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Efficacy of the Nitrification Inhibitor 3,4 Dimethylpyrazol Succinic Acid (DMPSA) when Combined with Calcium Ammonium Nitrate and Ammonium Sulphate—A Soil Incubation Experiment

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Abstract: The efficacy of the new nitrification inhibitor 3,4 dimethylpyrazol succinic acid (DMPSA) was tested with calcium ammonium nitrate (CAN) and ammonium sulphate (AS) fertilisers in an incubation experiment using a sandy loam soil and a sandy textured soil. The experiment was conducted over 80 days. For AS fertiliser, inclusion of DMPSA resulted in significantly less NO_3^--N present after 19 days in both soils. In the case of CAN, inclusion of DMPSA resulted in significantly less NO_3^--N present after 45 days in the sandy loam soil and after 30 days in the sandy soil. DMPSA is effective nitrification inhibitor when combined with CAN and AS, with a mean reduction of 61% and 58%, respectively, in the average daily nitrification rate over the study period. Over the 80-day incubation period in the sandy loam soil, only 35% NH_4^+-N was converted to NO_3^--N for AS + DMPSA compared to 88% for AS. In the sandy soil, 92% NH_4^+-N was converted to NO_3^--N for AS compared with only 9% for AS + DMPSA by day 80. The results demonstrate that DMPSA is an effective nitrification inhibitor when combined with CAN and AS.

Keywords: nitrogen fertiliser; ammonium; nitrate; nitrification inhibitor; incubation; soil texture

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1. Introduction

Soil mineral nitrogen (N) availability is a key driver of the agronomic productivity of non-leguminous crops. Nitrogen-based fertilisers are widely used globally during intensification of agricultural systems to meet the growing demand for agricultural productivity [1]. Plant utilization of fertiliser N can frequently be less than 50% [2–4], with losses from agricultural soils to the environment [5,6]. Reactive fertiliser N (ammonium (NH_4^+), nitrate (NO_3^-)) used in agricultural production systems is associated with soil acidification, soil and water quality issues, and greenhouse gas (GHG) emissions [7]. Ammonia or ammonium, in excess of plant demand, is converted to other forms of N through the nitrification–denitrification processes, increasing GHG emissions as nitrous oxide (N_2O). N fertiliser can also be linked to the negative environmental impacts through ammonia (NH_3) volatilization and NO_3^- leaching [8].

Nitrification is the biological oxidation of NH_4^+ to NO_2^- and further oxidation to NO_3^- by the action of ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria in the presence of oxygen [9,10]. During an intermediate step in the nitrification process, AOB can produce nitric oxide and N_2O by reduction of NO_2^- under fully oxic conditions if NO_2^- concentrations are high [11]. Nitrate produced by the nitrification process is utilised as an electron acceptor with organic carbon (acting as an electron donor) in the denitrification process under low oxygen availability. In this nitrification–denitrification

process, N_2 gas is the endpoint, but intermediate step losses include the release of the important and potent greenhouse gas N_2O [12].

Grassland is one of the most important types of land use in the European Union [13]. Two-thirds of Ireland's land area is covered by natural or agricultural grasslands, which is the highest proportion in Europe [14]. In Ireland, calcium ammonium nitrate (CAN) is the dominant form of straight N fertiliser applied to grassland [14]. Nitrogen applied as CAN is susceptible to environmental loss of NO_3^- and N_2O [6,15].

Plant available forms of N are NH_4^+ or NO_3^-, which are absorbed by plants for their growth and development. Nitrate, an anion, is mobile and vulnerable to loss whereas NH_4^+, a cation, is not lost as easily from the soil and is taken up by plants for direct use in plant protein formation. Therefore, it is often desirable to manage the nitrification process to retain N-fertiliser in the plant-available NH_4^+ form to limit NO_3^- leaching and N_2O loss [16]. In recent years, interest has increased in nitrification inhibitors (NIs) as a useful mitigation tool for reducing environmental loss and increase N use efficiency.

Nitrification inhibitors have been developed for the control of agronomic N dynamics and as a means to reduce N_2O emissions from agricultural soils. Therefore, the use of NIs is a promising strategy to limit nitrification and denitrification losses by delaying the conversion of NH_4^+ to NO_3^-. Dimethylpyrazol (DMP)-based NIs, in particular, diciandyamide (DCD) and 3,4 dimethylpyrazol phosphate (DMPP), have recently been used in agricultural soil to minimise N-loss and increase the N-use efficiency [17–20]. The mode of action of DCD and DMPP is to restrict the first step of nitrification process by (1) interacting with the ammonia monooxygenase (AMO) enzyme [21] and (2) behaving as metal chelator by removing copper, which is a co-factor of AMO, thus avoiding the oxidation of NH_4^+ and decreasing the NO_3^--N content in the soil [22–24]. DCD rates are relatively high compared to DMPP. Moreover, DCD is highly mobile in the soil, which can lead to it moving away from the NH_4^+ fertiliser by leaching, thus reducing its efficiency as an NI [24,25]. Compared to DCD, DMPP overcomes the above-mentioned disadvantage of moving away from the fertiliser as it has the same mobility as NH_4^+ in the soil, which allows these two compounds to remain together. However, the use of a pyrazole compound in DMPP makes DMPP more volatile, which is one of the major disadvantages of the DMPP. To reduce the volatility and to stabilise the compound, manufacturers have developed a new DMP-based inhibitor 3,4 dimethylpyrazol succinic acid isomeric mixture (DMPSA), which holds a succinic residue bonded to the pyrazole ring. The novel NI DMPSA combines the well-known inhibitory effect of DMP with the slow release behavior of succinic acid [26]. For the release of the reactive compound of succinic acid, which is an organic acid, it needs to be microbially degraded, which results in less volatilisation of DMP and a prolonged and smoother availability of DMP in the soil [27,28]. Therefore, this new inhibitor differs from DMPP in the succinic group bonded to the pyrazole ring. Another advantage of DMPSA is that it is stable under basic conditions, thus allowing it to be combined with a broader range of fertilisers than DMPP. These additional fertilisers include CAN, ammonium sulphate (AS) or diammonium phosphate, which is not possible with DMPP [26]. Recio et al. [29] found a 71% reduction in N_2O emissions when urea was mixed with DMPSA in rainfed wheat in silty clay loam soil in Madrid, Spain. However, despite the potential of DMPSA to be combined with other fertilisers, information on its performance in combination with other fertilisers such as CAN and AS, which are commonly used in Europe, is minimal or absent. Until now, the novel DMPSA as NI has only been assessed under Mediterranean conditions [27,29–32] in sandy clay loam soil. To our knowledge, there is no published literature currently available regarding the efficacy of the novel NI DMPSA in combination with CAN and AS under Northern European conditions. The current laboratory incubation study tests the hypothesis that the use of DMPSA as an NI in combination with (a) CAN and (b) AS will reduce NO_3^- formation significantly, retaining applied NH_4^+ in the NH_4^+ form for longer compared to the standard untreated CAN and AS fertilisers in two different pasture soils.

2. Materials and Methods

2.1. Soil Sample Collection and Preparation

The soil used in the incubation experiment was collected from two different locations at the Teagasc, Environment, Soils and Land Use Experiment Station located in Co. Wexford, Ireland (52°18′ N; 6°30′ W). The land management of these soils was pasture with swards dominated by perennial ryegrass (*Lolium perenne* L.) for both soils. Both soil types are classified as a Stagnic Cambisol [33], and the textures of the soils are classified as sandy loam soil (52% sand, 34% silt, 14% clay) and sandy soil (73% sand, 17% silt, 10% clay) (Table 1).

Table 1. The initial physical and chemical characteristics of the soil used in the incubation study.

Soil Properties	Soil 1	Soil 2
Sand %	52	73
Silt %	34	17
Clay %	14	10
Textural class	Sandy loam	Sandy
Bulk density (g cm^{-3})	1.4	1.3
Soil pH	5.3	5.7
Organic matter (%)	5.3	3.7
Organic carbon (%)	2.1	1.4
Total N (%)	0.22	0.13
NH_4^+-N (mg kg^{-1})	4.6	1.2
NO_3^--N (mg kg^{-1})	11.2	13.7
Available * P (mg/L)	2.1	9.2
Available * K (mg/L)	56	70
Available * Mg(mg/L)	96	76
SO_4^{2-} (mg/L)	1.2	0.0

* by Morgan's extract (described in the Supplementary Materials), which is the standard soil test in Ireland.

From each location, approximately 60 kg of surface soil was collected from the upper 10 cm from four different points. Field-moist soil was sieved to pass through a 4 mm sieve to remove the coarse rocks and plant materials and to facilitate thorough mixing of the soils. In the final preparation, the soil samples were passed through a 2 mm sieve, mixed thoroughly again to ensure the homogeneity of soil, and stored at 4 °C (1 day) prior to use for the incubation study.

A sub-sample from two soil types of 500 g was taken, dried at 40 °C until a constant weight was reached, and passed through a 2 mm sieve. Soil physical and chemical characteristics were determined (Table 1).

2.2. Design of the Incubation Experiment

The incubation experiment was conducted between 28 January 2020 and 4 May 2020 at the Teagasc Johnstown Castle (JC) Research Centre, Co. Wexford, Ireland to determine the formation of NO_3^--N in two sets of soil over 80 days in response to DMPSA (EuroChem Agro Iberia S.L.) use in combination with CAN and AS. Destructive sampling to measure mineral N occurred on eleven sampling dates (including the day before the fertiliser application). There were five fertiliser treatments (including a control) and two soil types (sandy loam and sandy soil). The incubation was conducted at a constant temperature (15 °C) and at a moisture content of 50% water-filled pore space (WFPS). In total there were four replications per treatment, which resulted in a total of the 376 experimental units used in the experiment. Prior to the application of fertiliser, a baseline of soil mineral N was established from the two soils 14 days prior to the application of fertiliser and on the day of the fertiliser application. Five fertiliser treatments were established: Calcium ammonium nitrate (CAN), CAN+ nitrification inhibitor (DMPSA), ammonium sulphate (AS), AS + nitrification inhibitor (DMPSA), and a control (unfertilised). The rate of DMPSA

applied to the fertilsier by mass was 0.15%. CAN contains 27% N half of which is in NO_3^--N and half in NH_4^+-N form. In the case of AS, the N content is 21%, all of which is in NH_4^+-N form with 24% SO_4^{2-}S.

A known weight of fresh soil samples (equivalent to 100 g oven-dry soil) was weighed into 250 mL clear polystyrene jars that served as incubation chambers (38 cm^2 surface area). Water equilibration was followed without drying of the soil to reduce the potential for an artificial microbial flush. Incubation jars were covered with a drilled lid to minimise moisture loss from the soil but allow gaseous exchange. All the incubation jars were pre-incubated at 15 °C for 14 days prior to the addition of experimental treatments to allow time for microbial activity levels to equilibrate. During the pre-incubation period, the moisture content of the soil was checked and adjusted to approximately 50% WFPS. The required WFPS was maintained throughout the experimental period by checking it two days per week by weighing the jars and adding the required amount of deionised water when the water loss was greater than 0.5 g per jar. During this whole process, care was also taken not to disturb the soil, either through shaking or stirring. This procedure was maintained throughout the incubation period. After the pre-incubation period, fertiliser treatments were applied at a rate equivalent to 80 kg N ha^{-1} (305 mg N kg^{-1} soil). Fertiliser was added in granular form followed by a light stirring using a wooden stick to incorporate the fertiliser to 2 cm soil depth in the incubation jars to promote soil-fertiliser contact. Incubation jars for each of the eleven extraction time points (−14, 0, 3, 5, 8, 12, 19, 30, 45, 60, and 80 days) were randomly allocated to a 15 °C (constant) incubator (LMS Model 300A; Series 3). Within each shelf, the incubation jars for each time periods were fully randomised.

2.3. Extraction and Analysis

The soils were destructively sampled on day −14 (two weeks before fertiliser application), on 0 (before fertiliser application), and subsequently on day 2, 5, 8, 12, 18, 30, 45, 60, and 80 after fertiliser application. At each sample date, soils of all incubated treatments were analysed for mineral NH_4^+-N and NO_3^--N. Mineral N extraction was using a 2M KCl solution following the standard protocol at Teagasc, JC [34]. Briefly, 20 g of soil sample was mixed with 100 mL of 2 M KCl (1:5 ratio), shaken for 1 h in a reciprocating shaker at 160 rpm, filtered through Whatman® No. 1 filter paper, and stored at −8 °C before analysis. The soil extracts were analysed for NH_4^+-N concentration [35] and total oxidised N, which is a total of NO_3^--N and NO_2^--N with a colorimetric analysis by using an Aquakem 600 discrete analyser (Thermo Electron OY, Vantaa, Finland [36]). As NO_2^--N was minimal (mostly null); total oxidised N was considered to be NO_3^--N in this manuscript.

Part of each sample during each measurement was oven-dried (105 °C) for 24 h to calculate the gravimetric moisture to determine the soil dry matter content to express mineral N concentrations on a dry soil basis and to calculate WFPS.

2.4. Calculation

Concentrations of NH_4^+-N and NO_3^--N were converted from mg l^{-1} to mg kg^{-1} dry soil by using the moisture content of each sample.

The rate of N-nitrification was calculated as the difference between NO_3^--N concentrations between sampling days. Net N nitrification (mg kg^{-1} dry soil day^{-1}) was calculated by the following equation [37,38]:

$$\text{Net N nitrification} = (N1 - N0)/T \quad (1)$$

where N1 (mg N kg^{-1} dry soil) is the NO_3^--N concentration in the incubated sample, N0 is the NO_3^--N concentration in the initial sample, and T is incubation period (d).

The changes of NH_4^+ to NO_3^- (%) on day 80 were calculated by the following equation:

$$\text{Changes in ammonium (\%)} = (A2 - A80) * 100/A2 \quad (2)$$

where A2 = NH_4^+-N concentration on day 2, A80 = NH_4^+-N concentration on day 80.

Mineral N mass balance is reported as the total mg N recovered for each treatment in the sandy loam and sandy soil on day 80. For each treatment, total N recovered as mineral N was based on the difference between the measured total mineral N (NH_4^+ and NO_3^-) and that detected in unfertilized or control soil.

The soil water-filled pore space (WFPS) was calculated as Linn and Doran [39] described:

$$\text{Water-filled pore space (WFPS)} = 100 \times (\text{volumetric moisture content}/\text{total soil porosity}) \quad (3)$$

where volumetric moisture content = ((weight of wet soil-weight of dry soil)/weight of dry soil)) × bulk density of the soil × 100, total soil porosity = (1 − (soil bulk density/soil particle density)) × 100, and the soil particle density was assumed to be 2.65 g cm^{-3}.

2.5. Statistical Analysis

To evaluate the effect of the fertiliser N treatments over the incubation period on NH_4^+-N and NO_3^--N, a repeated-measures analysis of variance (ANOVA) was conducted for each soil type using the PROC GLIMMIX procedure of SAS (© 2002–2010; SAS Institute Inc., Cary, NC, USA). Differences between the treatments' effect on changes (%) in NH_4^+-N to NO_3^--N after 80 days were tested by ANOVA with the general linear model (GLM) procedure in SAS. The significant F-value was determined at the 95% level ($p \leq 0.05$). In the event of significance in ANOVA, means were compared using the F-protected least significant difference (LSD) test. Normality of the data set was examined by a PROC UNIVARIATE test. The test of equality of error variance of the PROC GLM procedure was used to test the homogeneity of variance, and all the data met the assumptions without transformation. Results are reported as means ± 1 standard error (SE).

3. Results

3.1. Soil Mineral N Dynamics in Different Soils during Incubation

To study the role of the nitrification inhibitor (DMPSA) on NH_4^+-N and NO_3^--N dynamics, mineral N concentrations were monitored over time for each treatment in the sandy loam and sandy soil. A significant treatment effect over time was observed for NH_4^+-N and NO_3^--N concentrations (Table 2). A significant time by treatment interaction on NH_4^+-N and NO_3^--N concentration was detected ($p \leq 0.02$). At the beginning of the experiment, prior to fertiliser application, the NH_4^+-N concentrations were 1.4 mg and 1.2 mg NH_4^+-N kg^{-1} dry soil in the sandy loam and sandy soil, respectively. During the 80-day incubation, the concentrations of NH_4^+-N remained largely unchanged in the control treatments of both soils (Figure 1).

Table 2. Effect of fertiliser types, time, and their interaction on soil ammonium and nitrate concentrations in the two soil types.

Experimental Factor	NH_4^+N		NO_3^--N	
	p Value	df	*p* Value	df
Sandy loam soil				
Fertiliser type	***	4	***	4
Time	***	8	***	8
Fertiliser type X time	*	32	*	32
Sandy soil				
Fertiliser type	***	4	***	4
Time	***	8	***	8
Fertiliser type X time	*	32	*	32

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; ns is not significant ($p > 0.05$).* denotes the significance level of the ANOVA for that effect. df is degrees of freedom.

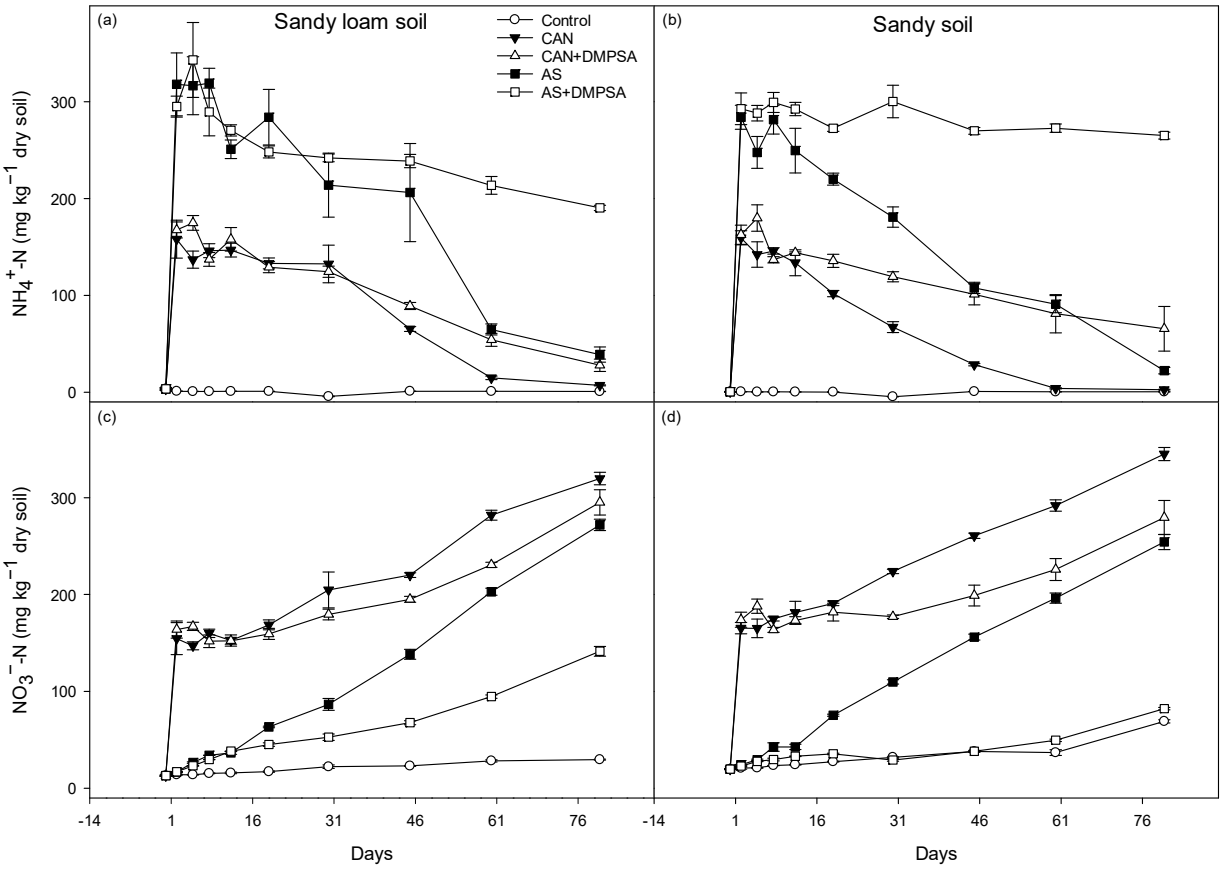

Figure 1. Changes in the NH_4^+-N and NO_3^--N concentrations during the 80-day incubation period at 15 °C in a sandy loam soil (**a**,**c**) and a sandy soil (**b**,**d**) receiving different fertiliser treatments. Error bars represent the mean ± 1 SE (n = 4).

At the beginning of the experiment, the concentration of NO_3^--N in the control treatment was 11 mg NO_3^--N kg^{-1} dry soil in the sandy loam soil and significantly increased to 30 mg NO_3^--N kg^{-1} dry soil after the 80-day incubation period. In the sandy soil, the control NO_3^--N concentration increased significantly from 14 mg NO_3^--N kg^{-1} dry soil at the beginning of the experiment to 69 mg NO_3^--N kg^{-1} dry soil by day 80. After the addition of fertiliser N, the soil NH_4^+-N concentration increased significantly for all fertiliser treatments and subsequently declined over time in both soil types. However, a significant divergence in the decline of NH_4^+-N and an increase in NO_3^--N was observed as a result of DMPSA inclusion for both CAN and AS fertilisers.

3.1.1. Sandy Loam Soil

In the sandy loam soil, a significant ($p \leq 0.05$) interaction between treatment and time was detected for the NH_4^+-N and NO_3^--N concentrations (Table 2). In this soil, AS and AS + DMPSA showed significantly ($p \leq 0.05$) higher NH_4^+-N concentrations compared to CAN, an NH_4^+-and NO_3^--containing fertiliser (Table S1). From day 60 onwards, a significant difference in NH_4^+-N concentration was detected between CAN and CAN + DMPSA (Figure 1).

The NH_4^+-N concentrations for the CAN treatment decreased to a level not significantly different to the control level by day 60. In contrast, the addition of DMPSA with CAN maintained a significantly ($p \leq 0.05$) higher NH_4^+-N concentration than the CAN and control treatment on day 60, which continued until the end of the experiment (day 80) (Figure 1, Table S1). In the case of AS, the inclusion of DMPSA resulted in a trend of higher NH_4^+-N retention (Figure 1) with significant divergence detected from day 60 to the end of the experiment (Table S1). Both treatments (AS, AS + DMPSA) resulted in significantly higher NH_4^+-N retention compared to the control treatment until the end of the exper-

iments. Overall, the addition of DMPSA to AS fertiliser resulted in the highest level of retained NH_4^+-N (190 mg N kg^{-1} dry soil) on day 80 (Figure 1). In contrast to the decrease in NH_4^+-N over time, NO_3^--N concentrations increased over time in all treatments. However, the trend of the increase was affected by fertiliser treatment (Figure 1). The CAN formulations, which contain 50% of its N as NO_3^--N unsurprisingly showed a significantly higher NO_3^--N concentration on day two than the AS formulations (Figure 1, Table S1). From day 45 onward, the NO_3^--N concentrations in the CAN + DMPSA treatment were significantly lower than those of CAN. For AS, showing an earlier significant effect than with CAN, the addition of DMPSA resulted in a lower NO_3^--N concentration from day 19 onwards. Overall, on day 80, the inclusion of DMPSA with AS resulted the lowest level of NO_3^--N accumulation among all treatments.

3.1.2. Sandy Soil

A significant ($p \leq 0.05$) fertiliser treatment by time interaction was detected in the sandy soil for NH_4^+-N and NO_3^--N concentrations (Table 2). In the sandy soil, AS formulations showed significantly ($p \leq 0.05$) higher NH_4^+-N concentrations compared to CAN formulations on day 2 after fertiliser application (Figure 1, STable 1). No significant difference in NH_4^+-N concentration was found between CAN and CAN + DMPSA until day 19 after fertiliser application (Figure 1). The NH_4^+-N concentrations in CAN gradually declined from 159 mg kg^{-1} dry soil to a level not significantly different from the control level (4 mg N kg^{-1} dry soil) by day 60. In contrast, while NH_4^+-N levels also declined for the CAN + DMPSA treatment from day 19, NH_4^+ levels were significantly ($p \leq 0.05$) higher compared to the control from day 19 onwards and remained so until the end of the experiment. In the case of AS, NH_4^+-N levels fell more slowly where DMPSA was included, being significantly higher by day 12 compared to AS and remaining at significantly higher levels throughout the experiment. Overall, only AS + DMPSA had a significantly ($p \leq 0.05$) higher NH_4^+-N concentration, which was 99%, 75%, and 91% higher than that of CAN, CAN + DMPSA and AS, respectively on day 80 (Figure 1).

The NO_3^--N concentrations showed an increasing trend in all treatments (Figure 1). On day 2 after fertiliser application, AS treatments had significantly lower NO_3^--N concentrations compared to CAN treatments (Figure 1). The inclusion of DMPSA with CAN resulted in significantly ($p \leq 0.05$) lower NO_3^--N concentrations compared to CAN by day 30, with divergence observed until the end of the experiment. However, AS + DMPSA exhibited significantly lower NO_3^--N concentrations compared to AS beginning from eight days after application. At the end of the experiment AS + DMPSA had the lowest NO_3^--N concentration of all treatments.

3.2. Changes in the Total Mineral Nitrogen in the Soil

The nitrification rate for treatments and soils over time are presented in Table 3. The nitrification rate for the control treatments ranged from −0.02 to 0.5 mg NO_3^--N kg^{-1} dry soil day^{-1} in the sandy loam soil and from −0.08 to 0.82 mg NO_3^--N kg^{-1} dry soil day^{-1} in the sandy soil. In the sandy loam soil, the rate of nitrification ranged from −2.45 to 4.3 mg NO_3^--N kg^{-1} dry soil day^{-1} for CAN (mean 0.19) and from −5.1 to 3.22 mg NO_3^--N kg^{-1} dry soil day^{-1} (mean 0.08) when DMPSA was included with CAN, whereas the nitrification rate of the AS treatment ranged from 0.6 to 4.31 mg NO_3^--N kg^{-1} dry soil day^{-1} (mean 0.35) and from 0.7 to 2.34 mg NO_3^--N kg^{-1} dry soil day^{-1} (mean 0.2) for the AS + DMPSA treatment (Table 3).

Table 3. Soil net nitrification rate (values are the average rate between a sampling day and the previous day).

	Net Nitrification Rate Calculated from NO_3^--N Formation (mg NO_3^--N kg^{-1} Dry Soil Day^{-1})									
	Sandy Loam Soil					Sandy Soil				
Days	**Control**	**CAN**	**CAN + DMPSA**	**AS**	**AS + DMPSA**	**Control**	**CAN**	**CAN + DMPSA**	**AS**	**AS + DMPSA**
D 2	0.50	-	-	1.81	1.96	0.57	-	-	2.27	1.55
D 5	−0.02	−2.45	1.07	3.30	2.09	0.06	−0.07	4.65	1.72	1.63
D 8	0.47	4.30	−5.11	2.50	2.12	0.82	3.25	−8.14	4.41	0.62
D 12	0.11	−1.88	0.11	0.60	2.18	0.22	1.65	2.42	0.03	0.87
D 19	0.19	2.31	1.01	3.84	0.98	0.45	1.34	1.23	4.68	0.36
D 30	0.47	3.28	1.82	2.12	0.70	0.41	3.01	−0.42	3.12	−0.58
D 45	0.05	1.02	1.04	3.45	1.00	0.40	2.45	1.46	3.08	0.60
D 60	0.35	4.13	2.38	4.31	1.80	−0.08	2.09	1.79	2.68	0.76
D 80	0.06	1.89	3.22	3.45	2.34	1.61	2.66	2.68	2.90	1.63
Average *	0.03	0.19	0.08	0.35	0.20	0.06	0.25	0.09	0.34	0.09

* Average daily soil nitrification rate during the periods between sampling. Within a column, values indicate the average soil nitrification rate (mg NO_3^--N kg^{-1} dry soil day^{-1}), which was calculated from NO_3^--N formation between the sampling day and the previous day.

In the sandy soil, the nitrification rate ranged from −0.07 to 3.25 mg NO_3^--N kg^{-1} dry soil day^{-1} (mean 0.25) for CAN and from −8.1 to 4.65 mg NO_3^--N kg^{-1} dry soil day^{-1} (mean 0.09) for CAN + DMPSA. For AS, the rate was 0.03 to 4.68 (mean 0.34) versus −0.58 to 1.63 mg NO_3^--N kg^{-1} dry soil day^{-1} (mean 0.09) for AS + DMPSA (Table 3). The highest average soil nitrification rate during the experimental period was observed for AS. The inclusion of DMPSA reduced the mean nitrification rate for both AS and CAN. In the absence of fertiliser addition, the control treatment had the lowest nitrification rate in both soil types (Table 3).

At the end of the experiment, when averaged across the treatments, the use of AS + DMPSA led to the highest NH_4^+-N concentrations of 190 and 265 mg NH_4^+-N kg^{-1} dry soil in the sandy loam and sandy soil, respectively. The use of CAN led to the highest NO_3^--N concentrations of 320 and 345 mg N kg^{-1} dry soil in the sandy loam and sandy soil, respectively (Figure 1). In the sandy loam soil by the end of the 80-day incubation, 95% of mineral N had nitrified to NO_3^--N for CAN and 88% for AS. In the sandy soil, it was 98% nitrification for CAN and 92% AS (Figure 2). However, with the use of DMPSA, the changes in NH_4^+ to NO_3^- (%) for CAN + DMPSA and AS + DMPSA were 83% and 35% in the sandy loam soil and 60 and 9% in the sandy soil (Figure 2). In the sandy loam soil, the conversion of NH_4^+ to NO_3^- for CAN was reduced by a factor of 1.14 with the inclusion of DMPSA. In AS, the reduction factor was 2.5 with the inclusion of DMPSA. In the sandy soil, DMPSA reduced the rate of change of NH_4^+ to NO_3^- by a factor of 1.6 and 10 when included with CAN and AS, respectively.

At the end of the experiment, the potential loss of N from the system was less than 10% for all the treatments in both the sandy loam and sandy soil except the AS treatment. The highest N loss from the system was observed for the AS treatment in the sandy soil (30%). The inclusion of DMPSA with AS reduced this loss to 9% only (Figure 3). Nitrogen loss from the system was higher in the sandy soil compared to the sandy loam soil for all the treatments.

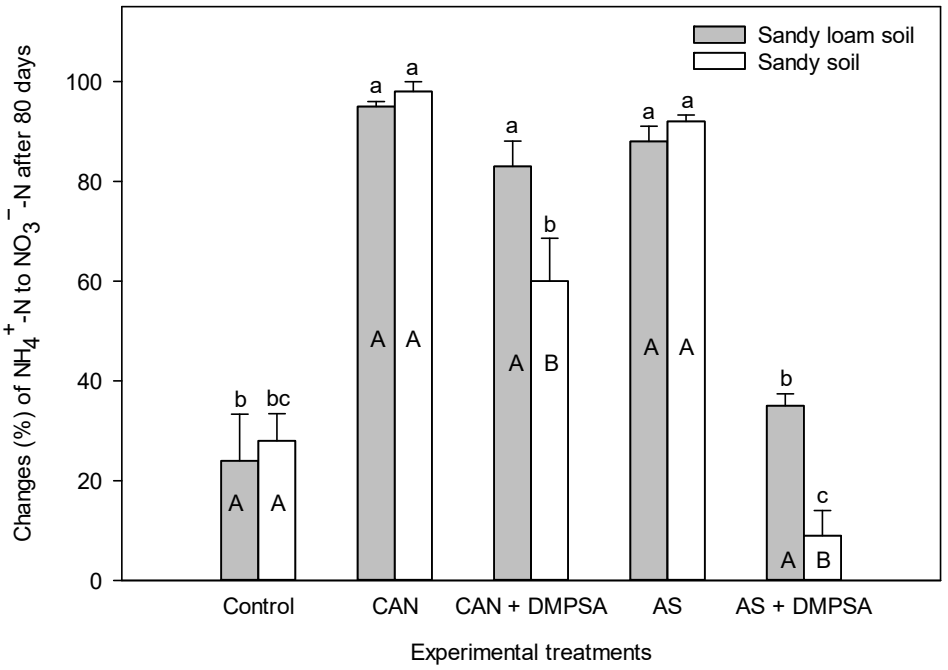

Figure 2. Change (%) in NH_4^+-N to NO_3^--N (%) at the end of an 80-day incubation period in a sandy loam and a sandy soil. Different lower case letters indicate a significant difference ($p \leq 0.05$) between different treatments within a soil type. Mean comparison based on the F-protected LSD test ($p \leq 0.05$).

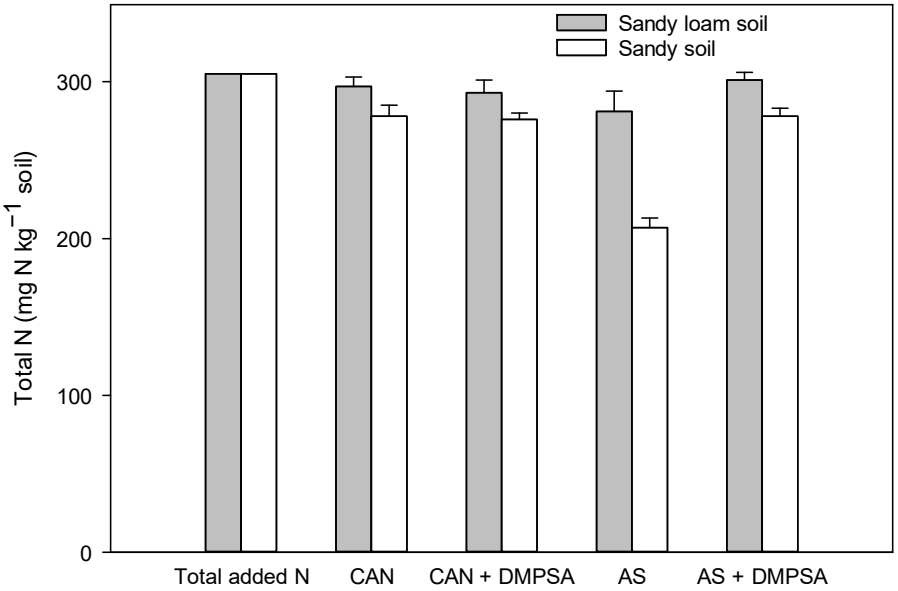

Figure 3. Total mineral N (NH_4^+ and NO_3^-) available in the sandy loam and sandy soil on day 80 (last sampling day). Total added N (added as fertiliser N) is presented in the first bar. Values presented are net of mineralisation in the control treatment over the incubation study. Error bars represent the mean ± 1 SE (n = 4).

4. Discussion

4.1. Efficacy of DMPSA as a Nitrification Inhibitor (Treatment Interaction with Time)

This study showed that the use of DMPSA with the NH_4^+-containing fertilisers effectively slowed the nitrification rate and resulted in the retention of N in the NH_4^+ form for a longer period of time compared to the standard fertiliser. Our results demonstrated

that NO_3^- levels in the soil were kept at a lower level when DMPSA was utilised. With the addition of DMPSA to both CAN and AS fertilisers, NH_4^+-N was sustained at a higher concentration until the end of the experiment compared to the standard fertilisers in these temperate grassland soils. (Figure 1). Guardia et al. [27], in a maize study in Spain, detected a delaying effect of DMPSA on NH_4^+ oxidation when ammonium nitrate fertiliser with DMPSA was used compared to ammonium nitrate without the addition of DMPSA. In the first step of nitrification, NH_4^+ is oxidised to hydroxylamine (NH_2OH) by AMO [40] and afterwards to NO_2^- by hydroxylamine oxidoreductase [40]. The delaying effect of DMPSA on NH_4^+ oxidation, which depressed the activity of the AMO enzyme, is the first enzymatic step of nitrification in soil [41,42]. The mechanism of delayed NH_4^+ oxidation to hydroxylamine, which is further oxidised into NO_2^- and then NO_3^- [42], is supported by the results of the current study, which show it to be effective in retaining NH_4^+-N in two temperate grassland soils with a relatively high organic carbon content. The reduced NO_3^--N concentrations at the end of the experiment when DMPSA was used (Figure 1), is evidence of its effectiveness in decreasing the nitrification rate when combined with CAN and AS fertilisers. By limiting NO_3^--N availability via the inhibition of nitrification, DMPSA potentially inhibits the denitrification process. As a result, the potential for N losses through NO_3^- leaching and denitrification is much reduced along with the potential of the production of N_2O during nitrification, which can mitigate climate change by potentially reducing global warming potential. Guardia et al. [27,43] and Torralbo et al. [26] also found that lower N_2O was produced when DMPSA was used with urea due to its direct and indirect effect on denitrification.

This present study also demonstrates that a significant effect of DMPSA (delayed conversion of NH_4^+) was observed after 60 days (both CAN and AS formulations) in the sandy loam soil, compared with 19 and 12 days for CAN- and AS-based formulations in the sandy soil used in this experiment. This result indicated that soil differences in the efficacy of DMPSA with respect to nitrification inhibition (Figure 1) should be considered when developing applications for different markets or soils. The long delaying (80 days) effect of DMPSA on nitrification might be caused by the slow release behavior of the succinic group in DMPSA [24,32,44]. Succinic acid is an organic acid [27,28] that needs microbial degradation before the active inhibiting substance (DMP) is released, potentially resulting in an extended inhibitory effect of DMPSA compared to other NIs.

In contrast to our finding, Recio et al. [30] found that after NI application with CAN fertiliser, DMPSA was highly effective shortly after fertiliser application but after three weeks, its effect was low, which may be the effect of soil temperature in the Mediterranean region [43,45]. This may also highlight the differences that can occur between the soils of a maize production system in Spain compared to DMPSA effects on N dynamics in temperate grassland soils.

4.2. Temporal Effect of DMPSA on Soil N Dynamics

This study clearly demonstrated that DMPSA had a prolonged nitrification inhibition effect when combined with NH_4^+-containing fertilisers. This effect could be useful in reducing environmental losses where fertiliser is applied to meet crop requirements over extended growing periods. Such practices are already common in cereals, maize, and grass silage settings. For example, 80 days after fertiliser application (the end of the experiment), only 9% of NH_4^+-N was converted to NO_3^--N in the case of AS + DMPSA in the sandy soil and 35% in the sandy loam soil (Figure 2). As DMPSA can retain fertiliser N in the form that is less vulnerable to loss (NH_4^+-N) for longer, there may be potential to reduce the application frequency in some systems, thus providing labour saving to farmers.

As NH_4^+ is also a plant available N source, delayed nitrification due to DMPSA is unlikely to have a negative effect on plant growth and development. Sometimes, NH_4^+ is indeed considered a preferable N source for plants [46,47] because NH_4^+ uptake and assimilation are less costly than NO_3^- uptake and assimilation from a plant energetic point of view [48,49]. Unlike NO_3^-, which is very mobile and easily moved by water, NH_4^+-N

is relatively less mobile in the soil and generally has lower leaching loss susceptibility (49). The use of DMPSA with mineral fertilisers allows the continuous release of nutrients (NH_4^+) into the root zone over a longer period of time compared to standard fertiliser.

4.3. Efficacy of the Nitrification Inhibitor in Different N Formulations (Treatment Effect)

With AS, a greater level of NH_4^+-N was presented compared to CAN, thus DMPSA had a greater effect on reducing the nitrification rate in AS. At the beginning of the experiment, the NO_3^--N concentrations in CAN formulations were significantly higher than those in the AS formulations, a consequence of CAN containing 50% of its N as NO_3^-. At the end of the experiment, AS + DMPSA delayed the oxidation of NH_4^+ in the soil and prevented the build-up of soil NO_3^-, thus showing higher efficiency compared to the CAN-based formulation in both soils. In line with our results, Pacholski et al. [28] also found that DMPSA mixed with urea retained NH_4^+ in the soil for a longer period compared to urea without DMPSA in an incubation trial in a silt loam soil in Germany. Scheer et al. [50] found that after application of 120 kg urea-N ha^{-1} with DMPP, a significant reduction in the NO_3^- level and an increase in the level of soil NH_4^+ were observed after 100 days in a broccoli production system. Our study showed similar long duration suppression of nitrification over 80 days.

4.4. Efficacy of the Nitrification Inhibitor in Different Soil Types

In the present study, differences in the level of DMPSA performance were observed between soil textures. In the sandy loam soil, significant differences in NH_4^+-N concentration were observed after 60 days owing to DMPSA use (for both CAN and AS), whereas in the sandy soil, it took just 12 days for AS + DMPSA and 19 days for CAN + DMPSA to show a significant divergence in NH_4^+-N levels compared to their standard AS and CAN counterparts. The apparently greater efficacy of DMPSA observed in the sandy-textured soil may be associated with the lower soil organic matter (3.7%) and clay concentrations (10%) compared with the sandy loam soil. In the sandy loam soil, the higher soil organic matter (5.3%) may stimulate an increase in *Nitrosomonas* sp. increasing nitrification, which might reduce the efficacy of NIs in this soil [51]. The current study provides evidence that the efficacy of DMPSA with respect to nitrification inhibition can be influenced by soil. This is an important finding in addition to evidence of efficacy for its practical use in agricultural systems (Figure 1). Our results show that DMPSA efficacy is also subject to the soil effects on efficacy, which have been noted for other NIs. For example, Ruser and Schulz et al. [18] reported differences in the inhibitory effect of DMPP among different soils. They found that relative NO_2^--N formation decreased and the efficacy of DMPP increased in soils with more sand, which is in agreement with the effects we observed for DMPSA. In general, the efficiency of NIs appear to be influenced by soil organic matter and soil texture [52]. Cahalan et al. [53] and Singh et al. [20] reported that the NI DCD has lower effectiveness and persistence as soil organic matter increases and as soil texture becomes finer. Another explanation by Barth et al. [54] is that the DCD and DMPP efficiency is reduced in fine or clay textured soils due to the NI being adsorbed on clay and organic matter surfaces and thus becoming less available for microbial activity [55]. In the present study, the sandy loam soil had higher soil organic matter than the sandy soil, which in addition to the finer texture may explain the lesser though significant effect of DMPSA on nitrification inhibition in this soil.

5. Conclusions

The novel nitrification inhibitor DMPSA was found to be effective in reducing the nitrification rate of calcium ammonium nitrate and ammonium sulphate fertilisers in grassland soils of sandy loam and a sandy texture. When used with high NH_4^+-N-containing fertiliser such as AS, DMPSA retained the majority of the total N pool in the NH_4^+ form over a relatively long period of at least 80 days. As a result, the potential for N losses through NO_3^- leaching and denitrification are reduced along with the potential for production of

N_2O during nitrification. In the case of fertilisers such as CAN, which contains both NH_4^+ and NO_3^-, a significant divergence in soil NO_3^- levels with the use of DMPSA was noted at 30 to 45 days after application vs. after just 19 days with AS. These findings indicate that DMPSA is more likely to provide benefits where CAN applications are applied to meet crop requirements over longer periods. This may present opportunities for reduced frequency of application compared with the standard practice. Soil was also observed to affect the nitrification rate, so differences in efficacy can be expected across different soils. Overall, this laboratory incubation study indicates that DMPSA offers the potential to reduce NO_3^- and nitrification associated environmental losses and thus improve the environmental credentials of conventional NH_4^+-N containing fertilisers. There is also potential that benefits would be greatest in situations where application frequency is reduced, reducing farm labour requirements. However, such strategies do require in-field evaluation.

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