



## Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions under different temperature and moisture conditions

Sergio Menéndez<sup>a,\*</sup>, Iskander Barrena<sup>b</sup>, Igor Setien<sup>b</sup>, Carmen González-Murua<sup>b</sup>, José María Estavillo<sup>b</sup>

<sup>a</sup> Institute of Agro-biotechnology (IdAB), UPNa-CSIC-GN, 31192 Mutilva Baja, Navarra, Spain

<sup>b</sup> Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU), Apdo. 644, E-48080 Bilbao, Spain

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### ABSTRACT

Agricultural intensification has led to the use of very high inputs of nitrogen fertilizers into cultivated land. As a consequence of this, nitrous oxide (N<sub>2</sub>O) emissions have increased significantly. Nowadays, the challenge is to mitigate these emissions in order to reduce global warming. Addition of nitrification inhibitors (NI) to fertilizers can reduce the losses of N<sub>2</sub>O to the atmosphere, but field studies have shown that their efficiency varies depending greatly on the environmental conditions. Soil water content and temperature are key factors controlling N<sub>2</sub>O emissions from soils and they seem to be also key parameters responsible for the variation in nitrification inhibitors efficiency. We present a laboratory study aimed at evaluating the effectiveness of the nitrification inhibitor 3,4-dimethylpyrazol phosphate (DMPP) at three different temperatures (10, 15 and 20 °C) and three soil water contents (40%, 60% and 80% of WFPS) on N<sub>2</sub>O emissions following the application of 1.2 mg N kg<sup>-1</sup> dry soil (equivalent to 140 kg N ha<sup>-1</sup>). Also the CO<sub>2</sub> and CH<sub>4</sub> emissions were followed to see the possible side effects of DMPP on the overall microbial activities. Nitrogen was applied either as ammonium sulfate nitrate (ASN) or as ENTEC 26 (ASN + DMPP). The application of ENTEC 26 was effective reducing N<sub>2</sub>O losses up to the levels of an unfertilized control treatment in all conditions. Nevertheless, the percentage of reduction induced by DMPP in the ENTEC treatment with respect to the ASN varied from 3% to 45% depending on temperature and soil water content conditions. At 40% of WFPS, when nitrification is expected to be the main process producing N<sub>2</sub>O, the increase of N<sub>2</sub>O emissions in ASN together with temperature provoked an increase in DMPP efficiency reducing these emissions from 17% up to 42%. Contrarily, at 80% of WFPS, when denitrification is expected to be the main source of N<sub>2</sub>O, emissions after ASN application decreased with temperature, which induced a decrease from 45% to 23% in the efficiency of DMPP reducing N<sub>2</sub>O losses. Overall, the results obtained in this study suggest that DMPP performance regarding N<sub>2</sub>O emissions reduction would be the best in cold and wet conditions. Neither CO<sub>2</sub> emissions nor CH<sub>4</sub> emissions were affected by the use of DMPP at the different soil water contents and temperatures.

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### 1. Introduction

Agricultural intensification has led to the use of high inputs of nitrogen fertilizers into cultivated land. As a consequence of this, losses by nitrate leaching and N<sub>2</sub>O emissions have increased significantly (Bouwman et al., 2002). Regarding gaseous emissions, soil microbial processes produce gases like CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> which are emitted to the atmosphere and play an important role in environmental terms due to their global warming potential (IPCC, 1997).

Nitrous oxide (N<sub>2</sub>O) has great importance as a greenhouse gas because it has a mean atmospheric residence time of more than 100

years. N<sub>2</sub>O warming potential depends on its life time. When a time horizon of 100 years for N<sub>2</sub>O is considered, its warming potential has been estimated to be 310 times higher than the CO<sub>2</sub> warming potential (Prather et al., 2001). Moreover, N<sub>2</sub>O is not only involved in the global warming effect, but it also contributes to the destruction of the ozone layer. Approximately 35% of the global annual N<sub>2</sub>O emission is attributed to agriculture (Isermann, 1994), being agricultural soils the major source of these emissions, which arise mainly from both anaerobic denitrification and aerobic nitrification microbial processes.

Methane (CH<sub>4</sub>) is also a greenhouse gas which contributes in a 15% to global warming (Christiansen and Cox, 1995). Its concentration is expected to rise from 1.72 ppb in 1994 to about 1.82 ppb in 2034 (IPCC, 1995) with the added risk that its warming potential in a time horizon of 100 years is 21 times higher than the CO<sub>2</sub>

\* Corresponding author. Fax: +34 94 816 89 30.

E-mail addresses: [sergio.menendez@unavarra.es](mailto:sergio.menendez@unavarra.es), [sergio.menendez@ehu.es](mailto:sergio.menendez@ehu.es) (S. Menéndez).

warming potential. The net soil – atmosphere CH<sub>4</sub> flux is the result of the balance between the offsetting processes of methanogenesis (CH<sub>4</sub> produced during decomposition of organic matter in anoxic conditions) (Woese et al., 1990) and methanotrophy (microbial CH<sub>4</sub> consumption in aerobic conditions) (Whalen and Reeburgh, 1996), although there is some evidence of an anaerobic pathway for CH<sub>4</sub> oxidation (Segers, 1998). So, depending on the environmental conditions (oxygen availability) soils can be a source or a sink of methane (Nykänen et al., 1995; Maljanen et al., 2003).

Over the past years, nitrification inhibitors have been presented as a tool to reduce N losses and increase fertilizer use efficiency (Slangen and Kerkhoff, 1984; Dittert et al., 2001; Di and Cameron, 2003; Boeckx et al., 2005; Pereira et al., 2010). One of these inhibitors is 3,4-dimethylpyrazole phosphate (DMPP) which has become a very popular and used inhibitor in the world in the last decade (Zerulla et al., 2001; Hatch et al., 2005). Several studies have demonstrated that DMPP not only increases crop yield in barley, maize and wheat (Linzmeier et al., 2001; Pasda et al., 2001), but it also reduces nitrous oxide emissions (Weiske et al., 2001; Hatch et al., 2005; Merino et al., 2005) without increasing the risk of enhanced ammonia volatilization (Menéndez et al., 2006, 2009; Li et al., 2009). Regarding the other greenhouse gases (CO<sub>2</sub> and CH<sub>4</sub>), opposite results have been described in the literature. While Menéndez et al. (2006, 2009) reported that CO<sub>2</sub> emissions were unaffected by DMPP, Weiske et al. (2001) described an unexpected reduction in CO<sub>2</sub> and CH<sub>4</sub> emissions induced by DMPP. In fact, these authors could not find an explanation for these reductions and reported that they were not able to confirm the reduction in CO<sub>2</sub> emission they have observed in the field when they performed a laboratory study with the same soil. There is no expected reason why DMPP should decrease CO<sub>2</sub> emissions. Even in the case that DMPP affected to CO<sub>2</sub> production/consumption by nitrifiers, it would be unlikely to observe any effect of DMPP on overall CO<sub>2</sub> soil fluxes, as nitrifiers represent only a small proportion of soil microorganisms. So, unless its application induced a great change in the soil activity and/or growth of microbial populations other than the nitrifiers, which is neither expected, CO<sub>2</sub> emissions are not presumed to change. In the case of CH<sub>4</sub> emissions, we could contemplate that DMPP may have an effect on methane monooxygenase activity. In this sense, it has been reported that methane monooxygenase structural similarity with ammonium monooxygenase could lead to its inhibition induced by some nitrification inhibitors (Bronson and Mosier, 1994). Furthermore, it has been described that high soil ammonium contents can induce an inhibition of methanotrophic activity (Jassal et al., 2011). So, the application of a nitrification inhibitor that induces an increase in soil ammonium content, could reduce CH<sub>4</sub> consumption rates, and an increase in CH<sub>4</sub> emission could be foreseen. Moreover, this effect would be also modulated in a different manner at different soil water contents, as methanotrophy is dominant in drier soils and methanogenesis in wetter soils.

The efficiency of DMPP in reducing N<sub>2</sub>O emissions in field experiments has been shown to vary from 0% up to 60% (Barth et al., 2001; Dittert et al., 2001; Zerulla et al., 2001; Macadam et al., 2003; Menéndez et al., 2006, 2009; Chen et al., 2010; Pereira et al., 2010) although the reason for this variation in efficiency is not fully understood. Both the fluctuations with time in the environmental conditions along these field experiments as well as the different experimental designs in each case make difficult to find the reasons for these variations in results. Two environmental factors have been described as key parameters responsible for this variation: soil water content and temperature. In this sense, the literature suggests that the efficiency of DMPP in reducing N<sub>2</sub>O emissions decreases at high soil water contents and temperature. With respect to the influence of soil water content Menéndez et al. (2009) described that DMPP was not effective reducing losses when soil water content was over 60% of

WFPS, and they attributed this lack of efficiency to the low emissions measured in comparison to those reported when WFPS was lower. Regarding the effect of temperature, Merino et al. (2005) described that in our edaphoclimatic conditions the duration of the effect of DMPP in autumn was longer than in spring, suggesting that a possible faster degradation of the molecule at higher soil temperatures might be responsible for a lower efficiency. So, the mechanism whereby soil water content and temperature influence the efficiency of DMPP reducing greenhouse gas emissions needs to be thoroughly studied. In this sense, a matter that needs to be analyzed is to what extent DMPP has been able to reduce losses up to unfertilized levels in the different experiments and this fact has not been taken into account at the time of interpreting the differences in the percentages of emission reductions observed with respect to conventional fertilizer. In the literature, authors might have not always used properly the terminology that distinguishes between DMPP's efficiency inhibiting nitrification and DMPP's efficiency reducing emissions.

In field conditions, it is difficult to ascertain the mechanism whereby soil water content and temperature influence the efficiency of DMPP reducing greenhouse gas emissions, due to the natural fluctuations in temperature and rainfall. Consequently, the aim of the present work was to evaluate the effect of temperature and soil water content on the performance of DMPP under laboratory conditions where both temperature and soil water content were thoroughly controlled.

## 2. Materials and methods

### 2.1. Experiment set-up

Soil was collected from a 0–20 cm layer of a typical cut grassland in the Basque Country (Northern Spain, 43°18'20" N, 3°53'0" W; 30 m a.s.l.). The soil was a poorly drained clay loam (34% fine sand, 3% coarse sand, 34% silt, and 29% clay) with a pH (1:2 H<sub>2</sub>O) of 6.6 and an organic matter content of 1.72%. Roots and stones were removed and the soil sieved at 4 mm before being dried at air temperature. The apparent density of the soil after sieving was also determined. One hundred g of dried soil were weighed and introduced in a 300 mL open pot. In order to reactivate soil microorganisms (Anderson and Domsch, 1973), soil was rehydrated and 0.5 g of glucose and 85 µg N kg<sup>-1</sup> dry soil (equivalent to 10 kg N ha<sup>-1</sup>) as ammonium sulfate nitrate (ASN) were added to each pot 21 days before treatments application.

The trial was designed as a split plot arrangement. Main factor was incubation temperature. Soil water content and fertilizer treatments were subfactors. Three fertilizer treatments were applied: a control treatment without fertilizer, a second one with ammonium sulfate nitrate (ASN 26%) and a third one consisting in the combination of ASN with DMPP, available in the market as ENTEC 26 (developed by BASF (Ludwigshafen, Germany) and commercialized by K + S Nitrogen (Mannheim, Germany)). Fertilizers were applied at a rate of 1.2 mg N kg<sup>-1</sup> dry soil (equivalent to 140 kg N ha<sup>-1</sup>). Nitrogen in ASN consisted of 7.5% nitric and 18.5% ammoniacal. In order to get a homogenous distribution of fertilizers in soil, they were dissolved in water and 5 mL volume of those solutions was applied with a pipette to the corresponding pots in order to get a rate of 140 kg N ha<sup>-1</sup> and a homogenous distribution of fertilizers in soil. Concurrently, each incubation temperature (10 °C, 15 °C and 20 °C) was subdivided into three sub-treatments at different soil water contents (40%, 60% and 80% of WFPS). Each sub-treatment was sub-subdivided into other three groups, according to the fertilizer applied. Water was added to all pots up to reach the humidity defined for each soil water content. Pots were covered with Parafilm in order to maintain soil humidity. Every 3 days, pots were weighted and water was added if it was necessary.

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