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Integrated soil fertility management drives the effect of cover crops on GHG emissions in an irrigated field

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Abstract

Agronomical and environmental benefits are associated with replacing winter fallow by cover crops (CC). Yet, the effect of this practice on nitrous oxide (N_2O) emissions remains poorly understood. In this context, a field experiment was carried out under Mediterranean conditions to evaluate the effect of replacing the traditional winter fallow (F) by vetch (*Vicia sativa* L.; V) or barley (*Hordeum vulgare* L.; B) on greenhouse gas (GHG) emissions during the intercrop and the maize (*Zea mays* L.) cropping period. The maize was fertilized following Integrated Soil Fertility management (ISFM) criteria. Maize nitrogen (N) uptake, soil mineral N concentrations, soil temperature and moisture, dissolved organic carbon (DOC) and GHG fluxes were measured during the experiment. The ISFM resulted in low cumulative N_2O emissions (0.57 to 0.75 kg N_2O-N ha^{-1}), yield-scaled N_2O emissions (3-6 g N_2O-N kg aboveground N $uptake^{-1}$) and N surplus (31 to 56 kg N ha^{-1}) for all treatments. Although CCs increased N_2O emissions during the intercrop period compared to F (1.6 and 2.6 times in B and V, respectively), the ISFM resulted in similar cumulative emissions for the CCs and F at the end of the maize cropping period. The higher C:N ratio of the B residue led to a greater proportion

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of N_2O losses from the synthetic fertilizer in these plots, when compared to V. No significant differences were observed in CH_4 and CO_2 fluxes at the end of the experiment. This study shows that the use of both legume and non-legume CCs combined with ISFM could provide, in addition to the advantages reported in previous studies, an opportunity to maximize agronomic efficiency (lowering synthetic N requirements for the subsequent cash crop) without increasing cumulative or yield-scaled N_2O losses.

1. Introduction

Improved resource-use efficiencies are pivotal components of a sustainable agriculture that meets human needs and protects natural resources (Spiertz, 2010). Several strategies have been proposed to improve the efficiency of intensive irrigated systems, where nitrate (NO_3^-) leaching losses are of major concern, both during cash crop and winter fallow periods (Quemada et al., 2013). In this sense, replacing winter intercrop fallow with cover crops (CCs) has been reported to decrease NO_3^- leaching via retention of post-harvest surplus inorganic nitrogen (N) (Wagner-Riddle and Thurtell, 1998), consequently improving N use efficiency (NUE) of the cropping system (Gabriel and Quemada, 2011). Furthermore, the use of CCs as green manure for the subsequent cash crop may further increase soil fertility and NUE (Tonitto et al., 2006; Veenstra et al., 2007) through slow release of N and other nutrients from the crop residues, leading to synthetic fertilizer saving.

From an environmental point of view, N fertilization is closely related with the production and emission of nitrous oxide (N_2O) (Davidson and Kanter, 2014), a greenhouse gas (GHG) with a molecular global warming potential c. 300 times that of carbon dioxide (CO_2) (IPCC, 2007). Nitrous oxide released from agricultural soils is

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mainly generated by nitrification and denitrification processes, which are influenced by several soil variables (Firestone and Davidson, 1989). Thereby, modifying these parameters through agricultural management practices (e.g. fertilization, crop rotation, tillage or irrigation) aiming to optimize N inputs, can lead to strategies for reducing the emission of this gas (Ussiri and Lal, 2012). In order to identify the most effective GHG mitigation strategies, side-effects on methane (CH_4) uptake and CO_2 emission (i.e. respiration) from soils, which are also influenced by agricultural practices (Snyder et al., 2009), need to be considered.

To date, the available information linking GHG emission and maize-winter CCs rotation in the scientific literature is scarce. The most important knowledge gaps include effects of plant species selection and CCs residue management (i.e. retention, incorporation or removal) (Basche et al., 2014). Cover crop species may affect N_2O emissions in contrasting ways, by influencing abiotic and biotic soil factors. These factors include mineral N availability in soil and the availability of carbon (C) sources for the denitrifier bacterial communities, soil pH, soil structure and microbial community composition (Abalos et al., 2014). For example, non-legume CCs such as winter cereals could contribute to a reduction of N_2O emissions due to their deep roots, which allow them to extract soil N more efficiently than legumes (Kallenbach et al., 2010). Conversely, the higher C:N ratio of their residues as compared to those of legumes may provide energy for denitrifiers, thereby leading to higher N_2O losses in the presence of mineral N from fertilizers (Sarkodie-Addo et al., 2003). Moreover, winter CCs can also abate indirect gaseous N losses through the reduction of leaching and subsequent emissions from water resources (Feyereisen et al., 2006). Thus, the estimated N_2O mitigation potential for winter CCs ranges from 0.2 to 1.1 kg N_2O ha^{-1} yr^{-1} according to Ussiri and Lal (2012).

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In a CC-maize rotation system, mineral fertilizer application to the cash crop could have an important effect on NUE and N losses from the agro-ecosystem. Different methods for calculating the N application rate (e.g. conventional or integrated) can be employed by farmers, affecting the amount of synthetic N applied to soil and the overall effect of CCs on N_2O fluxes. Integrated Soil Fertility Management (ISFM) (Kimani et al., 2003) provides an opportunity to optimize the use of available resources, thereby reducing pollution and costs from over-use of N fertilizers (conventional management). ISFM involves the use of inorganic fertilizers and organic inputs, such as green manure, aiming to maximize agronomic efficiency (Vanlauwe et al., 2011). When applying this technique to a CC-maize crop rotation, N fertilization rate for maize is calculated taking into account the background soil mineral N and the expected available N from mineralization of CC residues, which depends on residue composition. Differences in soil mineral N during the cash crop phase may be significantly reduced if ISFM practices are employed, affecting the GHG balance of the CC-cash crop cropping system.

Only one study has investigated the effect of CCs on N_2O emissions in Mediterranean cropping systems (Sanz-Cobena et al., 2014). These authors found an effect of CCs species on N_2O emissions during the intercrop period. After 4 years of CC (vetch, barley or rape)-maize rotation, vetch was the only CC species that significantly enhanced N_2O losses compared to fallow, mainly due to its capacity to fix atmospheric N_2 and because of higher N surplus from the previous cropping phases in these plots. In this study a conventional fertilization (same N synthetic rate for all treatments) was applied during the maize phase; how ISFM practices may affect these findings remains unknown. Moreover, the relative contribution of mineral N fertilizer, CC residues and/or soil mineral N to N_2O losses during the cash crop has not been assessed yet. In

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this sense, stable isotope analysis (i.e. ^{15}N) has emerged as a way to identify the source and the dominant processes involved in N_2O production (Arah, 1997). A comprehensive understanding of the N_2O biochemical production pathways and nutrient sources is crucial for the development of effective mitigation strategies.

The objective of this study was to evaluate the effect of two different CC species (barley and vetch) and fallow on GHG emissions during the CC period and during the following maize cash crop period in an ISFM system. An additional objective was to study the contribution of the synthetic fertilizer and other N sources to N_2O emissions using ^{15}N labelled fertilizer. We hypothesized that: 1) the presence of CCs instead of fallow would affect N_2O losses, leading to higher emissions in the case of the legume CC (vetch) in accordance with the studies of Basche et al. (2014) and Sanz-Cobena et al. (2014); and 2) in spite of the ISFM during the maize period, which theoretically would lead to similar soil N availability for all plots, the distinct composition of the CC residues would affect N_2O emissions. In order to test these hypotheses, a field experiment was carried out using the same management system for 8 years, measuring GHGs during the 8th year. To gain a better understanding of the effect of the management practices tested on the overall GHG budget of a cropping system, CH_4, CO_2 and yield-scaled N_2O emissions were also analyzed during the experimental period. The relative contribution of each N source (synthetic fertilizer or soil endogenous N, including N mineralized from the CCs) to N_2O emissions was also evaluated by ^{15}N-labelled ammonium nitrate (AN) in a parallel experiment.

2. Materials and methods

2.1. Site characteristics

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The study was conducted at "La Chimenea" field station (40°03′N, 03°31′W, 550 m a.s.l.), located in the central Tajo river basin near Aranjuez (Madrid, Spain), where an experiment involving cover cropping systems and conservation tillage has been carried out since 2006. Soil at the field site is a silty clay loam (*Typic Calcixerept*; Soil Survey Staff, 2014). Some of the physico-chemical properties of the top 0–10 cm soil layer, as measured by conventional methods, were: pH_{H2O}, 8.16; total organic C, 19.0 g kg^{-1}; $CaCO_3$, 198 g kg^{-1}; clay, 25%; silt, 49% and sand, 26%. Bulk density of the topsoil layer determined in intact core samples (Grossman and Reinsch, 2002) was 1.46 g cm^{-3}. Average ammonium (NH_4^+) content at the beginning of the experiment was 0.42±0.2 mg N kg $soil^{-1}$ (without differences between treatments). Nitrate concentrations were 1.5±0.2 mg N kg $soil^{-1}$ in fallow and barley and 0.9±0.1 mg N kg $soil^{-1}$ in vetch. Initial dissolved organic C (DOC) contents were 56.0±7 mg C kg $soil^{-1}$ in vetch and fallow and 68.8±5 mg C kg $soil^{-1}$ in barley. The area has a Mediterranean semiarid climate, with a mean annual air temperature of 14 °C. The coldest month is January with a mean temperature of 6 °C, and the hottest month is August with a mean temperature of 24 °C. During the last 30 years, the mean annual precipitation has been approximately 350 mm (17 mm from July to August and 131 mm from September to November).

Hourly rainfall and air temperature data were obtained from a meteorological station located at the field site (CR10X, Campbell Scientific Ltd, Shepshed, UK). A temperature probe inserted 10 cm into the soil was used to measure soil temperature. Mean hourly temperature data were stored on a data logger.

2.2 Experimental design and agronomic management

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Twelve plots (12m × 12m) were randomly distributed in four replications of three cover cropping treatments, including a cereal and a legume: 1) barley (B) (*Hordeum vulgare* L., cv. Vanessa), 2) vetch (V) (*Vicia sativa* L., cv. Vereda), and 3) traditional winter fallow (F). Cover crop seeds were broadcast by hand over the stubble of the previous crop and covered with a shallow cultivator (5 cm depth) on October 10th 2013, at a rate of 180 and 150 kg ha^{-1} for B and V, respectively. The cover cropping phase finished on March 14th 2014, with an application of glyphosate (N-phosphonomethyl glycine) at a rate of 0.7 kg a.e. ha^{-1}. All the CC residues were left on top of the soil. Thereafter, a new set of N fertilizer treatments was set up for the maize cash crop phase. Maize (*Zea mays* L., Pioneer P1574, FAO Class 700) was direct drilled on April 7th 2014 in all plots, resulting in a plant population density of 7.5 plants m^{-2}; harvesting took place on September 25th 2014. The fertilizer treatments consisted of AN applied on 2nd June at three rates: 170, 140 and 190 kg N ha^{-1} in F, V and B plots, respectively, according to ISFM practices. For the calculation of each N rate, the N available in the soil (which was calculated following soil analysis as described below), the expected N uptake by maize crop, and the estimated N mineralized from V and B residues were taken into account, assuming that crop requirements were 236.3 kg N ha^{-1} (Quemada et al., 2014). Estimated NUE of maize plants for calculating N application rate was 70% according to the NUE obtained during the previous years in the same experimental area. Each plot received P as triple superphosphate (45% P_2O_5, Fertiberia®, Madrid, Spain) at a rate of 69 kg P_2O_5 ha^{-1}, and K as potassium chloride (60% K_2O, Fertiberia®, Madrid, Spain), at a rate of 120 kg K_2O ha^{-1} just before sowing maize. All N, P and K fertilizers were broadcast by hand, and immediately after N fertilization the field was irrigated to prevent ammonia volatilization. The main crop previous to sowing CCs was sunflower (*Helianthus annuus* L., var. Sambro). Neither the sunflower nor the CCs were fertilized.

In order to determine the amount of N_2O derived from the N fertilizers, double-labelled AN ($^{15}NH_4{}^{15}NO_3$, 5 % atom ^{15}N, from Cambridge Isotope Laboratories, Inc., Massachusetts, USA) was applied on 2m x 2m subplots established within each plot at a rate of 130 kg N ha^{-1}. In order to reduce biases due to the use of different N rates (e.g. apparent priming effects or different mixing ratios between the added and resident soil N pools) the same amount of N was applied for all treatments. In each subplot, the CC residue was also left on top of the soil. This application took place on 26th May by spreading the fertilizer homogenously with a hand sprayer, followed by an irrigation event.

Sprinkler irrigation was applied to the maize crop in a total amount of 688.5 mm in 31 irrigation events. Sprinklers were installed in a 12m x 12m framework. The water doses to be applied were estimated from the crop evapotranspiration (ETc) of the previous week (net water requirements). This was calculated daily as ETc. = Kc × ETo, where ETo is reference evapotranspiration calculated by the FAO Penman–Monteith method (Allen et al., 1998) using data from the meteorological station located in the experimental field. The crop coefficient (Kc) was obtained using the relationship for maize in semiarid conditions (Martínez-Cob, 2008).

Two different periods were considered for data reporting and analysis: Period I (from CC sowing to N fertilization of the maize crop), and Period II (from N fertilization of maize to the end of the experimental period, after maize harvest).

2.3 *GHG emissions sampling and analyzing*

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Fluxes of N_2O, CH_4 and CO_2 were measured from October 2013 to October 2014 using opaque manual circular static chambers as described in detail by Abalos et al. (2013). One chamber (diameter 35.6 cm, height 19.3 cm) was located in each experimental plot. The chambers were hermetically closed (for 1 h) by fitting them into stainless steel rings, which were inserted at the beginning of the study into the soil to a depth of 5 cm to minimize the lateral diffusion of gases and to avoid the soil disturbance associated with the insertion of the chambers in the soil. The rings were only removed during management events. Each chamber had a rubber sealing tape to guarantee an airtight seal between the chamber and the ring. A rubber stopper with a 3-way stopcock was placed in the wall of each chamber to take gas samples. Greenhouse gas measurements were always made with barley/vetch plants inside the chamber. During the maize period, gas chambers were set up between maize rows.

During Period I, GHGs were sampled weekly or every two weeks. During the first month after maize fertilization, gas samples were taken twice per week. Afterwards, gas sampling was performed weekly or fortnightly, until the end of the cropping period. To minimize any effects of diurnal variation in emissions, samples were always taken at the same time of the day (10–12 am), that is reported as a representative time (Reeves et al., 2015).

Measurements of N_2O, CO_2 and CH_4 emissions were made at 0, 30 and 60 min to test the linearity of gas accumulation in each chamber. Gas samples (100 mL) were removed from the headspace of each chamber by syringe and transferred to 20 mL gas vials sealed with a gas-tight neoprene septum. The vials were previously flushed in the field using 80 mL of the gas sample. 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

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transported gas samples to a ^{63}Ni electron-capture detector (Micro-ECD) to analyze N_2O concentrations and to a flame ionization detector (FID) connected to a methanizer to measure CH_4 and CO_2 (previously reduced to CH_4). The temperatures of the injector, oven and detector were 50, 50 and 350ºC, respectively. The accuracy of the gas chromatographic data was 1% or better. Two gas standards comprising a mixture of gases (high standard with 1500 ± 7.50 ppm CO_2, 10 ± 0.25 ppm CH_4 and 2 ± 0.05 ppm N_2O and low standard with 200 ± 1.00 ppm CO_2, 2 ± 0.10 ppm CH_4 and 200 ± 6.00 ppb N_2O) were provided by Carburos Metálicos S.A. and Air Products SA/NV, respectively, and used to determine a standard curve for each gas. The response of the GC was linear within 200–1500 ppm for CO_2 and 2–10 ppm CH_4 and quadratic within 200–2000 ppb for N_2O.

The increases in N_2O, CH_4 and CO_2 concentrations within the chamber headspace were generally (80% of cases) linear (R^2> 0.90) during the sampling period (1h). Therefore, emission rates of fluxes were estimated as the slope of the linear regression between concentration and time (after corrections for temperature) and from the ratio between chamber volume and soil surface area (MacKenzie et al., 1998). Cumulative N_2O, CH_4 and CO_2, emissions per plot during the sampling period were estimated by linear interpolations between sampling dates, multiplying the mean flux of two successive determinations by the length of the period between sampling and adding that amount to the previous cumulative total (Sanz-Cobena et al., 2014). The measurement of CO_2 emissions from soil including plants in opaque chambers only includes ecosystem respiration but not photosynthesis (Meijide et al., 2010).

2.4 ^{15}N Isotope analysis

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Gas samples from the subplots receiving double-labelled AN fertilizer were taken after 60 min static chamber closure 1, 4, 9, 11, 15, 18, 22 and 25 days after fertilizer application. Stable ^{15}N isotope analysis of N_2O contained in the gas samples was carried out on a trace gas analyzer (using cryo-trapping and cryo-focusing) coupled to a 20/22 isotope ratio mass spectrometer (both from SerCon Ltd., Crewe, UK), at Rothamsted Research North Wyke. Solutions of 6.6 and 2.9 atom% ammonium sulphate [$(NH_4)_2SO_4$] were prepared and used to generate 6.6 and 2.9 atom% N_2O (Laughlin et al., 1997) which were used as reference and quality control standards. During the experiment, the mean natural abundance of atmospheric N_2O (0.369 atom% ^{15}N) was subtracted from measured enriched samples to calculate the atom percent excess. To obtain the N_2O flux that was derived from fertilizer ($N_2O - N_{dff}$), the Eq. (1) was used (Loick et al., 2016):

$$N_2O - N_{dff} = N_2O - N \times \left(\frac{N_2O\text{−}atom\ percent\ excess_{sample}}{atom\ percent\ excess_{fertilizer}}\right) \quad (1)$$

in which 'N_2O-N' is the N_2O emission from soil, '$N_2O - ape_{sample}$' is the ^{15}N atom% excess of emitted N_2O (being equal to '^{15}N atom% of measured samples' minus 0.369 atom% where 0.369 atom% is the mean natural ^{15}N abundance of '*background* N_2O' obtained in our experiment), and '$ape_{fertilizer}$' is the ^{15}N atom% excess of the applied fertilizer (Loick et al., 2016).

2.5 Soil and crop analyses

In order to relate gas emissions to soil properties, soil samples were collected at 0-10 cm depth during the growing season on almost all gas-sampling occasions, particularly after each fertilization event. Three soil cores (2.5 cm diameter and 15 cm

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length) were randomly sampled close to the ring in each plot, and then mixed and homogenized in the laboratory. Soil NH_4^+ and NO_3^- concentrations were analyzed using 8 g of soil extracted with 50 mL of KCl (1 M), and measured by automated colorimetric determination using a flow injection analyzer (FIAS 400 Perkin Elmer) provided with a UV-V spectrophotometer detector. Soil (DOC) was determined by extracting 8 g of homogeneously mixed soil with 50 mL of deionized water, and analyzed with a total organic C analyser (multi N/C 3100 Analityk Jena) equipped with an IR detector. The Water-Filled Pore Space (WFPS) was calculated by dividing the volumetric water content by total soil porosity. Total soil porosity was calculated according to the relationship: soil porosity = (1- soil bulk density/2.65), assuming a particle density of 2.65 g cm^{-3} (Danielson and Sutherland, 1986). Gravimetric water content was determined by oven-drying soil samples at 105 °C with a MA30 Sartorius ®.

Four 0.5m × 0.5m squares were randomly harvested from each plot, before killing the CC by applying glyphosate. Aerial biomass was cut by hand at soil level, dried, weighed and ground. A subsample was taken for determination of total N content. From these samples was determined CC biomass and N contribution to the subsequent maize.

At maize harvest, two 8 m central rows in each plot were collected and weighed in the field following separation of grain and straw. For aboveground N uptake calculations, N content was determined in subsamples of grain and biomass. Total N content on maize and CC subsamples were determined with an elemental analyzer (TruMac CN Leco).

2.6 Calculations and statistical analysis

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Yield-scaled N_2O emissions and N surplus in the maize cash crop were calculated as the amount of N_2O emitted (considering the emissions of the whole experiment, i.e. Period I + Period II) per unit of above-ground N uptake, and taking the difference between N application and above-ground N uptake, respectively (van Groenigen et al., 2010).

Statistical analyses were carried out with Statgraphics Plus 5.1. Analyses of variance were performed for all variables over the experiment (except climatic ones), for both periods indicated in section 2.2. Data distribution normality and variance uniformity were previously assessed by Shapiro-Wilk test and Levene's statistic, respectively, and transformed (log10, root-square, arcsin or inverse) before analysis when necessary. Means of soil parameters were separated by Tukey's honest significance test at $P<0.05$, while cumulative GHG emissions, YSNE and N surplus were compared by the orthogonal contrasts method at $P<0.05$. For non-normally distributed data, the Kruskal–Wallis test was used on non-transformed data to evaluate differences at $P<0.05$. Linear correlations were carried out to determine relationships between gas fluxes and WFPS, soil temperature, DOC, NH_4^+ and NO_3^-. Theses analyses were performed using the mean/cumulative data of the replicates of the CC treatments (n=12), and also for all the dates when soil and GHG were sampled, for Period I (n=16), Period II (n=11) and the whole experimental period (n=27).

3. Results

3.1 Cover crop (Period I)

3.1.1 Environmental conditions and WFPS

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Mean soil temperature during the intercrop period was 8.8°C, ranging from 1.8 (December) to 15.5°C (April) (Fig. 1a), which were typical values in the experimental area. Mean soil temperature during maize cropping period was 24.6°C, which was also a standard value for this region. The accumulated rainfall during this period was 215 mm, whereas the 30-year mean is 253 mm. Water-Filled Pore Space ranged from 40 to 81% (Fig. 1b). No significant differences were observed for WFPS mean values between the different treatments (P>0.05).

3.1.2 Mineral N and DOC and cover crop residues

Topsoil NH_4^+ content was below 5 mg N kg soil^{-1} almost of the time in Period I, although a peak was observed after maize sowing (55 days after CCs kill date) (Fig. 2a), with the highest values reached in B (50 mg N kg soil^{-1}). Mean NH_4^+ content was significantly higher in B than in F (P<0.05). Nitrate content increased after CCs killing, reaching values above 25 mg N kg soil^{-1} in V treatment (Fig. 2c). Mean NO_3^- content during Period I was significantly higher in the V plots than in the B and F plots (P<0.001). Dissolved Organic C ranged from 60 to 130 mg C kg soil $^{-1}$ (Fig. 2e). Average topsoil DOC content was significantly higher in B than in V and F (P<0.05). The total amount of cover crop biomass left on the ground was 540.5±26.5 and 1106.7±93.6 kg DM ha^{-1} in B and V, respectively. Accordingly, the total N content of these residues was 11.0±0.6 and 41.3±4.5 kg N ha^{-1} in B and V, respectively.

3.1.3 GHG fluxes

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Nitrous oxide fluxes ranged from -0.06 to 0.22 mg N m^{-2} d^{-1} (Fig. 3a) in Period I. The soil acted as a sink for N_2O at some sampling dates, especially for the F plots. Cumulative fluxes at the end of Period I were significantly greater in CC treatments compared to F (1.6 and 2.6 higher in B and V, respectively) (P<0.05; Table 1). Net CH_4 uptake was observed in all intercrop treatments, and daily fluxes ranged from -0.60 to 0.25 mg C m^{-2} d^{-1} (data not shown). No significant differences were observed between treatments in cumulative CH_4 fluxes at the end of Period I (P>0.05; Table 1). Carbon dioxide fluxes (data not shown) remained below 1 g C m^{-2} d^{-1} during the intercrop period. Greatest fluxes were observed in B although differences in cumulative fluxes were not significant (P>0.05; Table 1). Nitrous oxide emissions were significantly correlated to CO_2 fluxes (P<0.01, n=17, r=0.69) and soil temperature (P<0.05, n=17, r=0.55).

3.2 Maize crop (Period II)

3.2.1 Environmental conditions and WFPS

Mean soil temperature ranged from 19.6 (reached in September) to 32.3°C (reached in August) with a mean value of 27.9°C (Fig. 1a). Total rainfall during the maize crop period was 57 mm. Water-Filled Pore Space ranged from 19 to 84% (Fig. 1c). Higher mean WFPS values (P<0.01) were measured in B during some sampling dates.

3.2.2 Mineral N and DOC

Topsoil NH_4^+ content increased rapidly after N fertilization (Fig. 2b) decreasing to values below 10 mg N kg soil^{-1} from 15 days after fertilization to the end of the experimental period. Nitrate concentrations (Fig. 2d) also peaked after AN addition, reaching the highest value (170 mg N kg soil^{-1}) 15 days after fertilization in B (P<0.05). No significant differences (P>0.05) between treatments were observed in average soil NH_4^+ or NO_3^- during maize phase. Dissolved Organic C ranged from 56 to 138 mg C kg soil^{-1} (Fig. 2f). Average topsoil DOC content was 26 and 44% higher in B than in V and F, respectively (P<0.001).

3.2.3 GHG fluxes, Yield-Scaled N_2O emissions and N surplus

Nitrous oxide fluxes ranged from 0.0 to 5.6 mg N m^{-2} d^{-1} (Fig. 3b). The highest N_2O emission peak was observed 1-4 days after fertilization for all plots. Other peaks were subsequently observed until 25 days after fertilization, particularly in B plots where N_2O emissions 23 and 25 days after fertilization were higher (P<0.05) than those of F and V (Fig. 3b). No significant differences in cumulative N_2O fluxes were observed between treatments throughout or at the end of the maize crop period (Table 1), albeit fluxes were numerically higher in B than in V (0.05<P<0.10). Daily N_2O emissions were significantly correlated with NH_4^+ topsoil content (P<0.05, n=12, r=0.84).

As in the previous period, all treatments were CH_4 sinks, without significant differences between treatments (P>0.05; Table 1). Respiration rates ranged from 0.15 to 3.0 g C m^{-2} d^{-1}; no significant differences (P>0.05; Table 1) were observed among the CO_2 values for the different treatments. Yield-scaled N_2O emissions and N surplus are shown in Table 1. No significant differences were observed between treatments although these values were generally lower in V than in B (0.05<*P*<0.15).

Considering the whole cropping period (Period I and Period II), N_2O fluxes significantly correlated with WFPS (*P*<0.05, n=12, r=0.61), NH_4^+ (*P*<0.05, n=27, r=0.84) and NO_3^- (*P*<0.05, n=27, r=0.50).

3.2.4 Fertilizer-derived N_2O emissions

The proportion (%) of N_2O losses from AN, calculated by isotopic analyses, is represented in Fig. 4. The highest percentages of N_2O fluxes derived from the synthetic fertilizer were observed one day after fertilization, ranging from 34% (V) to 67% (B). On average, almost 50% of N_2O emissions in the first sampling event after N synthetic fertilization came from other sources (i.e. soil endogenous N, including N mineralized from the CCs). The mean percentage of N_2O losses from synthetic fertilizer throughout all sampling dates was 2.5 times higher in B compared to V (*P*<0.05). There were no significant differences between V and F (*P*>0.05).

4. Discussion

4.1 Role of CCs in N_2O emissions: Period I

Cover crop treatments (V and B) increased N_2O losses compared to F, especially in the case of V (Table 1). These results are consistent with the meta-analysis of Basche et al. (2014), which showed that overall CCs increase N_2O fluxes (compared to bare fallow), with highly significant increments in the case of legumes and a lower effect in

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the case of non-legume CCs. In the same experimental area, Sanz-Cobena et al. (2014) found that V was the only CC significantly affecting N_2O emissions. The greatest differences between treatments were observed at the beginning (13-40 days after CCs sowing), and at the end of this period (229 days after CCs sowing) (Fig. 3a). On these dates, the mild soil temperatures and the relatively high moisture content were more suitable for soil biochemical processes, which may trigger N_2O emissions (Fig. 1a, b) (Firestone and Davidson, 1989). Average topsoil NO_3^- was significantly higher in V (Fig. 2b), which was the treatment that led to the highest N_2O emissions. Legumes such as V are capable of biologically fixing atmospheric N_2, thereby increasing soil NO_3^- content with potential to be denitrified. Further, the mineralization of the most recalcitrant fraction of the previous V residue (which supplies nearly four times more N than the B residue, as indicated in section 3.1.2) together with high C-content sunflower residue could also explain higher NO_3^- contents in V plots (Frimpong et al., 2011), and higher N_2O losses from denitrification (Baggs et al., 2000). After CCs kill date, N release from decomposition of roots and nodules and faster mineralization of V residue compared to that of B (shown by NO_3^- in soil in Fig. 2c) are the most plausible explanation for the N_2O increases at the end of the intercrop period (Fig. 3a) (Rochette and Janzen, 2005; Wichern et al., 2008).

Some studies (e.g. Justes et al., 1999; Nemecek et al., 2008) have pointed out that N_2O losses can be reduced with the use of CCs, due to the extraction of plant-available N unused by previous cash crop. However, in our study lower N_2O emissions were measured from F plots without CCs during the intercrop period. This may be a consequence of higher NO_3^- leaching in F plots (Gabriel et al., 2012; Quemada et al., 2013), limiting the availability of the substrate for denitrification. Frequent rainfall during the intercrop period (Fig. 1a) and the absence of N uptake by CCs may have led to N losses through leaching, resulting in low concentrations of soil mineral N in F plots.

Nitrous oxide emissions were low during this period, but in the range of those reported by Sanz-Cobena et al. (2014) in the same experimental area. Total emissions during Period I represented 8, 10 and 21% of total cumulative emissions in F, B and V, respectively (Table 1). The absence of N fertilizer application to the soil combined with the low soil temperatures during winter – which were far from the optimum values for nitrification and denitrification (25-30 °C) processes (Ussiri and Lal, 2012) – may have caused these low N_2O fluxes. The significant positive correlation between soil temperature and N_2O fluxes during this period highlights the key role of this parameter as a driver of soil emissions (Schindlbacher et al., 2004; García-Marco et al., 2014).

4.2 Role of CCs in N_2O emissions: Period II

Isotopic analysis during Period II, in which ISFM was carried out, showed that a significant proportion of N_2O emissions came from endogenous soil N or the mineralization of crop residues, especially after the first days following N fertilization (Fig. 4). In this sense, even though an interaction between crop residue and N fertilizer application has been previously described (e.g. in Abalos et al., 2013), the similar proportion of N_2O losses coming from fertilizer in B and F (without residue) one day after N fertilization revealed the importance of mineral N harbored in soil micropores in the N_2O bursts after the first irrigation events.

As we hypothesized, although ISFM practices were adopted, the different CCs played a key role in the N_2O emissions during Period II. Barley plots had higher N_2O emissions than fallow or V-residue plots (at the 10% significance level; Table 1).

Further, a higher proportion of N_2O emissions was derived from the fertilizer in B-residue than in V-residue plots (Fig. 4). These results are in agreement with those of Baggs et al. (2003), who reported a higher percentage of N_2O derived from the ^{15}N-labeled fertilizer using a cereal (ryegrass) as surface mulching instead of a legume (bean). The differences between B and V in terms of cumulative N_2O emissions and in the relative contribution of each source to these emissions (fertilizer- or soil-N) could be explained by: i) the higher C:N residue of B (20.7±0.7 while that of V was 11.1±0.1, according to Alonso-Ayuso et al. (2014)) may have provided an energy source for denitrification (Sarkodie-Addo et al., 2003), increasing the reduction of the NO_3^- supplied by the synthetic fertilizer and enhancing N_2O emissions; ii) NO_3^- concentrations, which tended to be higher in B during the maize cropping phase, could have led to incomplete denitrification and larger N_2O/N_2 ratios (Yamulki and Jarvis, 2002); iii) the easily mineralizable V residue (with low C:N ratio) provided an additional N source for soil microorganisms, thus decreasing the relative amount of N_2O derived from the synthetic fertilizer (Baggs et al., 2000; Shan and Yan, 2013); and iv) V plots were fertilized with a lower amount of immediately available N (i.e. AN) than B plots, which could have resulted in better synchronization between N release and crop needs (Ussiri and Lal, 2012) in V plots. Supporting these findings, Bayer et al. (2015) recently concluded that partially supplying the maize N requirements with winter legume cover-crops can be considered a N_2O mitigation strategy in subtropical agro-ecosystems.

The mineralization of B residues resulted in higher DOC contents for these plots compared to the F or V plots (P<0.001). This was observed in both Period I (as a consequence of soil C changes after the 8-year cover-cropping management) and Period II (due to the CC decomposition). Although in the present study the correlation between

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DOC and N_2O emissions was not significant, positive correlations have been previously found in other low-C Mediterranean soils (e.g. Vallejo et al., 2006; López-Fernández et al., 2007). Some authors have suggested that residues with a high C:N ratio can induce microbial N immobilization (Frimpong and Baggs, 2010, Dendooven et al., 2012). In our experiment, a N_2O peak was observed in B plots 20-25 days after fertilization (Fig. 3b) after a remarkable increase of NO_3^- content (Fig. 2d), which may be a result of a re-mineralization of previously immobilized N in these plots.

The positive correlation of N_2O fluxes and soil NO_3^- content and WFPS during the whole cycle further supports the importance of denitrification process for explaining N_2O losses in this agro-ecosystem (Davidson et al., 1991; García-Marco et al., 2014). However, the strong positive correlation of N_2O with NH_4^+ indicated that nitrification was also a major process leading to N_2O fluxes, and showed that the continuous drying-wetting cycles during a summer irrigated maize crop in a semi-arid region can lead to favorable WFPS conditions for both nitrification and denitrification processes (Fig. 1c) (Bateman and Baggs, 2005). Emission Factors ranged from 0.2 to 0.6% of the synthetic N applied, which were lower than the IPCC default value of 1%. As explained above, ecological conditions during the intercrop period (rainfall and temperature) and maize phase (temperature) could be considered as normal (based on the the 30-year average) in Mediterranean areas. Aguilera et al. (2013) obtained a higher emission factor for high (1.01%) and low (0.66%) water-irrigation conditions in a meta-analysis of Mediterranean cropping systems.

4.3 Methane and CO_2 emissions

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As is generally found in non-flooded arable soils, all treatments were net CH_4 sinks (Snyder et al., 2009). No significant differences were observed between treatments in any of the two periods (Table 1), which is similar to the pattern observed by Sanz-Cobena et al. (2014). Some authors (Dunfield and Knowles, 1995; Tate, 2015) have suggested an inhibitory effect of soil NH_4^+ on CH_4 uptake. Low NH_4^+ contents during almost all of the CCs and maize cycle may explain the apparent lack of this inhibitory effect (Banger et al., 2012). However, during the dates when the highest NH_4^+ contents were reached in V and B (225 days after CCs sowing) (Fig. 3a), CH_4 emissions were significantly higher for these plots (0.12 and 0.16 mg CH_4-C m^{-2} d^{-1} for V and B, respectively) than for F (-0.01 mg CH_4-C m^{-2} d^{-1}) (data not shown). Similarly, the NH_4^+ peak observed two days after fertilization (Fig. 3b) decreased in the order V>F>B, the same trend as CH_4 emissions (which were 0.03, -0.04 and -0.63 mg CH_4-C m^{-2} d^{-1} in V, F and B, respectively; data not shown). Contrary to Sanz-Cobena et al. (2014), the presence of CCs did not increase CO_2 fluxes (Table 1) during Period I (which was longer than that considered by these authors), even though higher fluxes tended to be associated to B plots, probably as a consequence of higher root biomass and plant respiration rates in the cereal (B) than in the legume (V). The decomposition of CC residues and the growth of maize rooting system resulted in an increase of CO_2 fluxes during Period II (Oorts et al., 2007; Chirinda et al., 2010), although differences between treatments were not observed.

4.4 Yield-scaled emissions, N surplus and general assessment

Yield–scaled N_2O emissions ranged from 1.74 to 7.15 g N_2O-N kg aboveground N uptake^{-1}, which is about 1-4 times lower than those reported in the meta-analysis of -

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van Groenigen et al. (2010) for a fertilizer N application rate of 150-200 kg ha^{-1}. Mean N surpluses of V and F (Table 1) were in the recommended range (0-50 kg N ha^{-1}) by van Groenigen et al. (2010), while the mean N surplus in B (55 kg N ha^{-1}) was also close to optimal. In spite of higher N_2O emissions in V during Period I (which accounted for a low proportion of total cumulative N_2O losses during the experiment), these plots did not emit greater amounts of N_2O per kg of N taken up by the maize plants, and even tended to decrease YSNE and N surplus (Table 1).

Adjusting fertilizer N rate to soil endogenous N led to lower N_2O fluxes than previous experiments where conventional N rates were applied (Sanz-Cobena et al., 2012; Adviento-Borbe et al., 2007), in agreement with the study of Migliorati et al. (2014). Our results highlight the critical importance of the cash crop period on total N_2O emissions, and demonstrate that the use of either non-legume and –particularly- legume CCs combined with ISFM may provide an optimum balance between GHG emissions from crop production and agronomic efficiency (i.e. lowering synthetic N requirements for a subsequent cash crop, and leading to similar YSNE as a fallow).

The use of CCs has environmental implications beyond effects on direct soil N_2O emissions. For instance, CCs can mitigate indirect N_2O losses (from NO_3^- leaching). In the study of Gabriel et al. (2012), conducted in the same experimental area, NO_3^- leaching was reduced (on average) by 30% and 59% in V and B, respectively. Considering an emission factor of 0.075 from N leached (De Klein et al., 2006), indirect N_2O losses from leaching could be mitigated by 0.23±0.16 and 0.45±0.17 kg N ha^{-1} yr^{-1} if V and B are used as CCs, respectively. Furthermore, the recent meta-analysis of Poeplau and Don (2015) revealed a C sequestration potential of 0.32±0.08 Mg C ha^{-1} yr^{-1} with the introduction of CCs. These environmental factors together with CO_2 emissions associated to CCs sowing and killing, should be assessed in future studies in order to confirm the potential of CCs for increasing both the agronomic and environmental efficiency of irrigated cropping areas.

Conclusions

Our study confirmed that the presence of CCs (particularly V) during the intercrop period increased N_2O losses, but the contribution of this phase to cumulative N_2O emissions considering the whole cropping cycle (intercrop-cash crop) was low (8-21%). The high influence of the maize crop period over total N_2O losses was not only due to N synthetic fertilization, but also to CC residue mineralization and especially endogenous soil N. The type of CC residue determined the N synthetic rate in a ISFM system and affected the percentage of N_2O losses coming from N fertilizer/soil N as well as the pattern of N_2O losses during the maize phase (through changes in soil NH_4^+, NO_3^- and DOC concentrations). By employing ISFM, similar N_2O emissions were measured from CCs and F treatments at the end of the whole cropping period, resulting in low YSNE (3-6 g N_2O-N kg aboveground N uptake^{-1}) and N surplus (31 to 56 kg N ha^{-1}). Replacing winter F by CCs did not affect significantly CH_4 uptake or respiration rates neither during intercrop or maize cropping periods. Our results highlight the critical importance of the cash crop period on total N_2O emissions, and demonstrate that the use of either legume or non-legume CC combined with ISFM may provide an optimum balance between GHG emissions from crop production and agronomic efficiency.

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Table 1 Total cumulative N_2O-N, CH_4-C and CO_2-C fluxes, yield-scaled N_2O emissions (YSNE) and N surplus in the three cover crop treatments (fallow, F, vetch, V, and barley, B) at the end of both cropping periods. *P* value was calculated with Student's *t*-test and d.f.=9. (*) and S.E. denote significant at $P<0.05$ and the standard error of the mean, respectively.

	Treatment		N_2O kg N_2O-N ha^{-1}	CH_4 kg CH_4-C ha^{-1}	CO_2 kg CO_2-C ha^{-1}	Surplus kg N ha^{-1}	YSNE g N_2O-N kg aboveground N $uptake^{-1}$
End of Period I	F		0.05	-0.30	443.02		
	V		0.13	-0.28	463.01		
	B		0.08	-0.24	582.13		
	S.E.		0.03	0.07	46.33		
	F versus CCs	Estimate	-11.48	-11.45	-134.37		
		t-test	-2.5	-0.61	-1.00		
		P value	**0.03 (*)**	**0.56**	**0.34**		
	V versus B	Estimate	5.29	-6.23	-127.50		
		t-test	1.99	-0.57	-1.64		
		P value	**0.08**	**0.58**	**0.14**		
End of Period II	F		0.57	-0.46	2595.07	31.47	4.21
	V		0.48	-0.33	2778.84	13.72	3.06
	B		0.74	-0.35	2372.07	55.94	5.64
	S.E.		0.10	0.08	177.35	15.30	0.85
	F versus CCs	Estimate	-7.46	-23.69	83.36	-3.16	-0.12
		t-test	-0.30	-1.25	0.19	-0.08	-0.14
		P value	**0.77**	**0.24**	**0.86**	**0.94**	**0.89**
	V versus B	Estimate	-26.59	2.08	417.8	-38.67	-2.59
		t-test	-1.90	0.19	1.62	-1.79	-2.16
		P value	**0.09**	**0.85**	**0.14**	**0.11**	**0.06**

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Figure captions:

Figure 1. Daily mean soil temperature (°C) rainfall and irrigation (mm) (**a**) and soil WFPS (%) in the three cover crop (CC) treatments (fallow, F, vetch, V, and barley, B) during Period I (**b**) and II (**c**). Vertical lines indicate standard errors.

Figure 2a, b NH_4^+-N; **c, d** NO_3^--N; and **e, f** DOC concentrations in the 0–10 cm soil layer for the three cover crop (CC) treatments (fallow, F, vetch, V, and barley, B) during both cropping periods. The black arrows indicate the time of spraying glyphosate over the cover crops. The dotted arrows indicate the time of maize sowing. Vertical lines indicate standard errors.

Figure 3. N_2O emissions for the three cover crop (CC) treatments (fallow, F, vetch, V, and barley, B) during Period I (**a**) and II (**b**). The black arrows indicate the time of spraying glyphosate over the cover crops. The dotted arrows indicate the time of maize sowing. Vertical lines indicate standard errors.

Figure 4. Proportion of N_2O losses (%) coming from N synthetic fertilizer during Period II, for the three cover crop treatments (fallow, F, vetch, V, and barley, B). Vertical lines indicate standard errors. "NS" and * denote not significant and significant at P<0.05, respectively.