Spanish Journal of Agricultural Research
20 (3), e0304, 15 pages (2022)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2022203-18416
INIA-CSIC

RESEARCH ARTICLE

OPEN ACCESS

Greenhouse gas emissions associated to sprinkler-irrigated alfalfa under semi-arid Mediterranean conditions

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Abstract

Aim of the study: Alfalfa is one the most important forage legume crop worldwide but little information is available regarding to greenhouse gas emissions (GHG) under Mediterranean sprinkler-irrigated conditions.

Area of study: Middle Ebro valley, Spain

Materials and methods: The GHG emissions during two alfalfa growing periods (4th and 5th stands) were evaluated using both the static method chambers and two automatic chambers coupled with a photoacoustic equipment that measured short-term gas emissions.

Main results: Year-average CH_4 fluxes were -0.71 g C ha^{-1} day^{-1}, generally no significantly different from zero. Year-average N_2O flux was 3.96 g N ha^{-1} day^{-1} with higher fluxes associated to some specific large rainfall or irrigation events. Average cumulative emissions of 865 g N ha^{-1} $year^{-1}$ were found. We found short-term peaks of N_2O (up to 160 g N ha^{-1} day^{-1}) associated with high values of soil water filled pore space (WFPS) that can go unnoticed using the static chamber procedure. In spite of the higher soil NO_3^- concentration in the alfalfa-precedent field compared to the maize-precedent field, no significant differences in cumulative N_2O emissions were observed in the two-month period after alfalfa or maize residues incorporation.

Research highlights: Low GHG emissions were found in an irrigated alfalfa crop compared to N-fertilized crops but a deeper knowledge of the limiting factors of denitrification observed during some anoxic events (WFPS>90%) is necessary to properly quantify N_2O emissions in irrigated alfalfa.

Additional key words: nitrous oxide; methane; alfalfa termination

Abbreviations used: GHG (greenhouse gas); SMN (soil mineral nitrogen); SWC (soil water content; WFPS (water filled pore space)

Citation: Isla, R; Guillén, M; Medina, ET; Latorre, B; Quílez, D; Cavero, J (2022). Greenhouse gas emissions associated to sprinkler-irrigated alfalfa under semi-arid Mediterranean conditions. Spanish Journal of Agricultural Research, Volume 20, Issue 3, e0304. https://doi.org/10.5424/sjar/2022203-18416

Supplementary material (Figs. S1 and S2) accompanies the paper on SJAR's website.

Received: 20 May 2021. **Accepted**: 11 Jul 2022.

Funding agencies/institutions	Project / Grant
Spanish Ministry of Economy, Industry, and Competitiveness	AGL2013-49062-C4-3-R and AGL2017-84529-C3-2-R

Competing interests: The authors have declared that no competing interests exist.

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Introduction

The assessment of nitrous oxide (N_2O) emissions from agricultural systems is a global concern because they are the main source of global anthropogenic N_2O-emissions (IPCC, 2014). Most of the studies assessing N_2O emissions from agricultural systems have focused on nitrogen (N) fertilized crops due the correlation found between N application rates and N_2O emissions (Bouwman, 1996; Alvaro-Fuentes *et al.*, 2016). Legume crops are generally not N fertilized, due to their symbiosis with different strains of bacteria that are able to fix N, which has led to the belief that have a minor impact on N_2O emissions compared to N fertilized crops. The annual greenhouse gas (GHG) inventories only consider direct N_2O emissions from non N-fertilized legume crops when their residues are incorporated to the soil after crop termination (IPCC, 2006).

Alfalfa (*Medicago sativa* L.) is one of the most cultivated forage legumes in the world (Annicchiarico *et al.*, 2015) and has a significant role within the rotation in many agro-ecosystems due to their ability to break down soil-borne diseases, improve weed control, and supply a considerable amount of N to the following crop (Cela *et al.*, 2011).

First determinations of N_2O emissions in alfalfa, under non-irrigated conditions of Wisconsin (USA), reported cumulative emissions of 3.2 kg N ha^{-1} $year^{-1}$ (Goodroad *et al.*, 1984), lower than reported values in maize in the same area. Other studies found lower (Wagner-Riddle *et al.*, 1997 and Gelfand *et al.*, 2016: 1 kg N ha^{-1} $year^{-1}$) or similar (Mackenzie *et al.*, 1997: 1.18-2.16 kg N ha^{-1} $year^{-1}$; Osterholz *et al.*, 2014: 2.42-2.72 kg N ha^{-1} $year^{-1}$; Burger *et al.*, 2016: 2.3-5.3 kg N ha^{-1} $year^{-1}$) N_2O emissions than generally found in N fertilized crops. However, Ellert & Janzen (2008) under irrigated conditions found that the inclusion of alfalfa in the rotation with cereals increased significantly the N_2O emissions.

After the detailed review of Rochette & Janzen (2005), it has been widely accepted that N_2O emissions from legume crops derive mainly from the decomposition of root exudates during the crop growth period, and from plant residues after legume plow-down, minimizing the previous belief that significant N_2O losses were associated to the biological N fixation ‘per se’. This conclusion was corroborated by Ingram *et al.* (2015), who found no increases in N_2O emissions in highly aerobic dryland native prairies interseeded with alfalfa, in spite of the observed increases of soil mineral nitrogen (SMN). Schmeer *et al.* (2014) reported that, under the humid conditions of Northern Europe, N-fertilized perennial grasses emitted significantly larger amounts of N_2O compared to an alfalfa-based prairie.

Similarly to other crops, N_2O emissions in alfalfa varied greatly according to environmental and crop management conditions mainly related to water availability (rainfall and irrigation management) and stand age, since alfalfa fields are usually not N-fertilized. Thus, under Mediterranean and flooded irrigated conditions, Burger *et al.* (2016) found a two-fold increase in N_2O emissions in older alfalfa (5th year stand) compared to a young alfalfa (2nd year stand), with a large contribution of total emissions occurring immediately after the irrigation events that resulted in soil water filled pore space (WFPS) values above 80%. The higher N_2O emissions in older alfalfa stands were attributed to higher C and N release from root turnover.

As mentioned, a significant C and N turnover to the soil is produced when the alfalfa crop is plow-down. In this regard, Pu *et al.* (1999) in an incubation study with added residues of legumes showed high emissions of N gases immediately after waterlogging compared to soils without added residues, although N_2O constituted only a small portion (1-4%) of the total N gas emission. Other incubation studies (*e.g.*, Shelp *et al.*, 2000) with alfalfa residues suggested that after a short period of aerobic conditions the dissolved organic carbon is reduced, limiting the posterior N_2O emissions in a subsequent anaerobic period. Some field studies found that the incorporation of legume residues to the soil resulted in higher N_2O emissions compared to the incorporation of non-legume residues (Millar *et al.*, 2004; Muhammad *et al.*, 2011). In these studies, N_2O emission after residue incorporation was positively correlated with residue N content and negatively correlated with the C:N ratio. However, Zhong *et al.* (2011) found no significant differences in N_2O emissions between the soil incorporation of plant residues of pulses (grain-legume) and those of cereals.

In spite of the mentioned studies, the N_2O emissions associated to alfalfa have been much less documented than other N fertilized crops, specially under semi-arid Mediterranean climate and sprinkler-irrigated conditions. Most of the mentioned studies were conducted under continental climatic conditions and without irrigation, with the exception of the study of Burger *et al.*, (2016). Taken into account the significant role of the soil water content (SWC) and environmental conditions on the pattern of N_2O fluxes (Mateo-Marín *et al.*, 2020), more information should be collected to evaluate the global impact of irrigated alfalfa on GHG emissions in semi-arid Mediterranean climate and sprinkler-irrigated, where attainable yield is among the highest worldwide (Lindenmayer *et al.*, 2011; Cavero *et al.*, 2017). Besides, there is few information about CH_4 emissions from alfalfa (Ellert and Janzen, 2008). Therefore, the objectives of the present study were to: (1) evaluate annual N_2O and CH_4 emissions in a sprinkler-irrigated alfalfa field during two consecutive years; (2) monitor soil N_2O hourly fluxes during one alfalfa cutting period, and (3) assess the soil N_2O fluxes in early spring after plow-down the alfalfa crop. Although soil CO_2 fluxes were measured during the experiments, they were mostly considered as ancillary variable related to soil activity more than the focus of the study. The initial hypothesis to this study is that, on the basis of the absence of N fertilizer applications, the GHG emissions in alfalfa should be lower than those in N fertilized crops under similar edapho-climatic conditions. Furthermore, irrigation events and alfalfa termination are the periods more susceptible to large N_2O emissions due to the high SWC and large addition of organic N, respectively.

Material and methods

The study was conducted from January 2016 to April 2018 in a 0.7 ha field located in the CITA experimental field 'Soto Lezcano' in the middle Ebro river basin (Zaragoza, Spain). The climate is semiarid Mediterranean-Continental with annual averages of mean, maximum and minimum daily air temperatures of 14.1°C, 21.4°C and 8.3°C, respectively; yearly average precipitation of 319 mm; and yearly average reference evapotranspiration of 1239 mm (period 2004-2018). The 2016 year had an average air temperature pattern close to the historical average (Fig. 1), but with a total precipitation (439 mm), 38% higher than the historical average (2005-2019: 317 mm). However, the 2017 was a drier year (255 mm) with warmer conditions, especially during February, March, May, and June, with monthly average temperatures 1.5ºC higher than the historical average. The field has a deep soil (120 cm depth) with loam texture classified as Typic Xerofluvent (SSS, 2014) and the main physicochemical characteristics of the top soil are shown in Table 1. The soil is very homogeneous in depth and the volumetric SWC at field capacity (-0.033 MPa) and wilting point (-1.5 MPa) are 30.2% and 8.7%, respectively. Thus, considering the whole profile, the soil has 258 mm of total available water capacity. The alfalfa was sown on April 17, 2013 and the study involved the 4th and 5th year alfalfa stands. The field was irrigated using a solid-set sprinkler irrigation system and the weekly irrigation requirements were calculated with the Penman-Monteith reference evapotranspiration method using the alfalfa crop coefficients according to FAO procedures (Allen *et al.*, 1998). A total of 37 and 33 irrigation events were applied from March to October during 2016 and 2017, respectively, with and average duration of 2.5 h (pluviometry of 7.9 mm h^{-1}). Thus, a total amount of 632 mm (917 mm; including rainfall) and 758 mm (1000 mm; including rainfall) of water was applied in 2016 and 2017, respectively, to satisfy the crop evapotranspirative demand. The crop was managed according the standard practices for the area with conventional machinery to cut, and collect the alfalfa hay. No N fertilizer was applied to alfalfa during both growing seasons and previous years, although 161 kg $ha^{-1}$of P_2O_5 and K_2O were applied using a complex PK fertilizer at the beginning of each growing season. One herbicide treatment (Thifensulfuron-methyl; Harmony® 50 SX at 30 g ha^{-1}) was applied every year prior the regrowth of alfalfa to control some weeds. No special problems of pest and weeds were detected during the experiment. The alfalfa hay was harvested 5 and 6 times in 2016 and 2017, respectively. The yield was evaluated by hand cutting a 1-m^2 area at one and eight locations during 2016 and 2017, respectively, at each harvest date. The total aboveground matter was oven-dry at 65ºC and weighed to calculate the yield (kg dry matter ha^{-1}). A sub-

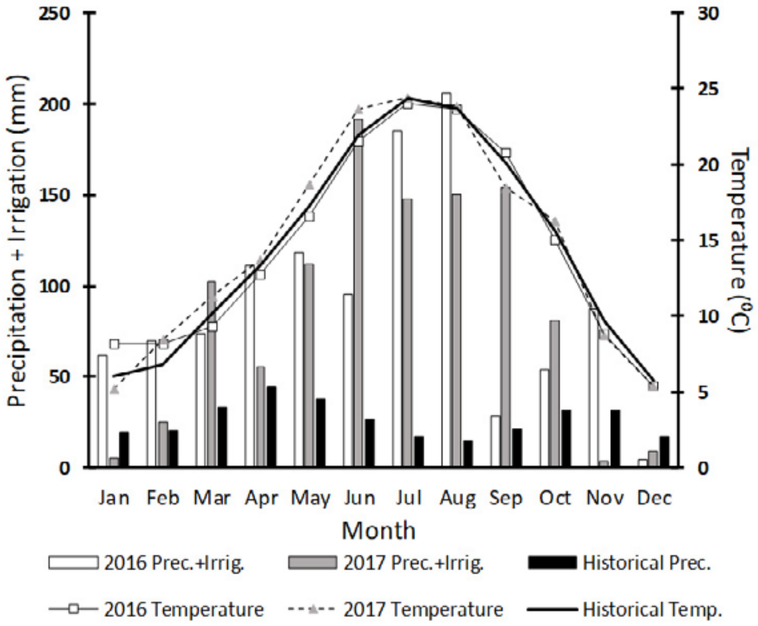

Figure 1. Mean monthly irrigation + precipitation and temperature (2016, 2017 and historical 2004-2019) for the CITA experimental field station at Montañana, Zaragoza.

Table 1. Top soil characteristics (mean ± SE; n=52) of the experimental field.

Soil characteristics (0-30 cm)	Mean±SE
Clay content (%)	11.7 ± 0.60
Sand content (%)	50.5 ± 0.99
Silt content (%)	37.8 ± 0.72
pH,water	8.1 ± 0.01
K (NH4Ac) (mg kg^{-1})	109.3 ± 2.06
P Olsen (mg kg^{-1})	10.4 ± 0.22
Ca (NH4Ac) (meq 100 g^{-1})	23.8 ± 0.15
EC (1:5$_{H2O}$) (dS m^{-1})	0.57 ± 0.02
Organic N (%)	0.10 ± 0.001
Organic matter (%)	1.38 ± 0.02

sample was ground for total N analysis by the combustion method (TruSpec CN, LECO, St. Joseph, MI, USA).

Static chambers experiment

Greenhouse gas emissions

Eight static closed no vented-chambers (similar to those of Holland *et al*., 1999) were used to measure soil CO_2, N_2O and CH_4 fluxes. The chambers were distributed in the field to capture the soil and crop variability. One polyvinyl chloride (PVC) collar was inserted 10 cm into the soil at each measurement point six days before the first sampling date. PVC chambers (19.7 cm height, 30 cm inner diameter; volume of 13.9 L) coated with a reflective bubble wrap material were fitted into the collars at the time of sampling. During 2016 the plants were removed, cutting periodically from the ground, but during 2017, the plants were left inside growing. Chambers were sampled every 3 to 4 weeks with 16 and 17 sampling times in 2016 and 2017, respectively. At each sampling date, 15 mL of air from inside each chamber were taken at 0, 30, and 60 min after chamber closure using a polypropylene syringe, and injected into 12-mL Exetainer® borosilicate glass vials (Labco Ltd., Lampeter, UK). Air samplings were mostly performed between 10:00 to 11:30 A.M. (Greenwich Mean Time) considering that soil temperature was the main factor driving daily changes in soil N_2O fluxes (Alves *et al*., 2012) and that soil temperature at that time was close to the daily average. Air samples were analyzed by gas chromatography using an Agilent 7890B chromatograph with electron-capture (ECD) and flame-ionization detector (FID). Soil fluxes of CO_2 (kg ha^{-1} day^{-1}), N_2O (g N ha^{-1} day^{-1}) and CH_4 (g C ha^{-1} day^{-1}) were calculated fitting a linear regression of the gas concentration in the chamber (corrected for air temperature) versus time. No significant differences (paired t-test; $p>0.05$) in soil N_2O fluxes were obtained using 0-30 min and 0-60 min closure times, which indicates no significant saturation effect. There was a total of 33 sampling dates during the two growing seasons, with an average sampling interval of 21 days. Cumulative emissions were calculated with the trapezoid rule, interpolating the averaged flux between consecutive sampling dates and multiplying it by the time period between the two samplings dates. The total N-scaled emissions were calculated as the total N_2O emission divided by the total N uptake of the aboveground alfalfa crop in each year.

Soil measurements

The topsoil (0-10 cm) was sampled to monitor SMN concentration in the upper part of the soil profile at every GHG sampling date (except in 3 dates), with a total of 29 dates. SWC was measured gravimetrically (drying at 105 ºC until constant weight) and a 10 g wet soil subsample was extracted with 30 mL of 2 N KCl after shaking on a reciprocal shaker for 30 min and filtering through cellulose filter. The soil extracts were analyzed by colorimetry using a segmented flow analyzer (AutoAnalyser3, Bran+Luebbe, Germany) to obtain nitrate (NO_3^-) and ammonium (NH_4^+) concentrations (mg N kg^{-1} soil). Topsoil moisture and temperature were also monitored continuously (3-min interval; averaged every 30 min) at the 5-cm depth in three locations of the field using SDI-12 Hydraprobe sensors (Stevens Water Monitoring Systems Inc., Beaverton, USA) connected to a data logger CR-200 (Campbell Scientific Ltd., Logan, USA). SWC values were calculated from the sensor readings with a calibration curve (R^2=0.81; n=28) obtained from soil sampling. Soil WFPS was calculated according to Linn & Doran (1984) as the quotient between volumetric soil water content and total soil porosity. Total soil porosity (44.5%) was estimated considering a particle density of 2.65 Mg m^{-3}, and the soil bulk density from 0 to 5 cm determined ‘in situ’ using the cylinder method (Grossman & Reinsch, 2002) as 1.47 Mg m^{-3}. Daily air temperature and precipitation were registered with an automated weather station located 100 m apart from the experimental site.

Automatic chambers experiment

A short-term experiment was carried out during the 2017 growing season to analyze the temporal variability of soil N_2O emissions during the period between the first and the second alfalfa harvest (April 26 to May 25). Two automated transient-state closed-system canopy chambers were used to continuously measure the gas exchange during the 29 days period. The chambers (Tecno El, Formello, Italy) were similar to that described by Steduto *et al.* (2002). It had a module that is a rectangular box with five transparent polycarbonate walls (1.5 mm thick), held together by a narrow aluminum angular frame. The chamber was open in the bottom, had a ground surface area of 0.75 m^2 (1.0 m × 0.75 m), and had a height of 0.5 m. It has a metal base that is inserted 5 cm into the soil. The chamber has four fans (Ebmpapst, Mulfingen, Germany) mounted in the corners which provide a total flux of 1.4 m^3 min^{-1}. The chamber top-cover has a hinge on one side, is usually open but can be moved to close the chamber in order to measure the gas exchange.

One thermocouple (Campbell Sci., TCBR-3, Shepshed, UK) not shielded was installed inside each chamber at half of its height to measure the air temperature at a 0.5 s interval.

The chamber top-cover was kept open for 30 min and closed for 30 min. The two chambers were synchronized so during the time that one was open the other was closed. During the time that the chamber was closed the four fans were stirring the air. A miniature diaphragm pump (model 15D1150, GAST, Benton Harbor, MI, USA) was used to continuously extract the air from inside of the chambers and to conduct it to a portable photoacoustic equipment (Innova 1412i Photoacustic Multigas Monitor) that analysed and stored temperature-corrected (25ºC) N_2O and CH_4 concentration. A flowmeter (Dwyer, model VFB-66-SSV-BFP, Michigan City, IN, USA) was used to get a constant flow of 5 L min^{-1} up to the Innova. The air was recirculated to the chamber. Gas samples were analyzed by the Innova every 2-min, so 15 gas samples were taken and analysed at each 30-min period. Gas was sampled alternatively from each closed chamber by means of a switch device that allowed to sample the gas from the chamber that was closed. Thus, a continuous gas exchange data set with a 30 min interval could be obtained during 29 days.

Although the photoacustic equipment (PA) was calibrated by the manufacturer company immediately prior to this study, we performed an empirical calibration versus the gas chromatograph equipment (GC) to ensure the comparability of the data obtained with the two analytical procedures. We observed a very good relationship [Eq. 1] between photoacoustic N_2O concentrations (N_2O_{PA}) and gas chromatography concentration (N_2O_{GC}), although the photoacoustic equipment was not enough sensitive to measure low concentrations (<1 mg N_2O m^{-3}; equivalent to fluxes of 10 g N-N_2O ha^{-1} day^{-1}). N_2O concentrations measured with the PA were adjusted to GC using Eq. [1].

$$N_2O_{GC} = 1.0829 \times N_2O_{PA} + 0.0231 \quad (R^2 = 0.99; N=34) \quad [1]$$

The N_2O fluxes were estimated as the slope of the linear regression of gas concentration versus time after closure. The N_2O fluxes were expressed as g N ha^{-1} day^{-1}. The cumulative fluxes (g N ha^{-1}) over the whole 29-days period were obtained similarly to the static chambers experiment, through integration fluxes between two sampling dates. Methane (CH_4) measurements from photoacoustic equipment did not provide enough accuracy and were not considered.

The SWC and soil temperature at 5 cm depth were monitored every 3 min in both chambers using field calibrated (R^2=0.97) SDI-12 Hydraprobe sensors (Stevens Water Monitoring Systems Inc., Beaverton, USA), similarly as described in the static chambers experiment.

The soil within each chamber was sampled 4 times along the experiment to determine the NO_3^- and NH_4^+ concentration (mg N kg^{-1}) in the topsoil (0-10 cm). The samples were analyzed using the same procedure already described.

During that period of 29 days, the average of daily mean, minimum, and maximum air temperature were 16.7, 8.52, and 24.5 ºC, respectively. A total of nine events of rain and two of irrigation occurred, with a total precipitation of 89.6 mm. The two irrigation events were on May 2 (39.5 mm) and May 17 (23.7 mm).

GHG fluxes after alfalfa termination and in an adjacent maize field

The alfalfa field was ploughed with a disk harrow and chisel on February 6, 2018, simultaneously with an adjacent field that had been cropped with maize during the previous 2016 and 2017 seasons. The maize field had been managed according to standard practices in the area with a N-fertilizer rate of 250 kg N ha^{-1} distributed in 50 kg N ha^{-1} at preplanting and two 100 kg N ha^{-1} side-dress applications at V6 and V14 maize growth stages. The maize development was correct with a grain yield of 13.5 Mg ha^{-1} (14% of humidity). The residues of alfalfa and maize in both fields were incorporated into the soil with the plough. Two days later, ten static closed no vented-chambers (similar to that described before) were installed in the two fields previously cropped with alfalfa (5 chambers) and maize (5 chambers). From February 14 to April 12 (57 days) the chambers were sampled 9 times to obtain soil GHG fluxes following the same method already described.

Previously to plough the fields, the total aboveground maize residue (stubble) was measured in one area of 5 m^2. In addition, the total alfalfa roots and crown residues in the topsoil (25 cm depth) were sampled in 8 locations of 0.46 m^2 each. The material was washed with distilled water to remove soil particles and dried to estimate the biomass of alfalfa residue. The total C and N of maize and alfalfa residues were analyzed by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA).

Statistical analysis

Analysis of variance was performed to evaluate the effect of the alfalfa *vs* maize crop residues on the analyzed variables. Comparisons among treatments were performed with Tukey's test. Repeated measure analysis along time, according to a first-order autoregressive structure model

AR(1), was performed to compare SMN and N_2O fluxes between alfalfa and maize fields after residue incorporation. A t-test was used to check if cumulative CH_4 fluxes were different from zero and to compare different variables between maize and alfalfa fields. In all tests, the level of significance considered by default was 95%. Soil GHG fluxes were transformed using the logarithm function when necessary to normalize their distributions and to homogenize the variances. Pearson correlation analysis was used to determine the relationship between the different variables included in the study such as N_2O fluxes and soil NO_3^- and NH_4^+ concentrations, soil temperature, and WFPS. Statistical analyses were performed using the STATGRAPHICS Centurion v.18.1.10.

Results

Static chambers experiment (2016-2017)

WFPS, soil temperature and SMN

A great temporal variability was observed in the daily topsoil WFPS (Fig. 2), that was mainly associated to the high number of irrigation events (36 and 33 in 2016 and 2017, respectively), very typical in sprinkler irrigated alfalfa. Thus, the daily average WFPS ranged from 24 to 80% with an average value of 54% (mode=59%), indicating that most of the time the soil was below field capacity (WFPS=67.7%), but during each irrigation event (or after a significant precipitation) the topsoil reached WFPS values above 70%. Considering the two growing seasons, about 10% of the time, the topsoil WFPS was higher than 70% (Fig. 3). The topsoil temperature ranged from 0.5 to 28.3ºC, with an average value of 14.9ºC.

The SMN in the topsoil (Fig. 4) was dominated by the NO_3^- form. Thus, the average NO_3^- content was 8.3 mg N kg^{-1} while the average NH_4^+ content was 1.5 mg N kg^{-1} (comprising less than 20% of the total SMN). Only at one sampling date (April 21, 2017), the soil NH_4^+ content was similar to the soil NO_3^- content. No relationship was observed between topsoil NO_3^- or NH_4^+ content with alfalfa phenology. Averaging over chambers and sampling dates, the soil NO_3^- content in 2017 (7.1 mg N kg^{-1}) was lower ($p<0.01$) than during 2016 (9.8 mg N kg^{-1}), and the opposite occurred for NH_4^+ content (2016: 1.13 mg N kg^{-1}; 2017: 1.9 mg N kg^{-1}). The relationship between soil NO_3^- content and soil temperature was not significant in both years but soil NO_3^- content was inversely related to WFPS (r=-0.40, $p<0.01$).

Soil GHG fluxes

Year-averaged soil CO_2 fluxes were higher in 2017 (74.9 kg C ha^{-1} day^{-1}; SE=3.9) than in 2016 (23.0 kg C ha^{-1} day^{-1}; SE=1.4). Soil CO_2 fluxes were significantly related to air temperature (r=0.76, $p<0.01$; data not shown). The cumulative fluxes of CO_2 during the 2016 season (no plants inside the chamber) were 7851 kg C $year^{-1}$ (SE=453, n=8), very close to the estimated C removed with the alfalfa hay in the same period (7402 kg C $year^{-1}$), and lower than the cumulative fluxes in 2017 (plants inside the chamber; Fig. S1 [suppl]).

CH_4 fluxes were low, ranging between -7.20 to 6.22 g C ha^{-1} day^{-1}, with an average value of -0.88 and -0.54 g C ha^{-1} day^{-1} in 2016 and 2017, respectively (Fig. 5). In 2016, only in 4 out of 16 sampling dates the flux was significantly

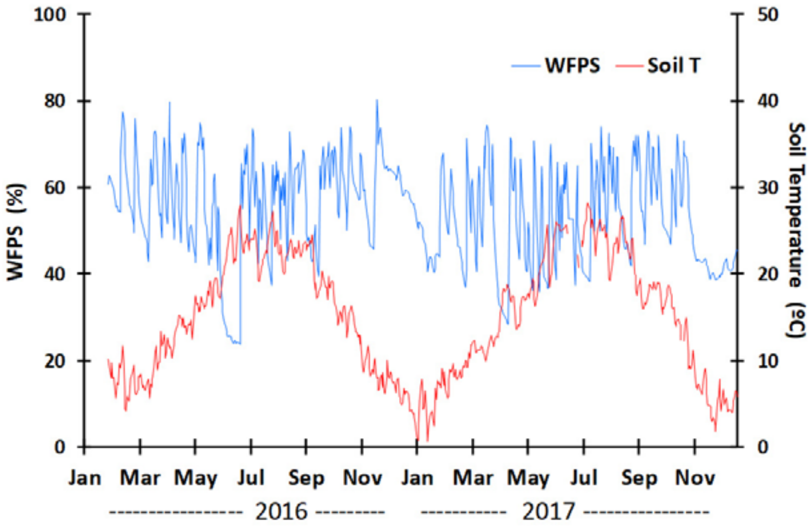

Figure 2. Evolution of daily water filled pore space (WFPS, %, 0-10 cm depth) and soil temperature (ºC, 5 cm soil depth) during 2016 and 2017. Values are the average of three soil sensors located in the alfalfa field.

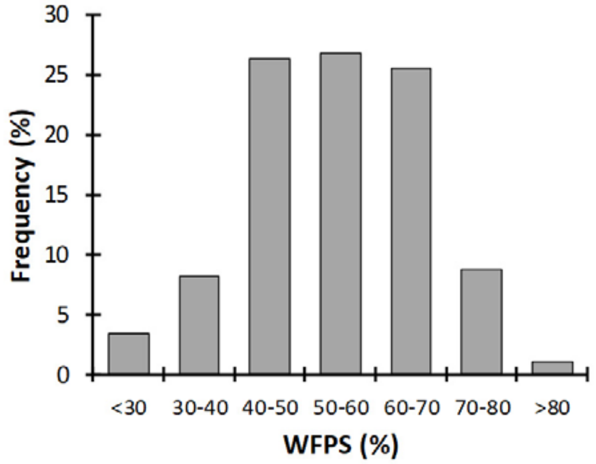

Figure 3. Histogram of frequencies of topsoil (0-10 cm depth) WFPS during 2016-2017 growing seasons.

(p<0.05) different from zero. In these cases, the flux was negative (average flux of -4.7 g C ha^{-1} day^{-1}). In 2017, only in 3 out of 17 samplings dates, the flux was significantly (p<0.05) different from zero, and similarly to 2016, in these two dates, the flux was negative (-1.39 g C ha^{-1} day^{-1}). The cumulative fluxes of CH_4 in 2016 and 2017 (Figs. 5a and 5c) were not significantly different from zero (p<0.05).

In the case of N_2O, in 28 out of 33 sampling dates, the N_2O flux was significantly (p<0.05) different from zero. No significant correlation was found (p>0.05) between N_2O fluxes and air temperature (data not shown). The year-averaged fluxes were 2.26 and 5.67 g N ha day^{-1} for 2016 and 2017, respectively. The higher average flux in 2017 was mainly due to a flux event recorded in one sampling date (April 21, 61.7 g N ha^{-1} day^{-1}; SE=8) that occurred 13 days after the first alfalfa harvest. This remarkable flux was consistent among the eight replicated chambers with values ranging from 42.4 to 83.9 g N ha^{-1} day^{-1}. That flux concurred with a high soil NH_4^+ content (12.2 mg N kg^{-1}; ranging between 6.1 and 22 mg N kg^{-1}) and a relatively high daily soil WFPS (average of 72%, ranging from 64 to 85% along the day) due to the 3-hour irrigation (24 mm) event applied the previous day of the measurements. However, the soil temperature during the mentioned event was only 16.7ºC. The four chambers located in places with soil NH_4^+ content greater than 10 mg N kg^{-1} presented an average N_2O flux of 82.8 g N ha^{-1} day^{-1}. The combined effect of WFPS and soil NH_4^+ content on N_2O fluxes is presented in Fig. S2 [suppl]. When the soil NH_4^+ content was higher than 5 mg N kg^{-1} and the WFPS was higher than 65%, the N_2O fluxes were greater than 50 g N ha^{-1} day^{-1}.

A differential relationship was found between N_2O and CO_2 fluxes depending on the top-soil water content (Fig. 6). Thus, when soil WFPS was lower than 60%, a linear relationship was observed, but the relationship became logarithmic when soil WFPS was higher than 60%.

The cumulative N_2O fluxes were 839 and 2226 g N ha^{-1} year^{-1} for 2016 and 2017, respectively (Fig. 5; Table 2). However, according to the results obtained from the automatic chambers experiment presented later in the document, the duration of N_2O peaks is usually very short (about 24 h). Therefore, considering the hypothesis that the duration of the high flux event was only of one day (instead of the effect on 47 days due to the low frequency GHG measurement), the corrected estimated cumulative N_2O emissions would be 890 g N ha^{-1} year^{-1} for the 2017 growing season.

Alfalfa yield

The total production of alfalfa hay is presented in Table 2, with an accumulated two-year value of about 28 Mg ha^{-1}. The aboveground dry matter of alfalfa contained 387 and 501 kg N ha^{-1} in 2016 and 2017, respectively. Overall, the total N-scaled nitrous oxide emissions were 2.17 y 1.78 g N kg^{-1} N for the two growing seasons.

Automatic chambers experiment (29 days)

Similarly to that observed in the static chambers experiment, the topsoil (0-10 cm) NH_4^+ content was lower than NO_3^- during the experiment, ranging from 0.82 to 3.21 mg N kg^{-1} with an average value of 1.5 mg N kg^{-1}. Soil NO_3^- content was significantly greater than NH_4^+, ranging from 8.3 to 18.3 mg N kg^{-1} and with an average value of 13.2 mg N kg^{-1}, with a significant increase (p<0.05) during the alfalfa growing period.

The evolution of topsoil (0-10 cm) WFPS was related to rainfall and irrigation events (Fig. 7). Thus, the

Table 2. Total dry matter of alfalfa (TDM), total N content on aboveground dry matter of alfalfa (TN), cumulative N_2O emission, and N_2O scaled emissions (mean ± standard error; when available).

Year	TDM (kg DM ha^{-1})	TN (kg N ha^{-1})	N_2O emission (g N ha^{-1})	N_2O scaled emissions (g N kg^{-1} N)
2016 (4th stand)	12359	387	839 ± 48	2.17
2017 (5th stand)	15855 ± 459	501 ± 12.9	890 ± 116	1.78
Total	28214	888	1729 ± 138	1.94

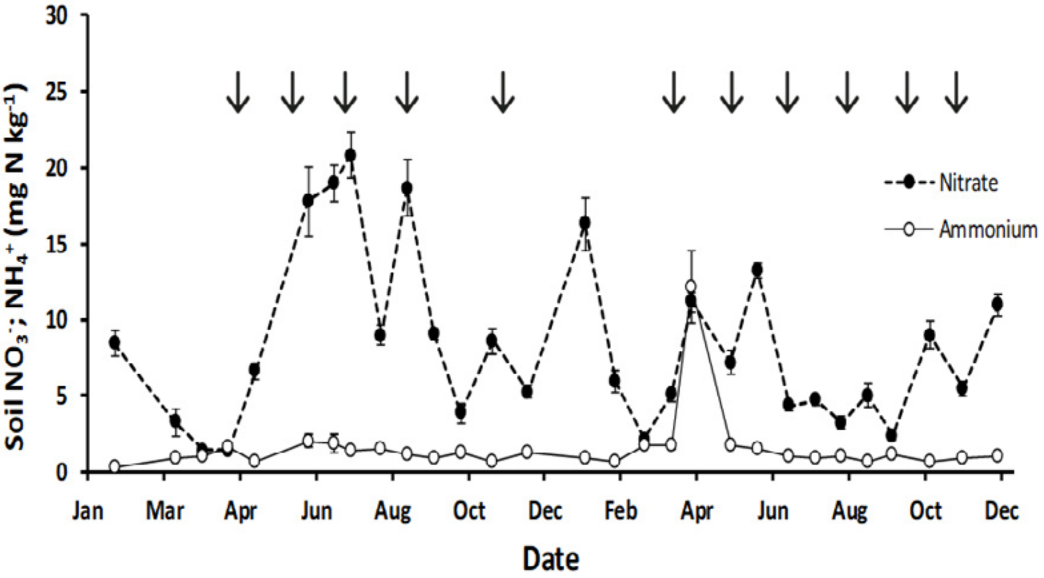

Figure 4. Evolution of soil mineral nitrogen (SMN, NO_3^- and NH_4^+) in the topsoil (0-10 cm depth) during 2016-2017 years. The vertical bar indicates the standard error (n=8). The arrows indicate the different harvest dates of alfalfa.

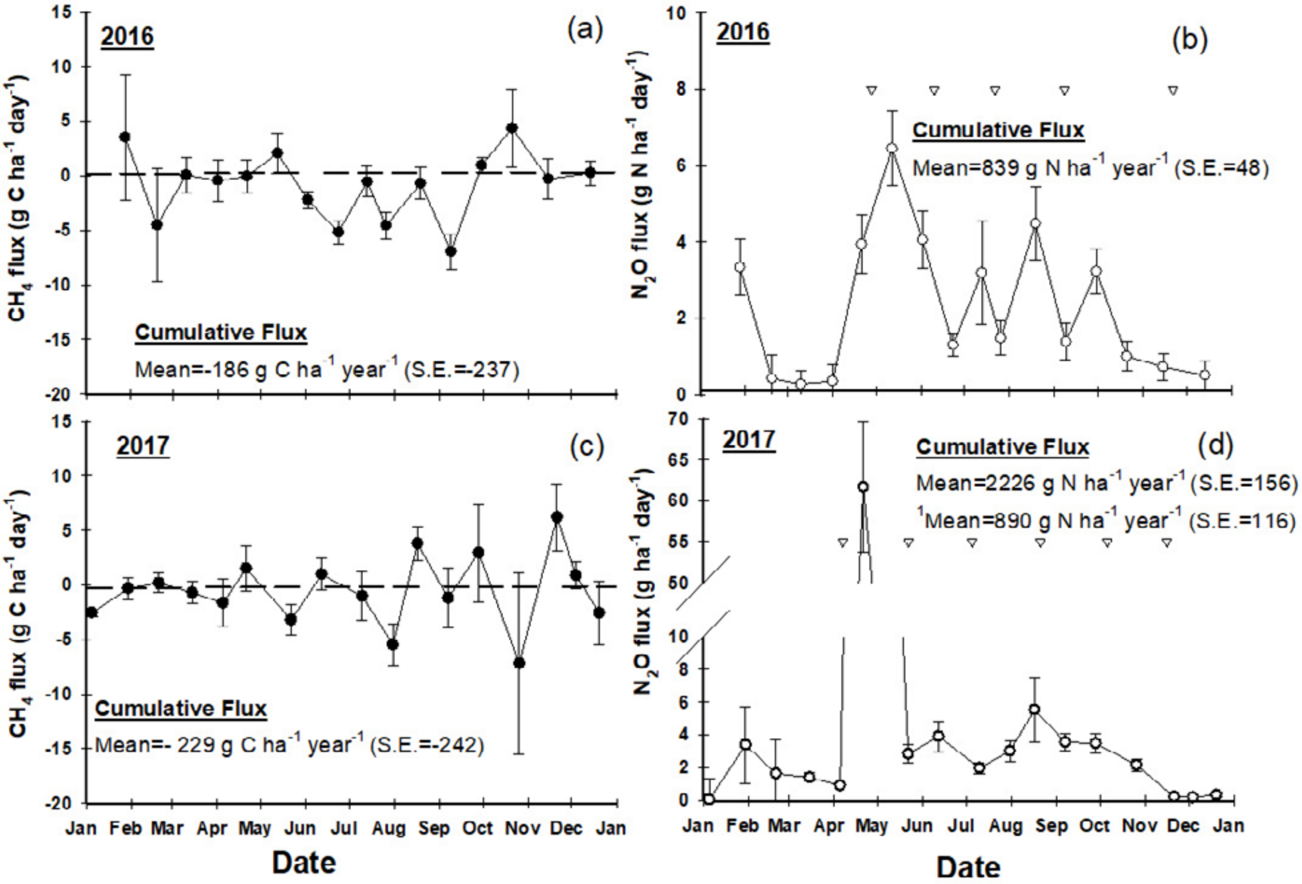

Figure 5. Evolution of methane (g C ha^{-1} day^{-1}) and nitrous oxide (g N ha^{-1} day^{-1}) fluxes during 2016 (a, b) and 2017 (c, d) in the alfalfa field. The vertical bars represent the standard error (n=8). The open triangles indicate the alfalfa harvest date. Mean cumulative emission is indicated in each figure. ^{1}Cumulative N_2O flux was estimated assuming a peak duration of one day (Fig 5-d).

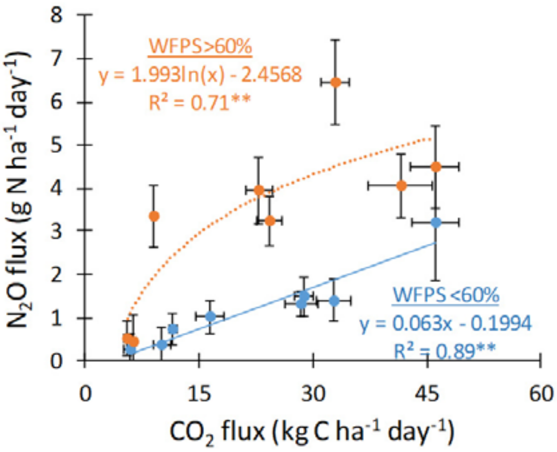

Figure 6. Relationship between N2O and CO2 fluxes for two different ranges of WFPS. Data are from the 2016 year, in which the plants were removed from the chambers. The bars indicate the standard error (n=8).

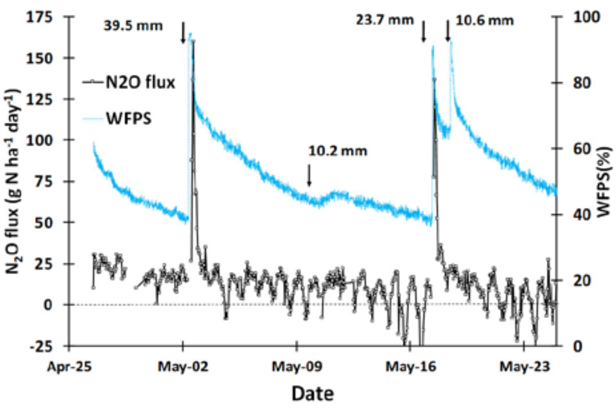

Figure 7. Hourly average N_2O flux (g N ha^{-1} day^{-1}) and 3-min average topsoil (0-10 cm) WFPS (%) during the period of April 26th to May 25th, 2017. The vertical arrows indicate the irrigation or rain events greater than 10 mm. The values are the average of the two chambers (C1 and C2).

two-chambers average WFPS ranged from 36 to 95% with a mean value of 51.7% (53.8 and 49.7% for chambers C1 and C2, respectively). Four peaks of topsoil WFPS were observed associated to events of irrigation or rain greater than 10 mm. In three of these events the WFPS reached values close to saturation.

The N_2O fluxes in the two chambers were very similar and thereby the hourly values were averaged (Fig. 7). The average hourly N_2O flux during the period of 29 days (26 April to 25 May) was 12.6 g N ha^{-1} day^{-1} (SE=0.67; n=653). Some negative N_2O fluxes (14% of the total measurements) were found at some specific days, but due to the low sensitivity of photoacoustic equipment at low N_2O concentrations, the accuracy and confidence of fluxes below 10 g ha^{-1} day^{-1} is low. However, the two N_2O peaks observed on May 2 (maximum 160 g N ha^{-1} day^{-1}; averaged over the two chambers) and May 17 (maximum 136 g N ha^{-1} day^{-1}; averaged over the two chambers) in both chambers had a short but significant duration, ranging from 6 to 24 hours. Averaging over the two chambers these peaks accounted for a total N_2O emission of 47.2 and 22.6 g N ha^{-1} associated to two irrigation events that increased the WFPS above 80%. It was noteworthy that another WFPS peak was measured after a rainfall occurred on May 18th (Fig. 7) but no peak of N_2O flux was observed.

GHG fluxes after alfalfa termination and in an adjacent maize field

The amount of residues (roots and crowns) in the alfalfa field was 4808 kg DM ha^{-1} (SE=623) with an average N content of 2.63% (SE=0.06). Thus, the total N incorporated into the topsoil with the alfalfa residues was 185 kg N ha^{-1} (SE=14.9). In the adjacent field, cropped the previous year with maize, a total of 10003 kg DM ha^{-1} of stubble with a N content of 0.55% was incorporated into the soil (55 kg N ha^{-1}). The C/N ratios of maize and alfalfa residues were 81.6 and 17.8, respectively.

A total of 144 mm of rain fell during the 57-day period (Febr. 14 to Apr. 12) included in the study (Fig. 8a). The topsoil (0-10 cm) WFPS ranged from 36 to 82% with an average of 62%, close to the 67% value corresponding to field capacity. WFPS changed over time according to rainfall events and there was a tendency of higher WFPS in the maize field (63.2%) compared to the alfalfa field (60.5%), although only in 2 out of 9 dates the difference was significant. The soil temperature at 5-cm depth ranged from 8 to 21ºC with an average value of 13.2ºC. No consistent significant differences in soil temperature were observed between maize and alfalfa fields along the whole period, but small (<1ºC) differences were found in three sampling dates.

Very low topsoil NH_4^+ content was found in the two fields (Fig. 8b) with values ranging from 0.4 to 2.7 mg N kg^{-1} and an average of 1.2 mg N kg^{-1}. No significant differences in soil NH_4^+ content were found between alfalfa and maize fields. However, the topsoil NO_3^- content in 5 out of 9 sampling dates was higher in the alfalfa field (average of 18.7 mg N kg^{-1}) than in the maize field (average of 10.2 mg N kg^{-1}).

Daily fluxes of CO_2 were significantly higher after alfalfa than after maize (Fig. 9); with a cumulative flux also significantly higher (510 kg C ha^{-1} for alfalfa *vs* 240 kg C ha^{-1} for maize) after 57 days of plow down the crop residues. The soil CH_4 flux was not significantly different from zero in 17 of the 18 samplings (9 dates × 2 plots). No significant differences of cumulative CH_4 fluxes between alfalfa and maize fields were found, with a tendency to CH_4 consumption (-3.1 g C-CH_4 ha^{-1} day^{-1}) by the soils during the measurement period. Soil N_2O fluxes ranged from 0 to 5 g N ha^{-1} day^{-1} except the last sampling date in the maize field

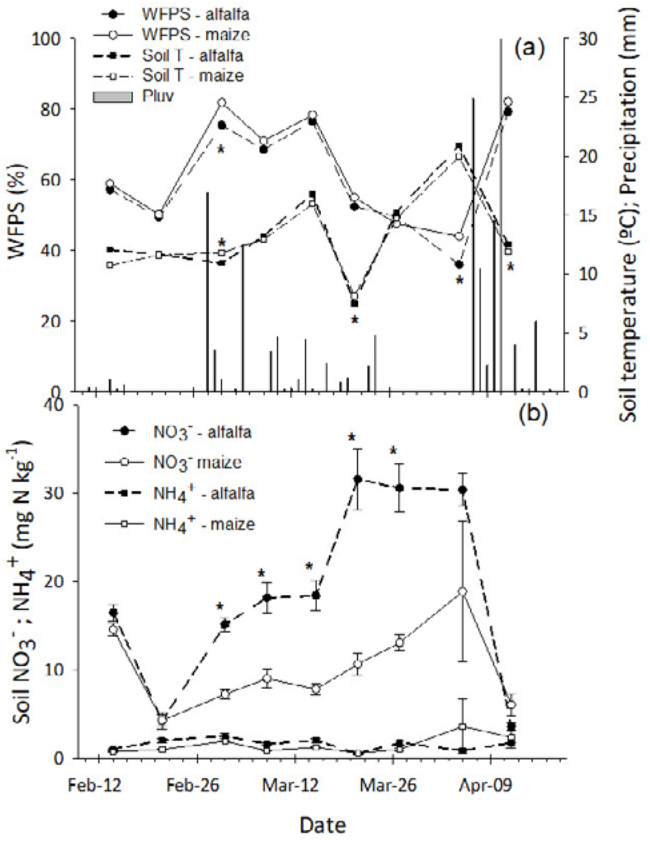

Figure 8. Evolution of (a) water filled pore space (WFPS,%), soil temperature (ºC), and precipitation; and (b) soil NO_3^- and NH_4^+ content (mg N kg^{-1}) in the topsoil (0-10 cm depth) of the maize and alfalfa (after plow-down) fields. For soil mineral content, the vertical bars indicate the standard error (n=5). The asterisk denotes the dates with significant differences ($p<0.05$; Tukey test) in NO_3^- content between alfalfa and maize fields.

(April 12) that showed a noticeable peak of 18.5 g N ha^{-1} day^{-1} (SE=8.21), that occurred after two consecutive days with significant irrigation (April 10th: 14.5 mm; April 11th: 30.6 mm). A great variability in N_2O flux was observed in the last sampling date at the maize field. Thereby, the N_2O flux ranged from 6.11 to 42.3 g N ha^{-1} day^{-1} among the five maize chambers. Only in one sampling date (February 21) the alfalfa field had a higher N_2O flux than the maize field. However, no significant differences were observed in the cumulative N_2O fluxes between the two fields either including or excluding the last sampling date.

Discussion

GHG emissions during the alfalfa growing period

Greenhouse gas fluxes are closely linked to soil microbial activity and root-soil turnover, and therefore related to alfalfa yield. The alfalfa yields and crop N content were typical for the region (Cavero *et al.*, 2017) and very similar to the values presented in the mentioned study performed close to our experimental site. At the conditions of the study, sprinkler irrigated alfalfa was not a significant source of CH_4 emissions. When fluxes were significantly different from zero they were mostly negative, suggesting a slight tendency to act as a CH_4 sink, probably due to the action of methanotrophic microbes under aerobic conditions. The seasonal average of CH_4 fluxes (-0.71 g C ha^{-1} day^{-1}; SE=0.55) was similar to the reported value of -1.08 g C ha^{-1} day^{-1} by Ellert & Janzen (2008). The predominant aerobic conditions under sprinkler irrigated, that usually applies low rates of water to crops, precludes significant CH_4 emissions as it has been reported under similar soil and irrigation management conditions in other crops such as maize (Alvaro-Fuentes *et al.*, 2016) and wheat (Mateo-Marín *et al.*, 2020). According to Walkiewicz *et al.* (2018), low soil NH_4^+ content increases CH_4 oxidation under aerated conditions, although the effect is very influenced by soil properties. Therefore, the low soil NH_4^+

content found in our study could partially explain the slight tendency to methanotrophic activity observed in the alfalfa crop in some specific days when the negative CH_4 flux was significant.

N_2O fluxes tended to be lower during winter (*i.e.*, from November to March), as found in other studies with other irrigated crops under similar climate conditions (Franco-Luesma *et al.*, 2020), where besides low soil temperature, irrigation is usually not applied during the winter. N_2O emissions in sprinkler-irrigated alfalfa under our Mediterranean conditions (865 g N ha^{-1} $year^{-1}$) were slightly lower than the reported under Midwestern US non-irrigated conditions (Goodroad *et al.*, 1984; Osterholz *et al.*, 2014) and similar to those obtained by Ellert & Janzen (2008) for sprinkler-irrigated alfalfa in Alberta (Canada) with similar soil texture, although with lower seasonal air temperature (6.2ºC). However, the N_2O emissions found in our study were clearly smaller than those reported by Burger *et al.* (2016) in a 5th year alfalfa stand (5.26 kg N ha^{-1} $year^{-1}$) and a 2th year alfalfa stand (2.26 kg N ha^{-1} $year^{-1}$) under similar climatic conditions but under flood irrigation. The shorter period of time where the SWC is above field capacity under sprinkler irrigation as compared to flood irrigation could be the reason of the lower N_2O emissions found in our experiment, as found in maize in a nearby experiment (Franco-Luesma *et al.*, 2020). Mateo-Marin (2020) reported 35% lower N_2O fluxes when wheat plants were included inside the chambers compared to trimmed plants. However, no significant effect was found in our study with alfalfa, with similar cumulative fluxes in 2016 and 2017, may be due to the deeper root system of alfalfa compared to wheat.

Although the daily average of WFPS in the topsoil during the two years was 54%, indicating the predominance of aerobic conditions in sprinkler-irrigated alfalfa, there were short periods of anaerobic conditions. Thus, over the two-year period of sprinkler irrigated alfalfa, during 1669 hours (79 days) the upper 10 cm of the soil presented WFPS values higher than 70%, and during a total of 187 hours (8 days) the topsoil presented a WFPS higher than 80%. A rapid response of N_2O fluxes to topsoil saturated conditions, due to irrigation or rainfall events, were observed in the automatic chambers experiment. There were also irrigation events that increased soil water content near to saturation but with no significant peaks of N_2O, indicating that availability of NO_3^- or labile carbon can be limiting N_2O losses by denitrification, or promoting complete denitrification, *i.e.*, N_2O reduction to N_2. However, Burger *et al.* (2016) did not find a relationship between dissolved organic carbon (DOC) concentration and N_2O fluxes in flooded-irrigated alfalfa, which suggest a complex interaction remaining to be clarified to fully understand the complex denitrification processes. Anyhow, only continuous or very frequent measurements can consistently detect these high peaks of N_2O flux, that are difficult to foresee in alfalfa because N fertilizer is not applied.

In agreement with the results reported by Beauchamp *et al.* (1996) and Burger *et al.* (2016), the largest N_2O fluxes observed in alfalfa (up to 263 g N ha^{-1} day^{-1}) were related to significant irrigation or rainfall events (>20 mm). These large fluxes had a very short duration since they dropped to one third after three hours, and are difficult to observe, unless continuous measurement is made. That short-time large fluxes, in absence of N fertilizer applications and high soil NH_4^+ content, can be associated to the N turnover from alfalfa roots and exudates, inducing both nitrification and denitrification and opportunity to N_2O emissions, depending on the SWC.

In both growing seasons, a large N_2O peak was observed at April-May, between the first and the second alfalfa harvest in the static chambers experiment. However, this early season peak of N_2O was much higher in 2017. A relatively long time period (28 days) without rain or irrigation, due to a problem with the pumping station, preceded this unique remarkable N_2O flux event. During this period, the soil experienced a sharp decrease in the WFPS (average of 29.7% from 13 to 19 April) and a sudden soil rewetting associated to irrigation the day before to the observed peak. Recent studies (Szukics *et al.*, 2010; Bergstermann *et al.*, 2011; Barrat *et al.*, 2021), described very rapid responses of nitrifiers and denitrifiers to changes in soil moisture, that can partly explain the unique high-emission event detected during our experiment. Main findings from these studies are that the N_2O pulses after rewetting are mainly related to the drought intensity before the rewetting event. As demonstrated by Zhu *et al.* (2013), the coincidence of low oxygen availability with large soil NH_4^+ concentration suggests that, the different ammonia oxidation pathways (nitrifier nitrification, nitrifier denitrification, and nitrification-coupled denitrification) can play a significant role in the observed N_2O peak. The observed peak in soil NH_4^+ could also be explained by an event happened at the end of 2016 growing season. Thus, in November 22 (2016) a significant regrowth of alfalfa was chopped and left into the soil surface due to impossibility to get natural hay process. The decomposition of a relatively large amount of plant residues with low C/N composition and high WFPS could trigger a raising in soil NH_4^+ as was described by López-Bellido *et al.* (2014) in Mediterranean conditions.

Our results also showed that the N_2O fluxes were significantly linked to CO_2 fluxes (C-mineralization) but the relationship differed according to the soil aeration status, as found by Samad *et al.* (2016). The observed year-averaged fluxes measured in the static chambers experiment were similar or lower than the basal fluxes from non N fertilized fields observed in other crops such as maize (mean=4.05 g N ha^{-1} day^{-1}; Alvaro-Fuentes *et al.*, 2016) and wheat (mean=2.80 g N ha^{-1} day^{-1}; Mateo-Marín *et al.*, 2020) under similar agroecological conditions.

In the static chambers experiment and manual air sampling, as probably happen in other similar studies, we were not able to sample during the hours immediately following

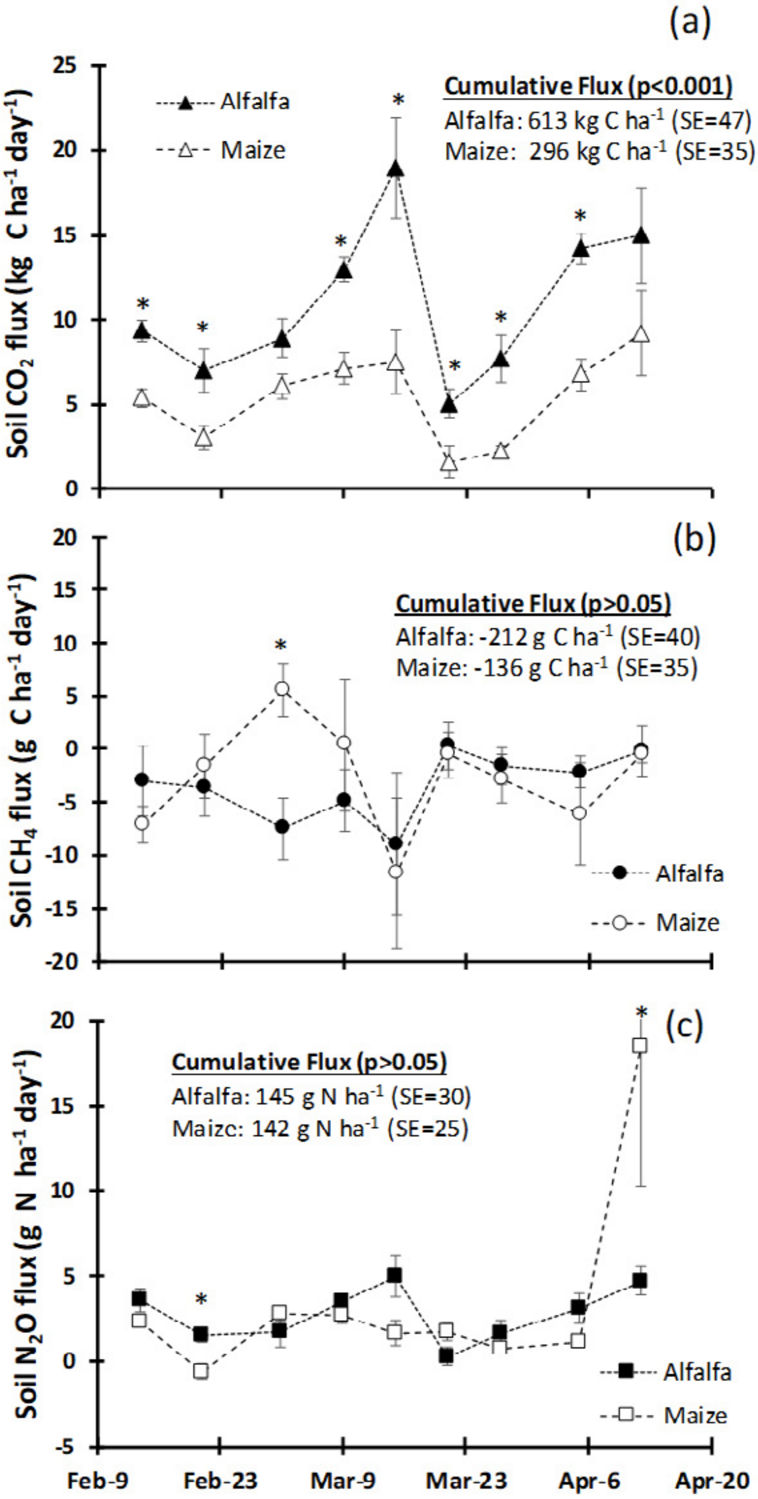

Figure 9. Evolution of (a) CO_2, (b) CH_4, and (c) N_2O daily fluxes of two adjacent fields during the fallow period (Febr-Apr) after a maize crop and alfalfa plow down. The vertical bars indicates the standard error (n=5). For a given sampling time the asterisk indicates when the difference was significant (p<0.05). The cumulative fluxes from February 14 to April 12 are presented.

irrigation or rainfall events due to the difficulty to move on saturated soils and to prevent human trampling on experimental areas near the chambers. This methodology can leave unnoticed short-duration N_2O peaks emissions that can be relatively frequent under sprinkler irrigated alfalfa (on average 34 irrigation events every year), leading to an underestimation of the cumulative N_2O emissions. However, as was showed in the 2017 alfalfa experiment, the static chamber methodology can also overestimate N_2O fluxes, attributing longer peak duration due to the large sampling interval. Therefore, the overall effect on cumulative emissions quite is unpredictable. However, the observed absence of N_2O peaks after some irrigation (or rainfall) events in which the WFPS surpassed 80-90% together with a significant lack of knowledge about denitrification kinetics (Samad *et al*., 2016) maintain considerable uncertainty about the overall contribution of these short-lived emission peaks in the whole growing season.

GHG emissions after alfalfa termination

The spring incorporation of a large amount of low C/N residue after alfalfa termination induced a higher microbiological activity (higher soil respiration rate) compared to the incorporation of a higher C/N residue observed in an adjacent maize cropped field. Pascault *et al.* (2013) demonstrated that the biochemical composition of the fresh residue added to the soil has a significant impact on the type of microbial communities that are stimulated to degrade the fresh organic matter with greater respiration rate after incorporating alfalfa residues compared to wheat residues with higher C/N ratio. In our experiment this effect was also observed with higher CO_2 emissions after incorporating alfalfa (613 kg C ha^{-1}; SE=47) than after incorporating maize (296 kg C ha^{-1}; SE=35) in the 57-days period after residue incorporation.

According to a recent meta-analysis (Charles *et al.*, 2017) the C/N ratio of crop residues has a significant impact on N_2O emissions. A lower C/N ratio results in a higher N_2O emission. However, contrary to the mentioned study and our initial hypothesis, N_2O emissions were not significantly affected by the type of residue, even when in the maize-stubble field the soil NO_3^- concentration was significantly lower than in the alfalfa-residue field. Although the N_2O fluxes were relatively low (< 5 g N-N_2O ha^{-1} day^{-1}), we cannot discard larger fluxes of other N gases such as N_2, specially at higher WFPS conditions and for high soil pH soils, as found by Pu *et al.* (1999). Large N_2 losses are unimportant from a GHG contribution perspective but are of interest if considered as N lost from the soil-crop system.

Conclusions

Our results show that the annual N_2O losses in sprinkler irrigated alfalfa in semi-arid Mediterranean conditions are clearly lower than those reported in N-fertilized maize (Alvaro-Fuentes *et al.,* 2016) or wheat (Isla *et al*., 2020) under similar environmental conditions. However, studies capable to capture more consistently episodic high N_2O fluxes associated to irrigation or rainfall events are necessary to obtain a more precise evaluation of N_2O emissions in alfalfa. For future studies in this crop, it is advisable to increase the gas sampling frequency to reduce the uncertainties, paying special attention in irrigation or rainfall events. In addition, due to the effect of soil water content on N_2O fluxes, specially associated to denitrification, the studies must also address the effect of irrigation practices such as irrigation system, doses and frequency. Recent work with maize has found that irrigation practices can affect N_2O fluxes (Franco-Luesma *et al*., 2019, 2020). Only with a deeper knowledge of the underlying mechanisms that result in soil N_2O flux in a worldwide crop such as alfalfa will be possible to decrease these emissions.

Acknowledgments

We acknowledge to J. Lampurlanes (University of Lleida, Spain) to design and provide a switch box that controlled gas sampling in the automatic chambers experiment. Thanks are also given to Jorge Alvaro-Fuentes (EEAD-CSIC, Spain) for the gas chromatography analysis support, and to the field and laboratory personnel of the Soils and Irrigation Department of CITA.

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References

Allen RG, Pereira LS, Raes D, Smith M, 1998. Crop evapotranspiration-Guidelines for computing crop water requirements. FAO Irrig Drain paper 56, Rome, 300(9).

Alvaro-Fuentes J, Arrúe JL, Cantero-Martínez C, Isla R, Plaza-Bonilla D, Quílez D, 2016. Fertilization scenarios in sprinkler-irrigated corn under mediterranean conditions: Effects on greenhouse gas emissions. Soil

Sci Soc Am J 80(3): 662-671. https://doi.org/10.2136/sssaj2015.04.0156

Alves BJR, Smith KA, Flores RA, Cardoso AS, Oliveira WRD, Jantalia CP, *et al*., 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N_2O flux from soils. Soil Biol Biochem 46: 129-135. https://doi.org/10.1016/j.soilbio.2011.11.022

Annicchiarico P, Barrett B, Brummer EC, Julier B, Marshall AH, 2015. Achievements and challenges in improving temperate perennial forage legumes. Crit Rev Plant Sci 34: 327-380. https://doi.org/10.1080/07352689.2014.898462

Barrat HA, Evans J, Chadwick DR, Clark IM, Le Cocq K, Cardenas LM, 2021. The impact of drought and rewetting on N_2O emissions from soil in temperate and Mediterranean climates. Eur J Soil Sci 72: 2504-2516. https://doi.org/10.1111/ejss.13015

Beauchamp EG, Bergstrom DW, Burton DL, 1996. Denitrification and nitrous oxide production in soil fallowed or under alfalfa or grass. Commun Soil Sci Plant Anal 27(1-2): 87-99. https://doi.org/10.1080/00103629609369546

Bergstermann A, Cardenas LM, Bol R, Gilliam LM, Goulding KWT, Meijide A *et al*., 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biol Biochem 43: 240-250. https://doi.org/10.1016/j.soilbio.2010.10.003

Bouwman AF, 1996. Direct emission of nitrous oxide from agricultural soils. Nutr Cycl Agroecosys 46(1): 53-70. https://doi.org/10.1007/BF00210224

Burger M, Haden VR, Chen H, Six J, Horwath WR, 2016. Stand age affects emissions of N_2O in flood-irrigated alfalfa: a comparison of field measurements, DNDC model simulations and IPCC Tier 1 estimates. Nutr Cycl Agroecosys 106(3): 335-345. https://doi.org/10.1007/s10705-016-9808-8

Cavero J, Faci JM, Medina ET, Martínez-Cob A, 2017. Alfalfa forage production under solid-set sprinkler irrigation in a semiarid climate. Agric Water Manage 191: 184-192. https://doi.org/10.1016/j.agwat.2017.06.018

Cela S, Salmeron M, Isla R, Cavero J, Santiveri F, Lloveras J, 2011. Reduced nitrogen fertilization to corn following alfalfa in an irrigated semiarid environment. Agron J 103(2): 520-528. https://doi.org/10.2134/agronj2010.0402

Charles A, Rochette P, Whalen JK, Angers DA, Changtigny MH, Bertrand N, 2017. Global nitrous oxide emission factors from agricultural soils after addition of organic amendments: A meta-analysis. Agr Ecosyst Environ 236: 88-98. https://doi.org/10.1016/j.agee.2016.11.021

Ellert BH, Janzen HH, 2008. Nitrous oxide, carbon dioxide and methane emissions from irrigated cropping systems as influenced by legumes, manure and fertilizer. Can J Soil Sci 88(2): 207-217. https://doi.org/10.4141/CJSS06036

Franco-Luesma S, Alvaro-Fuentes J, Plaza-Bonilla D, Arrúe JL, Cantero-Martinez C, Cavero J, 2019. Influence of irrigation time and frequency on greenhouse gas emissions in a solid-set sprinkler-irrigated maize under Mediterranean conditions. Agric Water Manage 221: 303-311. https://doi.org/10.1016/j.agwat.2019.03.042

Franco-Luesma S, Cavero J, Plaza-Bonilla D, Cantero-Martínez C, Tortosa G, Bedmar EJ, Álvaro-Fuentes J, 2020. Irrigation and tillage effects on soil nitrous oxide emissions in maize monoculture. Agron J 112: 56-71. https://doi.org/10.1002/agj2.20057

Gelfand I, Shcherbak I, Millar N, Kravchenko AN, Robertson GP, 2016. Long-term nitrous oxide fluxes in annual and perennial agricultural and unmanaged ecosystems in the upper Midwest USA. Global Change Biol 22(11): 3594-3607. https://doi.org/10.1111/gcb.13426

Goodroad LL, Keeney DR, Peterson LA, 1984. Nitrous oxide emissions from agricultural soils in Wisconsin. J Environ Qual 13(4): 557-561. https://doi.org/10.2134/jeq1984.00472425001300040010x

Grossman RB, Reinsch TG, 2002. Bulk density and linear extensibility. Methods of soil analysis: Part 4 physical methods. SSSA Book Series 2.1 5: 201-228. https://doi.org/10.2136/sssabookser5.4.c9

Holland EA, Robertson GP, Greenberg J, Groffman PM, Boone RD, Gosz JR, 1999. Soil CO_2, N_2O, and CH_4 exchange. In: Standard soil methods for long-term ecological research; Robertson GP *et al*. (eds). Oxford Univ Press, NY, pp: 185-201.

Ingram LJ, Schuman GE, Parkin TB, Mortenson M, 2015. Trace gas fluxes from a Northern mixed-grass prairie interseeded with Alfalfa. Plant Soil 386(1-2): 285-301. https://doi.org/10.1007/s11104-014-2264-9

IPCC, 2006. Agriculture, forestry and other land use Chapter 1: Introduction. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Intergovernmental Panel on Climate Change.

IPCC, 2014. Climate Change 2014: Mitigation of climate change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ Press, Cambridge, UK, and New York.

Isla R, Guillén M, Aibar J, Quílez D, 2020. Variabilidad espacial de emisiones de óxido nitroso y metano en una parcela comercial de trigo blando bajo sistema de riego por aspersión. VII Remedia Workshop, 22-23 Sep, Elche, Spain. http://hdl.handle.net/10532/5084

Lindenmayer RB, Hansen NC, Brummer J, Pritchett JG, 2011. Deficit irrigation of alfalfa for water-savings in the Great Plains and Intermountain West: A review and analysis of the literature. Agron J 103: 45-50. https://doi.org/10.2134/agronj2010.0224

Linn DM, Doran JW, 1984 Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am

J 48(6): 1267-1272. https://doi.org/10.2136/sssaj1984.03615995004800060013x

López-Bellido L, Muñoz-Romero V, Fernández-García P, López-Bellido RJ, 2014. Ammonium accumulation in soil: the long-term effects of tillage, rotation and N rate in a Mediterranean Vertisol. Soil Use Manag 30(4): 471-479. https://doi.org/10.1111/sum.12147

MacKenzie AF, Fan MX, Cadrin F, 1997. Nitrous oxide emission as affected by tillage, corn-soybean-alfalfa rotations and nitrogen fertilization. Can J Soil Sci 77(2): 145-152. https://doi.org/10.4141/S96-104

Mateo-Marín N, 2020. Efficacy of nitrification and urease inhibitors to reduce nitrogen losses under optimal management practices in irrigated Mediterranean agrosystems. Doctoral Thesis, University of Zaragoza, Zaragoza, Spain.

Mateo-Marín N, Quílez D, Guillén M, Isla R, 2020. Feasibility of stabilised nitrogen fertilisers decreasing greenhouse gas emissions under optimal management in sprinkler irrigated conditions. Agr Ecosyst Environ 290: 106725. https://doi.org/10.1016/j.agee.2019.106725

Millar N, Ndufa JK, Cadisch G, Baggs EM, 2004. Nitrous oxide emissions following incorporation of improved-fallow residues in the humid tropics. Global Biogeochem Cy 18(1): GB1032. https://doi.org/10.1029/2003GB002114

Muhammad W, Vaughan SM, Dalal RC, Menzies NW, 2011. Crop residues and fertilizer nitrogen influence residue decomposition and nitrous oxide emission from a Vertisol. Biol Fert Soils 47(1): 15-23. https://doi.org/10.1007/s00374-010-0497-1

Osterholz WR, Kucharik CJ, Hedtcke JL, Posner JL, 2014. Seasonal nitrous oxide and methane fluxes from grain- and forage-based production systems in Wisconsin, USA. J Environ Qual 43(6): 1833-1843. https://doi.org/10.2134/jeq2014.02.0077

Pascault N, Ranjard L, Kaisermann A, Bachar D, Christen R, Terrat S *et al.*, 2013. Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. Ecosystems 16(5): 810-822. https://doi.org/10.1007/s10021-013-9650-7

Pu GX, Saffigna PG, Strong WM, 1999. Potential for denitrification in cereal soils of northern Australia after legume or grass-legume pastures. Soil Biol Biochem 31(5): 667-675. https://doi.org/10.1016/S0038-0717(98)00154-0

Rochette P, Janzen HH, 2005. Towards a revised coefficient for estimating N_2O emissions from legumes. Nutr Cycl Agroecosys 73(2-3): 171-179. https://doi.org/10.1007/s10705-005-0357-9

Samad MS, Bakken LR, Nadeem S, Clough TJ, de Klein CAM, Richards KG *et al.*, 2016. High-resolution denitrification kinetics in pasture soils link N_2O emissions to pH, and denitrification to C mineralization. PLoS ONE 11(3): e0151713. https://doi.org/10.1371/journal.pone.0151713

Schmeer M, Loges R, Dittert K, Senbayram M, Horn R, Taube F, 2014. Legume-based forage production systems reduce nitrous oxide emissions. Soil Till Res 143: 17-25. https://doi.org/10.1016/j.still.2014.05.001

Shelp ML, Beauchamp EG, Thurtell GW, 2000. Nitrous oxide emissions from soil amended with glucose, alfalfa, or corn residues. Commun Soil Sci Plan 31(7-8): 877-892. https://doi.org/10.1080/00103620009370484

Soil Survey Staff, 2014. Keys to soil taxonomy, 12th ed. USDA-Natural Resources Conservation Service: Washington, DC.

Steduto P, Cetinkoku O, Albrizio R, Kanber R, 2002. Automated closed-system canopy-chamber for continuous field-crop monitoring of CO_2 and H_2O fluxes. Agric Forest Meteorol 111: 171-186. https://doi.org/10.1016/S0168-1923(02)00023-0

Szukics U, Szukics U, Abell GC, Hodl V, Mitter B, Sessitsch A *et al.*, 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. FEMS Microbiol Ecol 72(3): 395-406. https://doi.org/10.1111/j.1574-6941.2010.00853.x

Wagner-Riddle C, Thurtell GW, Kidd GK, Beauchamp EG, Sweetman R, 1997. Estimates of nitrous oxide emissions from agricultural fields over 28 months. Can J Soil Sci 77(2): 135-144. https://doi.org/10.4141/S96-103

Walkiewicz A, Brzezinska M, Bieganowski A, 2018. Methanotrophs are favored under hypoxia in ammonium-fertilized soils. Biol Fert Soils 54(7): 861-870. https://doi.org/10.1007/s00374-018-1302-9

Zhong Z, Nelson LM, Lemke RL, 2011. Nitrous oxide emissions from grain legumes as affected by wetting/drying cycles and crop residues. Biol Fert Soils 47(6): 687-699. https://doi.org/10.1007/s00374-011-0575-z

Zhu X, Burger M, Doane TA, Horwath WR, 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. P Nat Acad Sci 110(16): 6328-6333. https://doi.org/10.1073/pnas.1219993110