Effect of cover crops on greenhouse gas emissions in an irrigated field under integrated soil fertility management

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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. Our management (adjusted N synthetic rates due to ISFM) and pedo-climatic conditions resulted in low cumulative N_2O emissions (0.57 to 0.75 kg N_2O-N ha^{-1} yr^{-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 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 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 N use efficiency (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 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, it has been suggested that the higher C:N ratio of their residues as compared to those of legumes may provide energy (C) for denitrifiers, thereby leading to higher N_2O losses in the presence of mineral N-NO_3^- from fertilizers (Sarkodie-Addo et al., 2003). In this sense, the presence of cereal residues can increase the abundance of denitrifying microorganisms (Gao et al., 2016), thus enhancing denitrification losses when soil conditions are favorable (e.g. high NO_3^- availability and soil moisture after

74 rainfall or irrigation events, particularly in fine-textured soils) (Stehfest and Bouwman
75 2006; Baral et al., 2016). Besides, winter CCs can also abate indirect gaseous N losses
76 through the reduction of leaching and subsequent emissions from water resources
77 (Feyereisen et al., 2006). Thus, the estimated N_2O mitigation potential for winter CCs
78 ranges from 0.2 to 1.1 kg N_2O ha^{-1} yr^{-1} according to Ussiri and Lal (2012).

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

94 Only one study has investigated the effect of CCs on N_2O emissions in
95 Mediterranean cropping systems (Sanz-Cobena et al., 2014). These authors found an
96 effect of CCs species on N_2O emissions during the intercrop period. After 4 years of CC
97 (vetch, barley or rape)-maize rotation, vetch was the only CC species that significantly
98 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 this sense, stable isotope analysis (i.e. ^{15}N) represents a way to identify the source and the dominant processes involved in N_2O production (Arah, 1997). Stable Isotope techniques have been used in field studies evaluating N leaching and/or plant recovery in systems with cover crops (Bergström et al., 2001; Gabriel and Quemada, 2011; Gabriel et al., 2016). Furthermore, some laboratory studies have evaluated the effect of different crop residues on N_2O losses using ^{15}N techniques (Baggs et al., 2003; Li et al., 2016); but to date, no previous studies have evaluated the relative contribution of cover crops (which include the aboveground biomass and the decomposition of root biomass) and N synthetic fertilizers to N_2O emissions under field conditions. 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 in a parallel experiment.

2. Materials and methods

2.1. Site characteristics

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

148 semiarid climate, with a mean annual air temperature of 14 °C. The coldest month is
149 January with a mean temperature of 6 °C, and the hottest month is August with a mean
150 temperature of 24 °C. During the last 30 years, the mean annual precipitation has been
151 approximately 350 mm (17 mm from July to August and 131 mm from September to
152 November).

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

157

158 *2.2 Experimental design and agronomic management*

159 Twelve plots (12m × 12m) were randomly distributed in four replications of
160 three cover cropping treatments, including a cereal and a legume: 1) barley (B)
161 (*Hordeum vulgare* L., cv. Vanessa), 2) vetch (V) (*Vicia sativa* L., cv. Vereda), and 3)
162 traditional winter fallow (F). Cover crop seeds were broadcast by hand over the stubble
163 of the previous crop and covered with a shallow cultivator (5 cm depth) on October 10th
164 2013, at a rate of 180 and 150 kg ha^{-1} for B and V, respectively. The cover cropping
165 phase finished on March 14th 2014 following local practices, with an application of
166 glyphosate (N-phosphonomethyl glycine) at a rate of 0.7 kg a.e. ha^{-1}. Even though the
167 safe use of glyphosate is under discussion since years (Chang and Delzell, 2016), it was
168 used in order to preserve the same killing method in all the campaigns in this long-term
169 experiment under conservation tillage management. All the CC residues were left on top
170 of the soil. Thereafter, a new set of N fertilizer treatments was set up for the maize cash
171 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 ammonium nitrate 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 N use efficiency of maize plants for calculating N application rate was 70% according to the N use efficiency 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 ammonium nitrate ($^{15}NH_4{}^{15}NO_3$, 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*

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 and was covered with a radiant barrier reflective foil to reduce temperature gradients between inside and outside. A rubber stopper with a 3-way stopcock was placed in the wall of each chamber to take gas

221 samples. Greenhouse gas measurements were always made with barley/vetch plants
222 inside the chamber. During the maize period, gas chambers were set up between maize
223 rows.

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

230 Measurements of N_2O, CO_2 and CH_4 emissions were made at 0, 30 and 60 min
231 to test the linearity of gas accumulation in each chamber. Gas samples (100 mL) were
232 removed from the headspace of each chamber by syringe and transferred to 20 mL gas
233 vials sealed with a gas-tight neoprene septum. The vials were previously flushed in the
234 field using 80 mL of the gas sample. Samples were analyzed by gas chromatography
235 using a HP-6890 gas chromatograph equipped with a headspace autoanalyzer (HT3),
236 both from Agilent Technologies (Barcelona, Spain). Inert gases were separated by HP
237 Plot-Q capillary columns. The gas chromatograph was equipped with a ^{63}Ni electron-
238 capture detector (Micro-ECD) to analyze N_2O concentrations, and with a flame
239 ionization detector (FID) connected to a methanizer to measure CH_4 and CO_2
240 (previously reduced to CH_4). The temperatures of the injector, oven and ECD were 50,
241 50 and 350ºC, respectively. The accuracy of the gas chromatographic data was 1% or
242 better. Two gas standards comprising a mixture of gases (high standard with 1500 ±
243 7.50 ppm CO_2, 10 ± 0.25 ppm CH_4 and 2 ± 0.05 ppm N_2O and low standard with 200 ±
244 1.00 ppm CO_2, 2 ± 0.10 ppm CH_4 and 200 ± 6.00 ppb N_2O) were provided by Carburos
245 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

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 cryo-focusing gas chromatography unit coupled to a 20/20 isotope ratio mass spectrometer (both from SerCon Ltd., Crewe, UK). Ambient samples were taken occasionally as required for the subsequent isotopic calculations. 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. In order to calculate the atom percent excess (ape) of the N_2O

emitted in the sub-plots, the mean natural abundance of atmospheric N_2O from the ambient samples (0.369 atom% ^{15}N) was subtracted from the measured enriched gas samples. To obtain the N_2O flux that was derived from fertilizer ($N_2O - N_{dff}$), the following equation was used (Senbayram et al., 2009:

$$N_2O - N_{dff} = N_2O - N \times \left(\frac{N_2O_ape_{sample}}{ape_{fertilizer}}\right) \quad (1)$$

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, and '$ape_{fertilizer}$' is the ^{15}N atom% excess of the applied fertilizer (Senbayram et al., 2009).

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 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 subsequently filtering), 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

293 particle density of 2.65 g cm^{-3} (Danielson and Sutherland, 1986). Gravimetric water
294 content was determined by oven-drying soil samples at 105 °C with a MA30 Sartorius
295 ®.

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

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

306

307 *2.6 Calculations and statistical analysis*

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

313 Statistical analyses were carried out with Statgraphics Plus 5.1. Analyses of
314 variance were performed for all variables over the experiment (except climatic ones),
315 for both periods indicated in section 2.2. Data distribution normality and variance

316 uniformity were previously assessed by Shapiro-Wilk test and Levene's statistic,
317 respectively, and transformed (log10, root-square, arcsin or inverse) before analysis
318 when necessary. Means of soil parameters were separated by Tukey's honest
319 significance test at $P<0.05$, while cumulative GHG emissions, yield-scaled N_2O
320 emissions and N surplus were compared by the orthogonal contrasts method at $P<0.05$.
321 For non-normally distributed data, the Kruskal–Wallis test was used on non-
322 transformed data to evaluate differences at $P<0.05$. Linear correlations were carried out
323 to determine relationships between gas fluxes and WFPS, soil temperature, DOC, NH_4^+
324 and NO_3^-. Theses analyses were performed using the mean/cumulative data of the
325 replicates of the CC treatments (n=12), and also for all the dates when soil and GHG
326 were sampled, for Period I (n=16), Period II (n=11) and the whole experimental period
327 (n=27).

328

329 **3. Results**

330 *3.1 Cover crop (Period I)*

331 *3.1.1 Environmental conditions and WFPS*

332 Mean soil temperature during the intercrop period was 8.8°C, ranging from 1.8
333 (December) to 15.5°C (April) (Fig. 1a), which were typical values in the experimental
334 area. Mean soil temperature during maize cropping period was 24.6°C, which was also
335 a standard value for this region. The accumulated rainfall during this period was 215
336 mm, whereas the 30-year mean is 253 mm. Water-Filled Pore Space ranged from 40 to
337 81% (Fig. 1b). No significant differences were observed for WFPS mean values
338 between the different treatments ($P>0.05$).

339

340 *3.1.2 Mineral N and DOC and cover crop residues*

341 Topsoil NH_4^+ content was below 5 mg N kg $soil^{-1}$ most of the time in Period I,
342 although a peak was observed after maize sowing (55 days after CCs kill date) (Fig. 2a),
343 with the highest values reached in B (50 mg N kg $soil^{-1}$). Mean NH_4^+ content was
344 significantly higher in B than in F (P<0.05), but daily NH_4^+ concentrations between
345 treatments were only significantly different between treatments in one sampling date
346 (210 days after CCs sowing). Nitrate content increased after CCs killing, reaching
347 values above 25 mg N kg $soil^{-1}$ in V treatment (Fig. 2c). Mean NO_3^- content during
348 Period I was significantly higher in the V plots than in the B and F plots (P<0.001).
349 Dissolved Organic C ranged from 60 to 130 mg C kg $soil^{-1}$ (Fig. 2e). Average topsoil
350 DOC content was significantly higher in B than in V and F (10% and 12%, respectively,
351 P<0.01) but differences were only observed in some sampling dates. The total amount
352 of cover crop biomass left on the ground was 540.5±26.5 and 1106.7±93.6 kg dry
353 matter ha^{-1} in B and V, respectively. Accordingly, the total N content of these residues
354 was 11.0±0.6 and 41.3±4.5 kg N ha^{-1} in B and V, respectively.

355

356 *3.1.3 GHG fluxes*

357 Nitrous oxide fluxes ranged from -0.06 to 0.22 mg N m^{-2} d^{-1} (Fig. 3a) in Period
358 I. The soil acted as a sink for N_2O at some sampling dates, especially for the F plots.
359 Cumulative fluxes at the end of Period I were significantly greater in CC treatments
360 compared to F (1.6 and 2.6 higher in B and V, respectively) (P<0.05; Table 1). Net CH_4
361 uptake was observed in all intercrop treatments, and daily fluxes ranged from -0.60 to
362 0.25 mg C m^{-2} d^{-1} (data not shown). No significant differences were observed between

363 treatments in cumulative CH_4 fluxes at the end of Period I (P>0.05; Table 1). Carbon
364 dioxide fluxes (data not shown) remained below 1 g C m^{-2} d^{-1} during the intercrop
365 period. Greatest fluxes were observed in B although differences in cumulative fluxes
366 were not significant (P>0.05; Table 1) in the whole intercrop period, but soil respiration
367 was increased in B, with respect to F, from mid-February to the end of Period I. Nitrous
368 oxide emissions were significantly correlated to CO_2 fluxes (P<0.01, n=17, r=0.69) and
369 soil temperature (P<0.05, n=17, r=0.55).

370

371 *3.2 Maize crop (Period II)*

372 *3.2.1 Environmental conditions and WFPS*

373 Mean soil temperature ranged from 19.6 (reached in September) to 32.3°C
374 (reached in August) with a mean value of 27.9°C (Fig. 1a). Total rainfall during the
375 maize crop period was 57 mm. Water-Filled Pore Space ranged from 19 to 84% (Fig.
376 1c). Higher mean WFPS values (P<0.01) were measured in B during some sampling
377 dates.

378

379 *3.2.2 Mineral N and DOC*

380 Topsoil NH_4^+ content increased rapidly after N fertilization (Fig. 2b) decreasing
381 to values below 10 mg N kg $soil^{-1}$ from 15 days after fertilization to the end of the
382 experimental period. Nitrate concentrations (Fig. 2d) also peaked after AN addition,
383 reaching the highest value (170 mg N kg $soil^{-1}$) 15 days after fertilization in B (P<0.05).
384 No significant differences (P>0.05) between treatments were observed in average soil
385 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).

409 *3.2.4 Fertilizer-derived N_2O emissions*

410 The proportion (%) of N_2O losses from ammonium nitrate, calculated by
411 isotopic analyses, is represented in Fig. 4. The highest percentages of N_2O fluxes
412 derived from the synthetic fertilizer were observed one day after fertilization, ranging
413 from 34% (V) to 67% (B). On average, almost 50% of N_2O emissions in the first
414 sampling event after N synthetic fertilization came from other sources (i.e. soil
415 endogenous N, including N mineralized from the CCs). The mean percentage of N_2O
416 losses from synthetic fertilizer throughout all sampling dates was 2.5 times higher in B
417 compared to V (P<0.05) and was positively correlated with DOC concentrations
418 (P<0.05, n=12, r=0.71). There were no significant differences between V and F
419 (P>0.05).

420

421 **4. Discussion**

422 *4.1 Role of CCs in N_2O emissions: Period I*

423 Cover crop treatments (V and B) increased N_2O losses compared to F, especially
424 in the case of V (Table 1). These results are consistent with the meta-analysis of Basche
425 et al. (2014), which showed that overall CCs increase N_2O fluxes (compared to bare
426 fallow), with highly significant increments in the case of legumes and a lower effect in
427 the case of non-legume CCs. In the same experimental area, Sanz-Cobena et al. (2014)
428 found that V was the only CC significantly affecting N_2O emissions. The greatest
429 differences between treatments were observed at the beginning (13-40 days after CCs
430 sowing), and at the end of this period (229 days after CCs sowing) (Fig. 3a). On these
431 dates, the mild soil temperatures and the relatively high moisture content were more
432 suitable for soil biochemical processes, which may trigger N_2O emissions (Fig. 1a, b)

433 (Firestone and Davidson, 1989). Average topsoil NO_3^- was significantly higher in V
434 (Fig. 2b), which was the treatment that led to the highest N_2O emissions. Legumes such
435 as V are capable of biologically fixing atmospheric N_2, thereby increasing soil NO_3^-
436 content with potential to be denitrified. Further, the mineralization of the most
437 recalcitrant fraction of the previous V residue (which supplies nearly four times more N
438 than the B residue, as indicated in section 3.1.2) together with high C-content sunflower
439 residue could also explain higher NO_3^- contents in V plots (Frimpong et al., 2011), and
440 higher N_2O losses from denitrification (Baggs et al., 2000). After CCs kill date, N
441 release from decomposition of roots and nodules and faster mineralization of V residue
442 compared to that of B (shown by NO_3^- in soil in Fig. 2c) are the most plausible
443 explanation for the N_2O increases at the end of the intercrop period (Fig. 3a) (Rochette
444 and Janzen, 2005; Wichern et al., 2008).

445 Some studies (e.g. Justes et al., 1999; Nemecek et al., 2008) have pointed out
446 that N_2O losses can be reduced with the use of CCs, due to the extraction of plant-
447 available N unused by previous cash crop. However, in our study lower N_2O emissions
448 were measured from F plots without CCs during the intercrop period. This may be a
449 consequence of higher NO_3^- leaching in F plots (Gabriel et al., 2012; Quemada et al.,
450 2013), limiting the availability of the substrate for denitrification. Frequent rainfall
451 during the intercrop period (Fig. 1a) and the absence of N uptake by CCs may have led
452 to N losses through leaching, resulting in low concentrations of soil mineral N in F
453 plots.

454 Nitrous oxide emissions were low during this period, but in the range of those
455 reported by Sanz-Cobena et al. (2014) in the same experimental area. Total emissions
456 during Period I represented 8, 10 and 21% of total cumulative emissions in F, B and V,
457 respectively (Table 1). The absence of N fertilizer application to the soil combined with

458 the low soil temperatures during winter – which were far from the optimum values for
459 nitrification and denitrification (25-30 °C) processes (Ussiri and Lal, 2012) – may have
460 caused these low N_2O fluxes. The significant positive correlation between soil
461 temperature and N_2O fluxes during this period highlights the key role of this parameter
462 as a driver of soil emissions (Schindlbacher et al., 2004; García-Marco et al., 2014).

463

464 *4.2 Role of CCs in N_2O emissions: Period II*

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

474 As we hypothesized, the different CCs played a key role in the N_2O emissions
475 during Period II. Barley plots had higher N_2O emissions than fallow or V-residue plots
476 (at the 10% significance level; Table 1). Further, a higher proportion of N_2O emissions
477 was derived from the fertilizer in B-residue than in V-residue plots (Fig. 4). These
478 results are in agreement with those of Baggs et al. (2003), who reported a higher
479 percentage of N_2O derived from the ^{15}N-labeled fertilizer using a cereal (ryegrass) as
480 surface mulching instead of a legume (bean), in a field trial with zero-tillage
481 management. 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), favoring the reduction of the NO_3^- supplied by the synthetic fertilizer and enhancing N_2O emissions, as supported by the positive correlation of DOC with the proportion of N_2O coming from the synthetic fertilizer; 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. ammonium nitrate) 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 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 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. We hypothesized that management practices may have contributed to these low emissions, but other inherent factors such as soil pH should be also considered. Indeed, a higher N_2O/N_2 ratio has been associated to acidic soils, so lower N_2O emissions from denitrification could be expected in alkaline soils (Mørkved et al., 2007; Baggs et al., 2010).

4.3 Methane and CO_2 emissions

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 the whole Period I (which was longer than that considered by these authors), even though higher fluxes were associated to B (but not V) with respect to F plots in the last phase of the intercrop, probably as a consequence of higher root biomass and plant respiration rates in the cereal (B) than in the legume (V). Differences from fall to early-winter were not significant, since low soil temperatures limited respiration activity. 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

554 Yield–scaled N_2O emissions ranged from 1.74 to 7.15 g N_2O-N kg aboveground
555 N uptake^{-1}, which is about 1-4 times lower than those reported in the meta-analysis of -
556 van Groenigen et al. (2010) for a fertilizer N application rate of 150-200 kg ha^{-1}. Mean
557 N surpluses of V and F (Table 1) were in the recommended range (0-50 kg N ha^{-1}) by
558 van Groenigen et al. (2010), while the mean N surplus in B (55 kg N ha^{-1}) was also
559 close to optimal. In spite of higher N_2O emissions in V during Period I (which
560 accounted for a low proportion of total cumulative N_2O losses during the experiment),
561 these plots did not emit greater amounts of N_2O per kg of N taken up by the maize
562 plants, and even tended to decrease yield-scaled N_2O emissions and N surplus (Table 1).

563 Adjusting fertilizer N rate to soil endogenous N led to lower N_2O fluxes than
564 previous experiments where conventional N rates were applied (e.g. Adviento-Borbe et
565 al., 2007; Hoben et al., 2011; Sanz-Cobena et al., 2012; Li et al., 2015), in agreement
566 with the study of Migliorati et al. (2014). Moreover, CO_2 equivalent emissions
567 associated to manufacturing and transport of N synthetic fertilizers (Lal, 2004) can be
568 reduced when low synthetic N input strategies, such as ISMF, are employed. Our results
569 highlight the critical importance of the cash crop period on total N_2O emissions, and
570 demonstrate that the use of either non-legume and –particularly- legume CCs combined
571 with ISFM may provide an optimum balance between GHG emissions from crop
572 production and agronomic efficiency (i.e. lowering synthetic N requirements for a
573 subsequent cash crop, and leading to similar YSNE as a fallow).

574 The use of CCs has environmental implications beyond effects on direct soil
575 N_2O emissions. For instance, CCs can mitigate indirect N_2O losses (from NO_3^-
576 leaching). In the study of Gabriel et al. (2012), conducted in the same experimental area,
577 NO_3^- leaching was reduced (on average) by 30% and 59% in V and B, respectively.
578 Considering an emission factor of 0.075 from N leached (De Klein et al., 2006), indirect

579 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.

586

587 **Conclusions**

588 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 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}). 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 non-legume and –particularly– legume CCs combined

603 with ISFM could be considered as an efficient practice from both environmental and
604 agronomic points of view, leading to similar N_2O losses per kilogram of aboveground N
605 uptake as bare fallow.

606

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615

616 **References**

617 Abalos, D., Sanz-Cobena, A., Garcia-Torres, L., van Groenigen, J. W., and Vallejo, A.:
618 Role of maize stover incorporation on nitrogen oxide emissions in a non-irrigated
619 Mediterranean barley field. Plant Soil, 364(1-2), 357-371, 2013.

620 Abalos, D., Deyn, G. B., Kuyper, T. W., and van Groenigen, J. W.: Plant species
621 identity surpasses species richness as a key driver of N_2O emissions from
622 grassland. Glob. Change Biol., 20(1), 265-275, 2014.

623 Adviento-Borbe, M. A. A., Haddix, M. L., Binder, D. L., Walters, D. T., and
624 Dobermann, A.: Soil greenhouse gas fluxes and global warming potential in four high-
625 yielding maize systems. Glob. Change Biol., 13(9), 1972-1988, 2007.

626 Aguilera, E., Lassaletta, L., Sanz-Cobena, A., Garnier, J., and Vallejo, A.: The potential
627 of organic fertilizers and water management to reduce N_2O emissions in Mediterranean
628 climate cropping systems. A review. Agric. Ecosyst. Environ., 164, 32-52, 2013.

629 Allen, R. G., Raes, L. S, and Smith, D. M.: Crop evapotranspiration. Guidelines for
630 computing crop water requirements. Irrigation and Drainage, Paper 56. Rome, Italy:
631 FAO, 1998.

632 Alonso-Ayuso, M., Gabriel, J. L., and Quemada, M.: The kill date as a management tool
633 for cover cropping success. Plos One, 9(10), e109587, 2014.

634 Arah, J. R. M.: Apportioning nitrous oxide fluxes between nitrification and
635 denitrification using gas-phase mass spectrometry. Soil Biol. Biochem., 29(8), 1295-
636 1299, 1997.

637 Baggs, E. M., Rees, R. M., Smith, K. A., and Vinten, A. J. A.: Nitrous oxide emission
638 from soils after incorporating crop residues. Soil Use Manage., 16(2), 82-87, 2000.

639 Baggs, E. M., Stevenson, M., Pihlatie, M., Regar, A., Cook, H., and Cadisch, G.:
640 Nitrous oxide emissions following application of residues and fertiliser under zero and
641 conventional tillage. Plant Soil, 254(2), 361-370, 2003.

642 Baggs, E. M., Smales, C. L., and Bateman, E. J.: Changing pH shifts the microbial
643 sourceas well as the magnitude of N_2O emission from soil. Biol. Fert. Soils, 46(8), 793-
644 805, 2010.

Banger, K., Tian, H., and Lu, C.: Do nitrogen fertilizers stimulate or inhibit methane emissions from rice fields? Glob. Change Biol., 18(10), 3259-3267, 2012.

Baral, K. R., Arthur, E., Olesen, J. E., and Petersen, S. O.: Predicting nitrous oxide emissions from manure properties and soil moisture: An incubation experiment. Soil Biol. Biochem., 97, 112-120.

Basche, A. D., Miguez, F. E., Kaspar, T. C., and Castellano, M. J.: Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. J. Soil Water Conserv., 69(6), 471-482, 2014.

Bateman, E. J., and Baggs, E.M.: Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biol. Fert. Soils, 41(6), 379-388, 2005.

Bayer, C., Gomes, J., Zanatta, J. A., Vieira, F. C. B., de Cássia Piccolo, M., Dieckow, J., and Six, J.: Soil nitrous oxide emissions as affected by long-term tillage, cropping systems and nitrogen fertilization in Southern Brazil. Soil Till. Res., 146, 213-222, 2015.

Bergström, L. F., and Jokela, W. E.: Ryegrass Cover Crop Effects on Nitrate Leaching in Spring Barley Fertilized with (15)NH_4(15)NO_3. J. Environ. Qual., 30(5), 1659-1667, 2001.

Chang, E. T., and Delzell, E.: Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J. Environ. Sci. Heal. B, 51(6), 402-434, 2016.

666 Chirinda, N., Olesen, J. E., Porter, J. R., and Schjønning, P.: Soil properties, crop
667 production and greenhouse gas emissions from organic and inorganic fertilizer-based
668 arable cropping systems. Agric. Ecosyst. Environ., 139(4), 584-594, 2010.

669 Danielson, R. E., and Sutherland, P. L.: 18 Porosity. Methods of soil analysis: Physical
670 and mineralogical methods 9, 443, 1986.

671 Davidson, E.A.: Fluxes of nitrous oxide and nitric acid from terrestrial ecosystem, in:
672 Rogers, J. E., and Whitman, W. B. (Eds.), Microbial production and consumption of
673 greenhouse gases: Methane, Nitrous oxide and Halomethane, American Society of
674 Microbiology, Washington, pp. 219–236, 1991.

675 Davidson, E. A., and Kanter, D.: Inventories and scenarios of nitrous oxide emissions.
676 Environ. Res. Lett., 9(10), 105012, 2014.

677 De Klein, C., Novoa, R. S. A., Ogle, S., Smith, K. A., Rochette, P., Wirth, T. C., Mc
678 Conket, B. G., Walsh, M., Mosier, A., Rypdal, K., and Williams, S. A.: IPCC guidelines
679 for national greenhouse gas inventories, Volume 4, Chapter 11: N_2O emissions from
680 managed soils, and CO_2 emissions from lime and urea application. Technical Report 4-
681 88788-032-4, Intergovernmental Panel on Climate Change, 2006.

682 Dendooven, L., Patino-Zúniga, L., Verhulst, N., Luna-Guido, M., Marsch, R., and
683 Govaerts, B.: Global warming potential of agricultural systems with contrasting tillage
684 and residue management in the central highlands of Mexico. Agric. Ecosyst. Environ.,
685 152, 50-58, 2012.

686 Dunfield, P., and Knowles, R.: Kinetics of inhibition of methane oxidation by nitrate,
687 nitrite, and ammonium in a humisol. Appl. Environ. Microb., 61(8), 3129-3135, 1995.

688 Feyereisen, G. W., Wilson, B. N., Sands, G. R., Strock, J. S., and Porter, P. M.:
689 Potential for a rye cover crop to reduce nitrate loss in southwestern Minnesota. Agron.
690 J., 98(6), 1416-1426, 2006.

691 Firestone, M. K., and Davidson, E. A.: Microbiological basis of NO and N_2O
692 production and consumption in soil, in Andeae, M.O., Schimel, D.S. (Eds.), Exchange
693 of Trace Gases between Terrestrial Ecosystems and the Atmosphere Chichester: Wiley,
694 pp. 7-21, 1989.

695 Frimpong, K. A., and Baggs, E. M.: Do combined applications of crop residues and
696 inorganic fertilizer lower emission of N_2O from soil? Soil Use Manage., 26(4), 412-424,
697 2010.

698 Frimpong, K. A., Yawson, D. O., Baggs, E. M., and Agyarko, K.: Does incorporation of
699 cowpea-maize residue mixes influence nitrous oxide emission and mineral nitrogen
700 release in a tropical luvisol? Nutr. Cycl. Agroecosys., 91(3), 281-292, 2011.

701 Gao, J., Xie, Y., Jin, H., Liu, Y., Bai, X., Ma, D., Zhu, Y., Wang, C., and Guo, T.:
702 Nitrous Oxide Emission and Denitrifier Abundance in Two Agricultural Soils Amended
703 with Crop Residues and Urea in the North China Plain. Plos One, 11, e0154773, 2016.

704 Gabriel, J. L., and Quemada, M.: Replacing bare fallow with cover crops in a maize
705 cropping system: yield, N uptake and fertiliser fate. Eur. J. Agron., 34, 133-143, 2011.

706 Gabriel, J. L., Muñoz-Carpena, R., and Quemada, M.: The role of cover crops in
707 irrigated systems: Water balance, nitrate leaching and soil mineral nitrogen
708 accumulation. Agric. Ecosyst. Environ., 155, 50-61, 2012.

709 Gabriel, J. L., Alonso-Ayuso, M., García-González, I., Hontoria, C., and Quemada, M.:
710 Nitrogen use efficiency and fertiliser fate in a long-term experiment with winter cover
711 crops. Eur. J. Agron., 79, 14-22, 2016.

712 García-Marco, S., Ravella, S. R., Chadwick, D., Vallejo, A., Gregory, A. S., and
713 Cárdenas, L. M.: Ranking factors affecting emissions of GHG from incubated
714 agricultural soils. Eur. J. Soil Sci., 65(4), 573-583, 2014.

715 Grossman, R. B., and Reinsch, T.G.: 2.1 Bulk density and linear extensibility. Methods
716 of Soil Analysis. Part 4: Physical Methods, Soil Science Society of America, Madison,
717 USA, pp. 201-228, 2002.

718 Hoben, J. P., Gehl, R. J., Millar, N., Grace, P. R., and Robertson, G. P.: Nonlinear
719 nitrous oxide (N2O) response to nitrogen fertilizer in on-farm corn crops of the US
720 Midwest. Glob. Change Biol., 17(2), 1140-1152, 2011.

721 IPCC: Climate change 2007. The Physical Science Basis. Contribution of Working
722 Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate
723 Change, in: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B.,
724 Tignor, M., and Miller, H. L. (Eds.), Cambridge University Press, p. 996, 2007.

725 Justes, E., Mary, B., and Nicolardot, B.: Comparing the effectiveness of radish cover
726 crop, oilseed rape volunteers and oilseed rape residues incorporation for reducing nitrate
727 leaching. Nutr. Cycl. Agroecosys., 55(3), 207-220, 1999.

728 Kallenbach, C. M., Rolston, D. E., and Horwath, W. R.: Cover cropping affects soil
729 N_2O and CO_2 emissions differently depending on type of irrigation. Agric. Ecosyst.
730 Environ., 137(3), 251-260, 2010.

731 Kimani, S. K., Nandwa, S. M., Mugendi, D. N., Obanyi, S. N., Ojiem, J., Murwira,
732 Herbert K., and Bationo, A.: Principles of integrated soil fertility management. In:
733 Gichuri, M. P., Bationo, A., Bekunda, M. A., Goma, H. C., Mafongoya, P. L., Mugendi,
734 D. N., Murwuira, H. K., Nandwa, S. M., Nyathi, P., and Swift, M.J. (Eds.), Soil fertility
735 management in Africa: A regional perspective, Academy Science Publishers (ASP),
736 Centro Internacional de Agricultura Tropical (CIAT), Tropical Soil Biology and
737 Fertility (TSBF), Nairobi, KE, pp. 51-72, 2003.

738 Lal, R.: Carbon emission from farm operations. Environ. Int. 30, 981-990, 2004.

739 Laughlin, R. J., Stevens, R. J., and Zhuo, S.: Determining nitrogen-15 in ammonium by
740 producing nitrous oxide. Soil Sci. Soc. Am. J., 61(2), 462-465, 1997.

741 Li, N., Ning, T., Cui, Z., Tian, S., Li, Z., and Lal, R.: N_2O emissions and yield in maize
742 field fertilized with polymer-coated urea under subsoiling or rotary tillage. Nutr. Cycl.
743 Agroecosys., 102(3), 397-410, 2015.

744 Li, X., Sørensen, P., Olesen, J. E., & Petersen, S. O.: Evidence for denitrification as
745 main source of N_2O emission from residue-amended soil. Soil Biol. Biochem., 92, 153-
746 160, 2016.

747 López-Fernández, S., Diez, J. A., Hernaiz, P., Arce, A., García-Torres, L., and Vallejo,
748 A.: Effects of fertiliser type and the presence or absence of plants on nitrous oxide
749 emissions from irrigated soils. Nutr. Cycl. Agroecosys., 78(3), 279-289, 2007.

750 MacKenzie, A. F., Fan, M. X., and Cadrin, F.: Nitrous oxide emission in three years as
751 affected by tillage, corn-soybean-alfalfa rotations, and nitrogen fertilization. J. Environ.
752 Qual., 27, 698-703, 1998.

753 Martínez-Cob, A.: Use of thermal units to estimate corn crop coefficients under
754 semiarid climatic conditions. Irrigation Sci., 26, 335–345, 2008.

755 Meijide, A., Cárdenas, L. M., Sánchez-Martín, L., and Vallejo, A.: Carbon dioxide and
756 methane fluxes from a barley field amended with organic fertilizers under
757 Mediterranean climatic conditions. Plant Soil, 328(1-2), 353-367, 2010.

758 Migliorati, M. D. A., Scheer, C., Grace, P. R., Rowlings, D. W., Bell, M., and McGree,
759 J.: Influence of different nitrogen rates and DMPP nitrification inhibitor on annual N_2O
760 emissions from a subtropical wheat–maize cropping system. Agric. Ecosyst.
761 Environ., 186, 33-43, 2014.

762 Mørkved, P. T., Dörsch, P., and Bakken, L. R.: The N_2O product ratio of nitrification
763 and its dependence on long-term changes in soil pH. Soil Biol. Biochem., 39(8), 2048-
764 2057, 2007.

765 Nemecek, T., von Richthofen, J. S., Dubois, G., Casta, P., Charles, R., and Pahl, H.:
766 Environmental impacts of introducing grain legumes into European crop rotations. Eur.
767 J. Agron., 28(3), 380-393, 2008.

768 Oorts, K., Merckx, R., Gréhan, E., Labreuche, J., and Nicolardot, B.: Determinants of
769 annual fluxes of CO_2 and N_2O in long-term no-tillage and conventional tillage systems
770 in northern France. Soil Till. Res., 95(1), 133-148, 2007.

771 Poeplau, C., and Don, A.: Carbon sequestration in agricultural soils via cultivation of
772 cover crops–A meta-analysis. Agric. Ecosyst. Environ., 200, 33-41, 2015.

773 Quemada, M., Baranski, M., Nobel-de Lange, M. N. J., Vallejo, A., and Cooper, J. M.:
774 Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems
775 and their effects on crop yield. Agric. Ecosyst. Environ., 174, 1-10, 2013.

776 Quemada, M., Gabriel, J. L., and Zarco-Tejada, P.: Airborne hyperspectral images and
777 ground-level optical sensors as assessment tools for maize nitrogen fertilization. Remote
778 Sens., 6(4), 2940-2962, 2014.

779 Reeves, S., and Wang, W.: Optimum sampling time and frequency for measuring N_2O
780 emissions from a rain-fed cereal cropping system. Sci.Total Environ., 530, 219-226,
781 2015.

782 Rochette, P., and Janzen, H. H.: Towards a revised coefficient for estimating N_2O
783 emissions from legumes. Nutr. Cycl. Agroecosys., 73(2-3), 171-179, 2005.

784 Sanz-Cobena, A., Sánchez-Martín, L., García-Torres, L., and Vallejo, A.: Gaseous
785 emissions of N_2O and NO and NO_3^- leaching from urea applied with urease and
786 nitrification inhibitors to a maize (Zea mays) crop. Agric. Ecosyst. Environ., 149, 64-73,
787 2012.

788 Sanz-Cobena, A., García-Marco, S., Quemada, M., Gabriel, J. L., Almendros, P., and
789 Vallejo, A.: Do cover crops enhance N_2O, CO_2 or CH_4 emissions from soil in
790 Mediterranean arable systems? Sci. Total Environ., 466, 164-174, 2014.

791 Sarkodie-Addo, J., Lee, H. C., and Baggs, E. M.: Nitrous oxide emissions after
792 application of inorganic fertilizer and incorporation of green manure residues. Soil Use
793 Manage., 19(4), 331-339, 2003.

794 Schindlbacher, A., Zechmeister-Boltenstern, S., and Butterbach-Bahl, K.: Effects of soil
795 moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils.
796 J. Geophys. Res.-Atmos., (1984–2012), 109(D17), 2004.

797 Senbayram, M., Chen, R., Mühling, K. H., and Dittert, K.: Contribution of nitrification
798 and denitrification to nitrous oxide emissions from soils after application of biogas
799 waste and other fertilizers. Rapid Commun. Mass Sp., 23(16), 2489-2498, 2009.

800 Shan, J., and Yan, X.: Effects of crop residue returning on nitrous oxide emissions in
801 agricultural soils. Atmos. Environ., 71, 170-175, 2013.

802 Snyder, C. S., Bruulsema, T. W., Jensen, T. L., and Fixen, P. E.: Review of greenhouse
803 gas emissions from crop production systems and fertilizer management effects. Agric.
804 Ecosyst. Environ., 133(3), 247-266, 2009.

805 Soil Survey Staff: Keys to Soil Taxonomy, Washington, DC, USA: USDA, Natural
806 Resources Conservation Service, 2014.

807 Spiertz, J. H. J.: Nitrogen, sustainable agriculture and food security. A review. Agron.
808 Sustain. Dev.,30(1), 43-55, 2010.

809 Stehfest, E., and Bouwman, L.: N_2O and NO emission from agricultural fields and soils
810 under natural vegetation: summarizing available measurement data and modeling of
811 global annual emissions. Nutr. Cycl. Agroecosys., 74, 207-228, 2006.

812 Tate, K. R.: Soil methane oxidation and land-use change–from process to mitigation.
813 Soil Biol. Biochem., 80, 260-272, 2015.

814 Tonitto, C., David, M. B., and Drinkwater, L. E.: Replacing bare fallows with cover
815 crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N
816 dynamics. Agric. Ecosyst. Environ., 112(1), 58-72, 2006.

817 Ussiri, D., and Lal, R.: Soil emission of nitrous oxide and its mitigation, Springer
818 Science & Business Media, 2012.

Vallejo, A., Skiba, U. M., García-Torres, L., Arce, A., López-Fernández, S., and Sánchez-Martín, L.: Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts. Soil Biol. Biochem., 38(9), 2782-2793, 2006.

van Groenigen, J. W., Velthof, G. L., Oenema, O., van Groenigen, K. J., and van Kessel, C.: Towards an agronomic assessment of N_2O emissions: a case study for arable crops. Eur. J. Soil Sci., 61(6), 903-913, 2010.

Vanlauwe, B., Kihara, J., Chivenge, P., Pypers, P., Coe, R., and Six, J.: Agronomic use efficiency of N fertilizer in maize-based systems in sub-Saharan Africa within the context of integrated soil fertility management. Plant Soil, 339(1-2), 35-50, 2011.

Veenstra, J. J., Horwath, W. R., and Mitchell, J. P.: Tillage and cover cropping effects on aggregate-protected carbon in cotton and tomato. Soil Sci. Soc. Am. J., 71(2), 362-371, 2007.

Wagner-Riddle, C., and Thurtell, G.W.: Nitrous oxide emissions from agricultural fields during winter and spring thaw as affected by management practices. Nutr. Cycl. Agroecosys., 52(2-3), 151-163, 1998.

Wichern, F., Eberhardt, E., Mayer, J., Joergensen, R. G., and Müller, T.: Nitrogen rhizodeposition in agricultural crops: methods, estimates and future prospects. Soil Biol. Biochem., 40(1), 30-48, 2008.

Yamulki, S., and Jarvis, S.: Short-term effects of tillage and compaction on nitrous oxide, nitric oxide, nitrogen dioxide, methane and carbon dioxide fluxes from grassland. Biol. Fert. Soils, 36(3), 224-231, 2002.

841 **Table 1** Total cumulative N_2O-N, CH_4-C and CO_2-C fluxes, yield-scaled N_2O emissions and N surplus in the three cover crop treatments (fallow, F, vetch,
842 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
843 standard error of the mean, respectively.

844

	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}	Yield-scaled N_2O emissions 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**

845 **Figure captions:**

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

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

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

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

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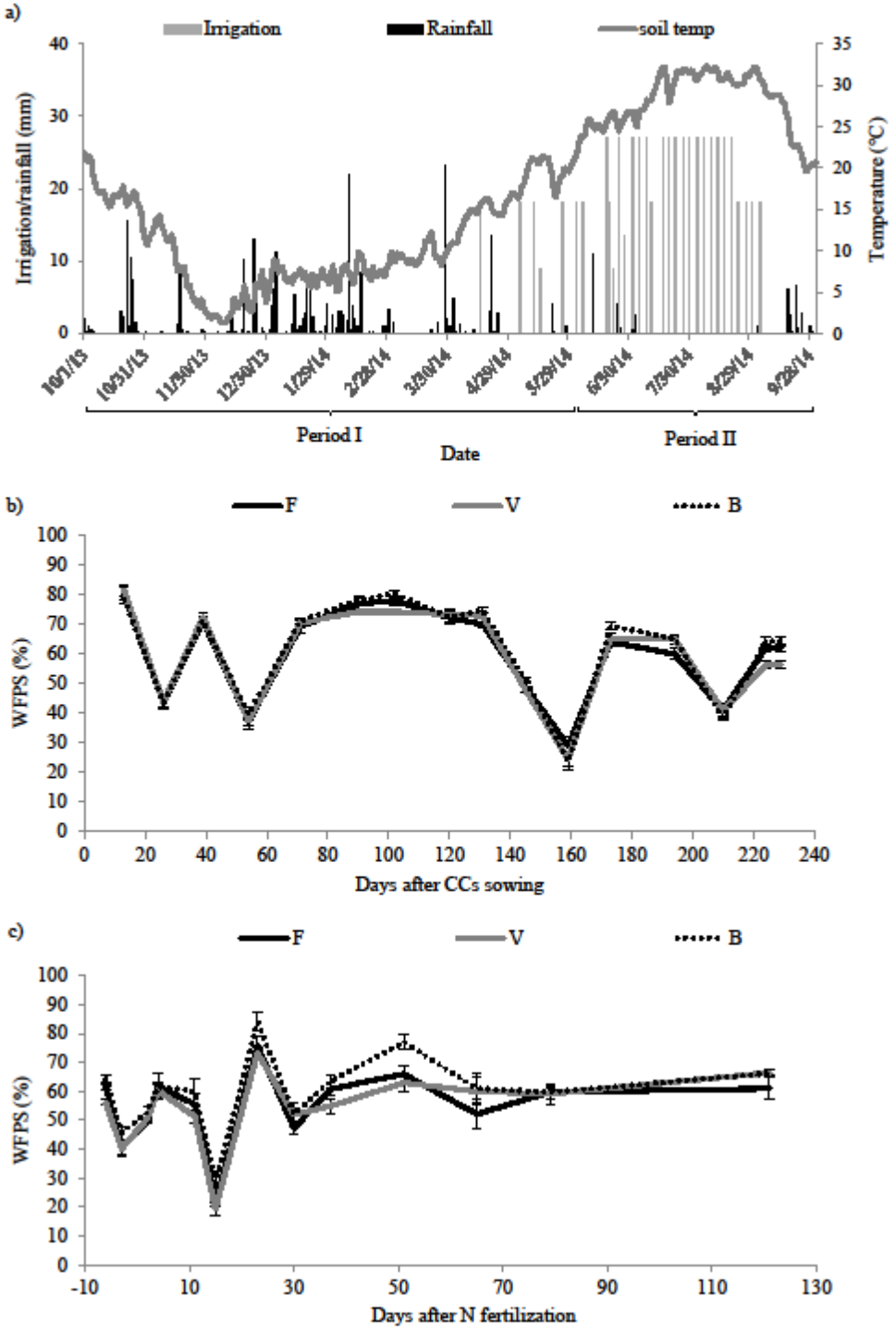

fig01

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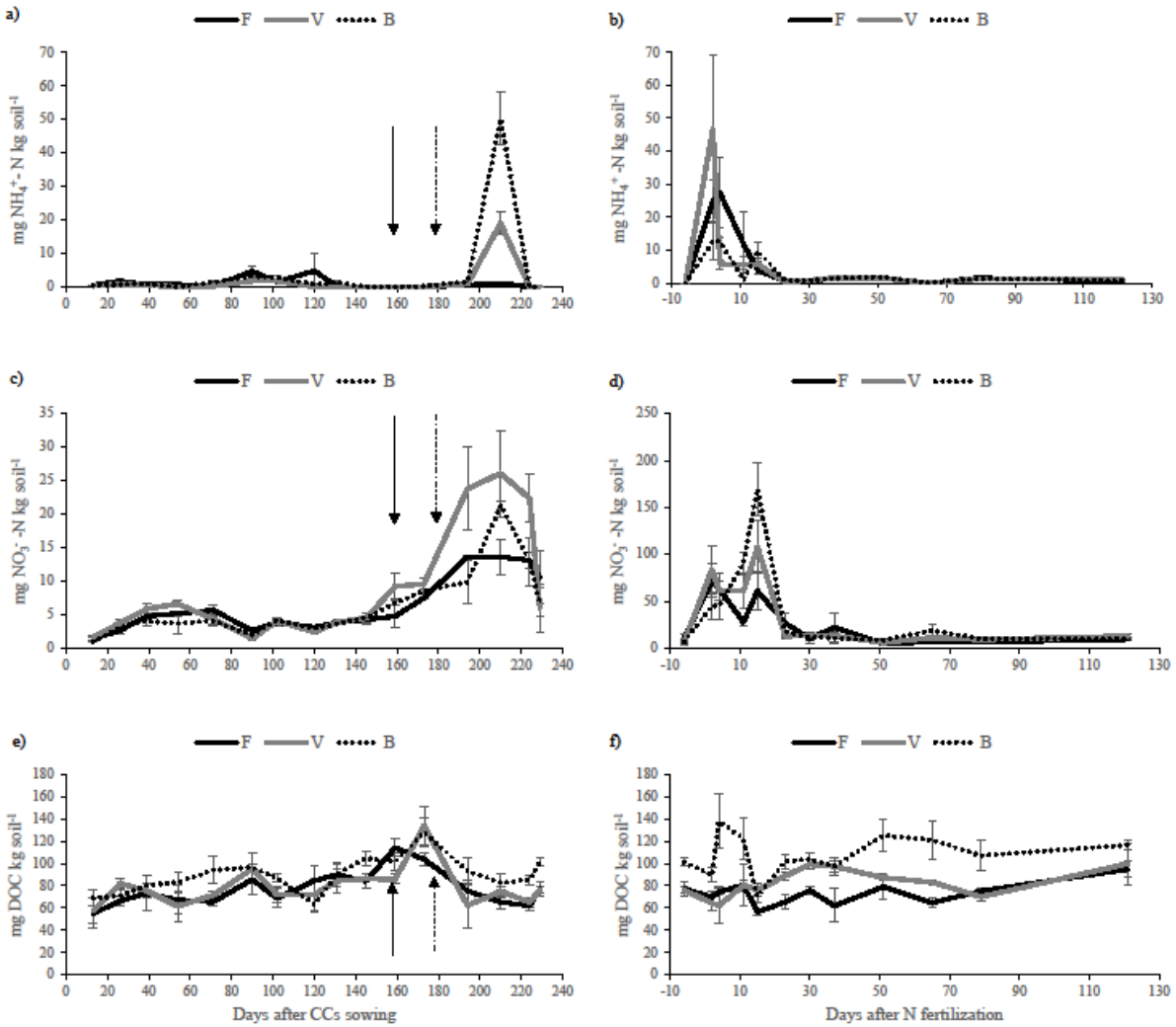
a)
F
V
B
mg NH_4^+-N kg soil^{-1}
b)
mg NH_4^+-N kg soil^{-1}
c)
mg NO_3^--N kg soil^{-1}
d)
mg NO_3^--N kg soil^{-1}
e)
mg DOC kg soil^{-1}
Days after CCs sowing
f)
mg DOC kg soil^{-1}
Days after N fertilization

fig02

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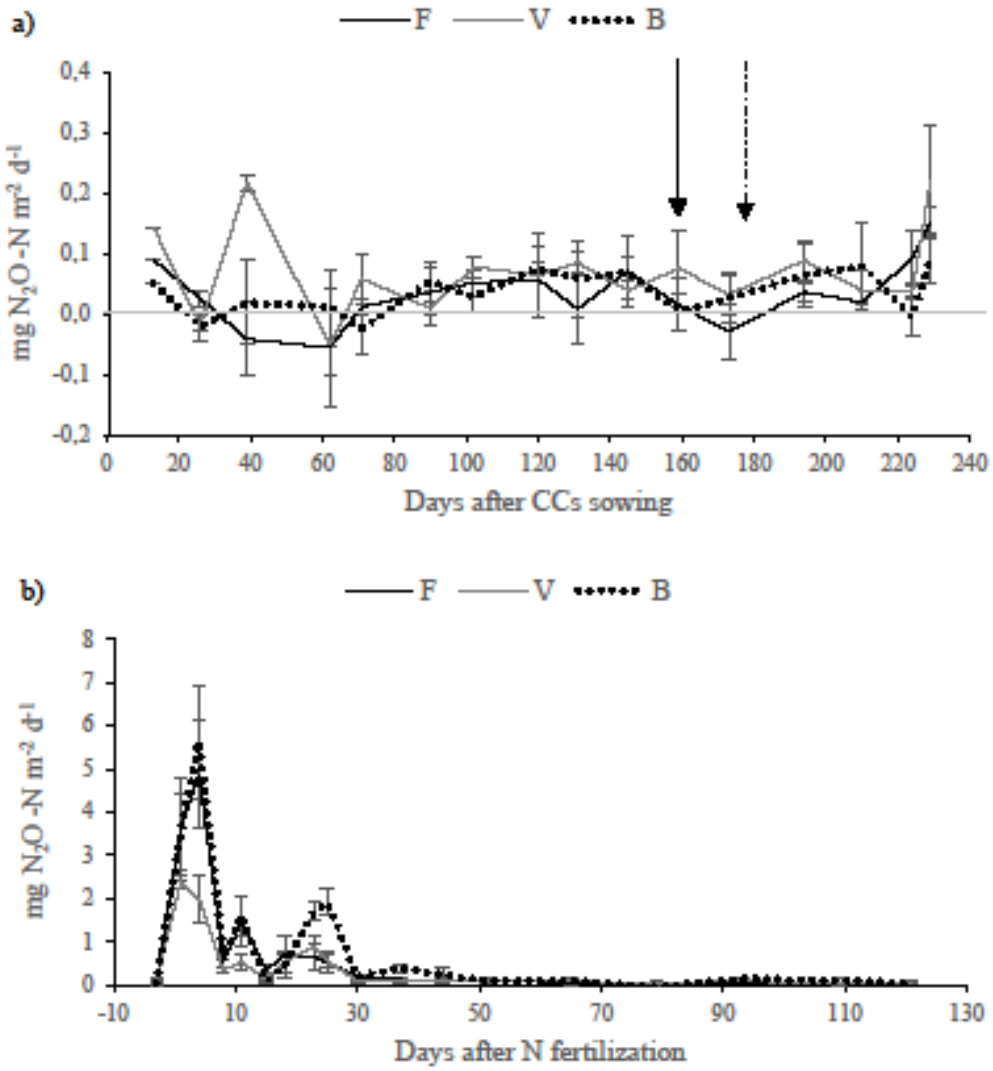

fig03

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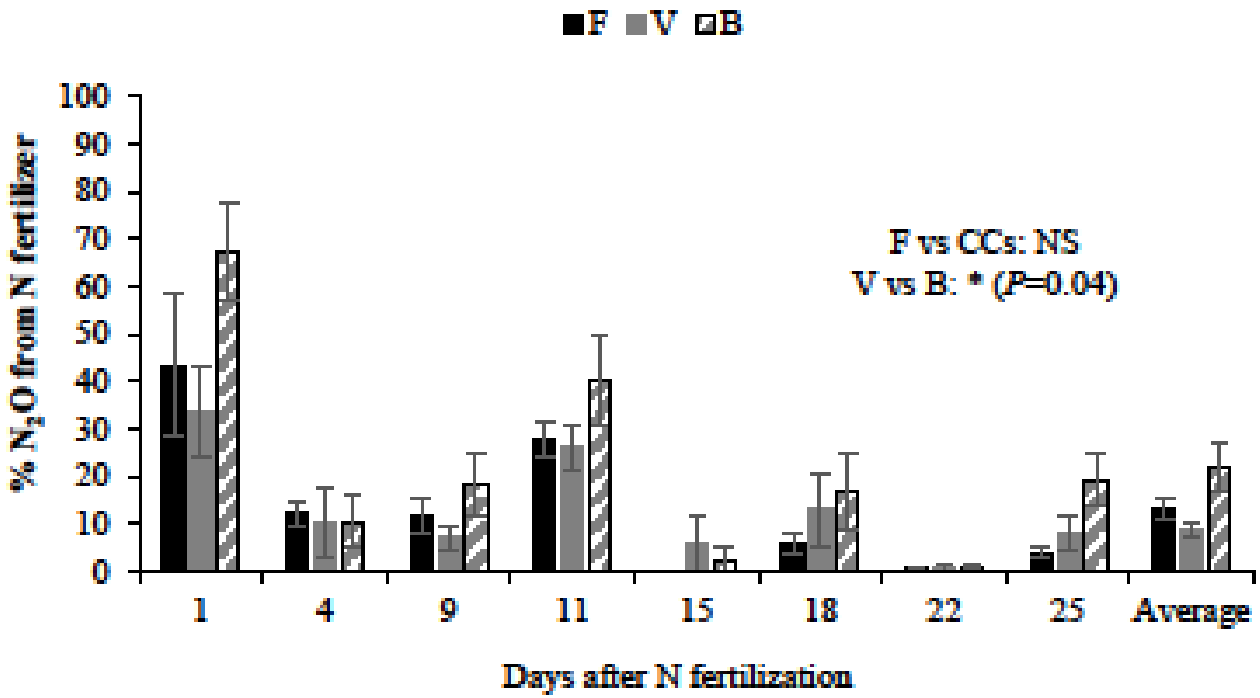

fig04

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