needle. Then, bottles were divided into two groups (with and without 8% (v/v) of acetylene). These laboratory incubations were undertaken at 25 °C and, after 0, 12 and 24 h; gas samples (10 mL) from each bottle were taken and stored in evacuated vials. N₂O concentrations were analyzed by gas chromatography as previously described.

Regarding the molecular biological analyses, DNA was extracted from three 250-mg aliquots of soil samples with the NucleoSpin Soil Kit (MACHEREY-NAGEL), according to the protocol of the manufacturer. The amount and quantity of DNA was assessed with a NanoDrop (ND-1000 Spectrophotometer) before PCR amplification and cloning. DNA soil samples were used on standard PCR-amplifications with specific primers for *nirK* and *nirS* genes (Table 4.1). *NirK* and *nirS* amplicons were gel purified, cloned using the pGEM-T Easy cloning kit (Promega) and introduced into *E. coli* strain DG-1. Plasmid DNA was extracted using the Nucleospin plasmid miniprep kit (Marcherey-Nagel) and the concentration determined by NanoDrop. The presence of the *nirK* and *nirS* fragments was verified by gel electrophoresis after restriction enzyme digestions and by sequence analysis.

Quantitative PCR assays were performed to calculate *nirK* and *nirS* genes abundance. Standard curves were generated by plotting the threshold cycle obtained in quantitative PCR reactions performed with a 7300 Real Time PCR System (Applied Biosystems) from serial dilutions between 10¹⁰ and 10³ copies of *nirK* and *nirS* containing plasmids linearized with *SalI*. The amplification efficiency (E) was estimated by using the slope of the standard curve according to the equation:

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$$E = \left(10^{-\frac{1}{\text{slope}}} \right) - 1$$

Quantitative PCR assays were carried out on 25 µl assay mixtures containing 10 ng of soil total DNA, 1 µM of each primer (Table 4.1) and 10 µl of SYBR green PCR Master Mix (Applied Biosystems). Thermal cycling conditions for the *nirK*, *nirS* are shown in Table 4.1 Thermal cycling, florescent data collection and data analysis were carried out with the 7300 Real Time PCR System sequence detection system (Applied Biosystems). For each treatment, three replicate DNA extractions from each soil were assayed. Two independent quantitative PCR assays were performed with these samples for each gene. Threshold cycles obtained with *nirK* and *nirS* primers were referred to copy number using the standard curves.

Table 4.1 Real Time PCR primers and thermal Cycling conditions used for quantification of different genes.

Primers	Sequence (5'-3')	Thermal conditions	Reference
<i>nirK</i>	ATYGGCGGVCA YGGCGA GCCTCGATCAGRTT RTGGTT	95°C, 15 min, 1cycle.	Henry et al. (2004)
		95°C for 15s, 63°C to 58 °C for 30s (-1°C by cycle), 72°C for 30s, 80°C for 15s, 6 cycles	
		95 °C for 15 s. 60 for 30s, 72 °C for 30s, 80 °C for 15s, 40 cycles.	
		95 °C for 15 s 60 to 95°C, 1 cycle.	
<i>nirS</i>	AACGGYSAAGGARACSGG GASTTCGGRTGSGTCTTSAYGAA	95°C, 15 min, 1cycle.	Throback, et al. (2004)
		95°C for 15s, 65°C to 60 °C for 30s (-1°C by cycle), 72°C for 30s, 80°C for 15s, 6 cycles	
		95 °C for 15 s. 60 for 30s, 72 °C for 30s, 80 °C for 15s, 40 cycles.	
		95 °C for 15 s 60 to 95°C, 1 cycle.	

At maturity, the yield of grain and above-ground biomass were determined by harvesting four randomly selected 0.7× 0.7 m squares from each plot. The plant components were dried in a 65 °C oven, weighed and ground for determination of N concentration (AOAC, 2000).

2.4. Calculations and statistical analysis

To facilitate discussion, cumulative N₂O and CH₄ fluxes were calculated in three different periods according to the fertilizer applications. The period I covered from the tillage practices in final-October to February. The period II covered from March to harvest. The period III covered from harvest (mid-June) to October, as intercrop period.

Statistical analyses were performed using Statgraphics Plus 5.1 (Manugistics 2000). The normality of the fluxes (N₂O and CH₄) and soil parameters (NH₄⁺-N, NO₃⁻-N and DOC, denitrification capacity and genes abundance) were verified using the Kolmogorov-Smirnov test. Where necessary, the data were log or square root transformed before analysis, such as *nirK* abundance and CH₄-C fluxes. Analysis of variance (two-way ANOVA, $P < 0.05$) was also carried out for all variables of the experiment in order to assess the effect of each tillage system and land-cover (fallow and wheat). The Least Significant Difference (LSD) test was used for multiple comparisons between means. For non-normally distributed data, the Kruskal-Wallis test was used on non-transformed data (NH₄⁺-N content) to evaluate differences at $P < 0.05$. Linear regression analyses ($P < 0.05$) were carried out to determine relationships between gas fluxes and soil parameters.

3. Results

3.1. Environmental conditions, soil moisture, mineral N and DOC

Mean soil temperature (Fig. 4.1a) ranged from 3.6 to 10.9 °C in period I and from 10.1 to 23 °C in period II. No significant differences between tillage systems ($P > 0.05$) were found in soil temperature. Total rainfall accounted for 177.5 mm and 280.3 mm during periods I and II, respectively, being close to the last 14-year mean (411.2 mm). During intercrop period (period III), soil temperature (Fig. 4.1a) ranged from 20.5 to 36.8 °C, and rainfall was 54.6 mm. Soil WFPS (Fig. 4.1b) in the upper soil layer (15 cm) was dependent on the rainfall events and the tillage practices, especially during period I when mean WFPS was significantly higher ($P < 0.05$) in NT (54.6%) than MT (45%) and CT (48%). However, mean WFPS in period II and for the whole experimental was not significantly different among tillage practices. The content of WFPS showed no significant differences ($P > 0.05$) between fallow and wheat subplots.

Fig.4.1 (a) Weekly means soil temperature (°C) and rainfall (mm), (b) Evolution of soil moisture content expressed as Water Filled Pore Space (WFPS, %), for no tillage (NT), minimum tillage (MT) and conventional tillage (CT), under the fallow (f) and wheat (w) subplots during the experimental period.

Soil NH_4^+ concentrations maintained below $4 \text{ mg NH}_4^+ - \text{N kg}^{-1}$ in all treatments in fallow (Fig. 4.2a). In the case of wheat, NH_4^+ content slightly increased (close to $10 \text{ mg NH}_4^+ - \text{N kg}^{-1}$) after each fertilization event (Fig. 4.2b). Soil NH_4^+ concentrations were similar ($P > 0.05$) among the tillage treatments but showed significantly higher values ($P < 0.01$) in wheat than fallow subplots, for periods I and II. Soil NO_3^- content in the fallow subplots (Fig. 4.2c) was generally low ($< 16 \text{ mg NO}_3^- - \text{N kg}^{-1}$) until April, when it increased in all tillage treatments. In the wheat subplots, NO_3^- content (Fig. 4.2d) increased during the period 15 to 20 days after each fertilization event, showing higher values ($P < 0.05$) than fallow subplots. Subsequently, NO_3^- content decreased in all wheat subplots in period II. Soil NO_3^- contents (Fig. 4.2c, d) were not different ($P > 0.05$) among the tillage systems but were significantly higher ($P < 0.05$) in wheat than in fallow during period I only.

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Soil DOC content (Fig. 4.2e, f) ranged from 23.6 to 56.8 mg C kg⁻¹; 18.1 to 40.9 mg C kg⁻¹ and 15.6 to 34.1 mg C kg⁻¹ for the NT, MT and CT plots, respectively. Soil DOC content was significantly higher ($P < 0.01$) in NT than MT and CT, whereas there were no differences ($P > 0.05$) between the fallow and wheat subplots.

Fig.4.2 (a, b) Soil $\text{NH}_4^+\text{-N}$, (c, d) $\text{NO}_3^-\text{-N}$ and (e, f) DOC concentrations for the three tillage systems: NT, MT and CT, in the fallow (f) and wheat (w) subplots, during the experimental period. Vertical lines indicate standard errors. Continuous arrow indicates the date of tillage practices and dotted arrow indicates the date of seeding and fertiliser application.

3.2. Denitrification capacity and gene densities

Denitrification capacity rates in the 0-15 cm soil layer (Table 4.2), expressed as $\text{N}_2\text{O}+\text{N}_2$ production, were not affected by the tillage practices ($P > 0.05$) and land-covers ($P > 0.05$) either, in March.

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The *nirK* copy numbers were higher than *nirS* copy numbers in the three tillage systems (Table 4.2). The *nirS* gene density was below the detection limit ($< 8 \times 10^3$ gene copies g^{-1} dry soil) in all tillage treatments (data not shown) as reported by Baudoin et al. (2009) under non-tilled and tilled soils. The *nirK* gene density was similar ($P < 0.05$) in the three tillage systems, with values ranging between 1×10^4 and 2×10^8 gene copies g^{-1} dry soil. The size of *nirK* genes was significantly higher ($P < 0.01$) in fallow than in wheat subplots. There were no correlations between *nirK* gene copies and denitrification capacity.

Several soil properties were measured in March together with denitrification capacity and denitrifier densities (Table 4.2). The effect of tillage system showed significant differences ($P < 0.05$) in MBC. The Cu content was similar among all treatments. No significant correlations were observed among denitrification capacity, *nirK* gene densities and soil properties.

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Table 4.2 The effect of tillage, crop rotation and tillage x crop interaction on denitrification capacity (mg N kg⁻¹ soil d⁻¹), densities of *nirK* gen and the soil properties (MBC and Cu content), in spring season.

Effect	Denitrification capacity (mg N kg ⁻¹ soil d ⁻¹)	<i>nirK</i> (gene copy numbers g ⁻¹ dry soil)	MBC (mg C kg ⁻¹ soil)	Cu (mg Cu kg ⁻¹ soil)
Tillage	ns	ns	*	ns
NT	5.52	4.53 x 10 ⁷	342.5 a	13.7
MT	4.66	8.91 x 10 ⁷	260.6 a	13.2
CT	3.41	1.12 x 10 ⁷	153.1 b	13.2
SE	0.67	0.81	26.6	0.4
<i>P-value</i>	0.2	0.61	0.02	0.67
Crop	ns	**	ns	ns
fallow	4.83	9.60 x 10 ⁷ a	240.3	13.4
wheat	4.23	1.31 x 10 ⁶ b	263.9	13.4
SE	0.93	0.74	38.2	0.4
<i>P-value</i>	0.67	0.001	0.68	0.9
Tillage x crop	ns	ns	ns	ns
SE	1.62	1.57	66.2	0.64
<i>P-value</i>	0.94	0.64	0.45	0.77

Within a column, means followed by the same letter are not significantly different according to Fisher's LSD at a 0.05 probability level. *P < 0.05; ** P < 0.01; ns=not significant. SE refers to standard error of the mean.

3.3. Fluxes of nitrous oxide and methane

Nitrous oxide emissions (Fig. 4.3a, b) were always below 0.4 mg N₂O-N m⁻² d⁻¹ in the three tillage systems under fallow and wheat. In the fallow subplots, N₂O fluxes ranged from -0.07 to 0.23 mg N₂O-N m⁻² d⁻¹, for NT-f, MT-f and CT-f, with slightly increased fluxes after rainfall events (Fig. 4.3a). In the wheat subplots, N₂O fluxes varied between -0.08 and 0.33 mg N₂O-N m⁻² d⁻¹, for NT-w, MT-w and CT-w (Fig. 4.3b). Several small peaks were measured along the experimental period, which were associated with fertilization events: 14 days after the 1st fertilization (0.21 and 0.11 mg N₂O-N m⁻² d⁻¹ in NT-w and CT-w, respectively), and 10 days after the 2nd fertilization (0.11, 0.15 and 0.19 mg N₂O-N m⁻² d⁻¹, for NT-w, MT-w and CT-w,

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respectively), also coinciding with rainfall events. Negative fluxes were observed in all treatments, especially for MT.

Cumulative N₂O emissions in the fallow subplots (Fig. 4.3c) during the experimental period were 30.7, 94.4 and 113.3 g N₂O-N ha⁻¹, for NT, MT and CT, respectively. Whereas cumulative N₂O emissions in the wheat subplots (Fig. 4.3d) were 46.2, 26.5 and 25.6 g N₂O-N ha⁻¹, for NT, MT and CT, respectively.

Total cumulative N₂O emissions (Table 4.3) were not significantly different ($P > 0.05$) among the tillage systems or between fallow and wheat ($P = 0.1$). The only difference was produced in period II, when N₂O emissions were significantly higher ($P < 0.05$) in fallow than in wheat subplots, and also, NT produced lower N₂O fluxes than MT/CT ($P < 0.05$). Fluxes of N₂O in period II were 59% and 18% of total N₂O emissions during the whole experiment in fallow and wheat, respectively. Nitrous oxide emissions were not significantly correlated ($P > 0.05$) with WFPS, soil temperature and the other soil parameters.

Fig.4.3 (a, b) Daily fluxes of N_2O and (c, d) cumulative N_2O emissions for the three tillage systems: NT, MT and CT, in the fallow (f) and wheat (w) subplots during the three distinct periods. Vertical lines indicate standard errors. Continuous arrow indicates the date of tillage practices and dotted arrow indicates the date of seeding and fertiliser application.

For the wheat subplots, N uptake from above-ground wheat biomass (grain and straw) was 61.2, 49.1 and 64.9 kg N ha⁻¹, for NT, MT and CT respectively; these values were not significantly different among tillage treatments. Yield-scaled N_2O emissions were 0.9, 0.6 and 0.6 g $\text{N}_2\text{O-N kg}^{-1}$ above-ground N uptake for NT, MT and CT, respectively, without significant differences ($P > 0.05$) among them.

The daily fluxes of CH_4 ranged from -0.75 to 1.02 mg $\text{CH}_4\text{-C m}^{-2} \text{d}^{-1}$ among the tillage systems (data not shown). Cumulative CH_4 emissions (Table 4.3) showed a net sink effect in all tillage treatments. No significant differences ($P > 0.05$) were seen for the cumulative CH_4 emissions from the different treatments. No significant correlations were found between soil CH_4 fluxes and soil parameters.

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Table 4.3 Effect of tillage, crop rotation and tillage x crop interaction on cumulative N₂O-N and CH₄-C emissions over the different periods of the experiment.

Effect	N ₂ O (g N ₂ O-N ha ⁻¹)				CH ₄ (g CH ₄ -C ha ⁻¹)
	period I	period II	period III	Total	Total
Tillage	ns	*	ns	ns	ns
NT	33.6	5.0 b	-0.2	38.4	-127.1
MT	23.3	37.4 a	-0.3	60.4	-241.4
CT	32.2	36.5 a	0.6	69.4	-111.1
SE	1.1	0.5	0.1	1.7	15.7
<i>P-value</i>	0.79	0.02	0.61	0.49	0.82
Crop	ns	*	ns	ns	ns
fallow	32.7	46.7 a	-0.03	79.5	-90.3
wheat	26.7	5.9 b	0.2	32.7	-229.5
SE	1.2	1.1	0.03	1.1	9.2
<i>P-value</i>	0.73	0.03	0.61	0.10	0.32
Tillage x crop	ns	ns	ns	ns	ns
SE	2.1	1.8	0.04	3.3	15.9
<i>P-value</i>	0.14	0.95	0.22	0.33	0.7

Within a column, means followed by the same letter are not significantly different according to Fisher's LSD at a 0.05 probability level. * P < 0.05; ns = not significant. S.E. refers to standard error of the mean.

4. Discussion

4.1. Effect of tillage practices on N₂O emissions

Tillage systems did not affect total N₂O emissions when considering the whole experimental period (Table 4.3). The lower N₂O fluxes emitted in period II by NT in comparison with MT and CT were not low enough to produce significant differences when the sum of the three periods was considered. This is possibly due to two factors: the similar soil denitrification capacity (Table 4.2) and also the similar mean WFPS (53%, 46% and 48% in NT, MT and CT, respectively) for the different tillage practices. Our results are also consistent with Dusenbury et al. (2008), who found that tillage systems did not affect N₂O losses for wheat-fallow rotation under semiarid conditions at the Great Plains in Canada, owing to similar moisture content among the tillage practices.

Several authors support the idea that the beneficial effect of tillage on N₂O emissions can be attributed to changes in soil structure, which in turn affects the aeration of the soil (Rochette,

2008; Plaza-Bonilla et al. 2013). In this regard, Six et al. (2004) found a lower volume of anaerobic microsites in NT compared with CT and MT, which could account for the lower N₂O production in NT systems. In our long-term experiment, Martin-Lammerding et al. (2011) observed a slight improvement of soil structure, measured as structural stability, in the upper soil layer of NT in comparison with CT, related to the higher SOC and particulate organic C contents for NT. Nevertheless, soil porosity was very similar for all tillage systems, as were the values of WFPS, which would justify the rare influence of the tillage on N₂O emissions in this study. Perhaps for these reasons, our results are not consistent with those reported by van Kessel et al. (2013), who concluded that long term NT practices (>10 years) reduced N₂O emissions compared with CT under dry climates. It is probable that this positive effect of NT is observed when NT produces significant changes in soil properties, particularly in WFPS for most of the year. However, our results agree with the conclusions of Rochette (2008), who assessed results from 25 field studies with CT and NT. This author pointed out that the impact of NT on N₂O was small in well-aerated soils, whereas NT only increased N₂O emissions in poorly-aerated soils. Using the criteria of Rochette (2008), our sandy-loam soil is a medium-aerated soil (good drainage combined with precipitation above 400 mm during the growing season) with a mean WFPS below 60%, both in NT and CT.

No tillage treatment had a higher DOC and MBC contents than CT. This was a consequence of the long-term application of crop residues on the soil surface for NT treatments, which increased organic C content in the topsoil. However, these differences in DOC were not enough to produce a significantly higher denitrification capacity in NT than in CT in the top soil (Table 4.2) in March. These contrast with those of Elmi et al. (2003) and Attard et al. (2011), who reported higher denitrification capacity in NT/MT than CT. This effect could be related to the similar *nirK* gene copy numbers found between tillage systems at that time. The densities of *nirK* gene (Table 4.2) measured in this study at that date were within the range (9.7×10^4 to 4.9×10^7 copies g⁻¹ dry soil) mentioned previously by Henry et al. (2004) and Rosa et al. (2014). Soil Cu content (measured at that time) was similar among tillage systems and no correlation with *nirK* abundance was found, whereas Enwall et al. (2010) pointed out a positive correlation between the *nirK* community structure and this micronutrient. These results suggest that tillage practices did not influence the size of denitrifier populations and denitrification capacity because of the few differences observed in soil properties, especially soil aeration and structure, and low N availability, supporting the lack of response of N₂O emissions to different tillage systems under semiarid conditions.

Although, N₂O emissions are similar among tillage systems, we may suggest that NT may be a good agricultural practice, because of the positive benefits related to the long-term soil C

sequestration, lower N₂O fluxes during period II and the reduction of production cost, as long as crop productivity is maintained. Particularly when considering that soils under semiarid Mediterranean areas are characterized by very low organic matter and fertility levels. Additionally, we speculated that the lack of differences on N₂O emissions between tillage systems may have been hidden by the low N input, and consequently limiting N₂O production.

4.2 Effect of land-cover on N₂O emissions

Annual N₂O losses were rather low ($< 0.2 \text{ kg N-N}_2\text{O ha}^{-1}$) for fallow and winter wheat subplots, mainly due to a low N fertilization rate ($< 60 \text{ kg N ha}^{-1}$) and the low rainfall ($< 500 \text{ mm}$) characteristic of rainfed Mediterranean agroecosystems. Cumulative N₂O emissions were below the range ($0.18\text{--}0.77 \text{ kg N-N}_2\text{O ha}^{-1} \text{ yr}^{-1}$) reported in other semiarid areas in North America by Kessavalou et al. (1998) and Dusenbury et al. (2008), in wheat–fallow rotation for NT and CT systems. However, similar results have been observed by Aguilera et al. (2013a), who carried out a meta-analysis in Mediterranean climate cropping systems and also reported low N₂O emission levels for winter cereals such as winter wheat ($0.3 \text{ kg N}_2\text{O ha}^{-1} \text{ yr}^{-1}$), attributed to low N fertilization rates applied to rainfed crops.

In order to compare GHG intensity with other agroecosystems (Van Kessel et al., 2013), yield-scaled N₂O emissions were calculated for wheat subplots. Our yield-scaled emissions were very low ($< 1 \text{ g N}_2\text{O-N kg}^{-1} \text{ N uptake}$) for all tillage treatments, several times lower than in other agroecosystems from the meta-analysis of van Groenigen et al. (2010). These authors reported that yield-scaled N₂O emissions ranged from 7.1 to 12.7 g N₂O-N kg⁻¹ N uptake for low N application rates ($< 100 \text{ kg N ha}^{-1}$). Cai et al (2013) also showed similar values, ranging from 4.2 to 8 g N₂O-N kg⁻¹ N uptake for a wheat crop in a wheat-maize rotation. An interesting result was that yield-scaled emissions in our low N system were even lower than those reported by van Groenigen et al. (2010) for treatments without fertilizer addition. Additionally, grain yield was optimum for these climatic and soil conditions, with a higher wheat grain yield (mean value of 3082 kg ha^{-1} for all tillage treatments) compared with previous years ($1743 \text{ kg grain ha}^{-1}$) (Martin-Lammerding et al., 2013). Regarding environmental and agricultural aspects, the strategy of adjusting the N rate (i.e. low N rate) to crop N demand seems to have worked well in this system and, therefore, this strategy should be implemented by farmers with rainfed crops in semiarid Mediterranean areas. On the other hand, the tillage system had no clear effect on yield-scaled emissions, due to the fact that no differences in N uptake and total N₂O fluxes were found between treatments.

Contrary to what we expected, cumulative N₂O fluxes for wheat subplots (Table 4.3) were lower in period II, when 40 kg N ha^{-1} was applied, than those in period I, when 16 kg N ha^{-1}

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¹ was applied. Mean wheat N uptake by grain and straw (58.4 kg N ha⁻¹) was similar to N fertilizer input (56 kg N ha⁻¹), as reported in other studies (i.e. Dusenbury et al., 2008). Since the most of this N was taken up by crop growth in period II, soil NO₃⁻ content rapidly reduced, reaching values below 10 mg NO₃⁻-N kg⁻¹ just three weeks after dressing fertilization (from April to June) (Fig. 4.2d). As a result of this low NO₃⁻ concentration, denitrification was probably not favoured, which may account for the low N₂O fluxes, even when WFPS was above 60% on some days. Additionally, the low average WFPS (<53%) for all tillage treatments may support the theory that part of the N₂O fluxes came from nitrification during period II. Nevertheless, the low content of applied NH₄⁺ (20 kg N ha⁻¹) may be a limiting factor that also contributed to the low magnitude of N₂O emissions. Nevertheless, further analysis should be carried out in order to clarify which mechanism was responsible for this source of N₂O.

The fallow subplots (Table 4.3) significantly enhanced cumulative N₂O emissions ($P < 0.05$) compared with wheat in period II, although no significant differences were seen in total N₂O emissions ($P = 0.10$) between fallow and wheat. In addition, N₂O emissions in period II were greater than in period I in all fallow treatments, contrary to what was seen in wheat subplots. The application of barley crop residues from the previous season and its subsequent mineralization may justify the N₂O fluxes in the fallow subplots (Mosier et al., 2006). Due to the poor quality of the barley residues (C:N ratio > 100), the mineralization of organic matter was slow during the first months after application, only increasing the NO₃⁻ content from April to June (Fig. 4.2c), concurrent with an increase in soil temperature during spring (Fig. 4.1a). This increase in NO₃⁻ content (>10 mg N kg⁻¹), together with WFPS values above 60% for almost 19 days during that period may have favoured denitrification, giving the higher N₂O emissions in the fallow than in the wheat subplots. The higher *nirK* copy numbers in the fallow compared with the wheat subplots ($P < 0.05$) in March, may have also contributed to the differences seen in N₂O emissions at that time. The presence of crop residues in fallow soils from the previous season may have favoured the increase of denitrifier populations. This is consistent with Bastida et al. (2009), who reported that the addition of organic amendments in soils increased the size of denitrifier populations targeted by *nirS*. Additionally, we speculated that denitrifier abundance, estimated by *nirK* gene copy numbers, might be less competitive in fertilised soils compared with fallow subplots. Wallenstein (2004) in a long-term forest experiment observed a lower *nirK* and *nirS* abundance in fertilized plots than in plots without N fertilization, which were associated with changes in soil C availability, moisture and pH. Similarly, Wolsing and Prieme (2004) reported a shift in denitrifier composition between plots with and without fertilizer in an agricultural soil due to direct effects of increased N. Nevertheless, densities of *nirK* should be considered with caution as quality information of microbial processes at a critical time (spring), because PCR approaches did not provide

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information on the active denitrifier populations (Philippot and Hallin, 2005). Besides, changes in denitrifier populations may also occur at different times depending on climatic, plant and soil conditions (Wolsing and Prieme, 2004); consequently, additional samples throughout the year would need to be taken in order to make definitive conclusions.

Negative N₂O fluxes were observed for both fallow and wheat subplots, especially when the soil NO₃⁻ content was low (<10 mg N kg⁻¹). We hypothesized that anaerobic microsites would have favoured the reduction of N₂O to N₂ by denitrification, despite the low WFPS (<65%) (Chapuis-Lardy et al., 2007). This effect has also been observed by Abalos et al. (2013) under similar climatic conditions and attributed to denitrification processes in which N₂O has been used as an electron acceptor by microorganisms, thereby producing negative fluxes of N₂O or decreasing net N₂O fluxes. However, we cannot dismiss the possibility that the consumption of N₂O was produced by nitrifier-denitrification as suggested by Schmidt et al. (2004), in which nitrifiers were able to produce N₂ from nitrite (NO₂⁻) under anoxic conditions inside the aggregates. Further studies should be carried out to explain differences in consumption processes.

Taking into account the N₂O emission results, fallow-winter wheat rotation may not be a suitable recommendation for farmers, even though N₂O fluxes in fallow were low, their contribution to total N₂O emissions was greater than the main crop. Furthermore, Aguilera et al. (2013a) pointed out that fallow and bare soils in cropping systems may release greater amounts of N compounds responsible for both direct and indirect N₂O emissions. However, analyzing the effect of fallow on other components of N cycle, such as the evolution of soil mineral N, the results of this experiment show a higher mineral N content in fallow than in wheat treatments at the end of crop period. During the summer the soil remains dry, this residual N could be available by the following crop in autumn, thus reducing the amount of N required for the following crop. Another potential benefit is a higher moisture content in fallow, which could be useful for the following crop, although this has not been observed in this experiment. Therefore, considering the benefits of legumes increasing N input through fixation, we suggest a decrease in the frequency of fallow cycles within the crop rotation sequence, replacing them by legume crops, especially for low N input systems where the amount of mineral N applied could limit crop yields.

4.3. Effect of tillage and land-cover on CH₄ fluxes

Overall, cumulative CH₄ fluxes resulted in low net uptake rates for all treatments (Table 4.3), with no significant differences among tillage treatments ($P > 0.05$) and between fallow and wheat subplots ($P > 0.05$). Our results are consistent with Dendooven et al. (2012) and Sanz-Cobena et al. (2014) under Mediterranean conditions. However, other authors (Ball et al., 1999; Ussiri et al., 2009) have reported that tillage decreased CH₄ uptake because the oxidizing bacteria were susceptible to soil disturbance, especially when tillage reduced structural stability and promoted soil anaerobic microsites. In our case, the low soil moisture content was maintained throughout the experimental period and the non-significant improvement of soil porosity in the NT system would justify no significant differences between the tillage treatments.

It has often been reported that the application of N fertilizer can reduce CH₄ oxidation capacity. Indeed, methanotrophic activity is partially inhibited by NH₄⁺ at the cellular level, due to competitive inhibition of the enzyme responsible for CH₄ oxidation (CH₄ monooxygenase) with the NH₃ monooxygenase (Dunfield and Knowles, 1995; Le Mer and Roger, 2001). In our study, this effect was not observed, and CH₄ oxidation was maintained at the same level in wheat as in fallow, probably due to the low rates of N fertilizer applied to the crop. This effect could be considered as an advantage of the low N input systems, because it maintains the CH₄ oxidation potential in soils.

5. Conclusions

Taking into account the results of this experiment, we can conclude that long-term tillage practices had no effect on N₂O and CH₄ fluxes in a coarse-textured soil under a Mediterranean climate. Cumulative N₂O emissions were low in fallow and wheat (< 0.2 kg N ha⁻¹), mainly attributed to the low N inputs as fertilizer or crop residues, and low soil moisture conditions. Variability of WFPS throughout the experimental period suggests that both nitrification and denitrification could have contributed to the N₂O emissions. Despite the higher DOC content in the NT topsoil, denitrification capacity was similar in all tillage systems, which could be related to the similar *nirK* copy numbers among tillage treatments in mid-March. This fact, together with the low moisture content throughout most of the experimental period, justified the lack of differences between N₂O emissions in all tillage systems. However, fallow showed higher N₂O emissions than wheat subplots, which was mainly due to the differences in mineral N between wheat and fallow, especially during April-May (period II). Densities of the *nirK* gene were significantly higher in fallow compared with wheat subplots, and were consistent with N₂O fluxes measured at the same time (period II). A sink for CH₄ was observed in all tillage

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treatments probably related to the low moisture content. No significant differences between fallow and wheat were observed in CH₄ fluxes. Based on the N₂O and CH₄ fluxes showed here, the fallow cycle within a crop rotation is not a good strategy to mitigate GHG emissions in rainfed agricultural systems. Hence, we propose a decrease in the frequency of fallow cycles within the crop rotation.

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**Conservation Agriculture practices reduce the global
warming potential of rainfed low N input semi-arid
agriculture**

1. Introduction

Agriculture contributes to 10-12% of the total global anthropogenic greenhouse gases (GHGs) (Stocker et al., 2013), through the release of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4). The global warming potential (GWP), which is a concept that integrates the radiative properties of all GHGs, expressed as CO_2 equivalents ($\text{CO}_2\text{-eq}$), is very dependent on N_2O emissions from agricultural crop systems. This gas, which is a by-product of microbial processes of nitrification and denitrification, (Firestone and Davidson, 1989), is released from soils after nitrogen (N) application (through fertilizers, manures or crop residues). By contrast, in aerated soils CH_4 uptake normally reduces GWP, because the amount of CH_4 oxidized by methanotrophic microorganisms is normally higher than the amount produced by methanogenic microorganisms (Chan and Parkin, 2001b). Additionally, agricultural practices that favour carbon (C) sequestration (Robertson et al., 2000) are also considered as valuable strategies to reduce the negative effect of GHG emissions associated with crop production. Therefore, agricultural management practices (e.g. tillage, fertilization and crop rotation) must integrate the reduction of soil GHG emissions and the increase of organic C content, while maintaining or enhancing crop yields to satisfy increasing global food demand.

Conservation agriculture, which involves crop rotations and reduced tillage (no tillage (NT) or minimum tillage (MT)), is currently common in Mediterranean climates due to its effects on preserving soil fertility and increasing the soil C sink (Kassam et al., 2012). These tillage practices often contribute to improve important abiotic parameters involved in the production and consumption of GHG from soils such as soil water content, aeration and soil organic C (SOC) (Martín-Lammerding et al., 2011; Plaza-Bonilla et al., 2014b; García-Marco et al., 2016) compared to conventional tillage (CT). However, contradictory results on N_2O and CH_4 fluxes have been reported (i.e. Pelster et al., 2011; Dendooven et al., 2012; Ball et al., 1999; Yonemura et al., 2014) due to interaction of tillage with several factors, e.g. soil type, climatic conditions (which determine the prevalence of nitrification or denitrification), nitrogen (N) fertilization rate, crop residues (type and management), and the duration of experiments (van Kessel et al., 2013).

The effect of crop rotations on GHG emissions is variable depending on rainfed/irrigated conditions, composition and management of previous crop residues (Malhi and Lemke, 2007), and mineral N remaining in soil from previous cropping phases. Cereal residues (high C:N ratio) can promote soil N immobilization when they are applied without an additional source of mineral N, consequently leading to a temporary reduction of N_2O fluxes (Huang et al., 2004). However, other authors (Sarkodie-Addo et al., 2003) have observed an enhancement of

denitrification losses when a mineral source is added together with high C:N ratio residues, providing an energy supply for denitrifying microorganisms. Addition of N fertiliser may also inhibit CH₄ uptake due to the interference of enzyme activity responsible for CH₄ oxidation (CH₄ monooxygenase) with NH₃ monooxygenase (Dunfield and Knowles, 1995), depending on N rate (Aronson and Helliker, 2010). Different quantities of crop residue are added to the soil under rotational wheat and wheat in monoculture systems, which can affect net N₂O and CH₄ production due to changes in soil C and N availability.

The influence of tillage and crop rotation on the stocks of SOC has been previously assessed, showing promising but contrasting results depending on management (e.g. type and duration of rotation) and experimental (e.g. depth, number of years since the beginning of the experiment) factors (Baker et al., 2007; Álvaro-Fuentes et al., 2014; Triberti et al., 2016). Thus, to identify whether conservation tillage practices (MT/NT and crop rotation) can mitigate both soil GHG emissions and net GWP is still unclear, particularly in semi-arid areas where the weight of direct N₂O losses is expected to be lower.

In rainfed semi-arid cropping systems, characterized by a high variability in the total amount and distribution of rainfall, low N input systems are being promoted in order to match N input to the expected N uptake by crops (Kimani et al., 2003), which may reduce the N surplus and also N losses (van Groenigen et al. 2010). Therefore, combining Conservation Agriculture practices with adjusted N-input is expected to provide an optimum balance between GWP and crop yields in semi-arid agro-ecosystems. In this context, the main objective of this study was to evaluate the effect of three long-term tillage systems (CT, MT and NT) and two cropping systems (wheat in monoculture and wheat in a 4-year rotation with fallow as preceding crop) on N₂O and CH₄ emissions over two campaigns. Additionally, crop yield, yield-scaled N₂O losses (YSNE) and GWP were evaluated. We hypothesized that: 1) considering climatic conditions of this experiment and the low N input, low N₂O emissions would be expected in all treatments; 2) emissions of N₂O and CH₄ in winter wheat in monoculture could be higher than in the rotational winter wheat, because of a combined effect of previous crop residues and N fertilizer application; and 3) NT would reduce net GWP as a result of the reduction of CO₂-eq emissions from farm operations and the increase of C stocks (Aguilera et al., 2013b).

2. Materials and methods

2.1. Site characteristics

A two-year study was carried out at “La Canaleja” Field Station (40° 32'N, 3° 20'W, 600 m), in Alcalá de Henares (Madrid, Spain), where a long-term tillage experiment began in

1994. Tillage systems and crop rotations including legumes and fallow have been assessed since that date. The soil was a sandy-loam *Calcic Haploxeralf* (Soil Survey Staff, 2010). The main physicochemical properties of the top soil layer (0-15 cm) were: sand, 50.8%; silt, 37.7%; clay, 11.5%; CaCO_3 , 41.6 g kg⁻¹; $\text{pH}_{\text{H}_2\text{O}}$, 7.9 and EC, 121.3 $\mu\text{S cm}^{-1}$. The site has a semiarid Mediterranean climate with dry summers. The 1994-2013 mean annual temperature and rainfall for this area were 13.5 °C and 402.7 mm, respectively.

2.2. Experimental design and management

The experiment was conducted from October 2011 to October 2013. The experimental design was a three-replicated split plot, divided into three main plots assigned to the three tillage systems (NT, MT and CT) in a randomized complete block design (Guardia et al., 2016a). Each of the main plots were further divided into five subplots (10 x 25 m) assigned in a completely randomized design to the phases of an annual crop rotation, involving fallow-wheat (*Triticum aestivum* L. var. Marius)–vetch (*Vicia sativa* L. var. Senda)-barley (*Hordeum vulgare* L. var. Kika), and also wheat in monoculture. In this study, we evaluated the effect of the three tillage systems mentioned above (tillage factor) and two cropping systems (cropping factor): wheat in rotation and wheat in monoculture; during two campaigns with different climatic (i.e. rainfall amount) and management conditions (i.e. rate of N fertilizer at dressing) (campaign factor): 2011/12 (campaign 1) and 2012/13 (campaign 2), resulting in eighteen subplots (3 plots x 2 subplots x 3 replicate blocks).

Moldboard (20 cm depth) and chisel ploughs (15 cm depth) were used in autumn (early-November 2011 and late-October 2012, for campaign 1 and 2, respectively) in CT and MT plots, respectively. Then, a cultivator pass was carried out for both tillage systems. Thus, crop residues were almost completely incorporated into the soil in CT, whereas under MT the previous season's crop residues covered approximately 30% of the plot surface. No tillage involved direct drilling and spraying with glyphosate (at a rate of 2 L ha⁻¹ of Sting Monsanto ®) for weed control, and previous season's crop residues were retained on the soil surface. Different types of crop residues were applied to the soil in the rotation treatment, depending on the phase of the rotation. Since wheat was preceded by fallow, the relatively small amount of biomass generated during that phase was left or incorporated into the soil surface of the following crop, winter wheat. By contrast, in wheat in monoculture, straw residue provided a greater N and C input (235 Mg C ha⁻¹; 20 kg N ha⁻¹) to the following crop of wheat. Rotational and monoculture wheat were sown on 26th November 2011 and 14th November 2012 in campaigns 1 and 2, respectively, with 210 kg seed ha⁻¹. Fertilizer was applied at seeding (16 kg N ha⁻¹ as NPK, 8-24-8) in both campaigns and at dressing as ammonium nitrate (NH_4NO_3 , 27-0-

0) on 22nd March 2012 and 11th March 2013. The N fertilization rate at dressing was calculated by taking into account the expected crop yield and soil mineral N content two weeks before fertilizer application (February). There was a higher than average nitrate (NO_3^- -N) content in the 0-15 cm soil at dressing fertilization in campaign 1 (27 mg NO_3^- -N kg^{-1}) than in campaign 2 (5.6 mg NO_3^- -N kg^{-1}), which resulted in different N rates in campaign 1 (11 kg N ha^{-1}) and 2 (54 kg N ha^{-1}). All treatments received post-emergency herbicide treatments (HerbimurDoble®) at a rate of 1.6 L ha^{-1} for both campaigns. Wheat was harvested on 10th June 2012 and 18th June 2013, for campaign 1 and 2, respectively.

2.3. GHG emissions sampling and analyzing

Fluxes of N_2O and CH_4 were measured from October 2011 to October 2013, using the static chamber technique as described in Guardia et al., (2016a). One chamber (diameter 35.6 cm, height 19.3 cm) was placed in each subplot and closed for 1 h. GHGs were sampled three times per week during the first and second week, then twice per week during the first month after fertilization events or during rainfall periods and then, every week or every two weeks until the end of the cropping period. After harvest, one gas sample was taken each month or after rainfall events. To minimize any effects of diurnal variation in emissions, samples were taken at the same time of day (10–12 am).

Gas samples (20 mL) were taken at 0, 30 and 60 min to test the linearity of gas accumulation in each chamber. Samples were analyzed by gas chromatography using a HP-6890 gas chromatograph equipped with a ^{63}Ni electron-capture detector (Micro-ECD) and a flame ionization detector (FID) to analyze N_2O and CH_4 , respectively. More details about the analysis and estimation of GHG fluxes can be found in Sanz-Cobena et al. (2014).

2.4. Soil, crop analyses and meteorological data

Composite soil samples were collected from each subplot at depths of 0-7.5 cm, 7.5-15 cm and 15-30 cm, air-dried and sieved to determine SOC (Nelson and Sommers, 1996). Bulk density was determined (Grossman and Reinsch, 2002) each year (Gómez-Paccard et al., 2015; Guardia et al., 2016a) for the same soil depths as SOC. In order to relate gaseous emissions to soil properties, soil samples were collected from 0-15 cm depths during the growing season on almost all gas-sampling occasions. Dissolved organic C (DOC) and soil mineral N concentrations (NH_4^+ -N and NO_3^- -N) were determined by extracting with deionized water or KCl (1M), respectively. Afterwards, DOC and mineral N contents were analyzed using a total organic C analyser (multi N/C 3100 Analytik Jena) or a flow injection analyzer (FIAS 400

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Perkin Elmer), respectively, as described in Guardia et al. (2016a). The water-filled pore space (WFPS) was determined and calculated as explained in Tellez-Rio et al. (2015a).

Hourly rainfall and air temperature data were obtained from a meteorological station located at the field site. Soil temperature was measured in each tillage system by inserting a temperature probe 15 cm into the soil. Mean hourly temperature data were stored on a data logger. Grain yield and above-ground biomass were measured by harvesting two randomly selected areas (0.5 x 0.5 m²) from each subplot and total N determined (TruMac CN Leco elemental analyzer).

2.5. YSNE, N surplus and GWP calculations

Yield-scaled N₂O emissions, expressed as g N₂O-N per kg of N uptake, were calculated based on the approach described by van Groenigen et al. (2010), using total above-ground N uptake (wheat grain and straw). The N surplus was calculated as N fertilizer application minus above-ground N uptake (van Groenigen et al., 2010). The sink of SOC (' Δ SOC') in the top 30 cm of soil and CO₂ emissions from fuel used in farm operations (e.g. tillage, herbicide and fertilizer application, seeding, harvest) and from manufacturing inputs (operation GHG emission + input GHG emission) were calculated as described by Guardia et al. (2016a). The ' Δ SOC' component, as an indicator of the soil C balance, was calculated taking the difference in SOC stocks between M-CT (as the baseline, since it represents the conventional and traditional management in comparison to the evaluated Conservation Agriculture practices) and the other treatments. To avoid the bias associated with changes in bulk density, the comparison of C stocks was made on a fixed soil mass basis, as described in Ellert and Bettany (1995). Default values of GHG emissions derived from farm operations and manufacturing inputs have been reported by West and Marland (2002), Lal (2004) and Snyder et al. (2009).

2.6. Statistical analysis

Statistical analyses were carried out with Statgraphics Plus 5.1. Analyses of variance (two-way ANOVA) were performed for almost all variables in the experiment for both campaigns (except climatic ones). A three-way ANOVA was also carried out in order to assess the effect of each campaign and the possible interactions among factors (campaign, tillage and crop). The normality and variance uniformity of data were assessed by the Shapiro-Wilk test and Levene's statistic, respectively, and log-transformed before analysis when necessary. Means were separated by Tukey's honest significance test at $P < 0.05$. For non-normally distributed data (mean soil NH₄⁺ content and YSNE in campaign 1 in the three-way ANOVA), the Kruskal–Wallis test was used on non-transformed data to evaluate differences at $P < 0.05$.

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Linear regression analyses were carried out to determine relationships between cumulative gas fluxes and soil parameters, with a 95% significance level.

3. Results

3.1. Environmental conditions, soil C and mineral N contents

Total rainfall was 194 mm in campaign 1 and 369 mm in campaign 2 (Fig. 5.1a), with a mean rainfall in the experimental area (1994-2013) of 365 mm. The soil WFPS values (Fig. 5.1b) in NT treatments were often higher than those in CT or MT. For NT the number of days with WFPS above 50% was 25-48 and 72-88 days in campaign 1 and 2, respectively; whereas those for CT were 10-15 and 25-35 days; and those for MT were 4-8 days in both campaigns.

Fig. 5.1 (a) Weekly mean soil temperature (°C) and rainfall (mm) and (b) evolution of soil water-filled pore space (WFPS, %) for the 0-15 cm top soil layer in the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) for rotational wheat (W) and wheat in monoculture (M), during both crop campaigns.

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Topsoil NH_4^+ content (Fig. 5.2a, b) peaked after each fertilization event. Average NH_4^+ values did not show significant differences between tillage and cropping systems, but were significantly smaller ($P < 0.05$) in campaign 1 than in campaign 2. The soil NO_3^- content in the topsoil (Fig. 5.2c, d) also increased after fertilization events in both campaigns, and no differences between cropping systems or tillage treatments were reported. Despite lower N application rates, the average NO_3^- content was higher in campaign 1 than in campaign 2 ($P < 0.05$).

Fig. 5.2 (a, b) NH_4^+ -N; (c, d) NO_3^- -N; and (e, f) DOC concentrations in the 0–15 cm soil layer during both crop campaigns for the different tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) treatments. Data are provided separately for rotational wheat (W, right) and wheat in monoculture (M, left) treatments. The arrows indicate the dates of application of synthetic N. Vertical lines indicate standard errors.

The DOC content of the topsoil (0–15 cm) is represented in Fig. 5.2e, f. The mean DOC content for NT (taking into account the whole crop period) was significantly higher than that for MT and CT (27 and 50% for campaign 1; 36 and 42% for campaign 2, respectively). No significant differences were found between cropping systems and campaigns. The SOC content

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in the upper soil layer was significantly increased after 17 years under NT, as opposed to MT and CT (Table 5.1). The highest SOC concentrations in the 15-30 cm layer were observed in CT ($P < 0.05$), but the SOC stock of the three soil layers (0-30 cm) was significantly higher in the NT treatment. With regards to the cropping effect, wheat in monoculture also tended to increase SOC content compared with rotational wheat ($0.05 < P < 0.10$).

Table 5.1 SOC content (g C kg^{-1}) in the 0-7.5, 7.5-15 and 15-30 cm soil layers, total SOC content (Mg C ha^{-1}) in the 0-30 cm depth of the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping system (rotational wheat, W, and monoculture wheat, M).

Depth (cm)	SOC (g kg^{-1})			Total SOC (Mg ha^{-1})
	0-7.5	7.5-15	15-30	0-30
Tillage	$P = 0.001$	$P = 0.516$	$P = 0.035$	$P = 0.033$
NT	11.2 b	5.8	4.6 a	28.8 b
MT	6.7 a	5.4	4.6 a	22.9 a
CT	5.7 a	5.4	5.3 b	22.9 a
S.E.	0.7	0.3	0.2	1.4
Crop	$P = 0.186$	$P = 0.008$	$P = 0.117$	$P = 0.070$
W	7.2	4.9 a	4.7	23.1
M	8.5	6.1 b	5.1	26.6
S.E.	0.6	0.2	0.1	1.1
Tillage x crop	$P = 0.713$	$P = 0.130$	$P = 0.098$	$P = 0.308$

Different letters within columns indicate significant differences by applying the Tukey's honest significance test at $P < 0.05$. Standard Error (S.E.) is given for each effect.

3.2. N_2O and CH_4 emissions

Nitrous oxide fluxes (Fig. 5.3) ranged from -0.18 to $0.46 \text{ mg N}_2\text{O -N m}^{-2} \text{ d}^{-1}$. Highest emissions were recorded after seeding and top-dressing fertilization, although negative N_2O fluxes were measured on several occasions. With regards to cumulative emissions (Table 5.2), significant interactions of the campaign factor with both tillage (significant at $P < 0.10$) and crop were reported. In campaign 1, cumulative N_2O emissions were significantly lower for NT than those for MT and CT, while no significant crop effect or tillage*crop interactions were found. In campaign 2, higher cumulative N_2O emissions ($P < 0.05$) were observed in the wheat in monoculture (with respect to rotational wheat), with no significant effect of tillage or the interaction of factors. Total cumulative N_2O fluxes were greater ($P < 0.05$) in campaign 2 than in campaign 1. The ratio of $\text{N}_2\text{O-N}$ emitted per unit of mineral N applied was significantly greater ($P < 0.05$) during campaign 1 (0.52%) than during campaign 2 (0.28%).

Fig. 5.3 Fluxes of $\text{N}_2\text{O-N}$ during both crop campaigns for the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping systems: (a) rotational wheat (W), and (b) wheat in monoculture (M). The arrows indicate the dates of application of synthetic N. Vertical lines indicate standard errors.

Methane emissions ranged from -1.32 to $0.46 \text{ mg CH}_4\text{-C m}^{-2} \text{d}^{-1}$ (data not shown). In both campaigns, net CH_4 oxidation (Table 5.2) was significantly lower in the wheat in monoculture than in rotational wheat, whereas no significant effect of tillage was reported ($P > 0.05$). In campaign 1, a significant and negative correlation was found between CH_4 fluxes and $\text{NH}_4^+\text{-N}$ content ($P < 0.05$, $n = 20$, $r = -0.52$). Methane emissions correlated with WFPS content in both campaigns ($P < 0.05$, $n = 20$, $r = 0.50$).

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Table 5.2 Total cumulative N₂O-N and CH₄-C fluxes, Grain-yield, Yield-scaled N₂O emissions (YSNE) and N surplus in the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping system (rotational wheat, W, and monoculture wheat, M), in campaign 1 and campaign 2, and during the two seasons of field experiment.

Effect	N ₂ O cumulative emission (g N ₂ O-N ha ⁻¹ yr ⁻¹)			Grain yield (kg grain ha ⁻¹)			CH ₄ cumulative emission (g CH ₄ -C ha ⁻¹ yr ⁻¹)			YSNE (g N ₂ O-N kg N up ⁻¹)			N surplus (Kg N ha ⁻¹)		
	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season	Camp. 1	Camp. 2	2-season
Tillage	*	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns	ns	ns
NT	72.0 a	181.6	130.3	2068	3233	2650	-670.6	-654.2	-662.4	1.7 a	3.4 b	2.5 a	21.6	-6.5	7.5
MT	158.1 b	191.5	174.9	1151	3241	2196	-694.8	-866	-780.7	5.3 b	3.1 ab	4.1 b	6.4	-4.4	1.0
CT	190.1 b	198.0	194.0	1530	3885	2708	-639.6	-708.5	-674.0	5.4 b	2.5 a	3.9 ab	12.0	13.2	12.6
S.E.	14.7	17.5	13.6	192	394	209	69.1	42.7	48.7	0.6	0.1	0.3	3.3	6.6	3.8
Crop	ns	*	ns	ns	ns	*	*	*	***	ns	**	*	ns	ns	ns
W	147.1	154.0 b	150.6	1832.5	3903.2	2868 b	-792.0 a	-919.9 a	-855.9 a	3.7	2.1 a	2.9 a	21.4	8.7	15.0
M	133.1	231.0 a	182.2	1333.6	3003.3	2169 a	-544.6 b	-566.2 b	-555.4 b	4.5	3.8 b	4.2 b	5.3	-7.2	-0.9
S.E.	28.0	18.0	12.5	240	322	171	62.6	82.7	29.7	0.9	0.3	0.4	4.9	7.2	4.0
Camp.			*			***			ns			*			*
1			140.1 a			1583 a			-668.4			4,1 b			13.3 b
2			192.7 b			3453 b			-743.1			3.0 a			0.8 a
S.E.			11.1			92			39.8			0.3			4.1
Till. x Crop	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Till. X Camp.			ns			*			ns			*			ns
Crop x Camp.			*			ns			ns			ns			ns

Different letters within columns indicate significant differences by applying the Tukey's honest significance test at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Standard Error (S.E.) is given for each effect.

3.3. Crop yield, YSNE and N surplus

Grain yield (Table 5.2) was significantly higher in campaign 2 than in 1 ($P < 0.001$). Crop yield for both campaigns (three-way ANOVA), showed a significant interaction ($P < 0.05$) between campaign and tillage: NT tended to increase (compared with CT) grain yield in the dry campaign 1 while the opposite tendency was observed in the normal campaign 2. On average, MT led to numerically (but not statistically) lower yields than NT and CT. Regarding cropping effect, grain yield in rotational wheat was significantly higher ($P < 0.05$) than that in wheat in monoculture.

In campaign 1, YSNE were significantly lower ($P < 0.05$) for NT than those for MT and CT (Table 5.2), whereas no significant differences were observed for the crop effect. Across both campaigns, MT and wheat in monoculture significantly increased YSNE as opposed to NT and rotational wheat, respectively. However, a significant interaction of tillage with the campaign factor was observed, since NT and CT were the tillage treatments with most mitigated YSNE in campaign 1 and 2, respectively. The values for N surplus were significantly lower ($P < 0.05$) in campaign 2 than campaign 1 (Table 5.2). There were no significant differences in N surplus values for the other effects (tillage, crop and interactions).

3.4. Global Warming Potential

The net GWP was significantly lower in NT than in MT and CT (Table 5.3). Wheat in monoculture tended to decrease the net GWP as a result of higher SOC contents, but differences were not statistically significant at 95% significance level. The GHG-GWP (soil N_2O and CH_4 fluxes) component was significantly affected by tillage and crop factors, since CT and wheat in monoculture significantly increased CO_2 -eq emissions compared with NT and rotational wheat, respectively. The GWP was higher during campaign 2 as a result of higher N fertilizer input (Fig. 5.4). The 'ΔSOC' component was generally lower in W than in the conventional M-CT, except under NT.

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Table 5.3 Estimated Global Warming Potential (GWP, kg CO₂ eq ha⁻¹ yr⁻¹) for the different tillage treatments (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping systems (rotational wheat, W, and monoculture wheat, M).

Global Warming Potential (GWP, kg CO ₂ eq ha ⁻¹ yr ⁻¹)			
Effect	GHG-GWP ^a	C sequestration ^b	Net GWP ^c
Tillage	<i>P</i> = 0.077	<i>P</i> = 0.008	<i>P</i> = 0.010
NT	58.6 a	-624.7 a	116.0 a
MT	89.8 ab	652.0 b	1444.8 b
CT	119.4 b	658.2 b	1546.7 b
S.E.	17.0	310.0	303.0
Crop	<i>P</i> = 0.009	<i>P</i> = 0.060	<i>P</i> = 0.078
W	58.4 a	600.8	1377.3
M	120.1 b	-143.8	694.4
S.E.	13.9	253.1	247.4
Campaign	<i>P</i> = 0.047	-	<i>P</i> = 0.189
1	67.5 a	-	806.1
2	111.0 b	-	1265.3
S.E.	13.9	-	247.4
Tillage x crop	<i>P</i> = 0.181	<i>P</i> = 0.332	<i>P</i> = 0.332
Tillage x campaign	<i>P</i> = 0.069	-	<i>P</i> = 0.988
Crop x campaign	<i>P</i> = 0.026	-	<i>P</i> = 0.883

Different letters within columns indicate significant differences by applying the Tukey's honest significance test at *P* < 0.05. Standard Error (S.E.) is given for each effect.

^a Sum of CO₂ equivalents from N₂O and CH₄ emissions, considering a 100-year horizon.

^b CO₂ equivalents from C sequestration, calculated taking the difference in SOC stocks between CT (as baseline) and the rest of tillage treatments, dividing it by the number of years since the experiment started (18) and considering the CO₂/C molar ratio.

^c Sum of CO₂ equivalents from N₂O and CH₄ emissions, C sequestration, operations and inputs.

Fig. 5.4 Relative contribution of each GHG source/sink to Net Global Warming Potential (GWP) in each tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping treatment (rotational wheat, W, and wheat in monoculture, M) during both crop campaigns.

Fig. 5.5 (a) Cumulative $\text{N}_2\text{O-N}$ emissions in campaign 1 and (b) campaign 2 for the different tillage (no tillage, NT, minimum tillage, MT, and conventional tillage, CT) and cropping (rotational wheat, W, and wheat in monoculture, M) treatments, from the beginning of the campaign to dressing fertilization (1st fertilization) and from dressing fertilization to the end of the campaign (2nd fertilization). Vertical lines indicate standard errors.

4. Discussion

4.1. Effect of campaign, tillage and crop systems on N_2O emissions

The main factor affecting N_2O emissions in this experiment was N input (from chemical fertilizer and crop residues), which was very dependent on the campaign and the soil moisture, which in turn was influenced by rainfall amount and distribution. In this context, N_2O fluxes were significantly higher in campaign 2 (with the highest N input and rainfall) (Table 5.2). Due

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to the complexity of factors and processes affecting the release of N₂O emissions, the effect of tillage and crop factors was not consistent throughout both campaigns, so that the interactions need to be analyzed in detail. Contrary to our hypothesis, tillage systems did not have any significant effect on N₂O emissions when the data from both campaigns are considered (Table 5.2). Our results were in agreement with those of Tellez-Rio et al. (2015a) and Guardia et al. (2016a) under similar climatic conditions. As observed for tillage, the crop effect did not influence N₂O emissions across the 2 campaigns. These results could be explained by the similar rates of synthetic N which was applied to both cropping systems, although a significant interaction (Table 5.2) with the campaign effect (i.e. higher N₂O losses in wheat in monoculture than in rotational wheat, but only in the second campaign) was observed. This interaction suggests that the effect of residues from previous crops can be comparable and even higher than that of synthetic fertilizers (Lenka and Lal, 2013), especially in calcareous soils and low N input semi-arid cropping systems. Additionally, the effect of tillage was not consistent in the two campaigns, since NT significantly reduced N₂O losses during campaign 1 but not during campaign 2 (normal precipitation and N input) compared with MT or CT. That caused the tillage*campaign interaction to be significant at 10% significance level.

The meta-analysis of van Kessel et al. (2013) reported a significant mitigation of N₂O emissions under NT in dry climates and long-term (> 10 years) studies. Lower emissions following long-term adoption of NT were explained as a result of the improvements of SOC content and porosity, thus reducing the formation of anaerobic microsites (Six et al., 2004). These lower emissions were generally observed under NT in our study for both campaigns in the rotational wheat system and also for monoculture wheat in campaign 1, supporting the results of van Kessel et al. (2013). Conversely, the results of monoculture in campaign 2 did not agree with this study, because relatively high N₂O fluxes, particularly after dressing fertilization were observed in the NT plots (Fig. 5.5). Therefore, we hypothesized that the influence of the climatic conditions (particularly rainfall) and tillage (incorporating/leaving the residue on surface) on the mineralization of previous crop residues (whose amount was different between cropping systems, as explained in section 2.2) drove the N₂O emission pattern in our experiment. In the case of rotational wheat, an important part of crop residue was presumably mineralized during fallow period (the previous year of rotational wheat growing phase), so N₂O fluxes may have been less dependent on the interaction of crop residue and mineral fertilizer than in continuous cropping of the winter wheat. However, in campaign 1, differences in N₂O emissions due to crop residue inputs were not observed between cropping systems. We hypothesized that the low rainfall amounts in campaign 1 limited soil water availability, particularly soil moisture content, which was not enough to promote intensive N mineralization and crop residues turnover, hence not stimulating N₂O production (Mutegi et al., 2010). The

number of days with a WFPS above 50%, which has been suggested as a threshold for highest N₂O losses (Linn and Doran, 1984; Li et al., 2016) was lower in campaign 1 (from 4 to 48 days) than in campaign 2 (from 7 to 88 days), depending on tillage system, supporting our findings.

By contrast, the N₂O emissions during campaign 2 were higher in wheat in monoculture than in rotational wheat. This effect could be a result of better environmental conditions for the mineralization of crop residues from the previous year (Chen et al., 2013). In monoculture wheat, a combination of residue inputs with a high C:N ratio (mean C:N ratio of 160.3) and mineral N fertilizer, both at seeding and dressing, may have stimulated denitrification losses from mineral N added to soil (Li et al., 2016), as residues provide an energy supply for denitrifying microorganisms (Sarkodie-Addo et al., 2003; Sanz-Cobena et al., 2014). This effect was particularly noticeable after dressing fertilization in the campaign 2, increasing fluxes in the monoculture wheat-NT treatment and changing the trend observed in the first campaign and the beginning of the second (Fig. 5.5). In summary, we hypothesized that the slower mineralization of non-incorporated wheat residues in NT (with respect to MT/CT) favored the N₂O release from the interaction of dressing synthetic N and the mineralization of wheat residues, during the season (spring) and the campaign (2, as opposed to the dry campaign 1) with more favorable conditions for mineralization (Abalos et al., 2013; Guardia et al., 2016a).

4.2. CH₄ emissions

In this long-term tillage study, cumulative emissions provided a net CH₄ sink in all tillage and cropping systems (Table 5.2), as generally reported in agricultural soils under semiarid conditions (Snyder et al., 2009). The negative correlation found between soil NH₄⁺ content and CH₄ fluxes ($P < 0.05$) in campaign 1 was in agreement with the meta-analysis of Aronson and Helliker (2010), which reported that low amounts of N (<100 kg ha⁻¹, as those of our experiment) tend to stimulate methanotrophy, while larger rates are inhibitory.

Tillage systems did not produce significant differences in CH₄ uptake in any campaign, which is consistent with results reported by Guardia et al. (2016a) and Tellez-Rio et al. (2015b), under semiarid Mediterranean conditions. However, some authors have suggested that the improvement of soil structure in NT, associated with increases in macroporosity and reduction of anaerobic microsites, can favor CH₄ consumption (Plaza-Bonilla et al., 2014b). Our results may have been a consequence of similar topsoil porosity in all tillage systems and the low soil moisture content maintained during campaign 1 (particularly) and 2.

Greater CH₄ oxidation ($P < 0.05$) was found in rotational wheat (preceded by fallow) than in wheat in monoculture in both campaigns. The incorporation of high C:N crop residues has been reported to increase CH₄ emissions (Le Mer and Roger, 2001), and that may have

partially offset the CH₄ oxidation in monoculture wheat subplots, where a higher amount of straw was retained/incorporated. This was also reported by Lenka and Lal (2013), who showed that CH₄ uptake capacity was increased in bare soil when compared to treatments with residue amendment.

4.3. Grain yield, YSNE and N surplus

Grain yield was affected by campaigns, which decreased almost 50% in the dry campaign 1 compared to campaign 2, due to the low rainfalls measured in campaign 1. The tillage*campaign interaction in wheat yields (see section 3.3) showed that the most productive tillage system was dependent on climate and management conditions (Table 5.2). Controversy still exists about crop yield declines in NT, but CT overall leads to higher crop yields in experiments with high water and nutrient availability (Chatskikh and Olesen, 2007), whereas in semiarid agro-ecosystems, the reductions in water evaporation and the enhancement of water availability and soil fertility achieved with NT adoption can result in higher yields (De Vita et al., 2007; Morell et al., 2011; Plaza-Bonilla et al., 2014b). These previous findings are in agreement with our results in the dry (<200 mm) campaign 1 and the normal (> 300 mm) campaign 2, and with the meta-analysis of van Kessel et al. (2013) or Pittelkow et al. (2015). Considering the average 2-campaigns data, MT resulted in numerically but not statistically lower yield than those of CT or NT. The increased weed pressure in this tillage system (Armengot et al., 2015) was also observed in our experimental site, and could explain this tendency. With regards to crop effect, wheat in monoculture significantly reduced grain yield compared with rotational wheat, especially in campaign 2. Our results confirm the positive effect of crop rotation on wheat yield under semi-arid conditions (López-Bellido and López-Bellido, 2001). Conversely, to elucidate the best management options for increasing farm returns, the whole rotation should be taken into account, since this type of rotation included a fallow (which may offset the increases in grain yields of the subsequent wheat phase) and a legume crop. In this sense, the possible cost abatement derived from incidences of pests (which are higher under continuous cropping of cereals) (Sanz-Cobena et al., 2017) or the payments that legumes are liable to receive due to new EU mandatory policy (e.g. crop diversification or surfaces of ecological interest) need to be considered, although are outside of the boundaries of this study.

The YSNE from our study were in the lowest range of values reported by van Groenningen et al. (2010), as previously found in semi-arid areas (Tellez-Rio et al., 2015b; Guardia et al., 2016a). Since grain yield was not high (compared with other wheat cropping areas), these low YSNE were a result of small N₂O losses, ranging from 0.07 to 0.23 kg N₂O ha⁻¹ yr⁻¹, compared to those (0.04-21.21 kg N₂O ha⁻¹) for European arable sites (Rees et al., 2013b).

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Besides the small N₂O emissions due to the low N fertilization rates, the low ratio of N₂O -N emitted per unit of mineral N applied (see section 3.2) confirms that the N₂O emission factors of rainfed semi-arid areas are much lower than the IPCC default value (Aguilera et al., 2013a; Cayuela et al., 2017). In this type of agro-ecosystem, N₂O emissions during winter are substantially limited by soil temperatures, while low WFPS is the main limiting factor for large N₂O losses during spring (when most growth of winter crops occurs). Additionally, low SOC contents (Ussiri and Lal, 2012) and high soil pH conditions (Baggs et al., 2010), as was the case for our experimental site, may have also contributed to low N₂O losses and YSNE. As a consequence, the mean values of N surplus (Table 5.2) were below the threshold (20-50 kg N ha⁻¹) of an exponential increase of YSNE (van Groenigen et al., 2010). Remarkably, N surplus was significantly higher in the first campaign, with the driest conditions, in spite of the lower rate of application of synthetic N. This would suggest that there was inefficient uptake of N uptake under water stress conditions, resulting in very low grain yields, without higher N₂O losses due to unfavorable soil WFPS, as explained above.

Our results highlight the importance of crop rotation as an effective YSNE mitigation strategy. Concerning the tillage*campaign interaction for YSNE, this was highly influenced by grain yields. Overall, NT significantly reduced YSNE as opposed to MT, which had no effect on area-scaled N₂O losses but was a less advantageous tillage management considering the YSNE ratio. In the campaign with less rainfall than the average, NT mitigated YSNE, as observed by van Kessel et al. (2013) for long-term studies under dry conditions, so it emerges as an interesting option in a global change context with increased aridity. In contrast, in normal rainfall campaigns CT arises as the most sustainable alternative for increasing grain yields while leading to similar N₂O losses as NT.

4.4. Global Warming Potential

Almost all treatments (except rotational wheat-NT) had positive GHG-GWP emission values (19-204 kg CO₂-eq ha⁻¹), showing that in spite of low N₂O fluxes, CH₄ oxidation did not offset N₂O losses (Fig. 5.4). As reported by previous studies (e.g. Aguilera et al., 2013b; 2015; Plaza-Bonilla et al., 2015; Abdalla et al., 2016), NT significantly increased the SOC content compared with CT (Table 5.3). This occurred despite the higher SOC content in the 15-30 cm layer in CT (as opposed to NT/MT), as suggested by Baker et al. (2007). The 'ΔSOC' was the main cause of the differences between tillage and crop treatments (Fig. 5.4), while CO₂-eq emissions from inputs and operations were significant but less important, a finding which is consistent with Aguilera et al. (2015) and Guardia et al. (2016a). Therefore, our results indicate that management practices which promote an increase in C stocks (e.g. NT) should be recommended in semi-arid areas. Supporting our findings, the recent meta-analysis of Abdalla

et al. (2016) pointed out that the abatement of CO₂-eq emissions through NT adoption is significantly higher in arid climates with low SOC content, as opposed to CT. Nitrous oxide (N₂O) emissions have shown to carry less weight in GWP estimates than in previous studies (Mosier et al., 2006; Adviento-Borbe et al., 2007), but uncertainties associated with SOC accumulation and its calculation (Guardia et al., 2016a) and the large climatic variability in rainfed semi-arid cropping areas, suggest that strategies that mitigate CO₂-eq from other GWP components (N₂O losses and inputs, e.g. by adjusting N rates) must be also considered.

Regarding the crop effect, wheat in rotation tended to decrease SOC accumulation and consequently to enhance GWP ($0.05 < P < 0.10$). Although the wheat phase of the rotation led to numerically higher CO₂-eq than wheat in monoculture, the widespread fallow-cereal-legume-cereal rotations provide further opportunities to mitigate the GWP during the legume (Guardia et al., 2016a) and fallow phases, when lower (or zero) fertilizer inputs are applied.

5. Conclusions

Our results showed that cumulative N₂O emissions and YSNE were low in this long-term experiment carried out under rainfed semiarid conditions with adjusted N inputs. Nitrous oxide emissions were increased in a year when rainfall was near normal in wheat in monoculture, as opposed to rotational wheat, and decreased in a drier than average year in NT, as opposed to MT/CT. Therefore, Conservation Agricultural practices (NT and rotation) resulted in similar or lower N₂O losses than conventional ones in this semi-arid low N input system. Grain yield and consequently YSNE were strongly affected by climatic variability, since NT and CT resulted in significantly higher productivities and lower YSNE in the dry and the normal campaigns, respectively. Wheat in rotation significantly mitigated YSNE, as opposed to wheat in monoculture. Higher SOC content caused NT to reduce Net GWP compared with the rest of tillage treatments. No-till should be recommended in semi-arid areas to mitigate the Net GWP of semi-arid agro-ecosystems, providing the opportunity to reduce YSNE in dry years and therefore in a global change scenario. By contrast, MT performed less well on the basis of YSNE and GWP balances. Wheat in rotation tended to increase Net GWP, but the abatement of YSNE and the opportunities for reducing input CO₂ emissions during other rotation phases (fallow and/or legume) may provide an optimum balance between grain yields and GHG mitigation. Due to the influence of soil and climatic variability, further studies in semi-arid areas are needed to confirm our results.

CHAPTER 6

Evaluation of the DNDC model for a cereal-legume rotation in conservation and conventional tillage under rainfed semiarid Mediterranean agroecosystem

1. Introduction

Agricultural practices, such as N fertilizer application and rates, tillage systems and crop rotation, are sources of greenhouse gas emissions to the atmosphere, in particular accounting for more than 80% of the anthropogenic N₂O emissions (IPCC, 2014). This potent greenhouse gas, N₂O, is the main responsible for the stratospheric ozone depletion, with a 100-y global potential c.300 times higher than carbon dioxide (Ravishankara et al., 2009). As management factors such as tillage, crop rotation and fertilization influence in the transformation of N in soil and can play an important role in the regulation and mitigation of N₂O emissions from agricultural soils (Snyder et al., 2009). Recently, European countries have set up bilateral agreement to reduce N₂O emissions from cultivated lands by 20% until 2020 (EU-Commission, 2014). Due to the harmful effect of N₂O, assessing the efficiency and sustainable of agricultural systems is crucial in order to fight against the threat of climate change on greenhouse gas emissions and at the same time meet the increasing demand for food and feed due to global population growing.

The dry Mediterranean basin regions are characterized by low organic matter content and scarcity of rainfall, irregularly distributed over the year (Kassam et al., 2012). These semiarid regions are widely used for rainfed crops, particularly cereal-legume rotation, and the adoption of conservation agriculture systems (Cantero-Martínez et al. 2007; Plaza-Bonilla et al., 2014a; Plaza-Bonilla et al., 2015). Compared to conventional systems, the use of conservation agriculture practices is an effective strategy to improve soil quality and fertility, increase soil organic carbon (SOC) stocks, reduce soil erosion, improve economic and environmental benefits and increase yields (Cantero-Martínez et al. 2007; Kassam et al., 2012). In the dry Mediterranean Europe, Spain spearheads the extension of adoption of conservation agriculture on 650,000 ha (FAO, 2011) and has doubled the surface area of cereal-forage rotation vetch rotation in the last four years (MAGRAMA, 2013). Conservation agriculture practices involve crop rotation and conservation tillage (No tillage (NT) and minimum tillage (MT)). The impact of tillage practices on soil N₂O emissions is highly variable depending on several factors such as: biophysical parameters, crop residue management and composition, soil texture and aeration (Rochette, 2008), the duration of tillage practices (Six et al., 2004) and climatic conditions (Van kessel et al., 2013). In fact, increases and decreases in N₂O emissions have been reported regarding the effect of NT and MT compared to conventional tillage (CT) (Plaza-Bonilla et al., 2014a, Guardia et al., 2016a). Moreover, the effect of crop rotation including legumes and the interaction effect between tillage and crop rotation on N₂O emissions under rainfed conditions is still poorly understood due to limited data availability. Previous studies have reported that N₂O fluxes from legume crops are associated to soil incorporation of legume residues and the N release from root exudates during growing season (Rochette and Janzen, 2005; Tellez-Rio et al., 2015a). Due to the limitation of field experiments, the use of the mechanistic models provide an

alternative method to identify N₂O mitigating practices while increasing or maintaining the crop yields, because they compare multiple agricultural management practices (Abalos et al., 2016).

Process-based biogeochemical models have proven to be useful tools to examine and better understand the processes involved in the C and N cycle in agricultural systems and processes involved in the production and consumption of GHG. One widely used mechanistic model is DeNitrification and DeComposition (DNDC), which was developed to predict and estimate greenhouse fluxes from agricultural soils (Li et al., 1992; Li, 2000). The DNDC model has been extensively tested and shown good agreement between measured and modelled data in many different ecosystems such as grassland, forest and cropland. The DNDC model has been tested for different field sites with contrasting tillage systems (Ludwig et al., 2011), straw incorporation (Cui et al., 2014) and crop rotation effect (Li et al., 2004). In some studies, the model has been applied successfully such as: Ludwig et al. (2011), Li et al. (2010) and Farahbakhshazad et al. (2008), whereas in other studies the use of DNDC model was less successful, for example: in Irish agriculture Abdalla et al. (2009) reported an underestimation of N₂O fluxes from reduced tillage in a sandy loam soil, due to an overestimation of the water filled pore space (WFPS) and the effect of SOC on the flux. However, the application of DNDC model in conservation and conventional tillage under semiarid Mediterranean conditions remains to be conducted for agricultural soils in Spain, in order to assess the impact of these agricultural practices on environmental N losses.

The objectives of this study were: i) Calibrate and validate the model for predicting N₂O emissions, yield, soil N content (NO₃⁻ and NH₄⁺) in two contrasting tillage systems (No tillage (NT) and conventional system (CT)) and in two phases of crop rotation (wheat (W) and vetch (V)) under semiarid conditions; ii) evaluate the best sustainable agricultural management practice in order to reduce N₂O emissions; iii) to assess the suitability of DNDC model to describe and predict the long term effects of tillage practices on crop yield, soil quality and N₂O emissions in a Mediterranean dryland farming.

2. Material and methods

2.1. Brief description of DNDC model

The DNDC model is a process-based simulation model which simulates C and N dynamics in agroecosystems and model version 9.5 (<http://www.dnrc.sr.unh.edu/>) was used in this study. The DNDC model consisted of two components incorporating six sub-models. Component 1 linked ecological drivers to soil environmental variables and consisted of the soil climate, crop growth and decomposition sub-models (Li et al., 1992). Component 2 linked soil environmental factors to trace gases and consisted of the sub-models: denitrification, nitrification and fermentation (Li et al., 2006).

Soil processes describing temperature, moisture, water flow, water uptake by plants, nitrification and denitrification are calculated on either a daily or hourly basis within the model, while DNDC outputs are provided on daily basis and annual reports (Congreves et al., 2016). The DNDC model can run at field or regional scales. In this study, the model was used in the field-scale mode. The main input parameters to run DNDC at field scale include: daily weather data (maximum and minimum temperature, precipitation, solar radiation and relative humidity), soil physical-chemical properties (texture, field capacity, wilting point, bulk density, pH, hydraulic conductivity, mineral N and organic carbon), and farming management practices (e.g. crop rotation, tillage, N fertilizer application details, planting and harvesting dates, tillage, irrigation, etc.). Model predictions include daily soil environmental factors (e.g., soil temperature and moisture) and gas fluxes (e.g., CO₂, N₂O) (Li, 2000). The differences in soil and climatic conditions that exist in performance of the DNDC model, reveals the necessity of model parameterization. Model validation against experimental data is a basic part of model development in order to prove model accuracy (Gilhepsy et al., 2014). A complete description and the last updates of the biogeochemical model DNDC can be found in the recently launched Global Research Alliance Modeling Platform (GRAMP), <http://gramp.org.uk/>.

2.2. Field experimental site and measurements

The dataset used in this study was obtained from three published studies done by Tellez-Rio et al. (2015a, 2015b, 2017) and Guardia et al. (2016a) in a long-term experiment carried out at “La Canaleja” Field Station (40° 32'N, 3° 20'W, 600 m), in Alcalá de Henares (Madrid, Spain) during 2011, 2012 and 2013. The site has a semiarid Mediterranean climate with dry summers and wet winters. Mean annual temperature and rainfall for this area were 13.5°C and 402.7 mm, respectively. According to the USDA soil taxonomy system (Soil Survey Staff, 2010), the soil is a loamy-sandy Calcic Haploxeralf. The main physicochemical properties of the top soil layer (0–15 cm) were: sand, 50.8%; silt, 37.7%; clay, 11.5%; CaCO₃, 41.6 g kg⁻¹; pH_{H2O}, 7.9 and EC, 121.3 mS cm⁻¹.

The experiment began in 1994 and was stabilised as split-plot design, divided into three main plots corresponding to the three tillage systems (NT, MT and CT) in a randomized complete three-block design. Each plot was further divided into five sub-plots (10 m x 25 m), assigned in completely randomized design to all phases of crop rotation involving fallow–wheat–vetch–barley and wheat in monoculture several crop rotations (cereal-legume-fallow) (Martin-Lammerding et al., 2011). From 2005, the annual crop rotation has been fallow–wheat (*Triticum aestivum* L. var. Marius)-vetch (*Vicia sativa* L. var. Senda)-barley (*Hordeum vulgare* L. var. Kika), for each tillage system (No tillage (NT) and conventional tillage (CT)). All phases of each rotation were present each year for all of the tillage treatment combinations and each rotation was cycled on its assigned plot.

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For this study, a three years dataset (October 2010 to September 2013) was evaluated for the tillage system (NT and CT) and for the following crop rotation:

- In 2010-2011: wheat and vetch (Tellez-Rio et al., 2015a, b);
- In 2011-2012: wheat (Tellez-Rio et al., 2017);
- In 2012-2013: wheat and vetch (Guardia et al, 2016a; Tellez-Rio et al., 2017).

Hourly rainfall and air temperature data were obtained from a meteorological station located at the field site. Soil ammonium (NH_4^+) and nitrate (NO_3^-) content were determined in each treatment-plot. Nitrous oxide emissions were measured in each plot using the closed chamber technique over the three years. The management practices such as the planting and harvesting dates, N fertilizer applications and tillage operations at Canaleja field site from 2011 to 2013 are described in Table 6.1. Further details of the measurements and experimental design of the Canaleja site can be found in Tellez-Rio et al. (2015a, b), Guardia et al. (2016a) and Tellez-Rio et al. (2017).

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Table 6.1 Details of the agricultural management practices for the campaigns: 2010-11, 2011-12, 2012-13 at Canaleja field site.

Agricultural practices	Campaign 2010-11				Campaign 2011-12			Campaign 2012-13		
	Fallow	Wheat	Vetch	Barley	Fallow	Wheat/ Barley	Vetch	Fallow	Wheat/ Barley	Vetch
Planting date	-	8 th Nov 10	8 th Nov 10	8 th Nov 10	-	26 th Nov. 11	26 th Nov. 11	-	14 th Nov. 12	15 th Nov. 12
Harvest date	-	16 th Jun 11	30 th May 11	25 th Jun 11	-	10 th Jun 12	1 th Jun 12	-	18 th Jun 13	12 th May 13
Tillage practices	NT: n.a. CT: 15 th Feb. 11	NT: n.a. CT: 29 th Oct. 11	NT: n.a. CT: 29 th Oct. 11	NT: n.a. CT: 29 th Oct. 11	NT: n.a. CT: 16 th Feb. 12	NT: n.a. CT: 8 th Nov. 11	NT: n.a. CT: 8 th Nov. 11	NT: n.a. CT: 18 th Feb. 13	NT: n.a. CT: 7 th Oct. 12	NT: n.a. CT: 7 th Oct. 12
Fertilizer application (kg N ha ⁻¹)	-	8 th Nov.10: 16 kg N/ha 20 th Mar. 11: 40 kg N/ha	-	8 th Nov.10: 16 kg N/ha 20 th Mar. 11: 40 kg N/ha	-	26 th Nov.11: 16 kg N/ha 22 th Mar. 12: 11 kg N/ha	-	-	14 th Nov.12: 16 kg N/ha 8 th Mar. 13: 54 kg N/ha	-

n.a.: not applicable.

2.3. Model calibration and validation

The model was calibrated using data of campaign 2010-11, by comparing the model output values with the measured input values of soil water content (SWC), soil NH_4^+ and NO_3^- content, crop yield and N_2O emissions. The input parameters were calibrated iteratively by testing a set of different values, ranging from ± 10 -25% the measured value. When each parameter was being calibrated, the other parameters were kept the same.

The calibrated parameters were:

1/ Adjustment of soil water dynamics using curve fitting: field capacity (FC), wilting point (WP), hydro-conductivity and porosity (calculated). For the NT system, optimum agreement was achieved by setting the WFPS at field capacity to 63% and the WFPS at wilting point to 20% and the hydro-conductivity to 0.1248 m h^{-1} . Whereas for the CT system, good agreement was achieved by setting the WFPS at field capacity to 60% and the WFPS at wilting point to 25% and the hydro-conductivity to 0.123 m h^{-1} (Table 6.2).

2/Crop yields: The calibration of crop yields, wheat and vetch, was achieved by adjusting and optimizing several combination of parameters: maximum biomass production, biomass fraction and the corresponding plant C:N ratios, thermal degree days and water demand (Table 6.3).

3/SOC: Using the cropping system history data for the plots of Canaleja Station, spin-up simulations were performed for stabilizing SOC content in both tillage system (NT and CT).

Table 6.2 Soil physico-chemical properties of Canaleja field site for the tillage systems: NT and CT.

Soil parameters	No tillage (NT)	Conventional tillage (CT)
Land-use type	Upland crop field	Upland crop field
Soil texture	Sandy loam	Sandy loam
Clay content (%)	0.119	0.119
Bulk density (g cm^{-3})	1.57	1.43
Soil pH	7.9	7.9
Field capacity (FC)	0.63	0.6
Wilting point (WP)	0.2	0.25
Hydrological conductivity (m hr^{-1})	0.1248	0.123
Porosity (%)	0.407	0.460
Initial soil organic C content (SOC) at surface soil (Kg C Kg^{-1})	0.00969	0.0065
Initial soil NO_3^- -N content (mg N kg^{-1})	6	6.6
Initial soil NH_4^+ -N content (mg N kg^{-1})	6.5	6

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Table 6.3 The calibrated crop parameters to the DNDC model at Canaleja field site.

Crop parameters	Winter wheat	Vetch (Hay legume)
Max. Biomass production (Kg C/ha) (grain)	2100	100
Biomass fraction (grain + leaf & stem + root)	0.5-0.21-0.21	0.01-0.4-0.4
Biomass C/N ratio (grain:leaf + stems: root)	35-140-140-140	15-15-15-30
Thermal degree days (TDD)	2000	2100
Water demand (g water g ⁻¹ DM)	140	200
N fixation index	1	2
Optimum temperature (°C)	22	21

Validation was done running the calibrated model for the wheat season (campaign 2011-2012) and for the vetch and wheat season (campaign 2012-13) in each tillage system (NT and CT), and comparing the model's outputs to the field measurements.

2.4. Model performance statistics

The performance of the measured and modelled values was undertaken following the methods defined in Ludwig et al., (2011) and Smith et al. (1997). Three deviation statistics were determined: the root mean square error (RMSE), model efficiency (EF), and relative error (E).

$$RMSE = \frac{100}{\bar{O}} \sqrt{\sum_{i=1}^n \frac{(Pi - Oi)^2}{n}}$$

$$EF = \frac{\sum_{i=1}^n (Oi - \bar{O})^2 - \sum_{i=1}^n (Pi - Oi)^2}{\sum_{i=1}^n (Oi - \bar{O})^2}$$

$$E = \frac{100}{n} \sum_{i=1}^n \frac{Oi - Pi}{Oi}$$

where O_i is the observed (measured) value, P_i is the predicted value, $\bar{O}$ is the mean of the observed data and n is the number of paired values. RMSE ranges from 0 to ∞ , EF from $-\infty$ to 1 and E from -1 to 1. For an ideal fit, RMSE and E equal zero and EF equals 1.

In addition to the degree of association between modeled and measured values was determined using the correlation coefficient (R) as described in Smith and Smith (2007).

3. Results and discussion

3.1. Evaluation of soil water content

The DNDC model calibration was evaluated using the climatic conditions for the campaign 2010-2011 (Fig. 6.1). In that campaign, mean air temperature ranged from -1.5°C to 30°C and total rainfall was 500 mm, which indicated a standard weather conditions for this Semiarid Mediterranean area. Whereas the weather conditions for the validation stages were particularly different: dry season for the campaign 2011-12 (total rainfall of 223 mm and mean temperature of 14.8 °C) and standard season for the campaign 2012-13 (total rainfall of 385 mm and mean temperature of 13.7 °C).

In general, the simulated soil water content (SWC) at 10 cm depth matched the measured values well for both NT and CT treatments (Fig. 6.2). For NT treatments (WNT and VNT), the RMSE, E and EF values were in general low (RMSE < 36 %, $-43 < E < -4.6$; and $-0.28 < EF < 0.73$) and the correlation coefficient values were relatively high ($R = 65-86\%$) (Table 6.4). For CT treatments (WCT and VCT), the RMSE, E and EF values were also low (RMS < 48 %, $-27 < E < -11$; and $EF < 0.55$) and the correlation coefficient values high ($R = 65-84\%$), Table 6.4.

Fig. 6.1 Climatic variables. Observed and modeled temperature (°C) and rainfall (mm) for: (a) the calibration stage (campaign 2010-11), and the validation stages (b) campaign 2011-12 and (c) campaign 2012-13.

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Table 6.4 Statistical analyses of the model performance simulating daily N₂O emissions, NO₃⁻ content and soil water content (SWC) for the calibration (campaign 2010-11) and validation stages (campaign 2011-12 and 2012-13), in the different treatments: wheat (WNT, WCT) and vetch (VNT and VCT).

	Daily N ₂ O emission			NO ₃ ⁻ content			SWC		
	Calibration	Val. 11-12	Val.12-13	Calibration	Val. 11-12	Val. 12-13	Calibration	Val. 11-12	Val. 12-13
WNT									
RMSE	599.02	491.14	651.74	73.09	8.09	70.12	18.78	8.17	18.90
R	-0.24	0.06	-0.29	0.50	0.84	0.65	0.76	0.79	0.86
E	314.27	-69.57	181.38	24.82	34.01	13.18	-4.60	-16.99	-10.12
EF	-6.27	-2.31	-5.76	-0.22	0.37	-0.01	0.54	0.50	0.73
WCT									
RMSE	646.03	237.13	453.92	77.54	64.74	75.68	21.42	48.72	25.51
R	0.03	-0.04	-0.45	0.64	0.72	0.70	0.78	0.69	0.84
E	309.72	-557.66	94.11	-7.79	5.23	-6.07	-11.78	-12.26	-27.89
EF	-3.91	-1.17	-2.09	0.22	-0.12	0.47	0.55	0.29	0.54
VNT									
RMSE	312.59		461.59	122.11		116.82	22.27		36.36
R	0.06	-	0.02	0.57	-	0.46	0.78	-	0.65
E	76.07		121.02	98.54		98.10	-28.71		-43.18
EF	-0.03		-0.08	-1.78		-2.17	0.46		-0.28
VCT									
RMSE	419.84		465.74	134.55		108.46	23.21		23.02
R	-0.05	-	-0.17	0.69	-	0.38	0.76	-	0.76
E	105.94		95.86	97.74		97.10	-14.84		-16.67
EF	-0.13		-0.14	-0.49		-3.37	0.51		0.47

RMSE = root mean square error; R = correlation coefficient; E = relative error; EF= Model efficiency.

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Analyzing the different periods in each campaign for NT and CT treatment, we observed that DNDC model overestimated slightly the simulated SWC compared to the measured data (Fig. 6.2), in calibration and validation stages. However, underestimation of SWC was observed for dry and hot periods for the calibration and validation stages. For example, the highest overestimation between observed and modelled SWC were detected in WCT and VNT plots from the May to June 2013 (Fig. 6.2 h, i), whereas underestimation was also obtained for season 2011-12 in WCT. In this last case, an underestimation of transpiration rather than evaporation (Molina-Herrera et al., 2016) probably occurred. Inaccuracies in the estimation of potential evapotranspiration (ET) were attributed to the Thornthwaite approach used by DNDC model (Ludwig et al., 2011), which based on average monthly temperature to calculate ET and this approach is less accurate than Penman-Monteith (Guo et al., 2007).

The deviations between model and measured SWC were also observed in other studies. Recently, He et al., (2017) reported that the DNDC model overestimated the SWC for NT and CT treatments in a clay loam soil during dry conditions, due to the incapacity of DNDC model to transport preferential water flow into the soil profile through clay cracks (Uzoma et al., 2015). Also, Abdalla et al. (2009) reported an overestimation of SWC for an Irish grassland sandy loam soil, under reduced and conventional tillage systems, which could affect to soil N turnover rate, limiting the contribution of nitrification and increasing of denitrification process.

CALIBRATION: CAMPAIGN 2010-11

Fig. 6.2 Measured and model SWC (%) in the different treatments: NT (WNT and VNT) and CT (WCT and VCT), during: (a-d) the calibration stage (campaign 2010-11).

VALIDATION: CAMPAIGN 2011-12 and 2012-13

Fig. 6.2 Measured and model SWC (%) in the different treatments: NT (WNT and VNT) and CT (WCT and VCT), during: the validation stages: (e-f) campaign 2011-12 and (g-j) campaign 2012-13.

3.2. Evaluation of crop yield

For the calibration stage (campaign 2010-11), the measured crop yields were covered well by the DNDC model simulated crop yields in NT (WNT and VNT) and CT treatments (WCT and VCT) (Table 6.5). The DNDC model underestimated the wheat grain yield for WNT (16%) and WCT (21%) treatments, while the vetch biomass yield was slightly overestimated for VNT (5%) and VCT (3.5%) treatments (Table 6.5). Statistical analysis of the yield performance showed in general low values: $RMSE < 38\%$, $-20 < E < 20$, $-30 < EF < 0.01$, for both NT (WNT and VNT) and CT (VNT and VCT)

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treatments. These results indicated nearly fair performance between measured and simulated crop yields.

For the validation stage of the campaign 2011-12, the simulated wheat crop yields were in good agreement with the measured wheat crop yield for the WNT and WCT treatments. The yield agreement showed the following statistics: $RMS < 36\%$, $E < 8$; and $-0.04 < EF < -0.26$, which indicated that the grain yield were reasonably well predicted with an average underestimation of 19.8% for WNT and 8.7% for WCT.

Conversely, for the validation stage of the campaign 2012-13, differences between the simulated and measured crop yield became larger in the wheat than in vetch crop (Table 6.5). The low values of RMSE ($< 15.7\%$), $E (> -15)$ and $EF (> -8.2)$ for VNT and VCT treatments indicated good agreement between simulated and measured vetch crop yields (Table 6.5). For wheat grain yields in this campaign, the model underestimated the crop yield by 40 % (WNT) and 45% (WCT). In spite of the underestimation model values of wheat grain yield for this campaign, the DNDC model predicted wheat grain yields within the range of values measured in the same field experimental site “La Canaleja”: 738-1278 kg C/ha for WNT and 958.5-1305 kg C/ha for WCT (Martin-Lammerding et al., 2013). In another field experiment nearby Canaleja field site (2 km) using a similar soil, Sanchez – Girón et al. (2004) reported mean values of wheat grain yields ranging from 1364-1426 kg C/ha in a loam soil. These values seem to indicate a good performance between model and measured wheat grain yields for the Canaleja field site.

Overall, the DNDC model was able to detect yearly variations and differences in crop yields between tillage treatments for both wheat and vetch crops during the calibration (campaign 2010-11) and validation stages (campaign 2011-12 and campaign 2012-13) due to the changes of temperature, rainfalls and N fertilization. For example: the modelled vetch crop yield was higher for VCT (5210 kg C/ ha) than VNT (4814 kg C/ ha) from 2010-11, whereas for the campaign 2012-13 the modelled vetch crop yield was higher for VNT (4237 kg C/ha) than VCT (3586 kg C/ha) (Table 6.5). In the case of wheat, the modelled grain yields were higher for WNT (929-1285 kg C/ha) than WCT (794-1127 kg C/ha) for the years 2011-13 (Table 6.5).

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Table 6.5 Statistical evaluation between simulated and measured (average $\pm$ standard error) crop yields for the calibration and validation stages under no tillage (NT) and conventional tillage (CT).

Crop	Treatment	Campaign	Measured yield (kg C/ha)	Simulated yield (kg C/ha)	RMSE	E	EF	Relative deviation (%)
Calibration								
Wheat	NT	2010-2011	1531 (65.1)	1285	22.95	13.32	-0.96	-16.07
Vetch	NT	2010-2011	4571 (227.0)	4814	38.6	-20.79	-0.02	5.31
Wheat	CT	2010-2011	1431 (9.4)	1127	21.37	21.17	-30.13	-21.22
Vetch	CT	2010-2011	5031 (228.1)	5210	35.08	-15.65	-0.01	3.56
Validation								
Wheat	NT	2011-2012	1158 (94.0)	929	36.99	8.85	-0.40	-19.81
Wheat	CT	2011-2012	869 (39.1)	794	19.39	6.01	-0.26	-8.76
Wheat	NT	2012-2013	1796 (142.9)	1072	47.45	36.64	-2.60	-40.33
Vetch	NT	2012-2013	3689 (61.0)	4237	15.73	-15.15	-8.16	14.84
Wheat	CT	2012-2013	1776 (72.9)	970.8	47.15	44.38	-12.35	-45.35
Vetch	CT	2012-2013	3315 (98.2)	3586	9.8	-8.49	-2.27	8.16

RMSE = root mean square error; E = relative error; EF= Model efficiency.

3.3. Evaluation of N mineral and N₂O emissions

Overall, the DNDC model performed poorly soil NH₄⁺ concentrations compared to observed data for all NT and CT treatments (data not shown). For the vetch plots, the model predicted low soil NH₄⁺ concentrations (< 0.5 kg N/ ha) compared to the measured values of NH₄⁺ content (< 4 kg N/ha), and there were not differences in measured soil NH₄⁺ content between tillage systems (Tellez-Rio et al., 2015a, b). For the wheat plots, simulated soil NH₄⁺ content were also underestimated with values lower than 1 kg N/ha and the DNDC model was not able to capture peaks of NH₄⁺ after fertilization events during autumn-winter and spring for WNT and WCT treatments (data not shown).

On the contrary, soil NO₃⁻ contents were relatively well predicted by the DNDC model, particularly for WNT and WCT, being detected a higher accuracy for WNT and WCT (RMSE < 77%, R = 0.5-0.84 %, -7 < E < 34 and EF < 0.5) compared to VNT and VCT (RMSE < 134%, R = 0.38-0.69%, E < 98, EF < -3.37), Table 6.4. For the wheat plots, the model was able to capture temporal variations of soil NO₃⁻ content after fertilizer applications (Fig. 6.3). The ability of the DNDC model to simulate the magnitude and temporal dynamic of soil NO₃⁻ contents was already been reported by Molina-Herrera et al. (2016) and Chirinda et al., (2011). For the vetch plots, the model was not able to detect soil NO₃⁻ content higher than 6 kg N/ha for VNT and VCT. In this case, the field measured NO₃⁻ values were below 10 kg N/ha for VCT and 25 kg N/ha for VNT (Fig. 6.3).

The deviations in soil N mineral (NH₄⁺ and NO₃⁻) content observed between measured and modelled data under NT and CT treatments have already been reported in other studies such as He et al. (2017) and Abdalla et al. (2014), which explained that the poor DNDC model performance of soil mineral N was due to the flaws in soil water balance module (a simple cascade approach) that could affect the water and nutrient upward flow and downward infiltration, especially under dry soil conditions where preferential flow through cracks can occur after rainfall.

CALIBRATION: CAMPAIGN 2010-2011

Fig. 6.3 Measured and model soil NO_3^- ($\text{kg NO}_3^- \text{-N ha}^{-1} \text{ d}^{-1}$) content in the different treatments: NT (WNT and VNT) and CT (WCT and VCT), during: (a-d) the calibration stage (campaign 2010-11).

VALIDATION: CAMPAIGN 2011-12 and 2012-13

Fig. 6.3 Measured and model soil NO_3^- ($\text{kg NO}_3^- \text{-N ha}^{-1} \text{ d}^{-1}$) content in the different treatments: NT (WNT and VNT) and CT (WCT and VCT), during: validation stages (e-f) campaign 2011-12 and (g-j) campaign 2012-13.

Generally, measured daily N_2O emissions were predicted with fair agreement for NT and CT treatments, simulated and measured N_2O emissions were increased after fertilizer application and following high rainfall events (Fig. 6.4). For the wheat plots, the highest N_2O peak emissions were well detected by the DNDC model after fertilizer application in both tillage systems (WNT and WCT) due to mainly an increase in the soil NO_3^- content detected by DNDC model, but the magnitude of these simulated pulses of N_2O emissions were overestimated by the DNDC model. For example: in the WCT plots simulated emission peak of N_2O was $0.05 \text{ Kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ compared to measured peak of N_2O ($0.002 \text{ Kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) by the end of March 2011; and for the case of WNT plots:

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simulated peak of N_2O ($0.017 \text{ Kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) and measured peak of N_2O ($0.0020 \text{ Kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) at mid-November 2010, (Fig. 6.4). For WNT and WCT treatments, simulated soil N_2O emissions returned to baseline emissions ($<0.0007 \text{ kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) 3-4 days or 4-7 days after firsts and second fertiliser applications, respectively (Fig. 6.4).

For the vetch plots, the DNDC model captured pulse emissions of N_2O after rainfall events in VNT and VCT, but the magnitude of these pulses were also slightly overestimated by the DNDC model, i.e.: observed pulse = $0.0015 \text{ kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$, simulated pulse = $0.0035 \text{ kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ in the VCT plots at the end of April 2011. The main factor responsible of these N_2O pulses was the SWC, which boosted from 23% to 63% due to heavy rainfall events (55 mm and 47 mm) characteristic of Mediterranean-climate areas. After those pulses, simulated N_2O emissions returned to the baseline emission ($0.0005 \text{ kg N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) in VNT and VCT plots (Fig. 6.4) in the campaign 2010-11.

The DNDC model was not able to simulate negative N_2O fluxes that were measured in the field experiment regardless of tillage system (WNT, VNT, WCT and VCT) due to low N input for the wheat plots, low available N mineral and WFPS content ($<58 \%$), in where the soil acted as a sink for N_2O emissions as described Abalos et al. (2013) and Tellez-Rio et al. (2015a). This shortcoming of the DNDC model was also reported by Abdalla et al. (2009), for a sandy loam soil under different tillage system, which may limit the good performance between model and measured cumulative N_2O emissions.

Although, DNDC estimated the timing of the main increases in soil daily N_2O emissions reasonably well, the statistics indicated a poor performance for simulating soil N_2O fluxes (Table 6.4), with high values of RMSE (RMSE $< 646 \%$ and RMSE < 465 , for the wheat and vetch plots, respectively), and low correlation coefficient ($R < 1\%$) for wheat and vetch plots. The poor performance of daily N_2O emissions, which was mainly caused by the poorly estimation of fluxes magnitude and frequent negative fluxes during several periods (Tellez-Rio et al., 2015a, b), was also observed by Bowman et al., (2010), who reported a low correlation between measured and simulated N_2O fluxes at daily scale in arable soils.

Simulated cumulative N_2O emissions (Table 6.6) ranged from 79.5 to 218.6 g $\text{N}_2\text{O-N ha}^{-1}$ and from 42.1 to 186.2 g $\text{N}_2\text{O-N ha}^{-1}$ for NT and CT treatments, respectively. As we expected, these predicted cumulative N_2O emissions were in the low range of N_2O emissions reported in other field experiments, such as: Jensen et al. (2012) for legume crops ($30\text{--}7090 \text{ g N}_2\text{O-N ha}^{-1}$) and Garcia-Marco et al. (2016) for cereal crop ($244\text{--}773 \text{ g N}_2\text{O-N ha}^{-1}$) in NT and CT systems. These predicted values overestimated N_2O fluxes measured using close chambers in these experiments, but especially

for wheat in campaign2010-11, for NT and CT. As commented previously, the simulated peaks promoted by fertilization events were poorly predicted by the model (Fig. 6.4).

CALIBRATION: CAMPAIGN 2010-11

Fig. 6. 4 Measured and model daily N_2O fluxes ($Kg\ N_2O-N\ ha^{-1}\ d^{-1}$) in the different treatments: NT (WNT and VNT) and CT (WCT and VCT), during: (a-d) the calibration stage (campaign 2010-11).

VALIDATION: CAMPAIGN 2011-12 and 2012-13

Fig. 6. 4 Measured and model daily N_2O fluxes ($Kg\ N_2O-N\ ha^{-1}\ d^{-1}$) in the different treatments: NT (WNT and VNT) and CT (WCT and VCT), during: validation stages (e-f) campaign 2011-12 and (g-j) campaign 2012-13.

In general, DNDC model predicted higher cumulative N_2O emissions for NT than CT treatments, whereas the observed data indicated contrasting results (Table 6.6), probably attributed to the overestimation of the magnitude of modeled daily N_2O fluxes in NT and CT, which warrant further model improvement. The overestimation between measured and model cumulative N_2O emissions could be due to a higher simulated SWC contents than observed values (Table 6.4), which may favour denitrification process, result in a increase in N_2O fluxes. This overestimation of cumulative N_2O emissions was higher than $100\ g\ N_2O-N\ ha^{-1}$ in most of wheat treatments independent on the campaign. Similar overestimations of the effects of cumulative N_2O emissions by DNDC have

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been reported by Abdalla et al. (2010), due to the model (DNDC) is very sensitive to soil organic carbon content (SOC) (Li et al., 1996, Beheydt et al., 2007). In this sense, Abdalla et al. (2009) reported a 20% increase in SOC corresponds to a 58% increase in N₂O flux.

Underestimation of predicted cumulative N₂O emissions (Table 6.6) were mainly observed in the vetch plots, i.e.: in VNT in the campaign 2010-11 (relative deviation = -21.6%), VNT and VCT in the campaign 2012-13 (relative deviation = -49.9% and -25.3%, respectively). In this case, underestimation of soil NO₃⁻ content together with overestimation of SWC occurred. Under these conditions, DNDC model probably overestimated the consumption of N₂O by denitrifiers due to this consumption normally happens when SWC and pH are high and soil NO₃⁻ content is low (Guardia et al., 2016a). This underestimation was also observed by Abdalla et al., (2012) in spring barley under reduced tillage and CT. Molina-Herrera et al. (2016) has pointed out that the differences between model and measured N₂O fluxes can be also explained by a large variability of physical and chemical properties which affect to soil N₂O emissions. The soil heterogeneity together with the often reduced number of measured field data (chambers) should be taken into account when comparing model and measured fluxes, because the predicted fluxes represent the mean of soil properties for a particular field study.

Overall, the performance between model and measured cumulative N₂O emissions suggest that the model for semiarid conditions could present some limitations in accounting for tillage management effects on soil N mineral, SWC, the availability of soil N and crop N uptake, and as a consequence DNDC predicted a poor performance of daily N₂O emissions. Thus, we suggest that further efforts are necessary to improve the parameterization for DNDC model under semiarid Mediterranean agroecosystems, in order to obtain a better performance between measured and model N₂O emissions. This target could make the model a more valuable and helpful tool to overcome some of the limitations of field studies and test mitigation options for reducing reactive N losses to the environment while sustaining or increasing crop yields. Additionally, we suggest that testing these datasets in other mechanistic models, such as DayCent (Alvaro-Fuentes et al., 2017) or CENTURY (Parton and Rasmussen, 1994), could provide better estimations of N₂O emission, particularly when assessing low rate of N₂O emissions as reported in this study. In this line, Alvaro-Fuentes et al. (2017) has successfully used the model Daycent to predict N₂O emissions and simulate future impacts of climate change on N₂O emissions in different land uses under typical Mediterranean conditions similar to our field experiment..

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Table 6.6 Measured (mean value± standard error) and model cumulative N₂O-N emissions (g N₂O-N ha⁻¹) for the different treatments: wheat (WNT and WCT) and vetch (VNT and VCT) during the calibration (campaign 2010-11) and validation stages (campaign 2011-12 and campaign 2012-13).

Season /treatment	Cumulative N ₂ O-N emission (g N ₂ O-N ha ⁻¹)			Relative deviation (%)
	Observed	Model	Difference	
2010 - 11				
WNT	49.80± 2.74	209.85	160.05	321.39
WCT	28.15± 2.95	148.58	120.43	427.81
VNT	51.10± 1.20	79.35	28.25	55.27
VCT	53.70± 1.41	42.09	-11.61	-21.62
2011-12				
WNT	74.90± 0.58	108.62	33.72	45.02
WCT	178.00± 0.38	89.74	-88.26	-49.59
2012-13				
WNT	123.50± 2.88	218.59	95.09	76.99
WCT	166.50± 1.04	174.22	7.72	4.64
VNT	191.10± 3.49	95.72	-95.38	-49.91
VCT	249.30± 2.46	186.23	-63.07	-25.30

4. Conclusions

Calibration and validation of DNDC model in rainfed Mediterranean agro-ecosystems showed a fair agreement for the SWC for both NT and CT treatments and it was able to capture yearly variations in crop yields between tillage treatments for both wheat and vetch crops, due to the changes of temperature, rainfalls and N fertilization. The soil NH₄⁺ content was poorly detected by the DNDC model for all NT and CT treatments, whereas soil NO₃⁻ contents were relatively well predicted by the DNDC model, being detected with a higher accuracy in wheat (WNT and WCT) than in vetch (VNT and VCT). The DNDC model reproduced the main pulses of N₂O emissions, after fertilizer application and following high rainfall events for NT and CT treatments, but the magnitude of these pulses were slightly overestimated by the DNDC. Overall, the DNDC model overestimated the daily N₂O fluxes for all treatments (WNT, WCT, VNT and VCT) which produced deviations between model and measured cumulative N₂O emissions for NT and CT treatments. The study demonstrated the potential of the model to predict yields and N₂O emissions and at the same time allowed to evaluate agricultural management options for N₂O mitigation in order to promote sustainable farming system. However, further investigations should be carried out to tackle the detected limitations to simulate soil N mineral and the magnitude of N₂O emissions under two contrasting tillage system.

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General discussion

The agriculture conservation systems in Mediterranean agro-ecosystems have been promoted in the last decades due to the potential benefits on the increase in soil organic C sink, reduce soil erosion and conserve more soil water availability. But it is necessary to consider other factors such as: agronomic productivity and environmental sustainability, in order to recommend the best combination of agricultural management practices and to achieve two challenges in the future: increase the food production to meet the increase in world population and reduce the environmental pollution.

To date, the effects of conservation agriculture practices (including tillage systems, crop rotations and its interaction) on GHG emissions are not consistent and controversy on this matter remains today. Thus, this thesis constitutes an overview of the effect of long-term agricultural conservation practices on GHG emissions under semiarid Mediterranean agro-ecosystems. Understanding and evaluating the process responsible of GHG emissions, studying the relationship between N_2O emissions and crop productivity through yield scaled N_2O emissions and calculate GHG-GWP balance between all possible GHG sources and sinks are crucial to adopt and recommend the optimal strategy for GHG mitigation in rainfed semiarid agro-ecosystems..

1. Effect of crop rotation on N_2O emissions

The crop rotation in semiarid Mediterranean agroecosystems usually involved cereal, legumes and fallow. The impact of crop rotation on N_2O emissions is attributed mainly to the following factors: N fertilization rates, type and management of crop residue from the previous crop in rotation and the interaction effect of tillage with the previous crop residues. But in our experiments, *what was more important: the effect of crop residue (presence or not of crop residues) from the previous crop or the effect on N mineral fertilization applied to the crops?*

1.1. Presence or not of crop residues in the previous crop

This effect could be analyzed for a same crop (wheat) analyzing rotational wheat (W), where the previous year was fallow, and monoculture wheat (M), where wheat straw (high C:N ratio) was applied into the soil the previous year (Chapter 5).

We found that rotational wheat (W) reduced significantly N_2O losses compared to wheat in monoculture (M) during the campaign 2012-13. This interaction can be explained by the differences in mineralization of previous crop residues. In W plots, an important part of crop residues was mineralized during fallow period, thus the N_2O losses were less dependent of the interaction of crop residue and mineral N fertilizer. However, higher N_2O losses in M were

caused by the interaction of dressing mineral N fertilizer and the mineralization of wheat residues (high C:N ratio), which favored more N₂O fluxes in M than in W. In agreement with Guardia et al. (2016a) and Sarkodie et al. (2003), the interaction of tillage with previous crop residues can explain those results. The application of high C:N residues in soils with low C content can stimulate N₂O losses driven by denitrification process. This effect was very dependent on the soil moisture and rainfall amount and distribution in each campaign. In fact, this finding was not observed in the dry campaign 2011-12 (rainfall = 194 mm). The drought experienced during that campaign, kept the soil dry for a large period of the year not promoting the mineralization of crop residues in M. Therefore, total N₂O emissions were similar in M and W for this campaign 2011-12.

1.2. Importance of N input

The application of mineral N fertilizer and organic N from residues of previous crop contributed to N₂O emission during crop period. But, what was most important: N organic source or mineral N input? This effect could be analyzed through the results of Chapter 4, where N₂O emissions in fallow (F) plots (in which barley crop residues were applied to the soil) were compared to a rotational wheat crop (W), without adding crop residues but with the application of 56 kg N ha⁻¹ as mineral fertilizer, during campaign 2010-11. We did not find differences between F and W on the annual total N₂O losses, but when fluxes were calculated in different periods according to fertilization application, differences in N₂O fluxes arised. In this sense, we noticed that in II period (covered from March to harvest) F increased N₂O emissions compared to W even after N fertilizer application. The application of barley crop residues from the previous season in F plots and its subsequent slow mineralization (Mosier et al., 2006) together with a WFPS above 60% may have favored denitrification process, producing higher N₂O losses in F than in W. Whereas, in W plots for this same II period, we found that N fertilizer input was quickly uptake by the crop, as NO₃⁻ content decreased below 10 mg NO₃⁻-N kg⁻¹, therefore, denitrification was not favored, which explained the low N₂O fluxes found in W. In this sense, Aguilera et al. (2013a) reported also that fallow periods can release greater amount of N compound responsible of indirect and direct N₂O losses. However, from an agronomic point of view, fallow periods increase soil moisture and soil mineral N content, which can be useful for the following crop and thus reducing the amount of N fertilizer for the subsequent crop. Based on N₂O fluxes during the fallow period, we recommend a reduction in the fallow periods and replacing fallows periods by legumes crops, especially in agro-systems with low availability in soil N.

In chapter 3, wheat residues were mineralized during a vetch crop, where mineral N was not applied. We found that the vetch crop produced higher N₂O losses particularly in MT than

wheat crop in NT, MT and CT, during the campaign 2010-11, confirming that mineralization of crop residues had an important effect on N₂O emissions.

2. Effect of Conservation tillage on GHG emissions under Mediterranean conditions

Overall, most of N₂O fluxes measured in this thesis were within the range of N₂O fluxes described in similar studies performed in semiarid Mediterranean agro-ecosystems (Aguilera et al. 2013a; Sanz-Cobena et al., 2014; Cayuela et al., 2017), which were low by adjusting fertilizer N rate to soil N conditions. The impact of tillage systems on N₂O was variable depending on: the campaign and the complex interaction between crop effect and management of crop residues (Table 7.1). Generally, our results showed no differences on N₂O emissions between NT/MT and CT during the campaigns 2010-11 and 2012-13, in agreement with those reported by Plaza-Bonilla et al. (2014a) and Guardia et al. (2016a,b).

However, when we analyzed the N₂O fluxes by crop and in each campaign, the differences in N₂O fluxes emerged and we found two exceptions that differed with these general results. Firstly, we found that MT in a vetch crop (preceded by wheat crop) increased significantly N₂O losses compared to NT and CT in the campaign 2010-11 (in Chapter 3). Those higher N₂O losses in MT were explained by the different N₂O flux patterns observed among tillage systems during the growth period of vetch, mainly in II period (early and full flowering stages and beginning of senescence, from January to mid-April) and III period (which included pod setting and grain filling to harvest (from the end of April to the beginning of June). In these two periods, soil conditions favored denitrification and nitrification process, which were responsible of soil N transformations and N₂O losses (Li and Lang, 2014). In this line, some studies have reported that the increase in N₂O fluxes at the end of vetch crop was produced as a consequence of low plant N uptake together with the increase in N soil associated to the decaying nodules and roots in this last stage of vetch growth (Yang and Cai, 2005), susceptible to be denitrified. Whereas other studies reported that the increase in N₂O losses was associated to denitrification process of sources such as: N rhizodeposition by legume roots (Wichern et al. 2008) and mineralization of labile legume N released from nodulated roots (Rochette and Janzen 2005).

Second exception, we found that NT system in wheat rotational (W) and in wheat in monoculture (M) mitigated significantly N₂O emissions compared to MT and CT during the dry campaign 2011-12 (rainfall < 200 mm) and with low fertilizer N input (27 kg N ha⁻¹), in Chapter 5, supporting the findings of the meta-analysis of Van kessel et al. (2013). Under long-term NT and dry climate, the literature indicate that the decrease in N₂O emissions in long-term NT was explained as a result of the improvements of SOC contents and porosity, leading to a reduction

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in anaerobic microsites (Six et al., 2004). Therefore, we hypothesized that the low rainfall limited the N mineralization and the crop residue turnover, as a consequence not encouraging N₂O production (Mutegei et al., 2010).

Based on these results, *do the conservation agricultural practices mitigate N₂O losses in rainfed Mediterranean agro-ecosystems?*

The lack of effect of conservation tillage on N₂O fluxes underscores the need to compare these practices with other GHG fluxes or with the GHG-GWP, as suggested by Guardia et al. (2016a). Regarding the GHG-GWP, almost all treatments (except rotational wheat -MT in campaign 2010-11 and rotational wheat -NT in campaign 2011-12) had a positive GHG-GWP emissions, revealing that in spite of low measured N₂O emissions, CH₄ uptake did not offset N₂O losses. Overall, CH₄ uptake was not affected by tillage practices in none of the campaigns, in agreement with Snyder et al. (2009) in agricultural soil under semiarid conditions, and showed low impact on CO₂-eq emissions, as observed by Guardia et al. (2016a). In addition to, GHG-GWP values were similar between tillage systems (Table 7.1), except in the campaign 2011-12, when NT in W and in M showed significantly low values of GHG-GWP compared to MT and CT, and in the campaign 2012-13, in which NT in W resulted in lower GHG-GWP than MT and CT. Therefore, considering the evaluation of GHG-GWP during several campaigns in different crops did not show a clear effect about what type of tillage system can mitigate global GHG emissions.

However, if we consider other factors such as the C sequestration (Δ SOC content) and net GWP, differences between tillage systems appeared as we reported in Chapter 5. No tillage system increased significantly C sequestration, which caused a significant decrease in Net-GWP in comparison with other tillage systems (MT and CT), as CO₂-eq emissions for inputs and operations in NT system were lowest than MT/CT and less important due to a reduction in the number of labour operations. This finding was in agreement with Guardia et al. (2016a) and Aguilera et al. (2015). Results from a recent meta-analysis (Abdalla et al., 2015) concluded that NT system significantly reduced CO₂-eq emissions and increased soil C stock particularly under arid and sandy soil conditions with low SOC content, supporting our findings. In spite of low weight of N₂O emissions in net-GWP and the uncertainties associated to the calculation of C sequestration (Guardia et al. 2016a), long-term NT system can be considered as a good strategy to mitigate CO₂-eq emissions and increase the generally low SOC content of Mediterranean soils compared to MT and CT systems in rainfed semiarid agro-ecosystems. In addition to, crop rotations that include legumes and fallow period are strategies that can also reduce net GWP potential because of the lower CO₂-eq emission from farm operations and inputs compared to continuous cropping of winter cereals, as already pointed out by Mosier et al. (2005) and

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Guardia et al. (2016a). Therefore, the combination of NT and crop rotation, including fallow and legumes, should be implemented due to the mitigation potential of CO₂-equivalent emissions and increase the soil C sequestration in Mediterranean agroecosystems.

Regarding the C sequestration, currently, there is a discussion in the literature about the importance of N in promoting additional C sequestration, because of the 0.4% targets of recent initiatives introduced in the last COP21 conference in Paris (<http://4p1000.org>). According to van Groeningen et al. (2017), the 4p1000 initiative is not feasible because the potential of SOC sequestration will depend on the availability of nutrients, particularly N, as soil organic matter contains C and N, with an average C/N ratio of 12 (Batjes, 1996). Additionally, it is not clear what will be the origin of the N (i.e.: N surpluses, N from crop residues, increase in N fertilizer rates to increase biomass production) by using it to sequester C in the soil (van Groeningen et al., 2017). An increase in N input could lead some risk of increasing N₂O emissions. So, we speculate that there may be a trade-off in providing additional N inputs between N₂O emissions and C sequestration. As N₂O emissions are very low in our study, could an increase in N input in NT systems promote a higher SOC sequestration without negative trade-offs concerning N₂O emissions under Mediterranean conditions? Future research should be focused on that matter in order to mitigate GHG emissions from agricultural soils.

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Table 7.1 Overview of total N₂O emissions and GHG-GWP in the different experiments of this Thesis, and the effect on tillage systems in each one.

CAMPAIGN	CROP	N ₂ O fluxes (g N ₂ O-N ha ⁻¹)			Differences between tillage systems?	GHG-GWP (kg CO ₂ eq ha ⁻¹ yr ⁻¹)			Differences between tillage systems?
		NT	MT	CT		NT	MT	CT	
2010-2011 Rainfall = 512mm Fertilization N rate = 16+ 40 kg N/ha (Chapter 3 and 4)	Fallow (F)	30.70	94.40	113.29	NO; <i>P</i> = 0.05	29.67	76.25	97.91	NO; <i>P</i> > 0.05
	Wheat (W)	46.2	26.5	25.6	NO; <i>P</i> > 0.05	18.96	-8.36	14.20	NO; <i>P</i> > 0.05
	Vecth (V)	51.1b	124.2a	53.7 b	YES; <i>P</i> = 0.045	35.49	71.93	28.45	NO; <i>P</i> > 0.05
2011-2012 Rainfall = 194 mm Fertilization N rate = 16 + 11 kg N/ha (Chapter 5)	Rotational wheat (W)	73.1a	191.7b	176.5b	YES; <i>P</i> = 0.025	-17.10a	96.53b	105.33b	YES; <i>P</i> = 0.031
	Wheat Monoculture (M)	70.9a	124.7b	203.8b	YES; <i>P</i> = 0.033	19.31a	68.22ab	132.81b	YES, <i>P</i> =0.042
2012-2013 Rainfall = 369 mm Fertilization N rate = 16 + 54 kg N/ha (Chapter 5)	Rotational wheat (W)	122.2	174.9	165.0	NO; <i>P</i> >0.05	27.93a	71.71b	66.02b	YES; <i>P</i> = 0.044
	Wheat Monoculture (M)	255.0	208.2	231.0	NO; <i>P</i> >0.05	204.29	122.59	173.29	NO; <i>P</i> > 0.05

Different letters within row indicate significant differences by applying the Least Significant Difference (LSD) test at *P* < 0.05.

3. Denitrifier abundance and activity in rainfed conservation agro-ecosystems

Conservation tillage systems (NT/MT) produce important changes on soil chemical N and C cycling, especially on denitrification process and the corresponding populations. Recently, amplification of functional bacterial genes represents a potential method to evaluate denitrifier abundance and their influence on N₂O emissions due to denitrification process in soils with different tillage management practices. Our results were focused on nitrite reductase, key enzyme regulating denitrification process through the reduction soluble NO₂⁻ into gas and encoded by *nirK* and *nirS* genes (Zumft, 1997).

In chapter 3 and 4, we found that the abundance of *nirK* gene was higher than *nirS* gene abundance, which was below the detection limit ($< 8 \times 10^{-3}$ gene copies g⁻¹ dry soil) in all tillage systems as reported by Baudoin et al. (2009). *NirK* density was within the range of 1×10^4 – 2×10^8 gene copies g⁻¹ dry soil, as previously observed by Henry et al. (2004) and Dandie et al. (2008). The response of denitrification capacity and denitrifier densities (through by *nirK* gene abundance) was variable depending on the interaction of tillage with crop rotation.

Denitrification capacity (N₂O+N₂) and *nirK* gene abundance was similar between tillage systems in fallow (F) and wheat in rotation (W). However, we found a significantly greater abundance of *nirK* gene in F than in W plots, which may explain the higher N₂O fluxes measured in F than in W at that time, concurrent with an increase of WFPS (>60 %) and NO₃⁻ content. For the same date, mean value of *nirK* densities in vetch crop was 1.75×10^7 gene copies g⁻¹ dry soil. We observed higher *nirK* gene abundance in F and V than in W at that critical period. Given these results, we speculated that the abundance of denitrifier might be less competitive in fertilized soils than in non fertilized soils (fallow or vetch). In this sense, Wallenstein (2004) and Wolsing and Prieme (2004) reported a higher denitrifier abundance in unfertilized plots than in fertilized plots, which were associated with changes in soil C availability, moisture and pH. As *nirK* is a member of multi-copper oxidase metalloprotein family (Cu-Nir), Cu content availability could be a decisive factor in this soil regulating denitrifier abundance. Taken into account that plants and soil microorganisms competed by soil micronutrients (i.e. Cu), the effect of plants and especially the interaction with other nutrients (such as N) could have an effect on this availability and denitrifiers population. However, in our experiment this hypothesis have not been validated because the total soil Cu content was similar for all plots, independent on tillage system and crop.

Tillage system affected to denitrification capacity and *nirK* gene abundance in vetch (Chapter 3), but not in fallow and wheat (Chapter 4). In this line, MT for vetch resulted in a notable higher denitrification capacity (N₂O+N₂) and *nirK* gene abundance compared with NT

and CT practices, suggesting better soil conditions for denitrifying bacteria under MT at a crucial time (spring). Although, there was not a clear cause that explained this enhancement in MT in vetch crop, the high density of denitrifiers in MT may justify the notable higher total N_2O emissions found in MT than in NT and CT.

These results should be considered with caution as a quality picture of soil denitrifier activity and abundance at a critical period (spring), because PCR approaches provide information on potential activity not real activity of denitrifier population (Philipot and Hallin, 2005). Furthermore, the relative abundance of denitrifier can change over time depending on climatic, soil and plant conditions (Wolsing and Prieme, 2004), additional soil samples through the crop and intercrop period should be analyzed in order to reach more robust conclusions.

4. Crop yield and Yield-scaled N_2O emissions in conservation tillage systems

Due to the concern of the decline in crop yields associated with the conservation tillage (NT/MT), studies that include crop yields and yield-scaled N_2O emissions result in good indicators of N_2O efficiency of cropping systems from both agronomical and environmental perspective.

In general, crop yields were very dependent on climatic conditions in each campaign (Table 7.2). In this sense, we observed that the mean grain yields in rotational wheat were: 3082 kg ha^{-1} (rainfall= 512 mm, campaign 2010-11), 1832 kg ha^{-1} (rainfall= 194 mm, campaign 2011-12), 3903 kg ha^{-1} (rainfall= 369 mm, campaign 2012-13). As in this semiarid Mediterranean area the last 10-year mean rainfall was 402 mm, the recent drought experienced during the campaign 2011-12 could influence on the grain yields in these agro-ecosystem.

Tillage practices also affected to crop yields, but they were dependent on the cropping systems and the climatic conditions. In Chapter 3 and 4, crop yield (grain yield or biomass yield) in wheat and vetch crop was not affected by tillage practices during the campaign 2010-2011. These results may be explained because that campaign was a standard year in terms of rainfall, particularly during the critical period of crop growing cycle (flowering and grain filling period at spring), which could have masked differences in soil water content and soil water use efficiency (Guardia et al., 2016a).

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Table 7.2 Summary of crop yields and Yield-scaled N₂O emissions in the different experiments of this Thesis.

CAMPAIGN	CROP	Grain yield (kg ha ⁻¹)/ Biomass yield (kg dry matter ha ⁻¹)			Differences between tillage systems?	YS-N ₂ O emissions (g N ₂ O/kg N above-ground N uptake)			Differences between tillage systems?
		NT	MT	CT		NT	MT	CT	
2010-2011	Rotational wheat (W)	3402	2666	3179	NO; <i>P</i> = 0.05	0.9	0.6	0.6	NO; <i>P</i> = 0.05
	Vetch (V)	9117	9053	10053	NO; <i>P</i> = 0.05	0.1b	0.4a	0.2b	YES; <i>P</i> = 0.029
2011-2012	Rotational wheat (W)	2314	1396	1787	NO; <i>P</i> = 0.05	1.3b	5.8a	4.0a	YES; <i>P</i> = 0.03
	Wheat Monoculture (M)	1822	905	1273	NO; <i>P</i> = 0.05	2.1b	4.7a	6.8a	YES; <i>P</i> = 0.041
2012-13	Rotational wheat (W)	3992	37670	3948	NO; <i>P</i> = 0.05	1.8	2.3	2.2	NO; <i>P</i> = 0.05
	Wheat Monoculture (M)	2474	2713	3822	NO; <i>P</i> = 0.05	4.9a	3.8ab	2.9b	YES; <i>P</i> = 0.018

Different letters within row indicate significant differences by applying the Least Significant Difference (LSD) test at *P* < 0.05.

Chapter 7

In Chapter 5, grain yield in W and M was significantly affected by the climatic conditions, especially rainfall amount. A reduction of almost 50% of grain yield was observed in the dry campaign 2011-12 compared to the normal campaign 2012-13 (> 350 mm), due to low rainfalls measured in that campaign (< 200 mm). Regarding of tillage effect, NT resulted in similar grain yields as CT in the two campaigns. Whereas MT reduced numerically grain yield in almost all crops (W and M) in both campaigns, probably attributed to weed pressure in MT, as previously reported by Armengot et al. (2015) and Guardia et al. (2016a). In addition to, we found a significant interaction between tillage and campaign (rainfall) in wheat yields: NT tended to increase the grain yields in the dry campaign while the opposite tendency was observed in the normal campaign 12/13. These findings were in agreement with the tendency reported in the literature, CT overall enhances greater crop yield in experiments with high water and nutrient availability (Chatskikh and Olesen, 2007), whereas in semiarid conditions, long-term NT may result in higher yield due to the decrease in water evaporations and the enhancement in water availability and soil fertility (De vita et al., 2007; Morell et al., 2011; Plaza-Bonilla et al., 2014a). Those results observed in all campaigns were in agreement with Van Kessel et al. (2013) and Pittelkow et al. (2015), no significant differences in crop yield were found between NT/MT and CT systems under rainfed conditions similar to those of our experiment (long-term tillage systems (> 10 years), dry climate, crop rotation with retention of crop residue and low N input).

Regarding of crop effect, wheat in monoculture decreased mean grain yield compared to wheat in rotation and averaged yield-scaled N_2O emissions were significantly higher in M than in W in the two campaigns (2011-12 and 12-13), Chapter 5, which underscores the beneficial effects of crop rotations in agronomic productivity compared to continuous cropping systems under semiarid conditions (Lopez-Bellido and Lopez –Bellido, 2001). However, in order to evaluate the best management options, it is necessary to take into account the whole rotation, which usually includes a fallow (which may increases the grain yields of the subsequent wheat phase) and a legume crop. In this sense, the possible cost abatement derived from incidences of pests (which are higher under continuous cropping of cereals) (Sanz-Cobena et al., 2016) or the greening payments that legumes are liable to receive due to new EU mandatory policy (e.g. crop diversification or surfaces of ecological interest) should be considered (Sanz-Cobena et al., 2017), although are outside of the boundaries of this thesis.

Yield-scaled N_2O emissions (YSNE) ranged between $0.6 - 6.85 \text{ g N}_2\text{O-N kg}^{-1} \text{ N uptake}$ for all tillage treatments (Table 7.2). Those results were in the lowest range of values for low ($< 100 \text{ kg N ha}^{-1}$) or even zero N input systems, reported by Van Groenigen et al. (2010), but in agreement with Guardia et al. (2016a) and Cai et al. (2013). These low YSNE values were

mainly attributed to the small N_2O fluxes ($0.026\text{--}0.255 \text{ kg N}_2\text{O-N ha}^{-1}$) due to low N fertilization rates compared to those ($0.04\text{--}21.21 \text{ kg N}_2\text{O-N ha}^{-1}$) for European arable sites (Rees et al., 2013b). Overall, we found that NT can mitigate YSNE in dry years ($< 200 \text{ mm}$) as previously reported by Van Kessel et al (2013), who found a significant reduction in YSNE in dry climates and long term NT tillage. Whereas in a normal year (rainfall $> 300 \text{ mm}$), YSNE in legumes and cereals in rotation were similar between NT and CT systems, in agreement with Guardia et al. (2016a). In contrast, CT in wheat in monoculture in a normal rainfall campaign (2012-13) resulted in a significantly lower YSNE compared to NT/MT systems, being the most sustainable alternative for increasing yields and leading to similar N_2O losses as NT.

5. Can the N_2O losses be predicted by the DNDC Model in semiarid rainfed conservation?

Process based models such as Denitrification and Decomposition model (DNDC) are powerful tools that help us to understand and predict the interaction effect of tillage systems and crop rotations on N_2O emissions and crop yield. Although, the national methodology is in accordance with Tier 1 and Tier 2 approaches established by IPCC (IPCC, 2006), still empirical emissions factors are used in order to evaluate the emissions from fertilized soils in Mediterranean agro-ecosystems (Lugato et al., 2010; Aguilera et al., 2013a; Sanz-Cobena, et al., 2016). Greenhouse data from models and inventory measurement systems are required by Tier 3 approach, but this approach is not available so far in Spain. Therefore, it is necessary to test the usefulness of DNDC for predicting N_2O emissions and improving the national GHG inventory in Spain toward Tier 3 approach.

Calibration and validation of DNDC model for NT and CT systems in rainfed Mediterranean crops showed that the model could be an effective tool to simulate soil water content (SWC) and crop yield in wheat and vetch. Overall, the DNDC model for NT and CT treatments overestimated slightly the simulated SWC compared to the measured data, both during calibration and validation stages. Probably, those inaccuracies were attributed to the estimation of potential evapotranspiration (ET) following Thornthwaite approach, which is based on average monthly temperature to calculate ET, and this approach is less accurate than Penman-Monteith (Guo et al., 2007). DNDC model was able to detect yearly variations and differences in crop yields between tillage treatments for both wheat and vetch crops due to the changes of temperature, rainfalls and N fertilization.

DNDC model poorly performed soil NH_4^+ concentrations for both NT and CT treatments. Soil NO_3^- contents were relatively well predicted particularly for wheat in NT and CT, whereas soil NO_3^- contents in vetch were underestimated in NT and CT. These inaccuracies in soil N mineral content were previously observed by He et al. (2017) and Abdalla et al.

(2014). They explained that the poor DNDC model performance of soil mineral N was due to the flaws in soil water balance module (a simple cascade approach) that could affect the water and nutrient upward flow and downward infiltration, especially under dry soil conditions where preferential flow through cracks can occur after rainfall when soil was dry.

Although, DNDC predicted the timing of the main increases in soil daily N₂O emissions reasonably well, after fertilizer application and following high rainfall events, the statistics indicated a poor performance of daily N₂O emissions for NT and CT treatments. That effect was mainly caused by the poor estimation of fluxes magnitude and frequent negative fluxes in several periods under NT and CT treatments. In this line, Bowman et al. (2010) also observed a low correlation between measured and simulated N₂O fluxes at daily scale in arable soils.

Overall, the DNDC model overestimated the daily N₂O fluxes for all treatments (WNT, WCT, VNT and VCT) which produced deviations between model and measured cumulative N₂O emissions in all treatments, in agreement with Beheydt et al. (2007) and Abdalla et al. (2010). These results suggested that more efforts are needed to improve the parameterization of DNDC model under semiarid Mediterranean agro-ecosystems in order to overcome the limitations of field studies and testing alternatives for decreasing N₂O losses while keeping or increasing the crop yields.

6. Future research

During our research we found a number of knowledge gaps that should be addressed by future field research.

In Chapter 3, we observed an increase in N₂O emissions at the end of vetch growth period, we speculated that effect was associated to the denitrification of sources such as N rhizodeposition by legume roots (Wichern et al. 2008) or mineralization of labile legume N released from nodulated roots (Rochette and Janzen 2005). Therefore, studies in laboratory and additional field measurement in different campaigns or in other legumes crop should be performance in order to obtain solid conclusions.

Additionally, the effect of nitric oxide (NO) in conservation agriculture should be assessed due to the environmental concern of this gas and the lack of studies focused on this potent N gas. Because NO is a highly reactive constituent of the troposphere and impacts human health and crop productivity (Skiba et al., 1997), and also contributes through its oxidation to the formation of acid rain (Pilegaard, 2013).

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Regarding the denitrifier gene abundance and activity, changes in the denitrifier community abundance due to tillage management and seasonal variations (Prieme and Wolsing, 2004) during crop and intercropping period should be assessed in order to complement the results of chapter 3 and 4. In addition to, analyses of different populations of denitrifying bacteria (i.e. *narG*, *napA*, *norB*, *norZ* and *nosZ*) encoding other steps of denitrification process can provide a better understanding of the relationship between activity and diversity of denitrifiers communities and can help to predict N losses on soils. Also, quantification and evaluation of nitrifiers population (i.e. *amoA*) can be useful as this nitrification process seems to play an important role in the N₂O emissions under semiarid Mediterranean agroecosystems.

Given that the concern of climate change and food security, the recent COP21 conference in Paris has launched an initiative of “4 per 1000” which aims at yearly 0.4% increase in the quantity of SOC contained in agricultural soils (<http://4p1000.org>). This initiative highlights that more research is necessary to identify and implement mitigation strategies and possible barriers to comply with that goal.

Due to IPCC default EF and TIER 1 approach generally overestimates the N₂O emissions in Mediterranean areas (Cayuela et al. 2017), the use of different mechanistic models, such as: DayCent (Alvaro-Fuentes et al. (2017), CENTURY (Parton and Rasmussen, 1994), APSIM (Vogeler et al., 2013) and DNDC (Gilhepsy et al., 2014), can provide reliable estimations of GHG emissions and improve the implementation of GHG national inventory in order to obtain accurate EFs for each regions. An important effort by modelers needs to be done in these areas through the calibration and validation models, specially DNDC and DayCent, in order to obtain a robust model for Spain, which could predict climate change impact on C and N dynamics, and, in turn, on N₂O emissions, and recommend the best agricultural management practices for mitigating those emissions. Future funding through national or international projects focusing on this topic are essential to achieve this goal.

CHAPTER 8

Conclusions

Based on the research objectives described in Chapter 2, the following conclusions can be drawn:

1. Emissions of N_2O in long-term conservation tillage systems (NT/MT) were overall similar to CT in a fallow-cereal-legume rotation under rainfed Mediterranean semiarid conditions. The lack of effect of conservation tillage on N_2O fluxes underscores the need to compare these practices with other GHG fluxes (i.e.: CH_4 oxidation, SOC stocks, inputs and farm operations) to recommend the best management practices.
2. Methane uptake was not affected by long-term tillage practices in agricultural soil under semiarid Mediterranean conditions. However, greater CH_4 oxidation was found in rotational wheat than in wheat in monoculture.
3. Tillage systems (NT, MT and CT) overall showed a positive GHG-GWP emission, revealing that in spite of low measured N_2O emissions, CH_4 uptake did not offset N_2O losses. Therefore, the evaluation of GHG-GWP during several campaigns in the different crops did not show a clear effect about what type of tillage system can mitigate global GHG emissions.
4. No tillage systems increased the SOC sequestration and reduced net GWP compared to CT. Therefore, the combination of NT systems and crop rotations, including cereal-legume crops, can be a good strategy to reduce $\text{CO}_2\text{-eq}$ emissions compared to MT and CT systems in rainfed semiarid agro-ecosystems. In addition to, NT can mitigate YSNE in dry years (rainfall < 200 mm).
5. Rotational wheat reduced N_2O losses and increased grain yield compared to wheat in monoculture and, in turn, produced an abatement of YSNE. Thus, the crop rotations including legumes could be considered as a good strategy for decreasing N_2O emissions and increasing CH_4 uptake and crop yield, together with the agronomic benefits associated to crop rotation (i.e.: reduction of weed population and use of pesticides, improvement of soil quality, decrease the use in N fertilizer through increasing N input by legumes N fixation).
6. Tillage systems affected to denitrification capacity ($\text{N}_2\text{O}+\text{N}_2$) and *nirK* gene abundance in vetch crop, as opposed to fallow and wheat, at a crucial time (spring). However, greater *nirK* density together with favourable conditions for denitrification was observed in fallow than in rotational wheat plots, which may explain the higher N_2O fluxes measured in fallow than in wheat at that time (spring). These findings highlight the usefulness and importance of combining molecular-based approaches with measurements of N_2O production in order to

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complement and understand microbial N transformations associated with agricultural management practices.

7. Overall, crop yields (rotational wheat, wheat in monoculture and vetch) were not affected by tillage systems under semiarid Mediterranean conditions. Low YSNE ($<7 \text{ g N}_2\text{O-N kg}^{-1} \text{ N uptake}$) were found for all tillage systems in these rainfed Mediterranean agro-ecosystem.

8. Calibration and validation of DNDC model for NT and CT systems showed a fair agreement in soil water content and crop yields for wheat and vetch in rainfed Mediterranean agro-ecosystems. But the DNDC model overall overestimated the daily N_2O fluxes for all treatments (WNT, WCT, VNT and VCT), producing deviations between model and measured cumulative N_2O emissions in NT and CT systems.

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