Gaseous nitrogen losses from pig slurry fertilisation: can they be reduced with additives in a wheat crop?

Noemí Mateo-Marín, Ramón Isla and Dolores Quílez

Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Unidad de Suelos y Riegos (asociada a EEAD-CSIC). Avda. Montañana 930, 50059 Zaragoza, Spain

Abstract

**Aim of study:** The use of pig slurry as fertiliser is associated with gaseous nitrogen (N) losses, especially ammonia (NH₃) and nitrous oxide (N₂O), leading to environmental problems and a reduction of its fertiliser value. This study evaluates, in an irrigated wheat crop, the effect of different additives mixed with pig slurry to decrease NH₃ and N₂O losses.

**Area of study:** Middle Ebro valley, Spain

**Material and methods:** The treatments were: i) non-N-fertilised control, ii) pig slurry (PS), iii) pig slurry with the urease inhibitor monocarbamide dihydrogen sulphate (PS-UI), iv) pig slurry with a microbial activator in development (PS-A), and v) pig slurry with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (PS-NI). Pig slurry was applied at a target rate of 120 kg NH₄⁺-N ha⁻¹. Ammonia volatilisation was measured using semi-opened static chambers after treatments application at presowing 2016 and side-dressing 2017. Nitrous oxide emissions were measured using static closed chambers after treatments application at the 2017 and 2018 side-dressing.

**Main results:** Ammonia volatilisation was estimated to be 7-9% and 19-23% of NH₄⁺-N applied after presowing and side-dressing applications, respectively. Additives were not able to reduce NH₃ emissions in any application moment. PS-NI was the only treatment being effective in reducing N₂O emissions, 70% respect to those in PS treatment. Crop yield parameters were not affected by the application of the additives because of the no effect of additives controlling NH₃ losses and the low contribution of N₂O losses to the N balance (<1 kg N₂O-N ha⁻¹).

**Research highlights:** The use of 3,4-dimethylpyrazole phosphate would be recommended from an environmental perspective, although without grain yield benefits.

**Additional key words:** ammonium volatilisation; microbial activator; nitrification inhibitor; nitrous oxide emission; urease inhibitor.

**Abbreviations used:** DM (dry matter); DMPP (3,4-dimethylpyrazole phosphate); EF (emission factor); MCDHS (monocarbamide dihydrogen sulphate); NI (nitrification inhibitor); NUE (nitrogen use efficiency); PS (pig slurry); PS-A (pig slurry with a microbial activator in development); PS-NI (pig slurry with the nitrification inhibitor 3,4-dimethylpyrazole phosphate); PS-UI (pig slurry with the urease inhibitor monocarbamide dihydrogen sulphate); REN (apparent N recovery efficiency); SOC (semi-opened free static chambers); UI (urease inhibitor).

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**Correspondence** should be addressed to Noemí Mateo-Marín: noemimateomarin@gmail.com

**Introduction**

Ammonia (NH₃) is an important atmospheric pollutant mostly emitted from agricultural sources. It leads to both environmental and socio-economic issues (EEA, 2019a). Ammonia reacts with atmospheric nitric and sulphuric acids to form fine particulate matter (PM₁₀⁻), considered a major environmental risk to human health (Hristov, 2011) since it is responsible for more than 410,000 premature deaths a year in Europe (EEA, 2019a). Besides, NH₃...
emission induces substantial environmental damages due to its effect on air pollution, soil acidification, water eutrophication, and loss of biodiversity (Vitousek et al., 1997; Ti et al., 2019).

Agriculture is responsible for the 92% of NH₃ emissions in Europe (EEA, 2019a), 80% of these emissions are attributable to livestock production systems and the remaining 20% is associated to synthetic fertilisers (EEA, 2016). In particular, spreading of manures and slurries for crop fertilisation causes 25% of these NH₃ emissions (EEA, 2019b). In the EMEP/EEA air pollutant inventory guidebook (EEA, 2019c) the emission factor for the calculation of ammonia emissions for pig slurry application to soil (0.45) is much higher than for N fertiliser (average 0.05). North-eastern Spain is a hotspot of NH₃ (Guevara et al., 2019) since this area gathers more than 15 million head of pigs (MAPA, 2020) comprising the 8% of total European pig livestock population (FAO, 2020).

The use of slurries as fertilisers could also imply nitrous oxide (N₂O) emissions that might be influenced by the highly mineralised nature of the nitrogen (N) contained in this product (Yagüe & Quílez, 2012) in comparison to other organic fertilisers. Nitrous oxide is the largest ozone-depleting substance (UNEP, 2013) and the third most contributing emission to the greenhouse gas effect because of its atmospheric lifetime (121 years) and its radiative properties (GWP-100 yr. of 265) (Myhre et al., 2013). The metanalysis of Aguilera et al. (2013) shows that pig slurry has the same potential to produce N₂O emissions than synthetic fertilisers and the 2019 refinement of the 2006 IPCC accounting methodology (IPCC, 2019) gives the same emission factor (0.5%) for both fertiliser types.

Gaseous N losses associated with slurry management can be controlled through physical and chemical procedures. Ammonia volatilisation can be decreased, for example, using trail hoses for the slurry application instead of the splash plate (Yagüe et al., 2019), using slurries with low dry matter (DM) content (Bosch-Serra et al., 2014), and acidifying slurries (Fangueiro et al., 2015). Sanz-Cobena et al. (2017) compiled a set of practices applicable for the Mediterranean area to organic fertilisation to mitigate N₂O emissions, some of them were also effective to reduce NH₃ volatilisation as an indirect contributor to N₂O emission. These practices include those devoted to decrease the contact surface between the fertiliser and the atmosphere (e.g. slurry injection into the soil or immediate incorporation of slurries into the soil after their application), adjusting N rates to actual crop uptake, and the use of inhibitors (nitrification inhibitors or urease inhibitors) as additives to fertilisers.

Nitrification inhibitors (NIs) are applied on ammonium-based fertilisers to delay the conversion of ammonium to nitrite by the depression of the Nitrosomonas activity (Zerulla et al., 2001a,b). Urease inhibitors (UIs) are considered for urea-based fertilisers since these substances delay the conversion of urea to ammonium (NH₄⁺) by the inhibition of the urease enzyme activity (Ussiri & Lal, 2013).

3,4-dimethylpyrazole phosphate (DMPP), one of the most extensively used NI (Abalos et al., 2014), has been traditionally blended into mineral fertilisers. However, a novel formulation based on DMPP (Vizura®) has been developed for liquid manure and biogas digestate. Monocarbamide dihydrogen sulphate (MCDHS; international patent WO 2007/132032 A1) is another substance marketed as urease inhibitor but there is no available information in the scientific literature to support its potential under field conditions. The manufacturing company also claims the protection of ammonium-N controlling pH levels and decreasing NH₃ volatilisation due to the micro-acidification produced in the hydrolysis of the MCDHS molecule, releasing protons H⁺.

In this context, the objective of this study was to evaluate in a wheat crop and under Mediterranean irrigated conditions, the effectiveness of the UI MCDHS, a soil microbial activator (developed in the Spanish Ministry of Science and Innovation project CDTI IDI-20170513), and the NI Vizura® to abate ammonia volatilisation and nitrous oxide emissions from surface applied pig slurry.

Material and methods

Site and experimental design

The trial was conducted at the experimental field ‘Soto Lezcano’ (middle Ebro Valley, Spain) during two wheat-growing seasons (2016/17 and 2017/18) under semiarid Mediterranean-continentall irrigated conditions. The climate is characterised by mean annual air temperature of 14.6 °C and mean annual precipitation and reference evapotranspiration of 318 mm and 1,243 mm, respectively (period 2004-2019). The trial was established on a deep (>120 cm) Typic Xerofluvent soil (Soil Survey Staff, 2014; Table 1) where bread wheat (Triticum aestivum L. cv. ‘Rimbaud’) was cultivated under sprinkler irrigation. Crop water needs were calculated weekly from the reference evapotranspiration (ET₀) estimated with the Penman-Monteith equation and the locally adapted crop coefficients (Kc) according to FAO procedures (Allen et al., 1998). Thereby, the crop received a total of 435 mm and 428 mm of water (rain plus irrigation) to supply the estimated crop ET of 429 mm and 383 mm, respectively, during the two cropping seasons.

The experiment had a randomised block design with four replicates and four treatments with a plot size of 2.0 × 3.6 m. The experiment was replicated three times: pre-sowing 2016, side-dressing 2017 (both in the same crop cycle), and side-dressing 2018. Slurry from fattening pigs was used in the three trials (Table 2) and the four evaluated
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Additives were provided by the manufacturing companies. The slurry was applied by hand to the experimental plots at a target rate of 120 kg NH₄⁺-N ha⁻¹ in all treatments (actual rates in Table 2). Nitrogen fertiliser treatments were: a) non-N application (Control); b) pig slurry (PS); c) pig slurry mixed with the urease inhibitor monocarbamide dihydrogen sulphate (PS-UI); d) pig slurry mixed with a soil microbial activator (PS-A) or with nitrification inhibitor 3,4-dimethylpyrazole phosphate (Vizura®; PS-NI). At side-dressing application in 2018, the PS-A was substituted by the PS-NI treatment. The urease inhibitor MCDHS was included as PS slurry additive treatment to assess the micro-acidification effect on ammonia losses declared by the manufacturing company that patented the inhibitor. The additives were applied according to the rate recommended by the manufacturing company: 2.5 L of MCDHS in 1 Mg of pig slurry, 2.5 kg of soil microbial activator in 1 Mg of pig slurry, and 3 L of Vizura® per hectare. Pig slurry was applied at presowing (14th November 2016) and at side-dress at tillering (7th April 2017 and 19th March 2018). At presowing in the two cropping seasons, 70 kg P₂O₅ ha⁻¹ and 150 kg K₂O ha⁻¹ were applied to avoid limitations of these two nutrients.

The crop was managed according to standard practices in the region. Wheat was sown on 30th December 2016 and 16th November 2017 at a plant density of 200 and 175 kg seed ha⁻¹, respectively. The crop was harvested at wheat maturity (4th July 2017 and 6th July 2018). Straw was hashed and incorporated to the soil before subsequent wheat seeding. Weeds, diseases, and pests were controlled and no special problems were detected during the experimental period.

### Table 1. Physicochemical characteristics of the soil at the beginning of the experiment.

|                      | 0-30 cm  | 30-60 cm |
|----------------------|----------|----------|
| Soil texture         | silt loam| silt loam|
| Sand (%)             | 32.5     | 31.1     |
| Silt (%)             | 50.5     | 51.9     |
| Clay (%)             | 17.0     | 17.0     |
| Stoniness (vol. %)   | 1        | 1        |
| Total nitrogen (Kjeldahl) (mg kg⁻¹) | 1,350 | 940 |
| Phosphorous (Olsen) (mg kg⁻¹) | 43 | 12.1 |
| Potassium (NH₄Ac) (mg kg⁻¹) | 408 | 231 |
| Organic matter (%)   | 1.84     | 0.92     |
| pH (1:2.5H₂O)        | 8.36     | 8.36     |
| Electrical conductivity (1:5H₂O) (dS m⁻¹) | 0.265 | 0.261 |

### Table 2. Physicochemical characteristics of the pig slurry and amount of nitrogen applied at presowing 2016, side-dressing 2017, and side-dressing 2018.

|                      | 2016/17 | 2017/18 |
|----------------------|---------|---------|
|                      | Presowing 2016 | Side-dressing 2017 | Side-dressing 2018 |
| Density (kg m⁻³)     | 1,030   | 1,034   | 1,022   |
| pH                   | -       | 7.6     | 7.8     |
| Electrical conductivity at 25 °C (dS m⁻¹) | 30.6 | 30.2 | 35.4 |
| Dry matter (kg DM m⁻³) | 41.4   | 73.4    | 31.6    |
| Organic matter (kg OM m⁻³) | 26.1  | 53.7    | 17.1    |
| Ammonium nitrogen (kg N m⁻³) | 3.2   | 4.5     | 4.0     |
| Organic nitrogen (kg N m⁻³) | 0.3   | 0.9     | 0.9     |
| Phosphorous (kg P₂O₅ m⁻³) | 0.6   | 0.3     | 0.6     |
| Potassium (kg K₂O m⁻³) | 4.0     | 4.3     | 4.5     |
| Ammonium-N (kg NH₄⁺-N ha⁻¹) | 141  | 157     | 158     |
| Total-N (kg N ha⁻¹)   | 155     | 208     | 191     |
Ammonia volatilisation

Ammonia volatilisation was measured at presowing 2016 and side-dressing 2017 using semi-opened free static chambers (SOC), similar to those of Araújo et al. (2009). Two polyethylene terephthalate chambers per plot (31.0-cm height and 10.3-cm inner diameter, 2-L volume bottle with the bottom cut) were located 2 cm above the soil surface and with a 2-cm diameter upper hole that ensured airflow. The removed bottom was situated 2 cm above the upper hole to avoid the entrance of rain and irrigation water into the chamber. Inside the chamber, there was an absorbent Spontex® Origin foam strip (Mapa Spontex Ibérica SA, Sant Cugat del Vallés, Spain) of 25.0 × 2.5 × 0.5 cm (length × width × thickness) and 0.087 g cm⁻³ (density) previously impregnated in acid solution (60 mL H₂SO₄, 1 mol dm⁻³ + glycerine (2% v/v)). The bottom end of the strip was immersed in a 100-mL plastic jar with 50 mL of the acid solution in permanent contact with the strip. The trapped ammonia in the foam strip was extracted with 250 mL of 2 M KCl. The extracts were analysed to determine ammonium concentration by colourimetry using a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

Samplings started the day of slurry application, foam strips were changed with a frequency ranging from twice a day during the first two days to once a week during the last weeks. Samplings were performed until 28 days after presowing fertilisation in 2016 (11 samplings) and 14 days after side-dressing fertilisation in 2017 (10 samplings) since spring weather conditions and irrigation management promoted that ammonia volatilisation stopped in a shorter timespan than in autumn. Ammonia volatilisation was not measured after side-dressing application in 2018.

Semi-open chambers are not able to measure absolute NH₃ emissions as the acid strips are not able to trap all the NH₃ emitted from the soil, so they need to be calibrated determining their efficiency trapping ammonia. Thus, the efficiency of the SOC trapping ammonia was determined in the laboratory using three solutions with known concentrations of ammonium (407 mg NH₄⁺-N L⁻¹, 289 mg NH₄⁺-N L⁻¹, and 237 mg NH₄⁺-N L⁻¹). SOCs were placed (by triplicate) over 100-mm diameter Petri dishes containing 30 mL of each of the three solutions. After 24 hours, the NH₄⁺-N trapped by foam strips and the volume and NH₄⁺-N concentration of the remaining solutions were quantified. SOC efficiency was defined as the ratio between NH₄⁺-N trapped by the foam and the volatilised NH₃ estimated as the difference between the initial amount of ammonium in the Petri dish and the remaining amount after 24 hours. Efficiency in the field could not be determined because the solution with the known concentration was systematically polluted.

Ammonia captured in the control treatment was subtracted from the ammonia captured at each PS treatment and then divided by the efficiency to obtain the amount of ammonia volatilised in the PS treatments.

Direct nitrous oxide emissions

Direct nitrous oxide emissions were measured in side-dressing 2017 and 2018 using a manual closed chamber methodology. The closed-chamber technique and the N₂O flux measurement procedure were similar to those described by Mateo-Marin et al. (2020). Shortly, polyvinyl chloride upper cover of chambers (18.5-cm height and 30.0-cm inner diameter) wrapped in reflective insulation film were set on collars inserted 10 cm into the soil, creating 13.1-L chamber headspace. At each sampling, 15 mL of inner air were taken using a polypropylene syringe at 0 and 60 min after chamber closure. The samples were injected into 12-mL Exetainer borosilicate glass vials (Model 038W, Labco). Samplings started roughly at the hour with the mean temperature of the day (between 9:30h and 11:00h GMT; Alves et al., 2012). Samples were analysed by gas chromatography using an Agilent 7890B gas chromatography system with HP-Plot Q column and electron-capture, flame-ionisation and methaniser detectors.

Direct nitrous oxide emission rates were calculated as the linear increment in gas concentration (corrected for the air temperature) in the chamber headspace and multiplied by the ratio between the chamber volume and the soil area covered by the chamber (MacKenzie et al., 1998).

In both seasons, nitrous oxide measurements started just before the pig slurry application at tillering and ended at harvest. The samplings had daily frequency after fertilisation (during six days) and then measurements were spreading from once a week to once every two weeks, with a total of 12 and 17 sampling dates in 2017 and 2018, respectively.

The N₂O emission factor (EF, %) was calculated as the ratio between the difference of cumulative N₂O emissions in fertilised and unfertilised N plots, and the amount of the N applied in the fertilised plots and multiplied by 100. The yield-scaled N₂O emission (YSN₂O; g N kg⁻¹ grain) is the ratio between the cumulative N₂O emissions and the grain yield.

Soil mineral nitrogen

Soil (0-15 cm and 15-30 cm depths) was sampled in the three experimental periods (presowing 2016, side-dressing 2017 and side-dressing 2018) with daily frequency the first 5 days after fertilisation and decreasing the frequency later to reach once a week at the end of the sampling period (total of 11, 12, and 15 sampling dates in presowing 2016, side-dressing 2017, and...
side-dressing 2018, respectively). Two soil core samples were taken per plot to make a composite sample for each depth. Samples were sieved (3 mm) and two subsamples were obtained. A subsample was dried at 105 °C until constant weight to determine gravimetric soil water content. Another subsample of 10 g of fresh soil was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through cellulose filter. Nitrate (NO₃⁻) and ammonium concentration in extracts were analysed by colourimetry using a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

**Productive parameters and efficiency in the use of nitrogen**

At wheat maturity, this plot was hand-harvested in two random areas of 0.54 m² to obtain the grain (adjusted to 120 g kg⁻¹) and aboveground biomass yield. Subsamples of grain and straw were dried at 65 °C and milled to obtain the grain and straw N concentration by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA).

Two parameters were used to compare the efficiency in the use of N between treatments, the nitrogen use efficiency (NUE) and the apparent N recovery efficiency (REₙ). NUE is the relation between the total aboveground N uptake and the N applied by fertilisation. REₙ is the increment in the aboveground N uptake due to the N application per unit of N applied (Eq. 1): 

\[
REₙ = \frac{Uₜ - U₀}{Fₜ}
\]  

[Eq. 1]

where \(Uₜ\) is the N uptake by aboveground biomass in the \(T\) treatment; \(U₀\) is the N uptake by aboveground biomass in the unfertilised control plot; and \(Fₜ\) is the amount of N applied in the \(T\) treatment. The mineral N contained in the applied slurry (i.e., NH₄⁺-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.

### Table 3

|          | C1   | C2   | C3   |
|----------|------|------|------|
| Initial (kg NH₄⁺-N ha⁻¹) | 15.5 | 11.1 | 9.1  |
| Final (kg NH₄⁺-N ha⁻¹)   | 0.113 (±0.001) | 0.088 (±0.004) | 0.076 (±0.001) |
| Absorbed (kg NH₄⁺-N ha⁻¹) | 4.01 (±0.23) | 2.67 (±0.05) | 2.11 (±0.11) |
| Efficiency (%)           | 26.0% (±1.5%) | 24.4% (±0.4%) | 23.5% (±1.3%) |

[a] Efficiency did not differ significantly between the three concentrations (Tukey’s test, \(p>0.05\)).

### Results

#### Ammonia volatilisation

In the study of efficiency, the amount of ammonia volatilised ranged between 8.9 and 15.4 kg N ha⁻¹ in the 24-hour period. The efficiency of the semi-opened chambers did not differ significantly among the three ammonium concentrations (Table 3). Mean efficiency was 24.6% ± 0.7% (mean ± SE).

There were substantial differences in the environmental conditions between the two measurement periods (Fig. S1 [suppl]). Thus, the average temperature and wind speed during the experimental period were 7.2 °C and 1.1 m s⁻¹ before presowing 2016, and 15 °C and 2.0 m s⁻¹ after side-dressing 2017.

The highest NH₃ peak was trapped at 4.5-6.5 h after pig slurry application (Fig. 1). Ammonia trapped in fertilised treatments (mean ± SE) were 2.40 ± 0.21 g N ha⁻¹ min⁻¹ (presowing 2016) and 7.24 ± 0.43 g N ha⁻¹ min⁻¹ (side-dressing 2017). At the following sampling (23-24 h after pig slurry application), NH₃ decreased to increase at the third sampling (27-31 h after pig slurry application) and decrease afterwards. A small emission peak was
trapped 10 days after the slurry application in side-dressing 2017. This peak is thought to be related to the degradation of the foam strips during the 5-day exposition period between 12th and 17th April.

No significant differences (repeated measure analysis) in \( \text{NH}_3 \) fluxes among the three pig slurry treatments were observed; however, all three treatments presented significantly higher fluxes than the non-fertilised control. The \( \text{NH}_3 \) fluxes were 280% and 479% higher in the fertilised treatments than in the Control for presowing 2016 and side-dressing 2017, respectively.

Cumulative ammonia volatilised after three, fourteen, and twenty-eight days after treatments application are presented in Table 4. During the first three days, \( \text{NH}_3 \) losses reached more than 50% of the total \( \text{NH}_3 \) emitted during the whole measurement period (51% for presowing 2016 and 65% for side-dressing 2017) and fourteen days after fertilisation, \( \text{NH}_3 \) losses represented 91% of the total measured losses of the period. No differences (\( p>0.05 \)) were found in the cumulative \( \text{NH}_3 \) emissions among fertiliser treatments for any period (3, 14, or 28 days, whatever it was the application moment, presowing or side-dress). Ammonia volatilisation losses in the period of fourteen days after pig slurry application were, on average, 34% larger (\( p<0.0001 \)) during side-dress than during presowing application.

**Direct nitrous oxide emission**

Soil temperature and water filled pore space (WFPS) at 10-cm depth during the \( \text{N}_2\text{O} \) samplings are shown in Fig. S2 [suppl]. Mean \( \text{N}_2\text{O} \) fluxes ranged from -0.5 g N ha\(^{-1}\) day\(^{-1}\) to 66.9 g N ha\(^{-1}\) day\(^{-1}\) for both cropping seasons (Fig. 2). Significant differences were observed between treatments: Control showed lower \( \text{N}_2\text{O} \) fluxes in comparison to the fertilised treatments in side-dressing 2017, and Control and PS-NI showed lower \( \text{N}_2\text{O} \) fluxes than PS and PS-A in side-dressing 2018 (repeated measure analysis; \( p<0.05 \)). The same behaviour was found in cumulative \( \text{N}_2\text{O} \) emissions. The \( \text{N}_2\text{O} \) emissions were on average 2.8 times higher in the PS treatments than in the non-fertilised control. However, in side-dressing 2018, the PS-NI

**Table 4.** Average cumulative ammonia volatilised\(^{[a]} \) (kg N ha\(^{-1}\); \( n=4 \)) and percentage respect to ammonium nitrogen applied with the pig slurry in the different treatments (PS: pig slurry, PS-UI: pig slurry + MCDHS, and PS-A: pig slurry + microbial activator). The results are presented separately by application moment (presowing 2016 and side-dress 2017) at three timespans (3, 14, and 28 days) after application.

|               | PS  | PS-UI | PS-A | DF | \( p \)-value |
|---------------|-----|-------|------|----|--------------|
| **Presowing 2016** |     |       |      |    |              |
| 3 days        | 5.4 (3.8%) | 5.3 (3.8%) | 6.6 (4.6%) | 6  | 0.197        |
| 14 days       | 9.0 (6.4%) | 9.6 (6.8%) | 11.2 (7.9%) | 6  | 0.181        |
| 28 days       | 9.7 (6.9%) | 10.5 (7.4%) | 12.1 (8.6%) | 6  | 0.158        |
| **Side-dressing 2017** |     |       |      |    |              |
| 3 days        | 17.9 (13.0%) | 19.0 (13.8%) | 20.8 (15.2%) | 6  | 0.695        |
| 14 days       | 26.4 (19.3%) | 28.9 (21.1%) | 31.6 (23.0%) | 6  | 0.609        |

\(^{[a]}\) Considering 24.6% as the efficiency of the method to trap the ammonia volatilised and discounting the amount of ammonia trapped in the control (background) treatment.
treatment decreased the \( \text{N}_2\text{O} \) emissions at the same level that the Control, with lower emissions than the PS and PS-UI treatments \((p<0.05)\). Although a large \( \text{N}_2\text{O} \) emission was observed in the PS-UI \((1,014 \, \text{g N ha}^{-1})\) during side-dressing 2018, it was not significantly different from that in the PS.

Crop season EFs for direct \( \text{N}_2\text{O} \) and \( \text{YS}_{\text{N}_2\text{O}} \) emissions did not show differences between treatments in side-dressing 2017, but in side-dressing 2018, the treatment with NI presented the lowest values (Table 5) for both variables. EFs were 92% and 97% significantly lower in PS-NI than in PS and PS-UI, respectively; and \( \text{YS}_{\text{N}_2\text{O}} \) were 71% and 87% significantly lower in PS-NI than in PS and PS-UI, respectively.

### Soil mineral nitrogen

After presowing application of PS, soil mineral N concentration was affected by treatments only in the top layer \((0-15 \, \text{cm in Table 6}; 15-30 \, \text{cm not shown})\). Differences were only significant between PS-UI and PS-A, and no effects of the additives mixed with the PS were detected. However, these differences were not noticed in the side-dress application \((2017)\) whatever it was the considered depth \((0-15 \, \text{cm or 15-30 cm})\). During the next season \((side-dressing 2018)\), soil nitrate concentration \((0-15 \, \text{cm})\) in PS-NI was 32% and 28% lower than in PS and PS-UI \((p<0.05)\), respectively; and soil ammonium concentration \((0-15 \, \text{cm})\) in PS-NI was 32% and 23% higher than in PS and PS-UI, respectively, but not significantly. The opposite behaviour of soil nitrate and ammonium concentrations generated no differences \((p>0.05)\) among treatments in soil mineral N content.

### Productive parameters and efficiency in the use of nitrogen

Table 7 shows that no differences in grain yield \((p>0.05)\) were found among treatments for any of the

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**Table 5.** Average \((n=4)\) cumulative direct nitrous oxide emissions \((\text{g N ha}^{-1})\), crop season emission factor (\%), and yield-scaled \( \text{N}_2\text{O} \) emission \((\text{g N kg}^{-1} \text{ grain})\) in the different fertiliser treatments. The results are presented separately by growing seasons with fertiliser application at side-dress (2016/17 and 2017/18). Values followed by the same letter were not significantly different \((p>0.05, \text{Tukey’s test})\).

|                      | Control | PS      | PS-UI   | PS-A   | DF | \( p \)-value |
|----------------------|---------|---------|---------|--------|----|---------------|
| **Side-dressing 2017** |         |         |         |        |    |               |
| \( \text{N}_2\text{O} \) emission \((\text{g N ha}^{-1})\) | 229.9 b | 610.6 a | 685.3 a | 661.5 a | 9  | <0.001        |
| Crop season EF (%)   | -       | 0.19    | 0.22    | 0.21   | 6  | 0.483         |
| \( \text{YS}_{\text{N}_2\text{O}} \) \((\text{g N kg}^{-1} \text{ grain})\) | -       | 0.11    | 0.13    | 0.13   | 6  | 0.312         |

|                      |         |         |         |        |    |               |
| **Side-dressing 2018** |         |         |         |        |    |               |
| \( \text{N}_2\text{O} \) emission \((\text{g N ha}^{-1})\) | 111.8 b | 460.9 a | 1014.4 a | 139.5 b | 9  | <0.001        |
| Crop season EF (%)   | -       | 0.24 ab | 0.60 a  | 0.02 b | 6  | 0.033         |
| \( \text{YS}_{\text{N}_2\text{O}} \) \((\text{g N kg}^{-1} \text{ grain})\) | -       | 0.07 a  | 0.15 a  | 0.02 b | 6  | 0.001         |

\[^{[a]}\] Differences were found when just the two treatments were analysed using Tukey’s test. This comparison was performed since the huge values of \( \text{N}_2\text{O} \) emissions in PS-UI hid differences. EF: emission factor; \( \text{YS}_{\text{N}_2\text{O}} \): yield-scaled \( \text{N}_2\text{O} \) emission.
three experimental periods. Aboveground biomass did not show effect of the treatments for the two periods of season 2016/17; however, in 2017/18 growing season, aboveground biomass in the Control treatment was 16% and 13% lower \((p<0.05)\) than in PS and PS-UI treatments, respectively. Total aboveground N was not affected in the three experiments by the fertiliser strategy, but grain N was influenced \((p=0.04)\) by the treatments when fertiliser was applied at tillering in 2017. Nitrogen use efficiency and recovery N efficiency did not show significant differences among treatments, independently of the season and the moment of N application (Table 7).

**Discussion**

**Ammonia volatilisation**

The hours that follow PS application are critical for \(\text{NH}_3\) losses. In this regard, the Directive (EU) 2016/2284 (EC, 2016) rightly suggests incorporating manures and slurries into the soil immediately or within four hours of spreading to reduce ammonia emissions from livestock manure. In this study, 2% and 7% of applied ammonium-N were volatilised as \(\text{NH}_3\) within the first 4.5 h and 6.5 h after the presowing and side-dress application, respectively. These values contrast with \(\text{NH}_3\) losses of 0.9% of total ammonium-N applied at presowing reported by Yagüe et al. (2019) the first 3.5 h after the spreading with trail-hose in a field located in the same region and under similar environmental conditions. According to the authors, soil moisture and pig slurry characteristics (dry matter) influence \(\text{NH}_3\) losses; high soil WFPS and high slurry DM boost \(\text{NH}_3\) volatilisation. These variables in conjunction with others as meteorological conditions, soil pH, soil management, and measurement method (Hafner et al., 2018) could explain the differences between both studies.

Weather conditions determined the \(\text{NH}_3\) evolution. Ammonia volatilisation was higher during diurnal hours (first and third samplings) than at the nighttime hours (second sampling) even when exposure times at night (average 18.5 hours) were longer than diurnal exposures (average 5.5 hours). Higher temperature and wind speed during diurnal hours increased the volatilisation (Fig. S1 [suppl]; mean thermal amplitude of 8.2 °C and mean wind speed amplitude of 2.3 m s\(^{-1}\)). The effect of the daily pattern of air temperature and wind speed on \(\text{NH}_3\) emissions was already observed by Li et al. (2018). Similarly, differences in weather conditions between application moments might have been one of the factors responsible for higher emissions at side-dressing 2017 than at presowing 2016 since at side-dress application the temperature and wind speed were higher than at presowing.

Slurries with low DM promote the infiltration into the soil, reducing \(\text{NH}_3\) volatilisation compared to slurries with a high DM which favour crust formation and lower infiltration rates (Bosch-Serra et al., 2014). In the present study, two contrasting slurries in term of DM, 41.4 kg DM m\(^{-3}\) (presowing 2016) and 73.4 kg DM m\(^{-3}\) (side-dressing 2017), had

| Table 6. Average (n=4) nitrate (\(\text{NO}_3^-\), mg N kg\(^{-1}\) soil), ammonium (\(\text{NH}_4^+\), mg N kg\(^{-1}\) soil) and mineral N (Nmin; mg N kg\(^{-1}\) soil) concentration from 0 to 15-cm depth in the one month after PS application in the fertiliser treatments (PS: pig slurry, PS-UI: pig slurry + MCDHS, PS-A: pig slurry + microbial activator, and PS-NI: pig slurry + DMPP). The results are presented separately by periods (presowing 2016, side-dressing 2017, and side-dressing 2018). Values followed by the same letter were not significantly different \((p>0.05, \text{Tukey’s test}).\) |
|---|---|---|---|---|---|---|
| **Presowing 2016** | PS | PS-UI | PS-A | DF | T\(^{[1]}\) | S\(^{[2]}\) |
| \(\text{NO}_3^-\) | 15.5 ab | 12.6 b | 18.2 a | 72 | \(<0.001\) | \(<0.001\) |
| \(\text{NH}_4^+\) | 35.1 | 34.1 | 41.0 | 72 | 0.654 | \(<0.001\) |
| Nmin | 50.6 ab | 46.8 b | 59.2 a | 72 | 0.001 | \(<0.001\) |
| **Side-dressing 2017** | PS | PS-UI | PS-NI | DF | T\(^{[1]}\) | S\(^{[1]}\) |
| \(\text{NO}_3^-\) | 11.1 | 11.8 | 14.8 | 72 | 0.053 | \(<0.001\) |
| \(\text{NH}_4^+\) | 2.0 | 2.7 | 2.0 | 72 | 0.276 | \(<0.001\) |
| Nmin | 13.1 | 14.5 | 16.8 | 72 | 0.124 | \(<0.001\) |
| **Side-dressing 2018** | PS | PS-UI | PS-NI | DF | T\(^{[1]}\) | S\(^{[1]}\) |
| \(\text{NO}_3^-\) | 8.4 a | 7.9 a | 5.7 b | 72 | \(<0.001\) | \(<0.001\) |
| \(\text{NH}_4^+\) | 11.8 | 12.7 | 15.6 | 72 | 0.301 | \(<0.001\) |
| Nmin | 20.1 | 20.7 | 21.4 | 72 | 0.948 | \(<0.001\) |

\(^{[1]}\) Repeated measure analysis considering the fertiliser treatment (T), sampling date (S), and their interaction (T×S). \(^{[2]}\) Nine, eight, and ten sampling dates for the 2016, 2017, and 2018 periods, respectively.
to be used. This fact could have also affected the comparison of NH₃ volatilisation between application moments.

Irrigation is another factor that could be relevant to compare presowing and side-dress applications. A short irrigation event of 2 mm was applied immediately after PS application in side-dressing 2017 to wash up the slurry placed on the canopy to avoid negative effects on leaves. This practice could have incorporated the ammonium slurry into the soil and reduced the potential for NH₃ volatilisation.

Apart from the cited variables, additives could alter NH₃ volatilisation. UIs are considered a strategy to reduce NH₃ emissions when they are added to urea-based fertilisers or manures since they delay the transformation of urea into ammonium (Sigurdarson et al., 2018). However, the addition of UI to pig slurry is a questionable strategy because of the high probability of fast transformation of urea into ammonium after excretion (Dai & Karring, 2014).

MCDHS was evaluated in the study due to the possibility that micro-acidification, through the hydrolysis of the MCDHS molecule, could reduce ammonia volatilisation or have potential effects over N dynamics (e.g., acting as NI). Nevertheless, the presence of dihydrogen sulphate in the molecule did not reduce the pH of the slurry (data not shown). Moreover, no changes were observed in soil mineral N concentrations, ammonia losses, nitrous oxide emissions, or yield in PS-UI treatment in comparison to PS treatment, which rejects effects due to MCDHS addition.

The microbial activator, PS-A, was able to maintain higher levels of nitrate in the soil than the urease inhibitor, PS-UI, but it was not able to show differences with PS treatment. Besides, despite the higher soil NO₃ concentration, it did not affect N₂O emissions.

### Table 7. Average (n=4) of productive parameters and N efficiency indexes in the different fertiliser treatments (Control, PS: pig slurry, PS-UI: pig slurry + MCDHS, PS-A: pig slurry + microbial activator, and PS-NI: pig slurry + DMPP). The results are presented separately by periods (presowing 2016 and side-dress 2017 and 2018). Values followed by the same letter were not significantly different (p>0.05, Tukey’s test).

|                                | Control | PS    | PS-UI | PS-A | DF  | p-value |
|--------------------------------|---------|-------|-------|------|-----|---------|
| **Presowing 2016**             |         |       |       |      |     |         |
| Grain (kg ha⁻¹)                | 7,237   | 6,139 | 5,695 | 6,410| 9   | 0.083   |
| Aboveground biomass (kg ha⁻¹)  | 14,454  | 13,542| 13,549| 14,037| 9 | 0.719  |
| Grain N (kg ha⁻¹)              | 153.9   | 122.4 | 118.8 | 134.4| 9   | 0.048[a]|
| Total aboveground N (kg ha⁻¹)  | 204.8   | 181.5 | 192.9 | 204.1| 9   | 0.466   |
| Nitrogen use efficiency        | -       | 1.29  | 1.37  | 1.45 | 6   | 0.525   |
| Apparent REₙ                  | -       | -0.16 | -0.08 | 0.00 | 6   | 0.525   |
| **Side-dressing 2017**         |         |       |       |      |     |         |
| Grain (kg ha⁻¹)                | 6,098   | 5,591 | 5,186 | 5,123| 9   | 0.087   |
| Aboveground biomass (kg ha⁻¹)  | 13,954  | 13,831| 13,141| 12,760| 9 | 0.529  |
| Grain N (kg ha⁻¹)              | 129.7 a | 119.9 ab| 110.3 ab| 107.2 b| 9 | 0.042  |
| Total aboveground N (kg ha⁻¹)  | 195.5   | 202.0 | 187.0 | 178.9| 9   | 0.384   |
| Nitrogen use efficiency        | -       | 1.19  | 1.13  | 1.31 | 6   | 0.241   |
| Apparent REₙ[b]                | -       | 0.04  | -0.05 | -0.12| 6   | 0.292   |
| **Side-dressing 2018**         |         |       |       |      |     |         |
| Grain (kg ha⁻¹)                | 5,938   | 6,837 | 6,471 | 6,245| 9   | 0.393   |
| Aboveground biomass (kg ha⁻¹)  | 14,263 b| 17,041 a| 16,473 a| 15,857 ab| 9 | 0.007  |
| Grain N (kg ha⁻¹)              | 99.2    | 122.2 | 108.3 | 107.7| 9   | 0.245   |
| Total aboveground N (kg ha⁻¹)  | 153.0   | 196.3 | 168.9 | 173.3| 9   | 0.136   |
| Nitrogen use efficiency        | -       | 1.65  | 1.33  | 1.43 | 6   | 0.104   |
| Apparent REₙ[b]                | -       | 0.36  | 0.13  | 0.17 | 6   | 0.210   |

[a] Significant effects of fertiliser treatments (p<0.05) from the analysis of variance procedure, but Tukey’s test did not show differences.  
[b] REₙ – Recovery efficiency

### Direct nitrous oxide emission

Few studies have evaluated the use of NI mixed with pig slurry to mitigate N₂O losses in the Mediterranean climate (Guardia et al., 2017). Under these climatic conditions, NI showed a promising potential to reduce N₂O emission.
conditions, Recio et al. (2018) assessed the use of the nitrification inhibitor DMPP when was added to preplanting pig slurry applications (rate of 50 kg N ha⁻¹) on both N₂O and NH₃ emissions, obtaining significant abating effect on direct N₂O emissions (decrease of 70% in a 22-days period after PS application) and no significant affection of cumulative NH₃ emissions and Chiodini et al. (2019) found between 62% and 94% reduction in N₂O emission in the crop season with emission factor being between two and four times lower when DMPP (Vizura®) was added to digested and injected in the soil in a maize crop. The present work corroborates the effect of DMPP mitigating N₂O emissions (decrease of 86%) with higher abatement potential than that reported by Guardia et al. (2017) at the same slurry rate (120 kg N ha⁻¹) and similar to the reported by Chiodini et al. (2019) both applied to maize crop. The inhibition of the activity of nitrifiers could be noticed from the lower soil NO₃⁻ and higher soil NH₄⁺ concentrations, although significant differences were only observed for nitrate in the 0-15-cm depth. In this regard, topsoil N processes are the most influential on N₂O emissions since N₂O produced in this layer can escape to the atmosphere (Yoh et al., 1997), whereas N₂O produced at deeper layers might not reach the soil surface (Neftel et al., 2000).

In this study, avoiding N₂O losses did not turn into a significant increment in N efficiency as could be expected: the less N₂O losses, the more N availability, and the more N absorption and N efficiency by plants. However, this fact was unnoticed in the efficiency indicators because of the low contribution of N₂O emissions to the N balance (<1 kg N₂O-N ha⁻¹) and the non-limiting soil N conditions proven by the high yields in unfertilised treatments during the two growing seasons.

In conclusion, important N losses due to ammonia volatilisation were observed after pig slurry fertilisation, lower after presowing (7-9% of NH₄⁺-N applied) than side-dressing application (19-23% of NH₄⁺-N applied) but none of the three additives evaluated in this work was effective to reduce them independently of the PS application moment, at presowing or at tillering. Nitrous oxide emissions were a minor component of the N balance (averaging 0.4% of NH₄⁺-N applied), although its high global warming potential highlighted the importance of DMPP reducing N₂O fluxes (roughly 70%) and, accordingly, yield-scaled N₂O emissions and N₂O emission factors. The use of pig slurry with additives had neither advantages nor disadvantages in terms of agronomic productivity and N use efficiency.

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