Agronomic and Environmental Implications of Substituting Pig Slurry for Synthetic Nitrogen in Mediterranean Wheat Systems

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Abstract: Using slurries as fertilizers is a promising strategy for the reuse of nutrients and striving towards a circular economy. This study aims to assess the agronomic productivity and the environmental effects on Mediterranean sprinkler-irrigated wheat during three consecutive years of (1) the use of pig slurry (PS) as a substitute for a synthetic fertilizer (urea, U) and (2) the addition of a urease inhibitor (monocarbamide dihydrogen sulfate, MCDHS) to pig slurry (PSI). A nitrogen (N) target rate of 120 kg NH_4^+-N ha^{-1} as U, PS, or PSI (main factor) was applied at tillering, and it was supplemented with N application at stem elongation (secondary factor). Grain yield, nitrogen use efficiency indexes, and nitrous oxide emissions were not significantly affected by the N source, U, or PS; in contrast to grain protein that was affected by the N source (lower protein content in PS). The higher unaccounted N from soil balance in PS compared to U fertilization could be associated with higher ammonia volatilization, although additional studies should confirm this hypothesis. Our study suggests that, under the considered cropping conditions, PS does not affect productivity or N_2O losses compared to U. The addition of MCDHS to pig slurry did not display agronomic or environmental benefits under the evaluated agro-environmental conditions.

Keywords: monocarbamide dihydrogen sulfate; nitrogen losses; nitrous oxide emissions; urea

1. Introduction

Wheat is one of the main cultivated cereals around the world, with 214.8 million hectares under production [1]. In Spain, wheat production represents 34% of grain cereal production [2], since the Mediterranean climate allows for producing high-quality wheat with adequate management practices [3], especially bread wheat, with more demanding quality standards.

Spain is the European country with the highest porcine livestock population, reaching 30.8 million heads in 2018 [4]. The slurry (PS) produced contains more than 1.3 million tons of nitrogen (N) and is applied as fertilizer to field crops, being the most common recycling method for this product. However, high rates of nutrients on farmland, usually desynchronized with crop demands, increase notably the risk of environmental pollution [5]. Pollution associated with PS application includes nitrate (NO_3^-) leaching, an increase in soil heavy metal concentrations (mainly zinc and copper), and ammonia (NH_3) and nitrous oxide (N_2O) emissions to the atmosphere [6–9]. Accordingly, different management practices can be implemented to reduce these environmental risks; e.g., PS applied at adequate rates leads to minimizing N losses by drainage [10] and early slurry incorporation with tillage after its application is recommended to control NH_3 volatilization [11].

The emission of N_2O from agricultural soils is mainly related to two biological processes, denitrification and nitrification, although some small N_2O emissions may be also produced through non-biological processes. Denitrification is the reduction of nitrate to molecular nitrogen (N_2) through the mediation of anaerobic denitrifiers; the process can be incomplete and some of the nitrogen is emitted as nitric oxide (NO) and N_2O [12]. The main factors affecting denitrification in soils are soil nitrate concentration, temperature, humidity, and labile carbon presence [13]. Denitrification is maximum at high soil nitrate concentrations, with temperatures in the range of 28–36 °C, when the soil water-filled pore space (WFPS) exceeds 60%, although in very wet soils (WFPS >90%) N_2 is the prevalent form of denitrification, and it is enhanced in soils with a ready supply of organic carbon, such as manure or crop residues [14]. However, PS has low C content, thus its application would be not expected to have a strong influence on N_2O emissions in comparison to synthetic N. Nitrification is the pathway in which ammonium is oxidized to nitrite, and then nitrite is reduced to nitrate; the process is mediated by autotrophic ammonia oxidizers, autotrophic nitrite oxidizers, and heterotrophic nitrifiers [15]. Nitrification needs soil ammonium, and it is optimum at a temperature between 25 and 30 °C, and when WFPS is around 55% for fine-textured soils, and around 40% WFPS for coarse-textured soils [16]. The main pathway of N_2O emission in aerobic Mediterranean cultivation is nitrification, with denitrification relevant only on the rare occasions when WFPS > 60%.

The meta-analysis of Aguilera et al. [8] showed that the use of organic fertilizers significantly reduced direct N_2O emissions in comparison to synthetic fertilizers, but this reduction depended on the type of product, being more effective in solid manure than in liquid slurry, where no reduction was observed. These authors identified some limitations and knowledge gaps of studies with organic fertilization that should be covered, such as measurements for longer periods or evaluation of yield and yield-scaled emissions with different types of products.

Adjusting N fertilizer rate and splitting the N application are well-known strategies to improve nitrogen use efficiency in wheat and reduce reactive N losses to the environment. Nitrogen application at tillering provides yield increase by increasing the number of ears, and latter application in the period of stem elongation-flowering increases the grain protein content [17]. Pig slurry application at the same nitrogen rate as that used for synthetic fertilizers can result in similar crop yields [18]. Although the traditional PS application to winter cereals is usually before sowing, PS application at the tillering stage expands its application time window and improves its usability [19] as this brings the N application time closer to crop uptake. The application of PS to wheat at tillering needs to be based on information about agronomic performance (yield and grain quality) for efficient recycling. However, there is a lack of information on PS efficiency in comparison to synthetic N in Mediterranean irrigated conditions. This incomplete knowledge is partly due to the high variability in PS compositions and environmental conditions. The yield response to PS application is additionally a key factor for the right assessment of N_2O emissions using the concept of yield-scaled emissions.

In the application at tillering, the slurry remains on the surface of the soil [20], increasing the risk of ammonia gaseous N losses. In this situation, practices such as immediately incorporating the slurry after its application or injecting slurries into the soil, which abate NH_3 and indirect N_2O emissions [21], are unfeasible, except in irrigated areas where PS can be incorporated by irrigation. The acidification of pig slurry is a solution to reduce NH_3 emissions during its application to the soil as fertilizer. The pH decrease during acidification reduces the concentration of NH_3 relative to NH_4^+ in the slurry reducing the risk for ammonia volatilization [22]. Monocarbamide dihydrogen sulfate (MCDHS; international patent WO 2007/132032 A1) has been classified as a urease inhibitor. Even when the pig slurry urea has been transformed into ammonium, according to the company that owns the MCDHS patent, the ammonium-N form is protected since the micro-acidification, due to hydrolysis of the MCDHS molecule, could reduce ammonia volatilization and affect soil N dynamic. However, this product has not been widely assessed and no information can be found in the scientific literature about its effects on the soil N dynamics.

In this context, the first objective of this study is to evaluate, in a bread wheat crop under semiarid Mediterranean irrigated conditions, the effect of substituting synthetic N fertilizer for pig

slurry on crop productivity, nitrous oxide emissions, emission factors, and yield-scaled emissions during three consecutive years. The second objective is to assess the effect of adding the novel urease inhibitor MCDHS to pig slurry on crop productivity, soil nitrogen dynamics, and nitrous oxide emissions through its acidification potential that protects ammonium-N form from ammonia losses.

2. Materials and Methods

2.1. Site and Experimental Design

The study was conducted in the experimental field "Soto Lezcano" (41°43′49″ N, 0°49′2″W) in the middle Ebro river basin (Zaragoza, Spain) on a Typic Xerofluvent soil [23]. The physicochemical characteristics of the soil are shown in Table 1. The climate of the region is semiarid Mediterranean-continental (mean annual air temperature of 14.6 °C; mean annual precipitation of 318 mm; mean annual reference evapotranspiration of 1243 mm; period 2004–2019).

Table 1. Main physicochemical soil characteristics at the beginning of the experiment (2015).

Soil Property	0–30 cm	30–60 cm	60–90 cm
Soil Texture	Silty-Loam	Silty-Loam	Loam
Sand (%)	32.5	31.1	38.2
Silt (%)	50.5	51.9	49.5
Clay (%)	17.0	17.0	12.3
Stoniness (%vol.)	1	1	1
Equivalent Calcium Carbonate (g kg^{-1})	40	41	39
Total Nitrogen (Kjeldahl) (mg kg^{-1})	1350	940	620
Phosphorous (Olsen) (mg kg^{-1})	43	12.1	< 5.0
Potassium (NH$_4$Ac) (mg kg^{-1})	408	231	101
Organic Matter (%)	1.84	0.92	0.50
pH (1:2.5$_{H2O}$)	8.36	8.36	8.28
Electrical Conductivity (1:5$_{H2O}$) (dS m^{-1})	0.265	0.261	0.307

Bread wheat (*Triticum aestivum* L. cv. 'Rimbaud') was cultivated under sprinkler irrigation during three growing seasons (2015/16, 2016/17, and 2017/18) following the management practices presented in Table 2. Irrigation requirements were calculated weekly from the reference evapotranspiration (ETo) estimated with the Penman–Monteith equation and the wheat crop coefficients according to the FAO procedure [24].

Table 2. General crop management practices of wheat during the three growing seasons.

Crop Management Characteristic	2015/16	2016/17	2017/18
Sowing Date	26/11/2015	30/12/2016	16/11/2017
Harvest Date	07/07/2016	04/07/2017	06/07/2018
Seed Rate (kg ha^{-1})	170	200	175
Date N Side-Dress at tillering	24/02/2016	21/03/2017	00/03/2018
Date N Side-Dress at Stem Elongation	05/04/2016	08/04/2017	24/04/2018
Irrigation + Rain (mm) [1]	380	435	428
Crop ET (mm)	416	429	383

[1] From sowing to harvest.

The experimental design was a split-plot with four replications. The main factor included three different fertilization strategies at tillering (Table S1): (a) urea at the rate of 120 kg N ha^{-1} (U120); (b) pig slurry at the target rate of 120 kg NH$_4^+$-N ha^{-1} (PS120); and (c) pig slurry mixed with the urease inhibitor monocarbamide dihydrogen sulfate at the target rate of 120 kg NH$_4^+$-N ha^{-1} (PSI120). The second factor of the split-plot consisted in four rates of synthetic N at stem elongation: 0 (AN0), 30 (AN30), 60 (AN60), and 90 (AN90) kg N ha^{-1} as ammonium nitrate (Table S1). Besides these, four

additional treatments were included with four different rates of N in form of urea at tillering: 0 (control), 60 (U60-AN0), 90 (U90-AN0), and 150 (U150-AN0) kg N ha^{-1}, with no N application at stem elongation (Table S1). These treatments allow for calculating the nitrogen fertilizer replacement value of the pig slurry in PS120 and PSI120.

The size of the experimental plots was 6.0 × 7.0 m for pig slurry treatments and 6.0 × 3.5 m for urea treatments. Pig slurry was applied using trail hoses and the dose was calculated according to its ammonium-N content measured in situ using Quantofix N-volumeter [25]. Slurry samples were collected for further analysis in the laboratory (Table 3). The application equipment was calibrated directly in the field to obtain the relationship between speed and dose of PS applied (weighing the tank before and after the application). Despite that, applying appropriate amounts of PS to reach target N rates was a challenge, and the actual N applied is shown in Table 3. The urease inhibitor MCDHS was mixed in the slurry tank according to the rate recommended by the manufacturing company (2.5 L per 1 Mg of fresh pig slurry).

Table 3. Physicochemical characteristics of slurry from fattening pigs (mean ± standard error; *n* = 3) and rates of pig slurry and nitrogen applied each year.

Pig Slurry Characteristic		2015/16	2016/17	2017/18
Density (kg m^{-3})		1018 ± 2	1020 ± 1	1012 ± 0
pH		-	7.6 ± 0.0	8.4 ± 0.0
Dry Matter (kg DM m^{-3})		24.1 ± 1.4	36.4 ± 0.9	12.7 ± 0.2
Organic Matter (kg OM m^{-3})		10.3 ± 0.7	21.6 ± 0.9	6.1 ± 0.2
Ammonium Nitrogen (kg N m^{-3})		2.8 ± 0.1	3.1 ± 0.1	2.2 ± 0.1
Organic Nitrogen (kg N m^{-3})		0.8 ± 0.1	0.8 ± 0.0	0.9 ± 0.2
Phosphorous (kg P$_2$O$_5$ m^{-3})		0.4 ± 0.1	0.8 ± 0.0	0.9 ± 0.5
Potassium (kg K$_2$O m^{-3})		2.8 ± 0.0	3.7 ± 0.1	3.4 ± 0.1
PS Rate [1]	(m^3 ha^{-1})	27.2	37.1	56.6
PS Rate	(kg NH$_4^+$-N ha^{-1})	74	114	118
PSI Rate [2]	(m^3 ha^{-1})	28.6	34.5	57.5
PSI Rate	(kg NH$_4^+$-N ha^{-1})	85	110	129

[1] PS: Pig slurry. [2] PSI: Pig slurry with MCDHS.

To analyze the effects of MCDHS on the soil N dynamics an additional experiment (hereafter called 'miniplots') was installed in the same field with the treatments control, PS120-AN0, and PSI120-AN0 in a randomized block design with four replications. The size of each experimental plot was 3.6 × 2.0 m. Pig slurry was applied manually in the miniplots, on the same day in both experiments (Table 2).

A short irrigation event (2 mm) was applied to incorporate N fertilizers into the soil. At presowing, 70 kg P$_2$O$_5$ ha^{-1} and 150 kg K$_2$O ha^{-1} were applied to avoid limitations of these nutrients. One month before presowing, the previous season's straw was incorporated into the soil using a harrow. The control of weeds, diseases, and pests was performed according to local management practices.

2.2. Measurements and Determinations

2.2.1. Soil Sampling

The soil of each plot was sampled before fertilizer application (15 February 2016; 20 February 2017; and 7 February 2018) and after harvest (14 July 2016; 25 September 2017; and 31 July 2018) at 0–30 cm, 30–60 cm, and 60–90 cm depths.

In the miniplots, the soil was sampled at depths of 0–15 cm and 15–30 cm 39 times from pig slurry application to harvest. The time interval between samplings increased from 24 h in the days that followed the application to 15 days at the end of the season.

Soil samples were sieved through a 3-mm mesh. One subsample was used to determine the soil water content by gravimetry (drying at 105 °C until constant weight). Another subsample (10 g of fresh soil) was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through a cellulose filter. NO_3^- and NH_4^+ concentrations in the extracts were determined by colorimetry using a segmented flow analyzer (AutoAnalyzer 3, Bran + Luebbe, Norderstedt, Germany).

2.2.2. Crop Sampling

Two subareas of 0.54 m^2 were randomly selected in each plot and hand-harvested to determine the harvest index and obtain the total biomass of the whole plot. An area 1.65 m wide and the length of the experimental plot (3.5 m for U treatment and 7.0 m for PS and PSI treatments) was mechanically harvested with a combine harvester to determine grain yield (reported on the basis of 120 g kg^{-1} moisture content). Grain and straw N contents were analyzed by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA) in samples previously dried at 65 °C and ground.

2.2.3. Nitrous Oxide Emissions

Four treatments were selected to measure N_2O fluxes to the atmosphere. These treatments were U120-AN30, PS120-AN30, and PSI120-AN30, which were considered *a priori* the treatments that would theoretically better cover wheat N requirements, and the control treatment that was necessary to calculate N_2O emission factors. Fluxes from the soil to the atmosphere were measured using static closed unvented chambers [26] of 18.5-cm height and 30.0-cm inner diameter and made in polyvinyl chloride. They were composed of a collar inserted 10 cm into the soil and an upper part wrapped by a reflective insulation film. Gas samples were taken 0, 20, 40, and 60 min after chamber closure in 2015/16 and 2016/17, and 0 and 60 min after chamber closure in 2017/18. Fifteen milliliters of air from the chamber headspace were injected into a 12-mL borosilicate glass vial (Model 038 W, Labco, Lampeter, UK) using a polypropylene syringe. Air samplings were started roughly at the hour of the day when soil temperature was closer to the mean temperature of the day (between 9:30 h and 11:00 h GMT) [27]. Samples were analyzed by gas chromatography using the same equipment and technique that were described in detail by Mateo-Marín et al. [28]: an Agilent 7890B gas chromatography system with HP-Plot Q column and electron-capture, flame-ionization and methanizer detectors.

Nitrous oxide fluxes were calculated as the linear increment in gas concentration within the chamber corrected by the air temperature and multiplied by the ratio between the chamber headspace and the soil area occupied by the chamber [29].

Samples were taken from 17/02/2016 to 27/11/2018. The frequency of N_2O samplings was daily just after fertilization events and then measurements were performed at longer intervals with a total of 18, 32, and 36 samplings for the 2015/16, 2016/17, and 2017/18 seasons, respectively. At each sampling date, soil moisture and soil temperature were measured at 5-cm depth using portable sensors (HH2 Moisture Meter Delta-T ML3 and ML2 ThetaProbe, and TME MM2000 Single Input Thermocouple Thermometer). Soil water-filled pore space was estimated as the quotient between volumetric soil water content and total soil porosity [30]. Soil porosity was calculated considering a particle density of 2.65 Mg m^{-3} and the soil bulk density was measured in situ using the cylinder method [31].

The cumulative emissions of N_2O were calculated for each year, for the period between sowing and sowing the next year, integrating the emissions over time. The average N_2O flux between two sampling dates was multiplied by the time interval between them to estimate the cumulative flux during the inter-sampling period and added for each year.

2.3. Data and Statistical Analysis

The grain yield response to N rates in synthetic fertilizer treatments was adjusted using the linear-plateau model [32] for each cropping season (Equation (1)):

$$\text{If } F < C; \ Y = a + b \times F$$
$$\text{If } F \geq C; \ Y = Y_{max} = a + b \times C \tag{1}$$

where Y is the grain yield (t ha^{-1}); F is the applied N rate (kg N ha^{-1}); a (intercept, t ha^{-1}) is the yield at 0 kg N ha^{-1}; b (t kg^{-1} N) is the increase in yield per unit increase in F; and C (kg N ha^{-1}) is the critical N rate or minimum N rate at which the maximum yield (Y$_{max}$) is obtained.

The nitrogen fertilizer replacement value (NFRV) of pig slurry treatments was calculated as the rate of synthetic N that produces the same yield than the PS120-AN0 and PSI120-AN0 treatments, and it was estimated from the response curve to the urea treatments for each season.

The efficiency in the use of nitrogen was evaluated using two indexes. The nitrogen use efficiency was calculated based on: i) the agronomic point of view (NUEb) as the ratio between the total N uptake by the aboveground biomass (kg ha^{-1}), and the N applied by urea fertilization or applied with pig slurry in ammonium-N form (kg ha^{-1}); and ii) the environmental point of view (NUEg) the ratio between the total N uptake by the grain (kg ha^{-1}), and the N applied by urea fertilization or applied with pig slurry in ammonium-N form (kg ha^{-1}). The mineral N contained in the applied slurry (i.e., NH$_4^+$-N) was used for the calculations, since it was considered that the contribution of pig slurry organic N and its residual effect was not substantial during the period of the experiment. The apparent recovery efficiency of N applied (RE$_N$) in total aboveground biomass is the increment in the aboveground N uptake due to the N application per mineral-N applied rate (Equation (2)):

$$RE_N = \frac{U_T - U_0}{F_T} \tag{2}$$

where U$_T$ (kg N ha^{-1}) is the N uptake by aboveground biomass in the T treatment; U$_0$ (kg N ha^{-1}) is the N uptake by aboveground biomass in the unfertilized control plot; and F$_T$ (kg N ha^{-1}) is the applied mineral-N in the T treatment.

The efficiency indicators were calculated and analyzed for two fertilizer strategies: (i) fertilizer treatments that showed the best agronomic response to the N application (120 kg N ha^{-1} at tillering and 0 kg N ha^{-1} at stem elongation), and (ii) treatments that, during the experiment design, were considered to better suit crop necessities (120 kg N ha^{-1} at tillering and 30 kg N ha^{-1} at stem elongation).

The grain protein content was calculated multiplying the total N content of the grain by the factor 5.7 [33].

The N$_2$O emission factor (EF, %) was calculated as the difference between N$_2$O emissions in fertilized and unfertilized (control) plots, divided by the total N applied in the fertilized plots, and multiplied by 100. The yield-scaled N$_2$O emission (YS$_{N2O}$; g N kg^{-1} grain) was determined as the ratio between the N$_2$O emissions and the grain yield of the plot.

A N balance was performed at each plot for soil depths of 0–90 cm, considering the conservation of mass [34], including the three growing seasons (from February 2016 to July 2018). Thus, the mean annual unaccounted N (N$_{unac\,O-I}$; kg N ha^{-1} yr^{-1}) or surplus N was calculated as the difference between N inputs (kg N ha^{-1}) and N outputs (kg N ha^{-1}) and divided by three years (Equation (3)) [5,19]:

$$N_{unac\,0-I} = (N_{is} + N_f + N_i + N_d) - (N_{fs} + N_u + N_{N2O}) \tag{3}$$

where N$_{is}$ (kg N ha^{-1}) is the initial soil mineral N; N$_f$ (kg N ha^{-1}) is the N applied with fertilizers; N$_i$ (kg N ha^{-1}) is the N applied with irrigation water; N$_d$ (kg N ha^{-1}) is the N input for deposition; N$_{fs}$ (kg N ha^{-1}) is the final soil mineral N; N$_u$ (kg N ha^{-1}) is the N uptake by aboveground biomass; and N$_{N2O}$ (kg N ha^{-1}) is the N$_2$O emission (if applicable). The N$_d$ was estimated as 12.5 kg N ha^{-1} yr^{-1}, using the value provided by Schwede et al. [35] for cropped land. The main components of unaccounted outputs of N are NH$_3$ volatilization, N oxides (NO, NOx) and N$_2$ emissions, N leaching, and immobilized N (including N in roots). N oxides emissions are very small [36], and N$_2$ emissions were considered unimportant since favorable conditions for its production were not reached during the experimental period (availability of highly enriched organic carbon and higher than 90% WFPS; [37]).

The $N_{unac\text{-}O\text{-}I}$ provides an indication of net N losses from the soil system when a positive value is obtained and a net input mostly coming from mineralization when a negative value is obtained.

To estimate the risk of nitrate leaching, the drainage was calculated at 0.9-m depth for each season using the simplified one-dimensional daily water balance described in Equation (4):

$$D = P + I - ETc + SW - FC \tag{4}$$

where D (mm) is the drainage; P (mm) is the precipitation; I (mm) is the irrigation; ETc (mm) is the estimated crop evapotranspiration using the Penman–Monteith reference evapotranspiration and FAO methodology [24]; SW (mm) is the soil water content measured by soil sampling by gravimetry; and FC (mm) is the soil field capacity, measured using Richards methodology [38]. Drainage is estimated when $P + I - ETc + SW$ exceeds field capacity. There were no visible signs of surface runoff and it was considered negligible.

Statistical analyses were performed using the SAS software University Edition (SAS Institute, Cary, NC, USA). The normal data distribution and uniformity of variance were verified using Shapiro–Wilk and Levene's test, respectively. Data were transformed using the Box–Cox transformation when basic model assumptions of normality and homogeneity of variance were not met. Transformed data were subjected to analysis of variance (MIXED procedure) and differences of means were compared with Tukey's test. A paired *t*-test was used to compare the soil N concentrations between treatments in the miniplots. A comparison of regression lines across groups was performed using a *F*-test. Repeated measure analysis along time, according to a first-order autoregressive structure model AR(1), was performed to compare N₂O fluxes among treatments for the period between the first fertilizer application and one month after the second application. In all tests, the default level of significance considered was 0.05.

3. Results

3.1. Productive Parameters

The maximum grain yield (Y_{max}, Figure 1) was higher in the first season (8357 kg ha⁻¹) than in the second and third seasons (5491 and 5543 kg ha⁻¹, respectively). No response of grain yield to N rates (urea) applied at tillering was observed in the first year (2015/16), while in 2016/17 and 2017/18, there was a positive response, and critical N rates were established at 34 and 59 kg N ha⁻¹, respectively (Figure 1). NFRV could not be quantified due to the low or lack of yield response to N application. The grain yield was affected by the N source ($p < 0.01$) in the first season (2015/16) since PS120-AN0 showed a 21% higher grain yield than U120-AN0 (Figure S1). In the three years, yield in PS120-AN0 and PSI120-AN0 was not lower than in U120-AN0, indicating that the fertilizer value of PS120 and PSI120 was at least similar to that of U120.
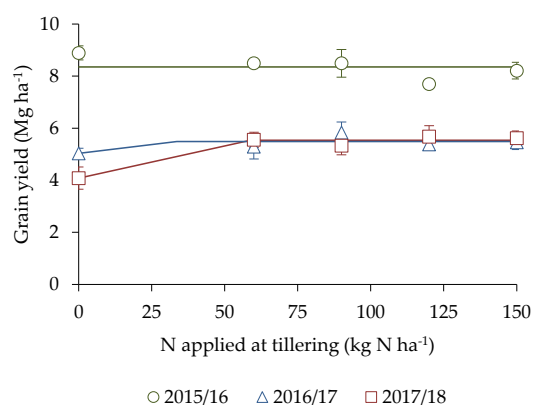

Figure 1. Grain yield response curves (linear-plateau model) to nitrogen application at tillering in the urea treatments in 2015/16, 2016/17, and 2017/18 seasons. Vertical bars indicate the standard error (*n* = 4).

The grain yield was not affected by a second synthetic-N side-dress application at stem elongation in any of the three seasons (Figure S1).

The second N application increased the grain protein content with a linear response as the N applied increases (Figure 2).
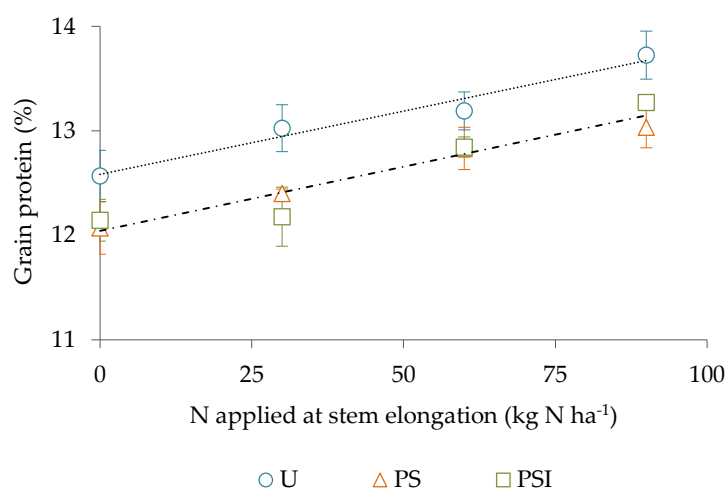

Figure 2. Relationship between the 3-year average grain protein (%) and the rate of synthetic-N applied at stem elongation (kg N ha^{-1}) for the different fertilizer strategies (U, PS, or PSI). Vertical bars indicate the standard error (*n* = 4).

For a given N application rate at stem elongation, U treatments presented higher (*p* < 0.05) grain protein content than treatments with pig slurry (PS and PSI), i.e., N source at tillering application determined differences in grain protein. These differences were also observed when the regression was performed with available N, defined as soil mineral N (SMN) from 0 to 30 cm in February in addition to the mineral-N fertilizer (Figure S2). The difference in available N between U and PS treatments to reach the average grain protein content (12.8%) was estimated at 28.8 kg N ha^{-1}.

There was no effect of treatments without N application at stem elongation on NUEb, NUEg, and RE$_N$ (Table 4). Moreover, no significant differences in these N efficiency indexes were observed among the treatments for the strategy with the second application of 30 kg N ha^{-1} (Table S2).

Table 4. Nitrogen use efficiency indexes (NUEb, NUEg, and RE$_N$; mean ± standard error; *n* = 4) for the fertilized treatments U120-AN0, PS120-AN0, and PSI120-AN0 in the three cropping seasons.

	U120-AN0	**PS120-AN0**	**PSI120-AN0**	*p*-**Value**
NUEb				
2015/16	2.79 ± 0.29	4.97 ± 0.94	4.56 ± 0.61	0.105
2016/17	1.85 ± 0.04	1.71 ± 0.06	1.91 ± 0.14	0.345
2017/18	1.48 ± 0.17	1.38 ± 0.13	1.35 ± 0.12	0.737
NUEg				
2015/16	1.29 ± 0.04	2.69 ± 0.38	2.23 ± 0.30	0.132
2016/17	0.96 ± 0.03	0.93 ± 0.04	1.02 ± 0.08	0.160
2017/18	0.90 ± 0.09	0.96 ± 0.11	0.89 ± 0.05	0.688
RE$_N$				
2015/16	−0.05 ± 0.29	0.12 ± 0.59	0.31 ± 0.10	0.836
2016/17	0.48 ± 0.04	0.26 ± 0.04	0.39 ± 0.13	0.367
2017/18	0.65 ± 0.17	0.52 ± 0.09	0.56 ± 0.09	0.657

3.2. Nitrous Oxide Emissions

Individual values of nitrous oxide fluxes ranged from −2.3 to 94.6 g N ha⁻¹ day⁻¹ in 2015/16, from −1.5 to 434.16 g N ha⁻¹ day⁻¹ in 2016/17, and from −2.5 to 197.5 g N ha⁻¹ day⁻¹ in 2017/18. Large variability in N_2O fluxes was observed among replicated chambers for a given sampling date and treatment. Standard errors ranged from 0.1 to 9.9 g N ha⁻¹ day⁻¹ in the control treatment and from 0.2 to 56.3 g N ha⁻¹ day⁻¹ in the three fertilized treatments.

Remarkable peaks of N_2O emissions (Figure 3) were observed mainly immediately after the first side-dress fertilizer application when soil temperature ranged from 12 °C to 21 °C and soil WFPS ranged from 45% to 70%.
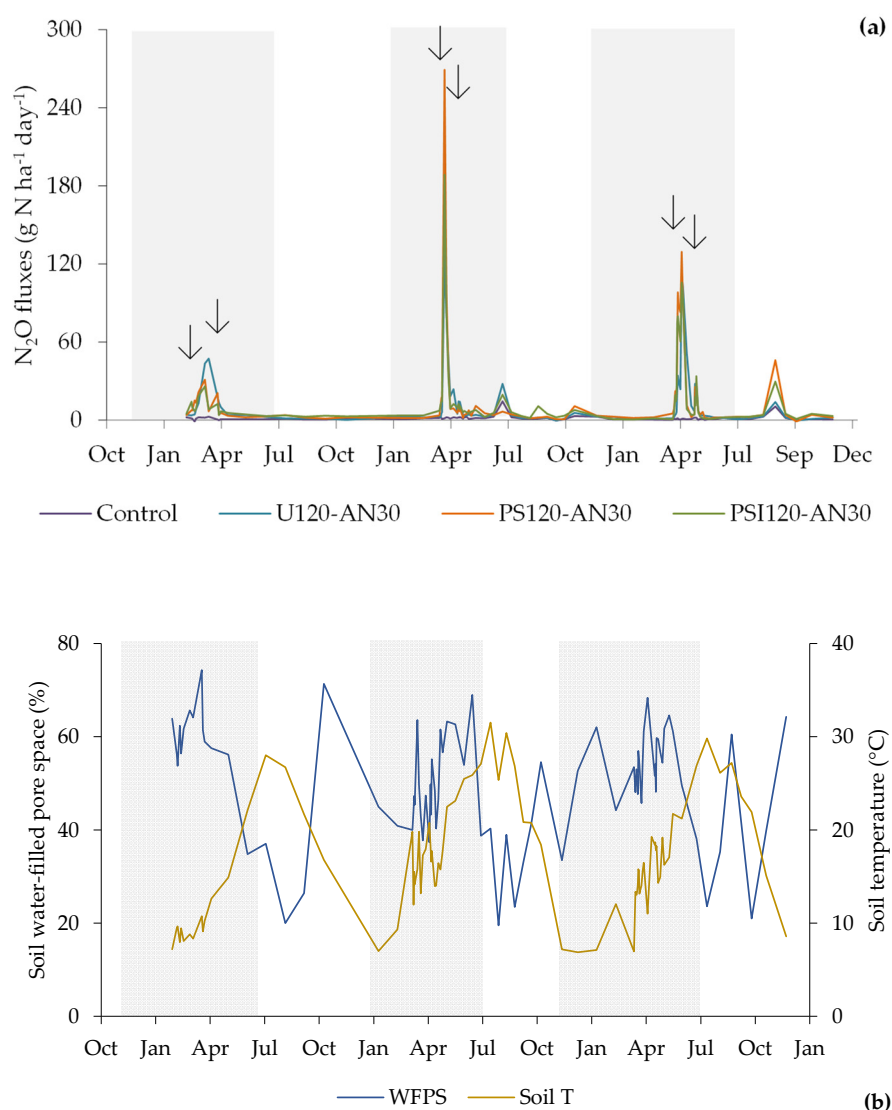
Figure 3. Temporal changes of (**a**) average N_2O fluxes (g N ha⁻¹ day⁻¹; *n* = 4) for each fertilizer treatment (control, U120-AN30, PS120-AN30, and PSI120-AN30) (**b**) soil temperature (T, °C) and water-filled pore space (WFPS, %) along the three growing seasons (2015/16, 2016/17, and 2017/18). Shadow areas show the period between presowing and harvest of each season. Arrows show fertilizer applications.

On average, 75% of N_2O was emitted during the cropping season (from sowing to harvest). The repeated measure analysis of N_2O fluxes from the first fertilizer application to one month after the second application did not show significant differences among the three fertilizer treatments in any of the three seasons. Significant differences in N_2O emissions were observed for the whole experiment

(2015–2018) among the control and the fertilized treatments (Table 5). These differences were also observed in each season, although during the second and third season, the differences between control and U treatment were not significant, due to the high variability between replicates. There were no significant differences related to the substitution of synthetic fertilizer (U120-AN30) for pig slurry (PS120-AN30) or to the addition of the urease inhibitor to the pig slurry (PSI120-AN30).

Table 5. Nitrous oxide emission (g N ha^{-1}; mean ± standard error; n = 4) in the four treatments (control, U120-AN30, PS120-AN30, and PSI120-AN30) for the three years (from sowing to the following sowing in 2015/16, 2016/17, and 2017/18) and the whole experiment (2015/18). Different letters in the same row indicate significant differences between treatments (Tukey's test; $p < 0.05$).

	Control	U120-AN30	PS120-AN30	PSI120-AN30	p-Value
N$_2$O (g N ha^{-1})					
2015/16	233 ± 86 b	1624 ± 650 a	1314 ± 330 a	1428 ± 587 a	<0.001
2016/17	576 ± 167 b	2101 ± 321 ab	2427 ± 447 a	2638 ± 715 a	0.009
2017/18	519 ± 127 b	2129 ± 400 ab	3094 ± 817 a	2538 ± 770 a	0.008
2015/18	1532 ± 387 b	6140 ± 1441 a	7262 ± 1390 a	7086 ± 2235 a	0.007

The N$_2$O EFs ranged between 0.91% and 1.42% (Table 6) and no significant differences among treatments for any of the cropping periods were observed. Averaging over treatments, values were 21% and 27% higher in the second and third season, respectively, compared to the first season EFs. Yield-scaled N$_2$O emissions were lower for the first season (average 0.16 ± 0.03 g N kg^{-1} grain) than for the other two seasons (Table 6). Mean YS$_{N2O}$ emission in 2016/17 (0.46 ± 0.05 g N kg^{-1} grain) and in 2017/18 (0.42 ± 0.05 g N kg^{-1} grain) were 184% and 159% higher than in 2015/16, respectively. Differences among the fertilized treatments were not detected in this parameter for any of the seasons ($p > 0.05$).

Table 6. EF (%) and YS$_{N2O}$ emission (g N$_2$O-N kg^{-1} grain) (mean ± standard error; n = 4) in the different fertilized treatments (U120-AN30, PS120-AN30, and PSI120-AN30) for the three years (2015/16, 2016/17, and 2017/18) and the whole experiment (2015/18). The data include the crop period (sowing to harvest) and the intercrop period (harvest to the following crop sowing).

	U120-AN30	PS120-AN30	PSI120-AN30	p-Value
EF (%)				
2015/16	0.93 ± 0.38	0.91 ± 0.25	0.92 ± 0.49	0.999
2016/17	1.02 ± 0.21	1.06 ± 0.23	1.26 ± 0.39	0.929
2017/18	1.07 ± 0.20	1.42 ± 0.56	1.01 ± 0.37	0.510
2015/18	1.02 ± 0.25	1.18 ± 0.26	1.12 ± 0.44	0.469
YS$_{N2O}$ (g N$_2$O-N kg^{-1} grain)				
2015/16	0.19 ± 0.08	0.15 ± 0.03	0.15 ± 0.06	0.919
2016/17	0.41 ± 0.05	0.49 ± 0.11	0.49 ± 0.12	0.964
2017/18	0.38 ± 0.05	0.48 ± 0.12	0.41 ± 0.11	0.763
2015/18	0.31 ± 0.06	0.36 ± 0.06	0.33 ± 0.10	0.893

3.3. Dynamic of Soil Mineral Nitrogen

Soil mineral nitrogen responded to N applications, reaching peaks of ~20 mg NO$_3^-$-N kg^{-1} soil and 20–35 mg NH$_4^+$-N kg^{-1} soil at 0–15-cm depth the day after the fertilization (Figure 4). These values went down until they reached, in two months, the same values of SMN observed in the control treatment. At the end of the first and third season, soil nitrate rose in the 0–15-cm depth (Figure 4).
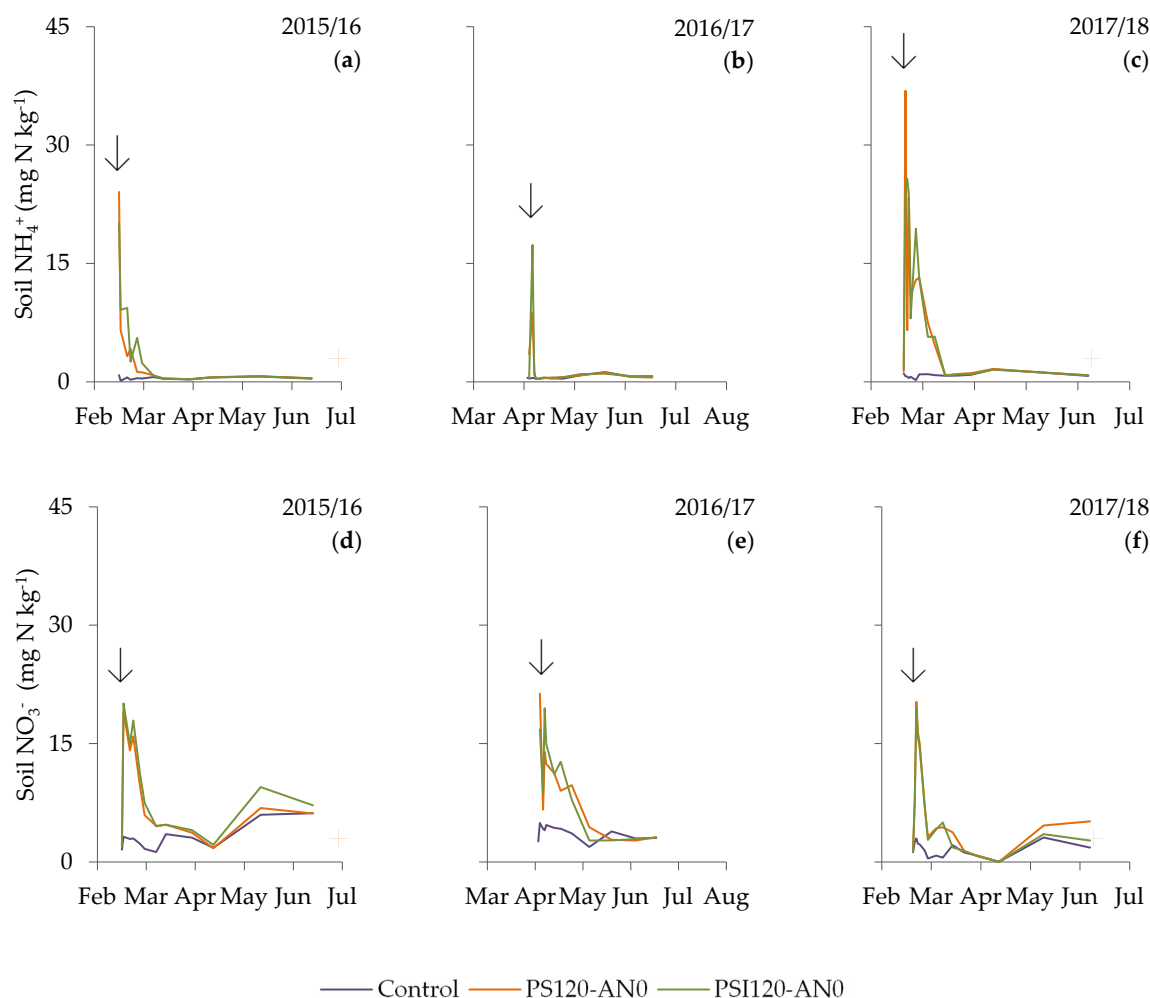
Figure 4. Soil ammonium concentration (**a–c**) and soil nitrate concentration (**d–f**) from 0 to 15-cm depth of the miniplots experiment. Arrows indicate N applications.

In the two months after the fertilization, the dynamic of SMN was similar in the PS and PSI treatments for all seasons, independent of the N form and soil depth (Table S3). The exception was the 2015/16 season when treatment with inhibitor presented 10% higher NO_3^- concentration than treatment without inhibitor. This difference, although significant, was relatively unimportant since it was detected only in one year and had no effect on total mineral N concentration (Table S3).

3.4. Nitrogen Balance

For the three years of the experiment, unaccounted N_{O-I} ($N_{unac\,O-I}$) was linearly related to the total N applied by fertilization (Figure 5), but the relationship changed depending on the fertilizer source. The inhibitor applied to the slurry did not affect the observed relationship and a pooled regression (PS + PSI) was considered. However, the urea treatments behaved differently ($p < 0.05$) than the PS treatments, with lower $N_{unac\,O-I}$ across the different ranges of applied N. Averaging over the different N rates, $N_{unac\,O-I}$ was 41 kg N ha^{-1} yr^{-1} lower in urea than in the slurry treatments.
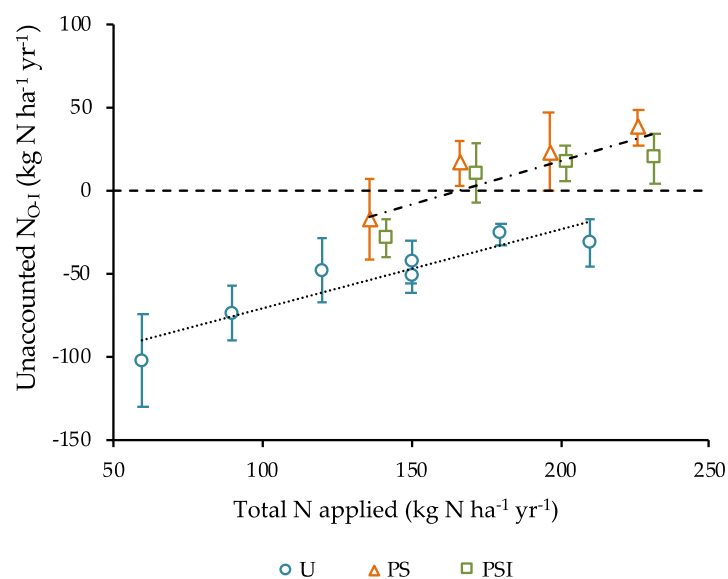
Figure 5. Relationship between the unaccounted N_{O-I} (kg N ha^{-1} yr^{-1}) obtained from the 0–90 cm soil N balance and the total N applied (kg N ha^{-1} yr^{-1}) for the whole experimental period. Vertical bars indicate the experimental standard error ($n = 4$).

According to the soil water balance, the volume of water drained below 0.9 m depth (from February to July) was zero for the first two seasons, and in the third season (2017/18), the drainage was estimated as 73 mm. In this case, 95% of this volume was concentrated after an unusually high rainfall period (83 mm) occurred between days 07/04/2018 and 11/04/2018, twenty days after the first side-dress N fertilization. Therefore, due to the small volume of drainage below the root zone, the total amount of N lost by leaching was relatively unimportant compared to other potential losses.

4. Discussion

The N source at tillering application did not affect grain yield and, as a consequence, pig slurry application produced yields similar to those obtained by fertilizing with urea. No grain yield reduction associated with the use of pig slurry substituting synthetic fertilizers has been reported in other studies for different crops [39–41]. A second N application as ammonium nitrate at stem elongation did not increase grain yield compared to a unique side-dress N application, which is in agreement with the inconsistent response of grain yield to variations in the timing and splitting of N fertilizer reported by López-Bellido et al. [42]. However, the second side-dress N application at stem elongation allowed an increase in grain protein, which corroborates previous studies like that by Debaeke et al. [17] who suggested that the split and late application of N guarantees a better distribution of N in the kernel. This increase in grain protein was observed when the N rates increased in the application at stem elongation even though they exceeded the critical N rate above which the maximum yield was obtained. Under similar irrigated Mediterranean conditions, Lloveras et al. [43] also reported that higher N rates are required to achieve high bread-making quality than to obtain the highest grain yield. Furthermore, grain protein was influenced in this study by the N source applied at tillering. Lower N rates at stem elongation were necessary to reach higher protein content when urea was used in the tillering side-dress application, compared to pig slurry. Even if the slurry was applied using trail hoses to reduce NH_3 volatilization—compared to the splash-plate method, for example [11]—the NH_3 losses were still expected to be higher in the slurry treatments than in the urea treatments. Therefore, higher N availability should be expected in urea treatments, with a subsequent increase in grain protein content. Under similar environmental conditions, Mateo–Marín et al. [44] measured ammonia losses derived from pig slurry applications comprising up to 28.5% of the N applied.

Unintentionally, the experiment took place under relatively high N availability conditions, associated with large N fertilizer rates relative to crop needs during the previous years, which led to a low grain yield response to N application for the three cropping seasons. Thus, the soil mineral N (0–30 cm depth) in the control treatment was relatively high before the first side-dress application, especially for the first and second year (2015/16: 59 ± 8 kg N ha^{-1}; 2016/17: 52 ± 5 kg N ha^{-1}; 2017/18: 27 ± 3 kg N ha^{-1}). NUEb values during the whole experiment exceeded the threshold of 0.9 proposed as an indicator of soil nutrient removal [45]. However, as only grain was exported from the plots, the depletion of soil N was not expected to be noteworthy. Soil N removal can also be accelerated by high RE$_N$ [46]; however, in this study, RE$_N$ values were lower than the mean value of 0.57 in the analysis of Ladha et al. [46]. After three consecutive years of growing wheat, the average RE$_N$ reached values within the normal range (0.50–0.80) in well-N-managed systems for cereal crops [47]. The lower values obtained for the first season can be explained by the absence of a response to the N application.

In this study, MCDHS did not affect N$_2$O emissions, as would be expected for the application of a urease inhibitor to pig slurry that has already transformed the urea-N to ammonium-N before the addition of the inhibitor. Nonetheless, the experiment allows discarding other potential effects associated with the presence of dihydrogen sulfate in the molecule, such as decreasing soil pH near the soil-fertilizer interphase, with a subsequent effect on N dynamics. Thus, the MCDHS did not reduce the pH of PS compared to the non-treated slurry (data not shown), which is in agreement with the absence of significant differences in soil mineral N content between both treatments (PI vs. PSI). The only difference in soil nitrate concentrations found (2015/16) between PS fertilizers (with and without MCDHS) was not consistent through soil depths and seasons, and the inorganic N forms (ammonium-N and mineral-N) were not affected by the addition of MCDHS. This absence of differences between treatments proves that the effect of the inhibitor on the soil N dynamics is not detectable; in fact, SMN evolution followed the same pattern, in terms of amounts and temporal dynamics, in both fertilizer treatments.

Nitrous oxide emissions responded to fertilizer application independently of the N source and mainly occurred under soil WFPS conditions (40–70%) that promoted nitrification [48], although in 23% of the dates WFPS exceeded 60%, and conditions were also ripe for denitrification. The absence of differences in N$_2$O emissions between urea and pig slurry might be attributed to the similar mineralized nature of the N forms they contain (urea-N in urea fertilizer and ammonium-N in pig slurry). Pig slurry did not promote higher emissions in the moments when denitrification processes were active, probably due to its low carbon content. Noticeable differences in the maximum N$_2$O flux peaks were observed among the three seasons, with a lower peak during the first year. This fact might be attributed to a rainfall event (24.5 L m^{-2}) which happened three days after the first fertilizer application of season 2015/16, displacing the mineral-N to deeper layers compared to the other seasons. The importance of the fertilizer position on N$_2$O emissions was demonstrated by Liu et al. [49], who reported between 40–70% higher fluxes when fertilizer was injected at 0–5-cm depth compared to fertilizer located at 10–15 cm.

Liu and Powers [50] indicated that N$_2$O EF for swine slurry application was similar to the default value (EF$_1$ = 1%) suggested by the IPCC [51]. According to a meta-analysis of Cayuela et al. [52], the organic-liquid fertilizers present the highest EF (0.85% ± 0.30, *n* = 30), compared to synthetic fertilizers, which presented EFs values generally lower than 1%. However, the 2019 Refinement to the 2006 IPCC Guidelines for National Gas Inventories [53] changed the default EF of "all N inputs in dry climates" to 0.5%. The present study did not show consistent differences in N$_2$O EFs between synthetic urea and slurry treatments, with values close to 1% after two N applications (120 kg N ha^{-1} and 30 kg N ha^{-1}). Nevertheless, the implications and benefits of rational recycling of nutrients from a growing porcine livestock population have to be considered comprehensively (i.e., life-cycle assessment) when comparing synthetic with organic fertilizers.

According to the water balance, the contribution of nitrate leaching to N losses was relatively low in the whole experiment, although the drainage produced in the third season, twenty days after the first side-dress N application, could have been produced in a critical moment for N leaching [6].

However, the low SMN content (13.6 kg N ha^{-1} from 0 to 30 cm the day before the rainfall event) rejects the possibility of large nitrate losses by leaching.

The more N applied, the more unaccounted N_{O-I}. The main components of unaccounted N_{O-I} were NH_3 volatilization and net mineralization. Since similar yields were obtained among treatments, no differences in N-immobilization due to straw incorporation should be expected. The trend to higher unaccounted N_{O-I} for slurry treatments compared to urea treatments agrees with lower grain N concentrations in pig slurry than in synthetic-N treatments, since more unaccounted N_{O-I} would mean less available N for the crop.

5. Conclusions

Pig slurry can replace the N necessary for bread wheat production under irrigated conditions without yield reductions and with similar nitrogen use efficiency compared to the synthetic urea fertilizer. Besides, the use of pig slurry does not increase the direct N_2O losses compared to the use of urea, one of the most popular synthetic fertilizers. However, higher uncertainties, probably associated with NH_3 volatilization losses, can jeopardize grain protein when slurry rates are not properly adjusted. Further studies should be performed to clarify the fate of the observed high N unaccounted values and to reduce N losses from irrigated agrosystems.

MCDHS added to pig slurry does not seem to have any agronomic or environmental benefit under the agro-environmental conditions of this study; thus, grain yield, N_2O emissions, EF, and YS_{N2O} were not affected by the addition of the inhibitor to pig slurry.

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