

# DMPSA application reduces N<sub>2</sub>O emissions from exogenous NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> sources in irrigated maize

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## Abstract

Agriculture is a major anthropogenic source of nitrous oxide (N<sub>2</sub>O). Application of nitrogenous (N) fertilizers enhances the emission of this greenhouse gas (GHG), mainly due to the biogenic processes of nitrification and denitrification. The use of nitrification inhibitors (NIs), that delay the microbial oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>, has been reported as a successful tool for mitigating N<sub>2</sub>O losses. In this context, a field experiment using <sup>15</sup>N labeled fertilizers was carried out in an irrigated Mediterranean maize field aiming to evaluate the effectiveness of the NI 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) on mitigating N<sub>2</sub>O emissions, and assess the processes involved in its production. The experiment, performed in 1 m<sup>2</sup> microplots, involved the application of <sup>15</sup>NH<sub>4</sub>NO<sub>3</sub> or NH<sub>4</sub><sup>15</sup>NO<sub>3</sub><sup>-</sup> (with and without DMPSA) to quantify the amount of N<sub>2</sub>O coming from exogenous NH<sub>4</sub><sup>+</sup>, exogenous NO<sub>3</sub><sup>-</sup> and endogenous soil N. The resulting N<sub>2</sub>O fluxes confirmed that the addition of DMPSA led to a significant (54%) abatement of this GHG. In this experiment, both nitrification and denitrification were partially inhibited by DMPSA. When NI was not applied, the largest N<sub>2</sub>O emissions were expected from NH<sub>4</sub><sup>+</sup> than from NO<sub>3</sub><sup>-</sup>-based fertilizers due to nitrification.

## Key Words

<sup>15</sup>N, nitrous oxide, nitrification inhibitor, 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture, Mediterranean climate, maize

## Introduction

Agriculture accounts for 12% of the global anthropogenic GHGs emissions and is considered the most important source of non-CO<sub>2</sub> (N<sub>2</sub>O and CH<sub>4</sub>) GHGs (IPCC 2014). The production or consumption of N<sub>2</sub>O is the result of different and simultaneous microbial processes, mainly nitrification and denitrification (Firestone and Davidson 1989). These processes are highly dependent on soil physico-chemical properties, which in turn can be easily modified by management practices and by climatic parameters (Snyder et al. 2009).

Many authors (Akiyama et al 2010, Abalos et al 2014) and IPCC Assessment Reports suggest that the application of N fertilizers including nitrification inhibitor compounds (NIs) could abate atmospheric N<sub>2</sub>O emissions and be an effective mitigation option. Nitrification inhibitors are compounds that delay the bacterial oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> in the soil for a certain period of time due to bacteriostatic effect on nitrifiers (*Nitrosomonas* bacteria), and consequently reducing N<sub>2</sub>O production during nitrification. Although NIs such as dicyandiamide (DCD) and 3, 4 dimethylpyrazol phosphate (DMPP) have been broadly evaluated under several climatic and management conditions, the effect of the new inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) on processes involved in N<sub>2</sub>O emissions remains unstudied. So, a field experiment with <sup>15</sup>N labelled N fertilizer was carried out with the aims: (1) quantifying the effect of application of DMPSA on N<sub>2</sub>O emissions from soils in an irrigated Mediterranean maize field and (2) assessing the processes involved in its production via quantification of the amount of N<sub>2</sub>O coming from exogenous NH<sub>4</sub><sup>+</sup>, exogenous NO<sub>3</sub><sup>-</sup> and endogenous soil N. Our main hypothesis was that DMPSA could reduce the emission of N<sub>2</sub>O as opposed to the addition of N fertilizer alone; and that the inhibition of oxidation of NH<sub>4</sub><sup>+</sup> could be the most effective strategy to mitigate N<sub>2</sub>O, particularly in this Mediterranean area, where nitrification is of major relevance (Aguilera et al. 2013).

## Methods

### *Field site and soil characteristics*

The field experiment was carried out at the field station “El Encín”, situated near Alcalá de Henares (Madrid,

Spain), in the middle of the Henares river basin, (latitude 40°32'N, longitude 3°17'W). According to the Soil Taxonomy of USDA, the soil was a *Calcic Haploxerept* with clayey loam texture (28% clay, 17% silt, and 55% sand) in the upper horizon (0-28 cm). The basic topsoil characteristics were: total organic carbon,  $8.2 \pm 0.4 \text{ g kg}^{-1}$ , total nitrogen,  $0.75 \pm 0.12 \text{ g kg}^{-1}$ , bulk density,  $1.41 \pm 0.03 \text{ Mg m}^{-3}$ , soil: water pH 7.6, and  $\text{CaCO}_3$ ,  $13.1 \pm 0.3 \text{ g kg}^{-1}$ . According to data from the meteorological station situated in the field, the mean annual average temperature and rainfall during the last 10 years was  $13.5^\circ\text{C}$  and 402 mm, respectively.

#### Experimental design and procedure

A randomized complete design with three replicates was established. Twelve microplots (1m x 1m) were used for this experiment. Each microplot received labeled  $^{15}\text{NH}_4\text{NO}_3$  or  $\text{NH}_4^{15}\text{NO}_3$  (25 atom % made from 98 atom % fertilizers (Campro Scientific GmbH, Berlin, Germany) and unlabeled  $\text{NH}_4\text{NO}_3$ ) and with and without DMPSA inhibitor (provided by EuroChem Agro). Those treatments were applied as a top-dressing (on 24<sup>th</sup> June) at a rate of  $180 \text{ kg total N ha}^{-1}$  (half as  $^{15}\text{NH}_4\text{NO}_3$  or  $\text{NH}_4^{15}\text{NO}_3$  and the other half as  $\text{NH}_4\text{NO}_3$ ). The proportion of DMPSA in the fertilizer was that recommended by the manufacturer, i.e. 0.8% of the  $\text{NH}_4^+$ -N applied. The treatments applied, their designation and application rate are summarized in Table 1.

**Table 1 Treatments applied in  $^{15}\text{N}$  microplots.**

Treatment applied	Designation	N rate (kg N ha <sup>-1</sup> )
$^{15}\text{NH}_4\text{NO}_3 + \text{NH}_4\text{NO}_3$	$^{15}\text{AN}$	90 + 90
$\text{NH}_4^{15}\text{NO}_3 + \text{NH}_4\text{NO}_3$	$\text{A}^{15}\text{N}$	90 + 90
$^{15}\text{NH}_4\text{NO}_3 + \text{NH}_4\text{NO}_3 + \text{DMPSA}$	$^{15}\text{AN} + \text{DMPSA}$	90 + 90
$\text{NH}_4^{15}\text{NO}_3 + \text{NH}_4\text{NO}_3 + \text{DMPSA}$	$\text{A}^{15}\text{N} + \text{DMPSA}$	90 + 90

A solution of 2 L of each treatment was prepared and homogeneously spread over the soil surface of the microplots with a hand sprayer. Phosphorous  $50 \text{ kg P ha}^{-1}$  as simple superphosphate ( $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ) and K fertilizers  $150 \text{ kg K ha}^{-1}$  as potassium sulfate ( $\text{K}_2\text{SO}_4$ ), both provided by EuroChem Agro, were applied to all treatments at the time of sowing. Maize (*Zea mays* L. FAO class 600) crop was sown in microplots on 17<sup>th</sup> April at a plant population density of  $7.5 \text{ plants m}^{-2}$ . Harvesting took place on 24<sup>th</sup> October of the same year. Irrigation was applied from sowing until 4<sup>th</sup> September by sprinkler method. A total amount of 705 mm of water was applied over 44 irrigation events.

During the experiment, gas, soil and plant samples were collected to measure the enrichment and amount of N. Gas samples from the microplots receiving labeled  $^{15}\text{NH}_4\text{NO}_3$  or  $\text{NH}_4^{15}\text{NO}_3$  fertilizer were taken after 60 min static chamber closure 3, 4, 6, 8, 10 and 13 days after fertilizer application. The concentration of  $\text{N}_2\text{O}$  was measured by GC using a HP-6890 gas chromatograph with a HTC-3 Headspace autosampler (Agilent Technologies, Spain). HP Plot-Q capillary columns transported gas samples to a  $^{63}\text{Ni}$  micro electron-capture detector ( $\mu\text{ECD}$ ). Helium as carrier and  $\text{Ar}/\text{CH}_4$  as make up gases were used.  $^{15}\text{N}$  enrichment of  $\text{N}_2\text{O}$  contained in the gas samples was measured using a TG2 trace gas analyser interfaced to a 20-22 isotope ratio mass spectrometer (both from SerCon Ltd., Crewe, UK) at Rothamsted Research North Wyke. Solutions of 6 and 30 atom% ammonium sulphate ( $(\text{NH}_4)_2\text{SO}_4$ ) were prepared and used to generate 6 and 30 atom %  $\text{N}_2\text{O}$  (Laughlin et al. 1997) and used as reference and quality control standards. During the experiment, the mean natural abundance of atmospheric  $\text{N}_2\text{O}$  (0.3663 atom%  $^{15}\text{N}$ ) was subtracted from the  $^{15}\text{N}$  enrichment of the samples to calculate the atom percent excess (ape). To obtain the  $\text{N}_2\text{O}$  flux that was derived from fertilizer ( $\text{N}_2\text{O} - N_{\text{diff}}$ ), the following equation was used:

$$\text{N}_2\text{O} - N_{\text{diff}} = (\text{N}_2\text{O} - N) \times \left( \frac{\text{N}_2\text{O} - \text{ape}_{\text{sample}}}{\text{ape}_{\text{fertilizer}}} \right)$$

In the above equation,  $\text{N}_2\text{O} - N$  is the  $\text{N}_2\text{O}$  emission from soil, ' $\text{N}_2\text{O} - \text{ape}_{\text{sample}}$ ' is the  $^{15}\text{N}$  atom% excess of emitted  $\text{N}_2\text{O}$  (being equal to ' $^{15}\text{N}$  atom% of measured samples' minus 0.3663 atom% where 0.3663 atom% is the mean natural  $^{15}\text{N}$  abundance of 'background  $\text{N}_2\text{O}$ ' obtained in our experiment), and ' $\text{ape}_{\text{fertilizer}}$ ' is the  $^{15}\text{N}$  atom% excess of the applied fertilizer (Loick et al. 2016).

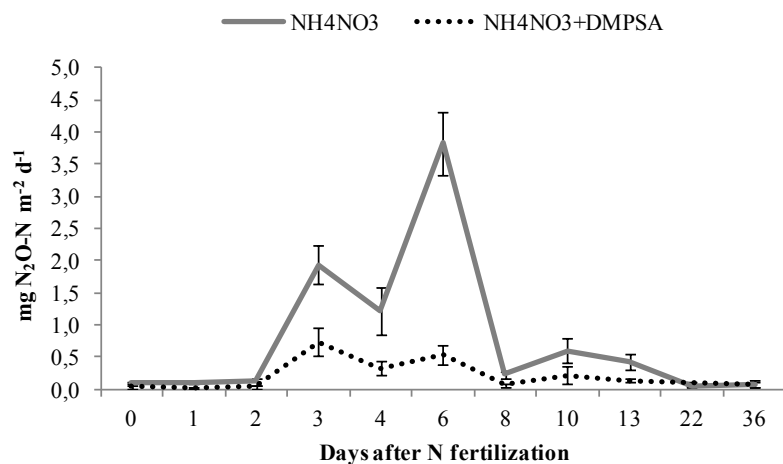
To obtain the  $\text{N}_2\text{O}$  flux that was derived from soil ( $\text{N}_2\text{O} - N_{\text{dfs}}$ ), the following equation was used:

$$N_2O - N_{df_s} = 100 - (N_2O - N_{df_{15AN}}) - (N_2O - N_{df_{A15N}})$$

In the above equation, ' $N_2O - N_{df_{15AN}}$  and  $N_2O - N_{df_{A15N}}$ ' is the  $N_2O$  flux that was derived from fertilizer  $^{15}NH_4NO_3$  ( $^{15}AN$ ) or  $NH_4^{15}NO_3$  ( $A^{15}N$ ) respectively.

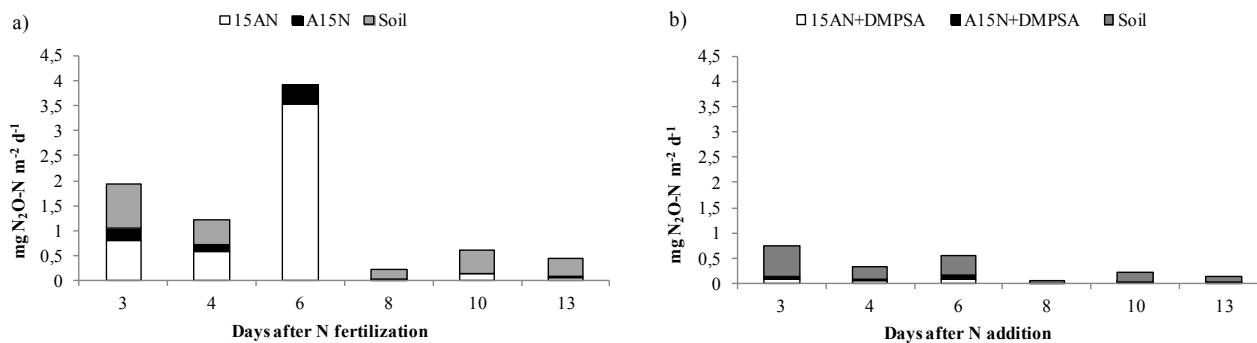
## Results

$N_2O$  fluxes measured in the microplots confirmed that the addition of DMPSA led to a significant (54 %) abatement of  $N_2O$  emissions (Figure 1).



**Figure 1.**  $N_2O$  emissions ( $mg\ N_2O\ m^{-2}\ d^{-1}$ ) in microplots during the first month after N fertilization. Vertical bars indicate standard errors.

The amount of  $N_2O$  coming from exogenous  $NH_4^+$ , exogenous  $NO_3^-$  and endogenous soil N during the week in which the peak occurred is shown in Figure 2.



**Figure 2.** (a) Amount of  $N_2O$  coming from  $^{15}AN$ ,  $A^{15}N$  and soil N in microplots without DMPSA addition and (b) amount of  $N_2O$  coming from  $^{15}AN$  and  $A^{15}N$  and soil N in microplots with DMPSA addition.

Our results revealed that exogenous  $NH_4^+$  ( $^{15}AN$ ) was the main source of  $N_2O$  emissions in the  $NH_4NO_3$  microplots without DMPSA application: 67% of  $N_2O$  losses came from  $^{15}AN$  treatment in two days in which emission peaks were observed (3 and 6 days after N addition). These results showed that  $NH_4^+$ -based fertilizers have the potential risk of increasing  $N_2O$  losses compared to  $NO_3^-$ -based sources under Mediterranean conditions. In agreement, the review of Aguilera et al. (2013) pointed out that nitrification is the major source of  $N_2O$  losses in low C-content Mediterranean soils. The amount of  $N_2O$  coming from  $^{15}AN+DMPSA$  treatment was much lower than in the  $^{15}AN$  treatment ( $P < 0.05$ ). The amount of  $N_2O$  released from soil mineral N was also numerically (but not statistically) higher in  $NH_4NO_3$  than in  $NH_4NO_3+DMPSA$  microplots. Furthermore, our results demonstrated the high effectiveness of NIs when soil conditions are favorable for  $NH_4^+$  oxidation, abating  $N_2O$  losses from both nitrification (directly) and denitrification (indirectly, by decreasing the availability of the substrate  $-NO_3^-$  for denitrifiers) (Firestone and Davidson 1989). The application of DMPSA also reduced  $N_2O$  emissions from exogenous  $NO_3^-$  ( $A^{15}N$ ) suggesting a possible effect of NI on denitrifier microorganisms. Further research is needed to elucidate this point.

## Conclusion

Our results demonstrate that application of DMPSA with ammonium nitrate provide a suitable option to improve environmental sustainability, diminishing N<sub>2</sub>O emissions. A large proportion of N<sub>2</sub>O losses came from the oxidation of NH<sub>4</sub><sup>+</sup>, the use of NO<sub>3</sub><sup>-</sup>-based fertilizers could also be considered as a potential N<sub>2</sub>O mitigation strategy under similar environmental conditions as those found in this irrigated Mediterranean maize field.

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