The effect of soil moisture at application on the behaviour of four nitrogen fertilisers in the presence of 3,4-dimethylpyrazole phosphate

Brooke Kaveney[1], Jason Condon[1], Greg Doran[1]

[1] Graham Centre for Agricultural Innovation, Wagga Wagga, NSW, 2650, www.grahamcentre.net, bkaveney@csu.edu.au

Abstract
A laboratory soil incubation experiment was conducted using the nitrification inhibitor 3,4-dimethylpyrazole phosphate in conjunction with four different agriculturally relevant nitrogenous fertilisers to examine the effects of soil moisture at application on inhibitor behaviour. Soil moisture influenced the mineral N concentrations of the nitrogen fertilisers. Fertilisers that were applied to moistened soils recorded significantly higher mineral N concentrations than when they were applied to dry soils. Of the four fertilisers applied to a moistened soil, urea had the lowest NH_4^+ and NO_3^- concentrations after a week of incubation. However, in dry soils both urea and UAN recorded significantly lower mineral N concentrations. Losses were attributed to volatilisation which occurs as urea is converted to NH_4^+. Regardless of the soil moisture conditions, DMPP effectively suppressed nitrification for all fertilisers. Fertilisers without DMPP recorded higher NO_3^- concentrations as the NH_4^+ was oxidised as part of the nitrification process. Experimental results demonstrate that different soil moisture conditions can influence the behaviour of nitrogen fertilisers but not DMPP.

Keywords
DMPP, urea, urea ammonium nitrate, ammonium nitrate, ammonium sulphate, moisture

Introduction
The nitrification inhibitor 3,4-dimethlypyrazole phosphate (DMPP) is used to slow nitrification rates and consequently mitigate nitrogen (N) losses from nitrate leaching and gaseous emissions associated with denitrification (Yang et al. 2016). DMPP can be used with a range of N-fertilisers include urea (Rowlings et al. 2016), ammonium sulphate (Tindaon et al. 2012) and ammonium-sulphate-nitrate (Kleineidam et al. 2011). Through the inhibition of nitrification, mineral N is maintained in the form of NH_4^+ for longer which potentially increases nitrogen use efficiency and plant availability of applied fertilisers.

In literature it is commonly reported in the methodology that DMPP and N fertiliser is applied to a soil that has already been pre-moistened and incubated. This is done to achieve equilibration in soil microbial processes before treatments are applied. The application of water to dry soil stimulates microbial processes including mineralisation, immobilisation and nitrification (Yu and Ehrenfeld 2009). This often results in flushes of nitrogen and carbon soon after water application (Mikha et al. 2005). Whilst applying DMPP and N fertilisers to a moistened soil in an experimental situation eliminates microbial fluctuations, it does not reflect the increasingly common field practice of sowing into dry soil.

Climate change has increased the variability of rainfall events that are required at the start of the growing season for crops to successfully germinate (Pook et al. 2009). Nitrogen fertilisers and DMPP that are generally applied at the time of sowing are consequently exposed to potentially dry soil conditions. Additionally, urea containing fertilisers must undergo urea hydrolysis before the DMPP can effectively inhibit nitrification. Nitrogen losses associated with urea hydrolysis are widely reported in literature (Gioacchini et al. 2002) with volatilisation losses accounting for 40 % of applied N lost (Rowlings et al. 2016). These losses can be reduced by the addition of soil water or irrigation (Dawar et al. 2011). In the absence of soil moisture in dry sowing, it is unclear if the application of DMPP with N fertilisers will affect nitrification inhibition. The purpose of this experiment was to quantify the mineral N recovery rates to determine if various agricultural nitrogen fertilisers effect the inhibitory performance of DMPP under two different soil moisture conditions. It was expected that decreased mineral N recoveries will occur for fertilisers containing urea and for dry incubated soils that will rapidly experience microbial flushes upon moisture addition.

Materials and methods
Soil was collected from the surface (0- 10 cm) of a pasture at Westmere, Victoria (-37°41’S, 142°54’E). Soil was mixed for homogenisation, air dried and sieved (< 2 mm). The Westmere soil was a sandy loam, pH

5.07 ($CaCl_2$), organic carbon 2.28 %, mineral N 0.17 % and cation exchange capacity (CEC) 10.30 cmol(+)/kg as determined by methods in Rayment and Lyons (2011). Two soil moisture treatments were applied; soil samples were moistened gravimetrically to a weight equivalent to -85 kPa (wet) or were left in an air-dried state (dry). Both treatments were incubated at 20°C in the dark for a week before treatment application. Four fertiliser treatments were applied; urea, urea ammonium nitrate (UAN), ammonium nitrate ($NH_4NO_3^-$) and ammonium sulphate ($(NH_4)_2SO_4$). Nitrogen was applied at an equivalent rate of 30 kgN/ha with and without DMPP at 0.5 kg/ha. A control of deionised water was also used. Dry incubation treatments were wet gravimetrically to a weight equivalent of -85 kPa after treatment application. Soil containers were incubated for a week after treatment application. Destructive sampling occurred at days 0 and 7. Sampled containers were mixed thoroughly and then soil was collected from each sample for mineral N and gravimetric water content. The concentration of NH_4^+ and NO_3^- was tested using 20 g of soil extracted in 100 mL of 1 M KCl-PMA shaken end-over-end for one hour with a subsequent settling period of 30 minutes. The supernatant was filtered through Whatman 42 filter paper and analysed colourimetrically using an automated SEAL AQ2 discrete analyser. Urea analysis was performed using the method described by Mulvaney and Bremner (1979). The incubation trial was conducted in a randomised block design with three replicates and results are reported as treatment minus control. Statistical analysis was performed using R (version 3.4.2). The effect and interaction of fertiliser x incubation x inhibitor on mineral N was examined using multi-way ANOVA. Results reported as statistically significant were done so at the 95% confidence interval.

Results

At sampling time zero there was no significant difference between fertiliser type, inhibitor use or incubation method. Recovery percentage for all treatments was approximately 100 %.

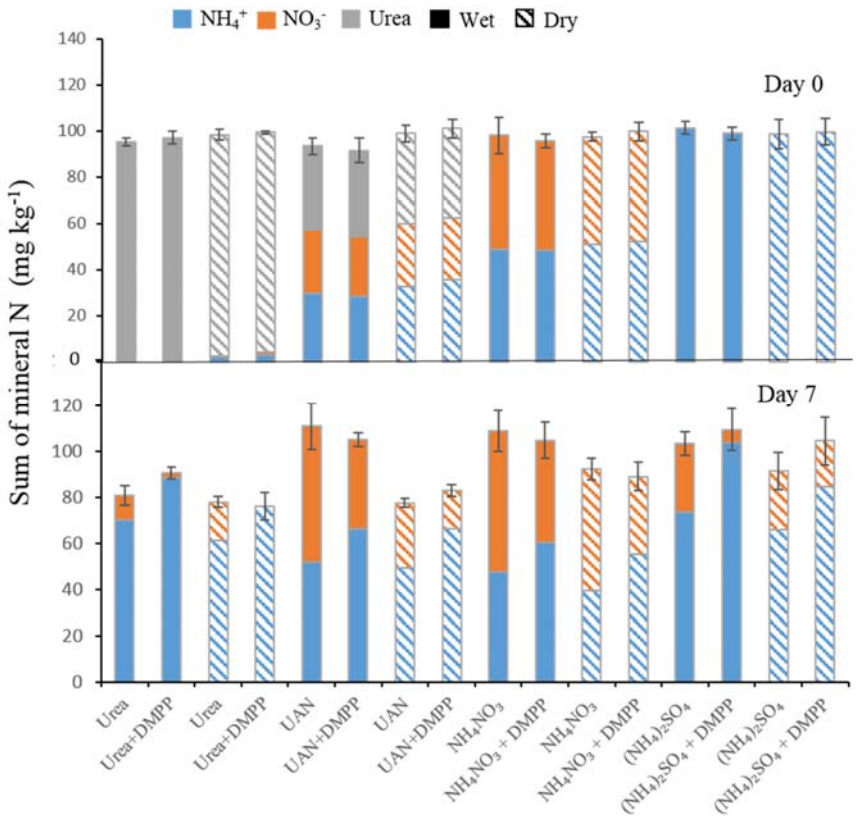

Figure 1: Mineral N (sum of NH_4^+, NO_3^- and urea) concentrations for each fertiliser, with and without DMPP, in both wet (solid columns) and dry (dashed columns) pre-incubated soils. Error bars represent the standard error of the means.

Due to the different nitrogen components of the various fertilisers the concentrations of NH_4^+ and NO_3^- were significantly different between fertilisers. Samples at day 7 showed a significant difference between wet and dry treatments (Figure 1). Except for $(NH_4)_2SO_4$, all fertiliser treatments that were wet prior to application had significantly higher total mineral N concentrations at day 7 than their dry-incubated counterparts. The

application of DMPP effectively inhibited nitrification for all fertilisers and maintained mineral N in the form of NH_4^+ at a significantly higher concentration than fertilisers without DMPP. When DMPP was not used, the NH_4^+ concentrations decreased whilst the NO_3^- concentrations increased. DMPP had no effect on the overall cumulative concentration of mineral N from fertilisers. Urea containing fertilisers underwent urea hydrolysis after application to the soil at day 0 and no urea was detected after a week of incubation. For wet soil treatments, mineral N of the urea treatments were significantly lower at day 7 than the other fertilisers used. Alternatively, in dry incubated conditions both urea and UAN had significantly lower mineral N concentrations.

Discussion

When fertilisers and the DMPP inhibitor were applied to a wet soil, the recovery of mineral N was greater than if they were applied to a dry soil. When a soil is moistened from a rainfall or irrigation event, changes in microbial dynamics ensue. A flush of carbon and nitrogen mineralisation occurs (Mikha et al. 2005) and nitrifying organisms increase in population. Many of these microbial changes occur within a week of addition of soil moisture (Iovieno and Baath 2008). In this experiment, the soils that were moistened had a week to equilibrate changes in microbial dynamics before fertilisers and inhibitor were applied. This is equivalent to a seasonal break rainfall event occurring on dry soil a week before sowing. An equilibrium in microbial processes had already been achieved before the nitrogen fertilisers and DMPP was applied at sowing. A large proportion of soil microorganisms would be operating at full capacity by the time N was added and thus the inhibitor would have maximal effect at inhibiting the nitrification of NH_4^+. For the dry soil, these microbial changes would have occurred simultaneously when fertiliser, DMPP and water was added. Fluctuations in microbial populations are occurring at the same time that DMPP is attempting to inhibit the oxidation of N fertiliser. Large losses of applied N fertiliser can also occur via immobilisation of N after the addition of rainfall (Rowlings et al. 2016). These changing microbial dynamics potentially contributed to the decreased recovery of mineral N applied to dry soils. Results indicate that decreased losses of mineral N occur when fertiliser and DMPP are applied onto moistened soil compared to dry soil. For maximised N availability to plants, applying fertilisers and DMPP to moistened soil will increased efficiency.

In dry incubated soil, fertilisers containing urea had lower mineral N concentrations than the NH_4^+ based fertilisers. For urea to become plant available it must first undergo the process of urea hydrolysis. This microbial reaction is catalysed by the enzyme urease and produces hydroxyl ions that increase the soil pH (Angus et al. 2014). This temporary increase in pH can promote NH_4^+ volatilisation where N is lost as NH_3 to the atmosphere. A 28-37 % loss of urea due to volatilisation was recorded by Soares et al. (2012) with a peak in N loss observed on the third sampling day. In field conditions, Rowlings et al. (2016) found that 40 % of applied urea was lost by volatilisation throughout the growing season when applied to an Australian pasture. This supports the findings established in this trial where significant losses in mineral N for wet and dry urea and dry UAN compared to other fertilisers was observed at sampling day 7 (Figure 1). It is postulated that volatilisation losses occurred for these urea containing fertilisers which resulted in decreased recoveries of mineral N. The urea treatment had greater losses as there was a higher concentration of urea present than in the UAN fertiliser.

Whilst applying N fertilisers to a moistened or dry soil appeared to affect the recovery of mineral N, soil moisture conditions did not affect the inhibition efficiency of DMPP. In both soil conditions the inhibitor supressed the activity of nitrifying organisms and maintained a large portion of mineral N as NH_4^+. This successful inhibition of nitrification by DMPP occurred across all four fertiliser types. By maintaining mineral N as NH_4^+, the risk of N losses like nitrate leaching and denitrification is decreased. The use of DMPP has been found to improve nitrogen use efficiency is a range of cropping and pasture situations when used in conjunction with N fertilisers (Rowlings et al. 2016).

Conclusion

Fertilisers that were applied to dry soil had decreased mineral N concentrations compared to fertilisers applied to moistened soils. It is proposed that moistened soils had equilibrated microbial activity and hence treatment application occurred on soil that had relatively stable microbial activity. It is probable that the dry soils experienced flushes of microbial changes simultaneously with the application of N fertilisers and water. Of the four fertilisers applied to moistened soil, urea had the lowest recovery whilst urea and UAN recorded the lowest recovery for fertilisers applied to dry soil. The inhibitor DMPP effectively supressed nitrification

for all four fertilisers regardless of the condition of soil moisture when DMPP was applied. To maximise N efficiency and plant availability, applying fertilisers with DMPP to moistened soil is preferable to the application on dry soil.

References

Angus, J. F., Gupta, V. V. S. R., Pitson, G. D. & Good, A. J. 2014. Effects of banded ammonia and urea fertiliser on soil properties and the growth and yield of wheat. *Crop and Pasture Science,* 65**,** 337-352.

Dawar, K., Zaman, M., Rowarth, J. S., Blennerhassett, J. & Turnbull, M. H. 2011. Urea hydrolysis and lateral and vertical movement in the soil: effects of urease inhibitor and irrigation. *Biology and Fertility of Soils,* 47**,** 139-146.

Gioacchini, P., Nastri, A., Marzadori, C., Giovannini, C., Vittori Antisari, L. & Gessa, C. 2002. Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. *Biology and Fertility of Soils,* 36**,** 129-135.

Iovieno, P. & Baath, E. 2008. Effect of drying and rewetting on bacterial growth rates in soil. *FEMS Microbiology Ecology,* 65**,** 400-407.

Kleineidam, K., Košmrlj, K., Kublik, S., Palmer, I., Pfab, H., Ruser, R., Fiedler, S. & Schloter, M. 2011. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. *Chemosphere,* 84**,** 182-186.

Mikha, M. M., Rice, C. W. & Milliken, G. A. 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. *Soil Biology and Biochemistry,* 37**,** 339-347.

Mulvaney, R. L. & Bremner, J. M. 1979. A modified diacetyl monoxime method for colorimetric determination of urea in soil extracts1. *Communications in Soil Science and Plant Analysis,* 10**,** 1163-1170.

Pook, M., Lisson, S., Risbey, J., Ummenhofer, C. C., Mcintosh, P. & Rebbeck, M. 2009. The autumn break for cropping in southeast Australia: trends, synoptic influences and impacts on wheat yield. *International Journal of Climatology,* 29**,** 2012-2026.

Rayment, G. E. & Lyons, D. J. 2011. *Soil chemical methods- Australasia,* Victoria, Australia, CSIRO.

Rowlings, D. W., Scheer, C., Liu, S. & Grace, P. R. 2016. Annual nitrogen dynamics and urea fertilizer recoveries from a dairy pasture using 15N; effect of nitrification inhibitor DMPP and reduced application rates. *Agriculture, Ecosystems & Environment,* 216**,** 216-225.

Soares, J. R., Cantarella, H. & Menegale, M. L. D. C. 2012. Ammonia volatilization losses from surface-applied urea with urease and nitrification inhibitors. *Soil Biology and Biochemistry,* 52**,** 82-89.

Tindaon, F., Benckiser, G. & Ottow, J. C. G. 2012. Evaluation of ecological doses of the nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) and 4-chloromethylpyrazole (ClMP) in comparison to dicyandiamide (DCD) in their effects on dehydrogenase and dimethyl sulfoxide reductase activity in soils. *Biology and Fertility of Soils,* 48**,** 643-650.

Yang, M., Fang, Y., Sun, D. & Shi, Y. 2016. Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethypyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. *Scientific Reports,* 6**,** 22075.

Yu, S. & Ehrenfeld, J. G. 2009. The effects of changes in soil moisture on nitrogen cycling in acid wetland types of the New Jersey Pinelands (USA). *Soil Biology and Biochemistry,* 41**,** 2394-2405.