Response to Reviewer #1 comments

1. To me this MS presents rather limited novelty to the study by Sanz-Cobena et al. (2014). Also the added 15N approach brings nothing really new to the current knowledge. The authors should therefore elaborate more clearly the novel and innovative character of their research.

We have tried to highlight in the Manuscript the novelty that our study has with respect to Sanz-Cobena et al. (2014). One of the main differences is the use of Integrated Soil Fertility Management (ISFM) in the current study as opposed to conventional fertilization in Sanz-Cobena et al. (2014). The results of the latter study hinted that the effects in soil N availability induced by contrasting cover-crops could represent an opportunity to adjust N fertilization for the cash crop accordingly, without significant yield penalties. This innovative point has now been highlighted in the title ("Effect of cover crops on greenhouse gas emissions in an irrigated field under integrated soil fertility management") and the introduction: "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."

With regards to the ^{15}N approach, we agree that there are some previous studies which have evaluated the interactive effects of different crop residues with N synthetic fertilization through ^{15}N methods (e.g. Baggs et al., 2003; Garcia-Ruiz and Baggs, 2007; Frimpong et al., 2011). Furthermore, ^{15}N has been used in different cover-cropping experiments (e.g. Bergstrom et al. 2001; Jayasundara et al., 2007; Gabriel and Quemada, 2011, Gabriel et al., 2016) but all of these studies were focused on plant recovery or N leaching. The study of Li et al. (2016) measured [15]N_2O after the application of different CC residues (including roots) and N synthetic fertilizer but under laboratory conditions. To our knowledge, no previous studies have evaluated the relative contribution of CC residues/soil N (which involve the aboveground biomass and the decomposition of root biomass) and N synthetic fertilizers to N_2O emissions under field conditions employing stable isotope techniques. We have elaborated more clearly this novel point in the introduction: "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."

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

Bergström, L. F., and Jokela, W. E.: Ryegrass Cover Crop Effects on Nitrate Leaching in Spring Barley Fertilized with (15)NH4(15)NO3. J. Environ. Qual., 30(5), 1659-1667, 2001.

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

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

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

Garcia-Ruiz, R., and Baggs, E. M.: N_2O emission from soil following combined application of fertiliser-N and ground weed residues. Plant Soil, 299(1-2), 263-274, 2007.

Jayasundara, S., Wagner-Riddle, C., Parkin, G., von Bertoldi, P., Warland, J., Kay, B., and Voroney, P.: Minimizing nitrogen losses from a corn–soybean–winter wheat rotation with best management practices. Nutr. Cycl. Agroecosys., 79(2), 141-159, 2007.

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

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

2. What is rather "non-innovative" is the fact, that the cover crops are killed chemically with glyphosate. This is somewhat disappointing for research in agricultural sustainability, as the safe use of glyphosate is under discussion since years. There are alternatives in place also for Mediterranean regions and might be found among farmers applying organic no-till agriculture. The authors should address this topic in the discussion section, that the application of glyphosate for cover crop management is disputable and alternative measures to remove the cover crops with smart methods are needed (e.g. European project TILMAN-ORG).

We agree and are aware that the application of glyphosate is under discussion since years, and now more than ever in the European Union it is a matter under the spotlight. However, the use of non-selective herbicides is a standard and broadly used method followed by conservation tillage growers for cover crop killing in Spain and many other regions. Another alternative for this kind of systems would be mowing but the adequate control is not always achieved, mainly in the case of legumes, in which regrowth is very common. The roller-crimper may be an alternative method but, as well, the legume killing effectiveness is under discussion. Therefore, the glyphosate use seemed an appropriate option that would ensure the killing in both barley and vetch treatments. Moreover, as the study was carried out in a long-term

experiment of cover cropping system, it was decided to maintain the same killing method each year. Clearly, further research is needed to investigate this interesting topic, but we considered that it did not fit in any of the subsections of the discussion. Therefore, in the Materials and Methods section of the revised manuscript we have included more information with regards to the use of glyphosate as the killing method in our study: "The cover cropping phase finished on March 14th 2014 following local practices, with an application of glyphosate (N-phosphonomethyl glycine) at a rate of 0.7 kg a.e. ha-1. Even though the safe use of glyphosate is under discussion since years (Chang and Delzell, 2016), it was used in order to preserve the same killing method in all the campaigns in this long-term experiment under conservation tillage management".

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.

3. Cover crop establishment: I am wondering that a hand broadcast technique is used for CC seeding. This might cause too many heterogeneities and influence yield-scaled N2O emissions. Please discuss.

In order to reduce economic costs to farmers interested in cover crops, a suitable choice for sowing would be the use of a centrifugal spreader. As the plot size was 12 x 12 m^2, the best way to emulate this type of sowing was by hand broadcasting. Results from several previous years and tests showed that this system ensures high homogeneity. Specifically, from cover crop emergence until its killing date, the ground cover was monitored by taking digital photos of four squares (0.5 x 0.5 m^2) marked in each plot and lately analyzed with a software based on colorimetry. At the first sampling date (23/10/2013), no differences were observed between vetch samples (ground coverage: 4.3% ± 0.2%), nor in barley (6.7% ± 0.5%).

4. The authors use too many and sometimes unnecessary abbreviations, please adapt.

We thank the reviewer for this remark. Some unnecessary abbreviations, e.g. ammonium nitrate (AN), yield-scaled N$_2$O emissions (YSNE), N use efficiency (NUE), dry matter (DM) have been removed. If the reviewer thinks that more abbreviations should be removed, we will do it.

5. Chambers for GHG sampling: I found it a bit too shallow to insert the stainless rings only 5 cm deep into the soil. There is a high risk of lateral N2O emission, when the rings/collars are inserted not deep enough (> 10 cm). Please explain.

We thank the reviewer for this comment and we agree that the stainless rings should have been inserted deeper. The rings we used had a height of approximately 10 cm and were inserted into the ground to a depth of ≥5 cm to get a practical height above soil surface of 4-5 cm needed to insert the chamber just above the ground, also preventing water accumulation in the soil surface due to irrigation. We have calculated our average air-filled porosity, which was slightly below 0.3 cm^3 cm^{-3}. Considering our chamber closure time, the average error may be slightly above 5% (since 6.2 cm is the adequate insertion depth for an air-filled porosity of 0.3 cm^3 cm^{-3} and one hour of closure time leading to an error of 5%) (Hutchinson and Livingston, 2001). In further experiments, we will adjust more accurately the insertion depth taking into account our experimental conditions, in order to reduce the error to a minimum.

Hutchinson, G. L., and Livingston, G. P.: Vents and seals in non-steady-state chambers used for measuring gas exchange between soil and the atmosphere. Eur. J. Soil Sci., 52(4), 675-682, 2001.

Response to Reviewer #2 comments

L. 1-2: The title "Integrated soil fertility management drives the effect of cover crops on GHG emissions in an irrigated field" is hard to understand, if not misleading; it gives the impression that we are dealing with a "mechanistic" which after all is not the case.
Even though the 15N experiment clearly showed that barley residues stimulated N2O emissions from AN fertilizer, the mechanisms behind remain elusive. This is a well conducted descriptive study, which should be reflected in the title. I suggest to change the title.

We agree with the reviewer's suggestion. We propose a new title more in line with descriptive studies: "Effect of cover crops on greenhouse gas emissions in an irrigated field under integrated soil fertility management".

L. 19: Cumulative N2O emissions were indeed low; but who can say whether this was due to ISFM? It was due to the low fertilization rates, perhaps, but this is not specific for ISFM and there was no control following principals other than ISFM.

We agree with the reviewer that low fertilization rates caused N_2O losses to be low, but these fertilization rates were a consequence of ISFM management, since the crop N requirements were partially supplied through soil inorganic N (measured after the CC killing) and N mineralization, thus reducing the amount of synthetic N. The specific pedo-climatic conditions of our study probably played a role too. The sentence has been changed for better understanding: "Our management (adjusted N synthetic rates due to ISFM) and pedo-climatic conditions resulted…" instead of "The ISFM resulted…"

L. 19. Cumulative N2O emissions lack time dimension

Thanks. This has been corrected (the units are now kg N_2O-N ha^{-1} yr^{-1}).

L. 67-69: This section sounds like making hypotheses after the event; if you want to make a point out of the fact that chemically mulched barley can lead to more N2O emissions during the cash crop phase because it fuels denitrification, offer some explanation why and when you would expect denitrification in a silty clay loam under irrigation. State more precisely that a stimulation of N2O emissions from denitrification by high C/N residues should strictly speaking only occur in the presence of ample nitrate, i.e. right after fertilization.

We thank the reviewer for this comment and we agree that this point should be better explained. More information and references have been added to this paragraph: "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

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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 rainfall or irrigation events, particularly in fine-textured soils) (Stehfest and Bouwman 2006; Baral et al., 2016)".

L. 127 ff.: Soil physico-chemical properties. The soil has a very high pH, high bulk density and low organic carbon. Being in its 8th year of intercropping versus winter fallow, should one expect differences in soil properties among these treatments? And could this explain slight differences in WFPS? Please comment or give soil properties per treatment.

On average, no significant differences between treatments were obtained with regards to soil WFPS. The higher values in B plots in some sampling dates could be a result of increased soil organic matter content in B plots (due to the high C:N residues in this long-term experiment), which could be associated to an enhancement of water-holding capacity (Dabney et al., 2001; Karhu et al., 2011; Hubbard et al., 2013). Since these higher WFPS values were found only in few sampling dates and mean contents did not differ between treatments, we have not discussed these issue in the manuscript, trying to avoid speculative statements.

Soil mineral N and DOC concentrations at the beginning of the experimental period were given in the manuscript for the different treatments. We did not expect differences between treatments in other physico-chemical properties (e.g. pH, texture) due to the different cover cropping treatments in the upper horizon, which was more influenced by the tillage system adopted (conservation tillage). These effects will be evaluated in further campaigns at the same experimental site.

Dabney, S. M., Delgado, J. A., and Reeves, D. W.: Using winter cover crops to improve soil and water quality. Commun. Soil Sci. Plan., 32(7-8), 1221-1250, 2001.

Karhu, K., Mattila, T., Bergström, I., and Regina, K.: Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity–results from a short-term pilot field study. Agric. Ecosyst. Environ., 140(1), 309-313, 2011.

Hubbard, R. K., Strickland, T. C., and Phatak, S.: Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil Till. Res., 126, 276-283, 2013.

L. 159: Why does ISFM maize with barley as intercrop receive 20 kg more N than with traditional winter fallow? Please explain.
L. 162: How was N mineralization from vetch and barley residues estimated?

In order to explain L159 and L162 comments, we will describe in detail the calculation that justifies the choice of different fertilizer doses. For this calculation, the soil inorganic N, N crop requirements, and N mineralization were taken into account as follows:
- Crop requirements (Nc) were 236.3 kg N ha^{-1} (Quemada et al., 2014).
- Soil inorganic N (N_{min}) was determined to 1-m depth in April, after the CC killing. Values obtained were: fallow = 47.7 kg N ha^{-1}; barley = 29.9 kg N ha^{-1}; vetch = 45.3 kg N ha^{-1}.
For the fallow treatment, the N mineralization ($N_{mineralization}$) considered was 71 kg N ha^{-1}, a value observed previous years in the same plots. For barley and vetch treatments, to this value was added the N coming from the mineralization of cover crop residues, estimated as 50% of

the cover crop N content. Biomass and %N concentration, necessary to calculate N content, were determined in each cover crop species at the killing moment.

Besides, an efficiency of Nitrogen use efficiency (Ef) of 70% was considered.

Therefore, the rate calculation was as follow:

$N_f = [N_c – (N_{min} + N_{mineralization})] / Ef$

N_f fallow = [236.3 – (47.7 +71)]/ 0.7 = 169.3 → 170 kg N ha^{-1}

N_f barley = [236.3 – (29.9 +74.6)]/ 0.7 = 188.3 → 190 kg N ha^{-1}

N_f vetch = [236.3 – (45.3 +90.5)]/ 0.7 = 143.5 → 140 kg N ha^{-1}

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

L. 170: Would you expect that ammonia volatilization at pH 8.2 differs in plots with and without mulched CCs, even after irrigation? Please comment

The presence of mulched CCs could have affected NH_3 volatilization, but we think that these losses were small (due to irrigation after fertilization and the type of N source –ammonium nitrate and crop residues-) (Sanz-Cobena et al., 2011; Bittman et al., 2014) with respect to those of N_2O, and the differences between treatments were, therefore, negligible.

Bittman, S., Dedina, M., Howard, C.M., Oenema, O., Sutton, M.A., 2014. Options for ammonia mitigation: guidance from the UNECE task force on reactive nitrogen. NERC/Centre for Ecology & Hydrology.

Sanz-Cobena, A., Misselbrook, T., Camp, V., Vallejo, A., 2011. Effect of water addition and the urease inhibitor NBPT on the abatement of ammonia emission from surface applied urea. Atmospheric Environment, 45(8), 1517-1524.

L. 220: PLOT columns are primarily for separating inert gases, not for "transporting"

We thank the reviewer's remark. The sentence has been changed: "Inert gases were separated by HP Plot-Q capillary columns. The gas chromatograph was equipped with a ^{63}Ni electron-capture detector (Micro-ECD) to analyze N_2O concentrations, and with a flame ionization detector (FID) connected to a methanizer to measure CH_4 and CO_2 (previously reduced to CH_4)".

L. 223: replace "detector" with "ECD". The FID is not heated.

Thanks. The change has been made.

L. 243: how was the temperature correction carried out? Opaque chambers deployed for 1 hour in a Mediterranean climate may lead to quite some heating of the chamber air. Did you measure temperatures within the chambers?

The chambers were all covered with radiant barrier reflective foil. In spite of this covering, the temperature inside the chamber increased compared to the temperature outside the chamber. For this reason, thermometers were placed inside three randomly selected

chambers during the closure period of each measurement and the fluxes were corrected for temperature. New information has been included to clarify this point: "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" and "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). Thermometers were placed inside three randomly selected chambers during the closure period of each measurement and the fluxes were corrected for temperature."

L. 256: for equation 1, Senbayram et al. (2009) should be cited and not Loick et al. (2016).

Ok, we have replaced Loick et al. (2016) by Senbayram et al. (2009).

Equ. 1 requires the knowledge of 15N atm% excess of emitted N2O (L. 257). This is not equal to the atm% of a sample collected after 1 hour chamber deployment minus the atm% at natural abundance (L. 258)! Senbayram applied this equation to a He-flushed closed flow-through system in which subsampled N2O directly relates to emitted N2O. In the present case, the sample is retrieved from a static chamber in which newly produced N2O mixes with abundant "old" N2O. A Keeling plot approach or some mixing calculation should be applied to derive the true 15N excess of soil emitted N2O before calculating the fraction of N2O derived from AN.

We have followed Senbayram et al. (2009) instructions for the sampling and calculations, and there is no other mixing equation needed. The same equation has been used in several previous studies, such as Lampe et al. 2006 (Sources and rates of nitrous oxide emissions from grazed grassland after application of ^{15}N-labelled mineral fertilizer and slurry) and Di and Cameron 2008 (Sources of nitrous oxide from ^{15}N-labelled animal urine and urea fertiliser with and without a nitrification inhibitor, dicyandiamide (DCD)).
The text of Senbayram refers to static chamber as follows: "For (N_2O) measurements, PVC chambers (60cm diameter × 25 cm height) were sealed onto the basal rings and gas samples were taken with 12-mL evacuated Exetainers, 0, 20 and 40 minutes after chamber closure."

L. 271: Did you filter the extract before DOC analysis? Which pore size?

Yes, the extract was filtered before DOC analysis using qualitative filter paper 1300/80 (Filter-Lab ®). This information has been added to the manuscript.

L. 323: : : : most of the time

Thanks. This has been corrected.

L. 325: add that the statistically significant difference in soil ammonium between treatments was found on one sampling date only.

Ok, this has been added to the sentence: "Mean NH_4^+ content was significantly higher in B than in F ($P<0.05$), but daily NH_4^+ concentrations between treatments were only significantly different between treatments in one sampling date (210 days after CCs sowing)".

L. 330: from figure 2e, it is not obvious that mean DOC contents were higher in B than in V, and if so, the difference was marginal. Besides, ordinary ANOVA on averaged time series data are not particularly helpful here. Did you use repeated-measure Anova?

We agree with the reviewer that differences in average contents were small, but with a high level of significance ($P<0.01$, this has been corrected in the text). New information has been included in the paragraph ("Average topsoil DOC content was significantly higher in B than in V and F (10% and 12%, respectively, $P<0.01$) but differences were only observed in some sampling dates"). We included Fig. 2 as a qualitative and informative representation of the evolution of mineral N and DOC. We tried a repeated-measure ANOVA, but the results did not provide useful information in addition to that of the figure and the average data, besides that the time*treatment interactions complicated the interpretation of the analysis.

L. 344. How can it be that CO2 emissions in plots with intercrops are only insignificantly higher than those in the fallow, of you include plants in your dark chambers and mulch half to 1 tons of dry matter per hectare. Any explanation? Was there a lot of weeds in the fallow? Please give details.

That was an unexpected result. During fall and early winter, low temperatures limited the growth of CCs, and soil respiration rates were small in all treatments. Conversely, from mid-February to the end of CC phase, differences between treatments were higher. We have carried out an ANOVA of average fluxes during this period, and CO_2 emissions were significantly higher in B treatment with respect to F, with V showing intermediate values. This information has been added to the text in the Results ("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) in the whole intercrop period, but soil respiration was increased in B, with respect to F, from mid-February to the end of Period I") and Discussion section ("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").

L. 388: as outlined above, I believe the absolute numbers for this proportion are wrong.
Interestingly, the proportions fluctuated strongly in time but less so across treatments.
Did you try to correlate the proportions with any of your ancillary variables (WFPS, temperature, NO3-)?

Thanks for this remark. Please see our answer to the comment on line 256. Following your suggestion, we have tried to correlate the proportions with these variables. We obtained a significant correlation between DOC content and the proportion coming from the synthetic fertilizer ($P<0.05$, $n=12$, $r=0.71$). These information has been added to the Results ("The mean

percentage of N_2O losses from synthetic fertilizer throughout all sampling dates was 2.5 times higher in B compared to V ($P<0.05$) and was positively correlated with DOC concentrations ($P<0.05$, n=12, r=0.71)") and the Discussion section ("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").

L. 447: the importance : : : for

Thanks. This has been corrected.

L. 447: not clear what you mean by "mineral N harbored in soil micropores"

The sentence has been changed. The new sentence is "…revealed the importance of soil mineral N contained in the micropores for the N_2O bursts after the first irrigation events, with respect to the N released from CC residues".

L. 449: I still don't understand what your finding of larger fertilizer derived N2O emission in B treatments has to do with ISFM, if ISFM denotes the simple fact that the three treatments received slightly different amounts of fertilizer N. Wouldn't you expect the same without ISFM?

We agree, the term "ISFM" is unnecessary here. The sentence "As we hypothesized, although ISFM practices were adopted, the different CCs played a key role in the N_2O emissions during Period II", has been changed to "As we hypothesized, the different CCs played a key role in the N_2O emissions during Period II".

L. 491 ff.: include soil pH in the discussion of possible reasons for the overall low emissions and emission factors

New information about the effect of soil pH on N_2O emissions has been included: "We hypothesized that management practices may have contributed to these low emissions, but other inherent factors such as the high soil pH could have played a role too. 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)".

L. 536 and 568: optimal balance between GHG emissions and agronomic efficiency provided by ISFM; I do not think you have evidence enough in your data to claim an optimal balance, as long as there is no control experiment receiving equal amounts of mineral fertilizers.

Thanks for your remark.

The following sentence has been deleted from de Manuscript: "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)".

New information and references about the effect of adjusting N synthetic rate has been added: "Adjusting fertilizer N rate to soil endogenous N led to lower N_2O fluxes than previous experiments conducted under similar environmental conditions where conventional N rates were applied (e.g. Adviento-Borbe et al., 2007; Hoben et al., 2011; Sanz-Cobena et al., 2012; Li et al., 2015), in agreement with the study of Migliorati et al. (2014). Moreover, CO_2 equivalent emissions associated to manufacturing and transport of N synthetic fertilizers (Lal, 2004) can be reduced when low synthetic N input strategies, such as ISMF, are employed".

The second sentence (in the Conclusions) has been changed for better understanding: "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 with ISFM could be considered an efficient practice from both environmental and agronomic points of view, leading to similar N_2O losses per kilogram of aboveground N uptake as fallow".

Effect of cover crops on greenhouse gas emissions in an irrigated field under integrated soil fertility management ~~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~~Our management (adjusted N synthetic rates due to ISFM) and pedo-climatic ~~resulted~~conditions resulted in low cumulative N_2O emissions (0.57 to 0.75 kg N_2O-N ha^{-1} yr^{-1}~~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 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 N use efficiencyNUE (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

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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

3

74 when soil conditions are favorable (e.g. high NO_3^- availability and soil moisture after

75 rainfall or irrigation events, particularly in fine-textured soils) (Stehfest and Bouwman

76 2006; Baral et al., 2016)Conversely, the higher C:N ratio of their residues as compared

77 to those of legumes may provide energy for denitrifiers, thereby leading to higher N_2O

78 losses in the presence of mineral N from fertilizers (Sarkodie Addo et al., 2003).

79 MoreoverBesides, winter CCs can also abate indirect gaseous N losses through the

80 reduction of leaching and subsequent emissions from water resources (Feyereisen et al.,

81 2006). Thus, the estimated N_2O mitigation potential for winter CCs ranges from 0.2 to

82 1.1 kg N_2O ha^{-1} yr^{-1} according to Ussiri and Lal (2012).

83   In a CC-maize rotation system, mineral fertilizer application to the cash crop

84 could have an important effect on N use efficiencyNUE and N losses from the agro-

85 ecosystem. Different methods for calculating the N application rate (e.g. conventional or

86 integrated) can be employed by farmers, affecting the amount of synthetic N applied to

87 soil and the overall effect of CCs on N_2O fluxes. Integrated Soil Fertility Management

88 (ISFM) (Kimani et al., 2003) provides an opportunity to optimize the use of available

89 resources, thereby reducing pollution and costs from over-use of N fertilizers

90 (conventional management). ISFM involves the use of inorganic fertilizers and organic

91 inputs, such as green manure, aiming to maximize agronomic efficiency (Vanlauwe et

92 al., 2011). When applying this technique to a CC-maize crop rotation, N fertilization

93 rate for maize is calculated taking into account the background soil mineral N and the

94 expected available N from mineralization of CC residues, which depends on residue

95 composition. Differences in soil mineral N during the cash crop phase may be

96 significantly reduced if ISFM practices are employed, affecting the GHG balance of the

97 CC-cash crop cropping system.

98   Only one study has investigated the effect of CCs on N_2O emissions in
99   Mediterranean cropping systems (Sanz-Cobena et al., 2014). These authors found an
100   effect of CCs species on N_2O emissions during the intercrop period. After 4 years of CC
101   (vetch, barley or rape)-maize rotation, vetch was the only CC species that significantly
102   enhanced N_2O losses compared to fallow, mainly due to its capacity to fix atmospheric
103   N_2 and because of higher N surplus from the previous cropping phases in these plots. In
104   this study a conventional fertilization (same N synthetic rate for all treatments) was
105   applied during the maize phase; how ISFM practices may affect these findings remains
106   unknown. Moreover, the relative contribution of mineral N fertilizer, CC residues
107   and/or soil mineral N to N_2O losses during the cash crop has not been assessed yet. In
108   this sense, stable isotope analysis (i.e. [15]N) represents a way to identify the source and
109   the dominant processes involved in N_2O production (Arah, 1997). Stable Isotope
110   techniques have been used in field studies evaluating N leaching and/or plant recovery
111   in systems with cover crops (Bergström et al., 2001; Gabriel and Quemada, 2011;
112   Gabriel et al., 2016). Furthermore, some laboratory studies have evaluated the effect of
113   different crop residues on N_2O losses using ^{15}N techniques (Baggs et al., 2003; Li et al.,
114   2016); but to date, no previous studies have evaluated the relative contribution of cover
115   crops (which include the aboveground biomass and the decomposition of root biomass)
116   and N synthetic fertilizers to N_2O emissions under field conditions.Moreover, the
117   relative contribution of mineral N fertilizer, CC residues and/or soil mineral N to N_2O
118   losses during the cash crop has not been assessed yet. In this sense, stable isotope
119   analysis (i.e. [15]N) has emerged as a way to identify the source and the dominant
120   processes involved in N_2O production (Arah, 1997). A A comprehensive understanding
121   of the N_2O biochemical production pathways and nutrient sources is crucial for the
122   development of effective mitigation strategies.

123   The objective of this study was to evaluate the effect of two different CC species

124   (barley and vetch) and fallow on GHG emissions during the CC period and during the

125   following maize cash crop period in an ISFM system. An additional objective was to

126   study the contribution of the synthetic fertilizer and other N sources to N_2O emissions

127   using ^{15}N labelled fertilizer. We hypothesized that: 1) the presence of CCs instead of

128   fallow would affect N_2O losses, leading to higher emissions in the case of the legume

129   CC (vetch) in accordance with the studies of Basche et al. (2014) and Sanz-Cobena et

130   al. (2014); and 2) in spite of the ISFM during the maize period, which theoretically

131   would lead to similar soil N availability for all plots, the distinct composition of the CC

132   residues would affect N_2O emissions. In order to test these hypotheses, a field

133   experiment was carried out using the same management system for 8 years, measuring

134   GHGs during the 8th year. To gain a better understanding of the effect of the

135   management practices tested on the overall GHG budget of a cropping system, CH_4,

136   CO_2 and yield-scaled N_2O emissions were also analyzed during the experimental

137   period. The relative contribution of each N source (synthetic fertilizer or soil

138   endogenous N, including N mineralized from the CCs) to N_2O emissions was also

139   evaluated by ^{15}N-labelled ammonium nitrate (AN) in a parallel experiment.

140

2. Materials and methods

141

2.1. Site characteristics

142

143   The study was conducted at "La Chimenea" field station (40°03′N, 03°31′W,

144   550 m a.s.l.), located in the central Tajo river basin near Aranjuez (Madrid, Spain),

145   where an experiment involving cover cropping systems and conservation tillage has

146   been carried out since 2006. Soil at the field site is a silty clay loam (*Typic Calcixerept*;

147 Soil Survey Staff, 2014). Some of the physico-chemical properties of the top 0–10 cm

148 soil layer, as measured by conventional methods, were: pH_{H2O}, 8.16; total organic C,

149 19.0 g kg^{-1}; $CaCO_3$, 198 g kg^{-1}; clay, 25%; silt, 49% and sand, 26%. Bulk density of

150 the topsoil layer determined in intact core samples (Grossman and Reinsch, 2002) was

151 1.46 g cm^{-3}. Average ammonium (NH_4^+) content at the beginning of the experiment

152 was 0.42±0.2 mg N kg soil^{-1} (without differences between treatments). Nitrate

153 concentrations were 1.5±0.2 mg N kg soil^{-1} in fallow and barley and 0.9±0.1 mg N kg

154 soil^{-1} in vetch. Initial dissolved organic C (DOC) contents were 56.0±7 mg C kg soil^{-1} in

155 vetch and fallow and 68.8±5 mg C kg soil^{-1} in barley. The area has a Mediterranean

156 semiarid climate, with a mean annual air temperature of 14 °C. The coldest month is

157 January with a mean temperature of 6 °C, and the hottest month is August with a mean

158 temperature of 24 °C. During the last 30 years, the mean annual precipitation has been

159 approximately 350 mm (17 mm from July to August and 131 mm from September to

160 November).

161 Hourly rainfall and air temperature data were obtained from a meteorological

162 station located at the field site (CR10X, Campbell Scientific Ltd, Shepshed, UK). A

163 temperature probe inserted 10 cm into the soil was used to measure soil temperature.

164 Mean hourly temperature data were stored on a data logger.

165

166 *2.2 Experimental design and agronomic management*

167 Twelve plots (12m × 12m) were randomly distributed in four replications of

168 three cover cropping treatments, including a cereal and a legume: 1) barley (B)

169 (*Hordeum vulgare* L., cv. Vanessa), 2) vetch (V) (*Vicia sativa* L., cv. Vereda), and 3)

170 traditional winter fallow (F). Cover crop seeds were broadcast by hand over the stubble

171 of the previous crop and covered with a shallow cultivator (5 cm depth) on October 10th

172 2013, at a rate of 180 and 150 kg ha^{-1} for B and V, respectively. The cover cropping

173 phase finished on March 14th 2014 following local practices, with an application of

174 glyphosate (N-phosphonomethyl glycine) at a rate of 0.7 kg a.e. ha^{-1}. Even though the

175 safe use of glyphosate is under discussion since years (Chang and Delzell, 2016), it was

176 used in order to preserve the same killing method in all the campaigns in this long-term

177 experiment under conservation tillage management. All the CC residues were left on top

178 of the soil. Thereafter, a new set of N fertilizer treatments was set up for the maize cash

179 crop phase. Maize (*Zea mays* L., Pioneer P1574, FAO Class 700) was direct drilled on

180 April 7th 2014 in all plots, resulting in a plant population density of 7.5 plants m^{-2};

181 harvesting took place on September 25th 2014. The fertilizer treatments consisted of

182 ammonium nitrate AN applied on 2nd June at three rates: 170, 140 and 190 kg N ha^{-1} in

183 F, V and B plots, respectively, according to ISFM practices. For the calculation of each

184 N rate, the N available in the soil (which was calculated following soil analysis as

185 described below), the expected N uptake by maize crop, and the estimated N

186 mineralized from V and B residues were taken into account, assuming that crop

187 requirements were 236.3 kg N ha^{-1} (Quemada et al., 2014). Estimated N use

188 efficiencyNUE of maize plants for calculating N application rate was 70% according to

189 the N use efficiencyNUE obtained during the previous years in the same experimental

190 area. Each plot received P as triple superphosphate (45% P_2O_5, Fertiberia$^{®}$, Madrid,

191 Spain) at a rate of 69 kg P_2O_5 ha^{-1}, and K as potassium chloride (60% K_2O, Fertiberia$^{®}$,

192 Madrid, Spain), at a rate of 120 kg K_2O ha^{-1} just before sowing maize. All N, P and K

193 fertilizers were broadcast by hand, and immediately after N fertilization the field was

194 irrigated to prevent ammonia volatilization. The main crop previous to sowing CCs was

195   sunflower (*Helianthus annuus* L., var. Sambro). Neither the sunflower nor the CCs were

196   fertilized.

197   In order to determine the amount of N_2O derived from the N fertilizers, double-

198   labelled ~~AN~~ ammonium nitrate ($^{15}NH_4^{15}NO_3$, 5 % atom ^{15}N, from Cambridge Isotope

199   Laboratories, Inc., Massachusetts, USA) was applied on 2m x 2m subplots established

200   within each plot at a rate of 130 kg N ha^{-1}. In order to reduce biases due to the use of

201   different N rates (e.g. apparent priming effects or different mixing ratios between the

202   added and resident soil N pools) the same amount of N was applied for all treatments. In

203   each subplot, the CC residue was also left on top of the soil. This application took place

204   on 26th May by spreading the fertilizer homogenously with a hand sprayer, followed by

205   an irrigation event.

206   Sprinkler irrigation was applied to the maize crop in a total amount of 688.5 mm

207   in 31 irrigation events. Sprinklers were installed in a 12m x 12m framework. The water

208   doses to be applied were estimated from the crop evapotranspiration (ETc) of the

209   previous week (net water requirements). This was calculated daily as ETc. = Kc × ETo,

210   where ETo is reference evapotranspiration calculated by the FAO Penman–Monteith

211   method (Allen et al., 1998) using data from the meteorological station located in the

212   experimental field. The crop coefficient (Kc) was obtained using the relationship for

213   maize in semiarid conditions (Martínez-Cob, 2008).

214   Two different periods were considered for data reporting and analysis: Period I

215   (from CC sowing to N fertilization of the maize crop), and Period II (from N

216   fertilization of maize to the end of the experimental period, after maize harvest).

217

218   *2.3 GHG emissions sampling and analyzing*

219   Fluxes of N_2O, CH_4 and CO_2 were measured from October 2013 to October

220 2014 using opaque manual circular static chambers as described in detail by Abalos et

221 al. (2013). One chamber (diameter 35.6 cm, height 19.3 cm) was located in each

222 experimental plot. The chambers were hermetically closed (for 1 h) by fitting them into

223 stainless steel rings, which were inserted at the beginning of the study into the soil to a

224 depth of 5 cm to minimize the lateral diffusion of gases and to avoid the soil disturbance

225 associated with the insertion of the chambers in the soil. The rings were only removed

226 during management events. Each chamber had a rubber sealing tape to guarantee an

227 airtight seal between the chamber and the ring and was covered with a radiant barrier

228 reflective foil to reduce temperature gradients between inside and outside. The rings

229 were only removed during management events. Each chamber had a rubber sealing tape

230 to guarantee an airtight seal between the chamber and the ring. A rubber stopper with a

231 3-way stopcock was placed in the wall of each chamber to take gas samples.

232 Greenhouse gas measurements were always made with barley/vetch plants inside the

233 chamber. During the maize period, gas chambers were set up between maize rows.

234   During Period I, GHGs were sampled weekly or every two weeks. During the

235 first month after maize fertilization, gas samples were taken twice per week.

236 Afterwards, gas sampling was performed weekly or fortnightly, until the end of the

237 cropping period. To minimize any effects of diurnal variation in emissions, samples

238 were always taken at the same time of the day (10–12 am), that is reported as a

239 representative time (Reeves et al., 2015).

240   Measurements of N_2O, CO_2 and CH_4 emissions were made at 0, 30 and 60 min

241 to test the linearity of gas accumulation in each chamber. Gas samples (100 mL) were

242 removed from the headspace of each chamber by syringe and transferred to 20 mL gas

243 vials sealed with a gas-tight neoprene septum. The vials were previously flushed in the

244   field using 80 mL of the gas sample. Samples were analyzed by gas chromatography

245   using a HP-6890 gas chromatograph equipped with a headspace autoanalyzer (HT3),

246   both from Agilent Technologies (Barcelona, Spain). Inert gases were separated by HP

247   Plot-Q capillary columns. transported gas samples to aThe gas chromatograph was

248   equipped with a ^{63}Ni electron-capture detector (Micro-ECD) to analyze N_2O

249   concentrations, and to with a flame ionization detector (FID) connected to a methanizer

250   to measure CH_4 and CO_2 (previously reduced to CH_4). The temperatures of the injector,

251   oven and detector ECD were 50, 50 and 350ºC, respectively. The accuracy of the gas

252   chromatographic data was 1% or better. Two gas standards comprising a mixture of

253   gases (high standard with 1500 ± 7.50 ppm CO_2, 10 ± 0.25 ppm CH_4 and 2 ± 0.05 ppm

254   N_2O and low standard with 200 ± 1.00 ppm CO_2, 2 ± 0.10 ppm CH_4 and 200 ± 6.00 ppb

255   N_2O) were provided by Carburos Metálicos S.A. and Air Products SA/NV, respectively,

256   and used to determine a standard curve for each gas. The response of the GC was linear

257   within 200–1500 ppm for CO_2 and 2–10 ppm CH_4 and quadratic within 200–2000 ppb

258   for N_2O.

259   The increases in N_2O, CH_4 and CO_2 concentrations within the chamber

260   headspace were generally (80% of cases) linear ($R^2 > 0.90$) during the sampling period

261   (1h). Therefore, emission rates of fluxes were estimated as the slope of the linear

262   regression between concentration and time (after corrections for temperature) and from

263   the ratio between chamber volume and soil surface area (MacKenzie et al., 1998).

264   Cumulative N_2O, CH_4 and CO_2, emissions per plot during the sampling period were

265   estimated by linear interpolations between sampling dates, multiplying the mean flux of

266   two successive determinations by the length of the period between sampling and adding

267   that amount to the previous cumulative total (Sanz-Cobena et al., 2014). The

268 measurement of CO_2 emissions from soil including plants in opaque chambers only

269 includes ecosystem respiration but not photosynthesis (Meijide et al., 2010).

270

271 *2.4 ^{15}N Isotope analysis*

272 Gas samples from the subplots receiving double-labelled AN fertilizer were

273 taken after 60 min static chamber closure 1, 4, 9, 11, 15, 18, 22 and 25 days after

274 fertilizer application. Stable ^{15}N isotope analysis of N_2O contained in the gas samples

275 was carried out on a cryo-focusing gas chromatography unit coupled to a 20/20 isotope

276 ratio mass spectrometer (both from SerCon Ltd., Crewe, UK). Ambient samples were

277 taken occasionally as required for the subsequent isotopic calculations. Solutions of 6.6

278 and 2.9 atom% ammonium sulphate $[(NH_4)_2SO_4]$ were prepared and used to generate

279 6.6 and 2.9 atom% N_2O (Laughlin et al., 1997) which were used as reference and

280 quality control standards. In order to calculate the atom percent excess (ape) of the N_2O

281 emitted in the sub-plots, the mean natural abundance of atmospheric N_2O from the

282 ambient samples (0.369 atom% ^{15}N) was subtracted from the measured enriched gas

283 samples. To obtain the N_2O flux that was derived from fertilizer ($N_2O - N_{dff}$), the

284 following equation was used (Senbayram et al., 2009:

285
$$N_2O - N_{dff} = N_2O - N \times \left(\frac{N_2O\_ape_{sample}}{ape_{fertilizer}} \right) \quad (1)$$

286 in which '$N_2O - N$' is the N_2O emission from soil, '$N_2O\_ape_{sample}$' is the ^{15}N

287 atom% excess of emitted N_2O, and '$ape_{fertilizer}$' is the ^{15}N atom% excess of the applied

288 fertilizer (Senbayram et al., 2009).

289 Gas samples from the subplots receiving double labelled AN fertilizer were

290 taken after 60 min static chamber closure 1, 4, 9, 11, 15, 18, 22 and 25 days after

12

fertilizer application. Stable ^{15}N isotope analysis of N$_2$O 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$_4$)$_2$SO$_4$] were prepared and used to generate 6.6 and 2.9 atom% N$_2$O (Laughlin et al., 1997) which were used as reference and quality control standards. During the experiment, the mean natural abundance of atmospheric N$_2$O (0.369 atom% ^{15}N) was subtracted from measured enriched samples to calculate the atom percent excess. To obtain the N$_2$O flux that was derived from fertilizer ($N_2O - N_{dff}$), the Eq. (1) was used (Loick et al., 2016):

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

in which 'N_2O-N' is the N$_2$O emission from soil, '$N_2O - ape_{sample}$' is the ^{15}N atom% excess of emitted N$_2$O (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$_2$O*' 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 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

314 8 g of soil extracted with 50 mL of KCl (1 M), and measured by automated colorimetric

315 determination using a flow injection analyzer (FIAS 400 Perkin Elmer) provided with a

316 UV-V spectrophotometer detector. Soil (DOC) was determined by extracting 8 g of

317 homogeneously mixed soil with 50 mL of deionized water (and subsequently filtering),

318 and analyzed with a total organic C analyser (multi N/C 3100 Analityk Jena) equipped

319 with an IR detector. The ~~Water~~water ~~Filled~~ filled ~~Pore~~ pore ~~Space~~ space (WFPS) was

320 calculated by dividing the volumetric water content by total soil porosity. Total soil

321 porosity was calculated according to the relationship: soil porosity = (1- soil bulk

322 density/2.65), assuming a particle density of 2.65 g cm^{-3} (Danielson and Sutherland,

323 1986). Gravimetric water content was determined by oven-drying soil samples at 105

324 °C with a MA30 Sartorius ®.

325 Four 0.5m × 0.5m squares were randomly harvested from each plot, before

326 killing the CC by applying glyphosate. Aerial biomass was cut by hand at soil level,

327 dried, weighed and ground. A subsample was taken for determination of total N content.

328 From these samples was determined CC biomass and N contribution to the subsequent

329 maize.

330 At maize harvest, two 8 m central rows in each plot were collected and weighed

331 in the field following separation of grain and straw. For aboveground N uptake

332 calculations, N content was determined in subsamples of grain and biomass. Total N

333 content on maize and CC subsamples were determined with an elemental analyzer

334 (TruMac CN Leco).

335

336 *2.6 Calculations and statistical analysis*

337   Yield-scaled N_2O emissions and N surplus in the maize cash crop were

338   calculated as the amount of N_2O emitted (considering the emissions of the whole

339   experiment, i.e. Period I + Period II) per unit of above-ground N uptake, and taking the

340   difference between N application and above-ground N uptake, respectively (van

341   Groenigen et al., 2010).

342   Statistical analyses were carried out with Statgraphics Plus 5.1. Analyses of

343   variance were performed for all variables over the experiment (except climatic ones),

344   for both periods indicated in section 2.2. Data distribution normality and variance

345   uniformity were previously assessed by Shapiro-Wilk test and Levene's statistic,

346   respectively, and transformed (log10, root-square, arcsin or inverse) before analysis

347   when necessary. Means of soil parameters were separated by Tukey's honest

348   significance test at $P<0.05$, while cumulative GHG emissions, ~~YSNE~~ yield-scaled N_2O

349   emissions and N surplus were compared by the orthogonal contrasts method at $P<0.05$.

350   For non-normally distributed data, the Kruskal–Wallis test was used on non-

351   transformed data to evaluate differences at $P<0.05$. Linear correlations were carried out

352   to determine relationships between gas fluxes and WFPS, soil temperature, DOC, NH_4^+

353   and NO_3^-. Theses analyses were performed using the mean/cumulative data of the

354   replicates of the CC treatments (n=12), and also for all the dates when soil and GHG

355   were sampled, for Period I (n=16), Period II (n=11) and the whole experimental period

356   (n=27).

357

358   **3. Results**

359   *3.1 Cover crop (Period I)*

360   *3.1.1 Environmental conditions and WFPS*

15

Con formato: Subíndice

361   Mean soil temperature during the intercrop period was 8.8°C, ranging from 1.8

362   (December) to 15.5°C (April) (Fig. 1a), which were typical values in the experimental

363   area. Mean soil temperature during maize cropping period was 24.6°C, which was also

364   a standard value for this region. The accumulated rainfall during this period was 215

365   mm, whereas the 30-year mean is 253 mm. Water-Filled Pore Space ranged from 40 to

366   81% (Fig. 1b). No significant differences were observed for WFPS mean values

367   between the different treatments ($P>0.05$).

368

369   *3.1.2 Mineral N and DOC and cover crop residues*

370   Topsoil NH_4^+ content was below 5 mg N kg soil^{-1} ~~almost~~ most of the time in

371   Period I, although a peak was observed after maize sowing (55 days after CCs kill date)

372   (Fig. 2a), with the highest values reached in B (50 mg N kg soil^{-1}). Mean NH_4^+ content

373   was significantly higher in B than in F ($P<0.05$), but daily NH_4^+ concentrations between

374   treatments were only significantly different between treatments in one sampling date

375   (210 days after CCs sowing). Nitrate content increased after CCs killing, reaching

376   values above 25 mg N kg soil^{-1} in V treatment (Fig. 2c). Mean NO_3^- content during

377   Period I was significantly higher in the V plots than in the B and F plots ($P<0.001$).

378   Dissolved Organic C ranged from 60 to 130 mg C kg soil^{-1} (Fig. 2e). Average topsoil

379   DOC content was significantly higher in B than in V and F (10% and 12%, respectively,

380    $P<0.$~~05~~01) but differences were only observed in some sampling dates. The total

381   amount of cover crop biomass left on the ground was 540.5±26.5 and 1106.7±93.6 kg

382   ~~DM~~ dry matter ha^{-1} in B and V, respectively. Accordingly, the total N content of these

383   residues was 11.0±0.6 and 41.3±4.5 kg N ha^{-1} in B and V, respectively.

384

385   *3.1.3 GHG fluxes*

386   Nitrous oxide fluxes ranged from -0.06 to 0.22 mg N m^{-2} d^{-1} (Fig. 3a) in Period

387 I. The soil acted as a sink for N_2O at some sampling dates, especially for the F plots.

388 Cumulative fluxes at the end of Period I were significantly greater in CC treatments

389 compared to F (1.6 and 2.6 higher in B and V, respectively) ($P<0.05$; Table 1). Net CH_4

390 uptake was observed in all intercrop treatments, and daily fluxes ranged from -0.60 to

391 0.25 mg C m^{-2} d^{-1} (data not shown). No significant differences were observed between

392 treatments in cumulative CH_4 fluxes at the end of Period I ($P>0.05$; Table 1). Carbon

393 dioxide fluxes (data not shown) remained below 1 g C m^{-2} d^{-1} during the intercrop

394 period. Greatest fluxes were observed in B although differences in cumulative fluxes

395 were not significant ($P>0.05$; Table 1) in the whole intercrop period, but soil respiration

396 was increased in B, with respect to F, from mid-February to the end of Period I ~~Carbon~~

397 ~~dioxide fluxes (data not shown) remained below 1 g C m^{-2} d^{-1} during the intercrop~~

398 ~~period. Greatest fluxes were observed in B although differences in cumulative fluxes~~

399 ~~were not significant ($P>0.05$; Table 1)~~. Nitrous oxide emissions were significantly

400 correlated to CO_2 fluxes ($P<0.01$, n=17, r=0.69) and soil temperature ($P<0.05$, n=17,

401 r=0.55).

402

403   *3.2 Maize crop (Period II)*

404   *3.2.1 Environmental conditions and WFPS*

405   Mean soil temperature ranged from 19.6 (reached in September) to 32.3°C

406 (reached in August) with a mean value of 27.9°C (Fig. 1a). Total rainfall during the

407 maize crop period was 57 mm. Water-Filled Pore Space ranged from 19 to 84% (Fig.

17

408   1c). Higher mean WFPS values ($P<0.01$) were measured in B during some sampling

409   dates.

410

411   *3.2.2 Mineral N and DOC*

412   Topsoil NH_4^+ content increased rapidly after N fertilization (Fig. 2b) decreasing

413   to values below 10 mg N kg soil^{-1} from 15 days after fertilization to the end of the

414   experimental period. Nitrate concentrations (Fig. 2d) also peaked after AN addition,

415   reaching the highest value (170 mg N kg soil^{-1}) 15 days after fertilization in B ($P<0.05$).

416   No significant differences ($P>0.05$) between treatments were observed in average soil

417   NH_4^+ or NO_3^- during maize phase. Dissolved Organic C ranged from 56 to 138 mg C kg

418   soil $^{-1}$ (Fig. 2f). Average topsoil DOC content was 26 and 44% higher in B than in V and

419   F, respectively ($P<0.001$).

420

421   *3.2.3 GHG fluxes, Yield-Scaled N_2O emissions and N surplus*

422   Nitrous oxide fluxes ranged from 0.0 to 5.6 mg N m^{-2} d^{-1} (Fig. 3b). The highest

423   N_2O emission peak was observed 1-4 days after fertilization for all plots. Other peaks

424   were subsequently observed until 25 days after fertilization, particularly in B plots

425   where N_2O emissions 23 and 25 days after fertilization were higher ($P<0.05$) than those

426   of F and V (Fig. 3b). No significant differences in cumulative N_2O fluxes were

427   observed between treatments throughout or at the end of the maize crop period (Table

428   1), albeit fluxes were numerically higher in B than in V ($0.05<P<0.10$). Daily N_2O

429   emissions were significantly correlated with NH_4^+ topsoil content ($P<0.05$, n=12,

430   r=0.84).

18

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~~ammonium nitrate, 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 ($P<0.05$) and was positively correlated with DOC concentrations (*$P<0.05$, n=12, r=0.71*). There were no significant differences between V and F ($P>0.05$).

4. Discussion

19

454   *4.1 Role of CCs in N_2O emissions: Period I*

455   Cover crop treatments (V and B) increased N_2O losses compared to F, especially

456   in the case of V (Table 1). These results are consistent with the meta-analysis of Basche

457   et al. (2014), which showed that overall CCs increase N_2O fluxes (compared to bare

458   fallow), with highly significant increments in the case of legumes and a lower effect in

459   the case of non-legume CCs. In the same experimental area, Sanz-Cobena et al. (2014)

460   found that V was the only CC significantly affecting N_2O emissions. The greatest

461   differences between treatments were observed at the beginning (13-40 days after CCs

462   sowing), and at the end of this period (229 days after CCs sowing) (Fig. 3a). On these

463   dates, the mild soil temperatures and the relatively high moisture content were more

464   suitable for soil biochemical processes, which may trigger N_2O emissions (Fig. 1a, b)

465   (Firestone and Davidson, 1989). Average topsoil NO_3^- was significantly higher in V

466   (Fig. 2b), which was the treatment that led to the highest N_2O emissions. Legumes such

467   as V are capable of biologically fixing atmospheric N_2, thereby increasing soil NO_3^-

468   content with potential to be denitrified. Further, the mineralization of the most

469   recalcitrant fraction of the previous V residue (which supplies nearly four times more N

470   than the B residue, as indicated in section 3.1.2) together with high C-content sunflower

471   residue could also explain higher NO_3^- contents in V plots (Frimpong et al., 2011), and

472   higher N_2O losses from denitrification (Baggs et al., 2000). After CCs kill date, N

473   release from decomposition of roots and nodules and faster mineralization of V residue

474   compared to that of B (shown by NO_3^- in soil in Fig. 2c) are the most plausible

475   explanation for the N_2O increases at the end of the intercrop period (Fig. 3a) (Rochette

476   and Janzen, 2005; Wichern et al., 2008).

477   Some studies (e.g. Justes et al., 1999; Nemecek et al., 2008) have pointed out

478   that N_2O losses can be reduced with the use of CCs, due to the extraction of plant-

20

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

21

after N fertilization revealed the importance of ~~soil~~ mineral N ~~harbored in~~ contained in the ~~soil~~ micropores ~~in~~ for the N_2O bursts after the first irrigation events, with respect to the N released from CC residues.

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), in a field trial with zero-tillage 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), ~~increasing~~ 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 ~~AN~~) than B plots, which could have resulted in better synchronization between N release and crop needs

22

528 (Ussiri and Lal, 2012) in V plots. Supporting these findings, Bayer et al. (2015) recently

529 concluded that partially supplying the maize N requirements with winter legume cover-

530 crops can be considered a N_2O mitigation strategy in subtropical agro-ecosystems.

531   The mineralization of B residues resulted in higher DOC contents for these plots

532 compared to the F or V plots ($P<0.001$). This was observed in both Period I (as a

533 consequence of soil C changes after the 8-year cover-cropping management) and Period

534 II (due to the CC decomposition). Although in the present study the correlation between

535 DOC and N_2O emissions was not significant, positive correlations have been previously

536 found in other low-C Mediterranean soils (e.g. Vallejo et al., 2006; López-Fernández et

537 al., 2007). Some authors have suggested that residues with a high C:N ratio can induce

538 microbial N immobilization (Frimpong and Baggs, 2010, Dendooven et al., 2012). In

539 our experiment, a N_2O peak was observed in B plots 20-25 days after fertilization (Fig.

540 3b) after a remarkable increase of NO_3^- content (Fig. 2d), which may be a result of a re-

541 mineralization of previously immobilized N in these plots.

542   The positive correlation of N_2O fluxes and soil NO_3^- content and WFPS during

543 the whole cycle further supports the importance of denitrification process for explaining

544 N_2O losses in this agro-ecosystem (Davidson et al., 1991; García-Marco et al., 2014).

545 However, the strong positive correlation of N_2O with NH_4^+ indicated that nitrification

546 was also a major process leading to N_2O fluxes, and showed that the continuous drying-

547 wetting cycles during a summer irrigated maize crop in a semi-arid region can lead to

548 favorable WFPS conditions for both nitrification and denitrification processes (Fig. 1c)

549 (Bateman and Baggs, 2005). Emission Factors ranged from 0.2 to 0.6% of the synthetic

550 N applied, which were lower than the IPCC default value of 1%. As explained above,

551 ecological conditions during the intercrop period (rainfall and temperature) and maize

552 phase (temperature) could be considered as normal (based on the the 30-year average)

23

553 in Mediterranean areas. Aguilera et al. (2013) obtained a higher emission factor for high

554 (1.01%) and low (0.66%) water-irrigation conditions in a meta-analysis of

555 Mediterranean cropping systems. We hypothesized that management practices may

556 have contributed to these low emissions, but other inherent factors such as soil pH

557 should be also considered. Indeed, a higher N_2O/N_2 ratio has been associated to acidic

558 soils, so lower N_2O emissions from denitrification could be expected in alkaline soils

559 (Mørkved et al., 2007; Baggs et al., 2010).

560

561   *4.3 Methane and CO_2 emissions*

562   As is generally found in non-flooded arable soils, all treatments were net CH_4

563 sinks (Snyder et al., 2009). No significant differences were observed between treatments

564 in any of the two periods (Table 1), which is similar to the pattern observed by Sanz-

565 Cobena et al. (2014). Some authors (Dunfield and Knowles, 1995; Tate, 2015) have

566 suggested an inhibitory effect of soil NH_4^+ on CH_4 uptake. Low NH_4^+ contents during

567 almost all of the CCs and maize cycle may explain the apparent lack of this inhibitory

568 effect (Banger et al., 2012). However, during the dates when the highest NH_4^+ contents

569 were reached in V and B (225 days after CCs sowing) (Fig. 3a), CH_4 emissions were

570 significantly higher for these plots (0.12 and 0.16 mg CH_4-C m^{-2} d^{-1} for V and B,

571 respectively) than for F (-0.01 mg CH_4-C m^{-2} d^{-1}) (data not shown). Similarly, the NH_4^+

572 peak observed two days after fertilization (Fig. 3b) decreased in the order V>F>B, the

573 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,

574 F and B, respectively; data not shown). Contrary to Sanz-Cobena et al.(2014), the

575 presence of CCs did not increase CO_2 fluxes (Table 1) during the whole Period I (which

576 was longer than that considered by these authors), even though higher fluxes were

24

577 associated to B (but not V) with respect to F plots in the last phase of the intercrop,

578 probably as a consequence of higher root biomass and plant respiration rates in the

579 cereal (B) than in the legume (V). Differences from fall to early-winter were not

580 significant, since low soil temperatures limited respiration activity~~Contrary to Sanz~~

581 ~~Cobena et al. (2014), the presence of CCs did not increase CO$_2$ fluxes (Table 1) during~~

582 ~~Period I (which was longer than that considered by these authors), even though higher~~

583 ~~fluxes tended to be associated to B plots, probably as a consequence of higher root~~

584 ~~biomass and plant respiration rates in the cereal (B) than in the legume (V)~~. The

585 decomposition of CC residues and the growth of maize rooting system resulted in an

586 increase of CO$_2$ fluxes during Period II (Oorts et al., 2007; Chirinda et al., 2010),

587 although differences between treatments were not observed.

588

589 *4.4 Yield-scaled emissions, N surplus and general assessment*

590 Yield–scaled N$_2$O emissions ranged from 1.74 to 7.15 g N$_2$O-N kg aboveground

591 N uptake^{-1}, which is about 1-4 times lower than those reported in the meta-analysis of -

592 van Groenigen et al. (2010) for a fertilizer N application rate of 150-200 kg ha^{-1}. Mean

593 N surpluses of V and F (Table 1) were in the recommended range (0-50 kg N ha^{-1}) by

594 van Groenigen et al. (2010), while the mean N surplus in B (55 kg N ha^{-1}) was also

595 close to optimal. In spite of higher N$_2$O emissions in V during Period I (which

596 accounted for a low proportion of total cumulative N$_2$O losses during the experiment),

597 these plots did not emit greater amounts of N$_2$O per kg of N taken up by the maize

598 plants, and even tended to decrease yield-scaled N$_2$O emissions~~YSNE~~ and N surplus

599 (Table 1).

600   Adjusting fertilizer N rate to soil endogenous N led to lower N_2O fluxes than

601   previous experiments where conventional N rates were applied (e.g. ~~Sanz-Cobena et al.,~~

602   ~~2012;~~ Adviento-Borbe et al., 2007; Hoben et al., 2011; Sanz-Cobena et al., 2012; Li et

603   al., 2015), in agreement with the study of Migliorati et al. (2014). Moreover, CO_2

604   equivalent emissions associated to manufacturing and transport of N synthetic fertilizers

605   (Lal, 2004) can be reduced when low synthetic N input strategies, such as ISMF, are

606   employed. Our results highlight the critical importance of the cash crop period on total

607   N_2O emissions, and demonstrate that the use of either non-legume and –particularly-

608   legume CCs combined with ISFM may provide an optimum balance between GHG

609   emissions from crop production and agronomic efficiency (i.e. lowering synthetic N

610   requirements for a subsequent cash crop, and leading to similar YSNE as a fallow).

611   The use of CCs has environmental implications beyond effects on direct soil

612   N_2O emissions. For instance, CCs can mitigate indirect N_2O losses (from NO_3^-

613   leaching). In the study of Gabriel et al. (2012), conducted in the same experimental area,

614   NO_3^- leaching was reduced (on average) by 30% and 59% in V and B, respectively.

615   Considering an emission factor of 0.075 from N leached (De Klein et al., 2006), indirect

616   N_2O losses from leaching could be mitigated by 0.23±0.16 and 0.45±0.17 kg N ha^{-1} yr^{-1}

617   if V and B are used as CCs, respectively. Furthermore, the recent meta-analysis of

618   Poeplau and Don (2015) revealed a C sequestration potential of 0.32±0.08 Mg C ha^{-1} yr^{-1}

619   with the introduction of CCs. These environmental factors together with CO_2

620   emissions associated to CCs sowing and killing, should be assessed in future studies in

621   order to confirm the potential of CCs for increasing both the agronomic and

622   environmental efficiency of irrigated cropping areas.

623

Con formato: Subíndice

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 yield-scaled N_2O emissions~~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~~non-legume and – particularly- legume CCs combined with ISFM ~~may~~ could be considered as an efficient practice from both environmental and agronomic points of view, leading to similar N_2O losses per kilogram of aboveground N uptake as bare fallow. ~~provide an optimum balance between GHG emissions from crop production and agronomic efficiency~~.

Con formato: Subíndice

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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}	Yield-scaled N_2O emissions ~~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**

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.