The effect of nitrification inhibitors on wheat crop performance on coarse-grained soils in Mediterranean environments

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Abstract
This research investigates whether nitrification inhibitors (NIs), including DMPP, DCD & nitrapyrin, are effective in preserving ammonium (NH_4^+) in soil, reducing the abundance of ammonia oxidising microorganisms in soil and improving crop performance in Mediterranean wheat cropping systems. Data from laboratory soil incubation studies and glasshouse pot trials demonstrated that, under controlled conditions, DMPP was highly effective at inhibiting nitrification and limiting the growth of ammonia oxidising bacteria (AOB) for over 100 days on coarse-grained soils common in the Western Australian wheatbelt. This, however, did not result in improvements in grain yield or quality in wheat (*Triticum aestivum*) cv. Mace which suggests that preservation of mineral N as NH_4^+ does little for crop N uptake. Under field conditions yield increases of ≈300 kg ha^{-1} were observed in the presence of DMPP in a small number of trials. These increases, however, only occurred on soils with a shallow coarse-grained layer (<30cm) over a heavy clay pan. It is likely that the clay layer in these soils slowed the movement of water (and N) thus allowing the crop access to fertiliser N for a longer period of time. Overall, this study demonstrates that NIs slow nitrification in a range of soils common in the study region and in some cases yield and NUE benefits can occur.

Key Words
3,4-dimethylpyrazole phosphate (DMPP); dicyandiamide (DCD); ammonium (NH_4^+)

Introduction
In an attempt to minimise N losses from fertiliser applications, compounds such as nitrapyrin, DCD and DMPP have all been identified as nitrification inhibitors (NIs) (Zerulla, Barth et al. 2001). These compounds inhibit nitrification by disrupting the ammonia monooxygenase (AMO) enzyme in ammonia oxidising microorganisms (McCarty 1999). In an agricultural sense, slowing nitrification should allow crops longer access to fertiliser N, and in some studies this has resulted in increased cereal grain yields or improved N use efficiency (NUE) (Pasda, Hähndel et al. 2001; Abalos, Jeffery et al. 2014). In addition, the effect of NIs on the abundance of ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) has only been researched minimally (Kleineidam, Košmrlj et al. 2011; Chen, Qi et al. 2015) and few studies have investigated whether changes in microbial abundances correlate with increased crop performance. Finally, few studies have investigated the effectiveness of NIs in Mediterranean environments or on predominantly coarse-grained soils that are common in the Western Australian wheatbelt. This research investigates the effectiveness of three common NIs (nitrapyrin, DCD and DMPP) under laboratory, glasshouse and field conditions in Mediterranean environments. The overall aim of this research was to determine the effectiveness of NIs in terms of: 1) allowing NH_4^+ to persist in the soil over time; 2) reducing abundances of ammonia oxidising microorganisms and 3) improving crop performance.

Methods
Laboratory Incubations
Two predominantly coarse grained soils (hydrosol & tenosol both >90% sand) from two locations within the Western Australian wheatbelt were used in laboratory incubation studies. Approximately 1 kg of soil, sieved to 2.0 mm, was added to an aluminium foil tray and watered to field capacity. All trays were fertilised with urea (CH_4N_2O) at a concentration of 100 mg N kg^{-1} soil. Nitrification inhibitors (nitrapyrin, DCD or DMPP) were added to selected trays at recommended rates (nitrapyrin = 5% of fertiliser N; DCD = 10% of fertiliser N; DMPP = 1% of fertiliser N). Inhibitors were not applied to trays which were urea-only controls. All treatments were prepared in triplicate (n=3) per soil type. Soils were incubated for 100 days at 25 ^{O}C and maintained at 15-20% moisture. At regular intervals samples were taken from each tray for the determination of mineral N concentrations (Rayment and Lyons 2011), potential nitrification rates (PNRs) (Hart, Stark et al. 1994) and abundances of AOA/AOB (O'Sullivan, Wakelin et al. 2012). A mixed model repeated

measures ANOVA (α=0.05) was used to determine whether the concentrations of mineral N species, PNR and abundances of AOB organisms changed as a result of soil type, inhibitor type and sampling time.

Glasshouse Pot Trials

Wheat (*Triticum aestivum*) cultivar Mace was grown in two coarse-grained soils (hydrosol & tenosol) and was fertilised at two N concentrations (50 & 150 mg N kg^{-1} soil as urea). Selected pots were amended with DCD or DMPP at the same rates as in the laboratory experiment (DMPP was applied as ENTEC™ urea, which is urea containing 1% DMPP in the granule). The remaining plants were grown as urea-only controls or as unfertilised controls. All treatments were prepared in triplicate (*n*=3) per soil type. Fertiliser N was applied as a split application with a basal rate applied at sowing (15 mg N kg^{-1} soil) and the remainder four weeks later. All plants were grown to maturity and grain yields and grain protein concentrations (Rayment and Lyons 2011) were determined. Soil samples were taken at regular intervals to determine soil mineral N concentrations, PNRs and abundances of AOA/AOB. A mixed model repeated measures ANOVA (α=0.05) was used to determine whether the concentrations of mineral N species and PNR changed as a result of soil type, inhibitor type and sampling time. Factorial ANOVAs (α=0.05) were used to determine whether grain yield or grain protein content were influenced by soil type, inhibitor treatment or the interaction between soil type and inhibitor treatment.

Field Trials

Wheat cv. Mace was grown in seven field trials at four locations across the Western Australian wheatbelt (Cunderdin, Merredin, Wongan Hills and Shenton Park) on a range of soil types (tenosols, chromosols, sodosols) and climates. In six of these trials (located at Cunderdin, Merredin and Wongan Hills) crops were fertilised at rates of 0, 30 & 60 kg N ha^{-1} as conventional urea or ENTEC™ urea applied as a split application (10 kg N ha^{-1} at sowing; remainder four weeks later) which is typical in the region. In an additional treatment, 60 kg N ha^{-1} ENTEC™ urea was applied entirely at sowing to determine if this practice is profitable. In the other trial (Shenton Park) N was applied at 180 kg N ha^{-1} as conventional urea, ENTEC™ urea or urea + 10% DCD. In this trial there were two further treatments with 180 kg N ha^{-1} as ENTEC™ urea and conventional urea + 10% DCD each applied entirely at sowing. All trials were set up in a randomised block design (*n*=3). Grain yields, grain protein concentrations and grain N recovery per hectare (NUE measure) were determined at maturity for all treatments. Soil mineral N concentrations, PNRs and abundances of AOA/AOB were determined periodically throughout the growing season for all treatments. Repeated measures ANOVAs (α=0.05) were used to determine if mineral N concentrations and PNRs differed as a result of the use of different fertiliser treatments over time. Single factor ANOVAs (α=0.05) were used to determine if grain yields, grain protein and NUE were influenced by the use of different fertiliser treatments.

Results and Discussion

Laboratory Incubations

The use of NIs had a significant effect (P<0.05) on soil mineral N concentrations (data not shown), PNRs and AOB abundances (Fig 1). Although all NIs were able to slow nitrification, DMPP held the PNR at less than 0.1 mg NO_3^- kg^{-1} soil hr^{-1} and held AOB abundances at <2.0E^4 for the 100 days whereas other inhibitors only reduced PNRs/AOB abundances for ~50 days (Fig 1).

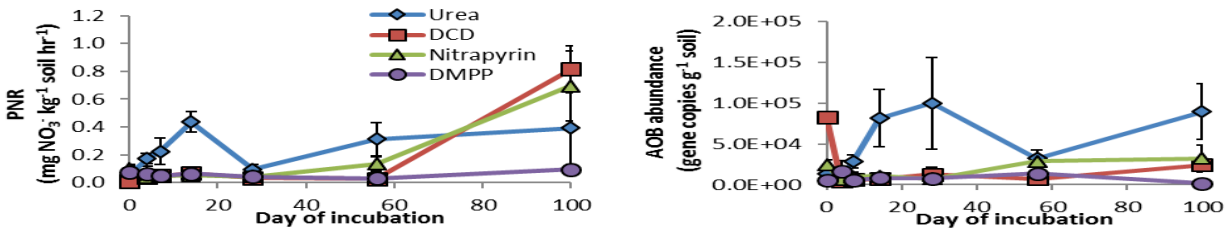

Figure 1. Potential Nitrification Rate (PNR - left) and Ammonia Oxidising Bacteria (AOB) abundances (right) in Williams soil (tenosol) from laboratory incubation experiment. Error bars represent the standard error of the mean (*n*=3).

Glasshouse Pot Trials

The application of DCD and DMPP (P<0.05) influenced soil mineral N concentrations (Fig 2), PNRs (data not shown) and AOB abundances (Fig 2). In this instance, particularly at high N applications (150 mg N kg^{-1} soil), DMPP slowed nitrification and limited abundances of AOB relative to when urea was applied on its

own or with DCD (Fig 2). Despite this, there was no significant (P<0.05) effect of DMPP or DCD on grain yield or grain protein content on either soil type (Fig 3).

Field Trials

There was a statistically significant (P<0.05) effect of different fertiliser treatments on grain yields of wheat cv. Mace in 3 of 7 trials (Table 1) and on grain protein and grain N recovery in some trials (data not shown). On two of the trials, statistically significant differences were attributed to grain yields being higher on all N fertilised plots than on unfertilised plots at Cunderdin (CUN 1 & 2) (Table 1), although on the CUN 2 trial ~300 kg ha^{-1} yield increases were observed when ENTEC^{TM} urea was applied relative to urea only (Table 1). On another trial at Wongan Hills (WH 2) there were significant effects of ENTEC^{TM} urea on grain yield with increases of ~300 kg ha^{-1} (Table 1). Across all seven trials, there were no significant (P<0.05) differences in NH_4^+ availability, PNRs or AOB abundances when different N fertiliser treatments were used (data not shown).

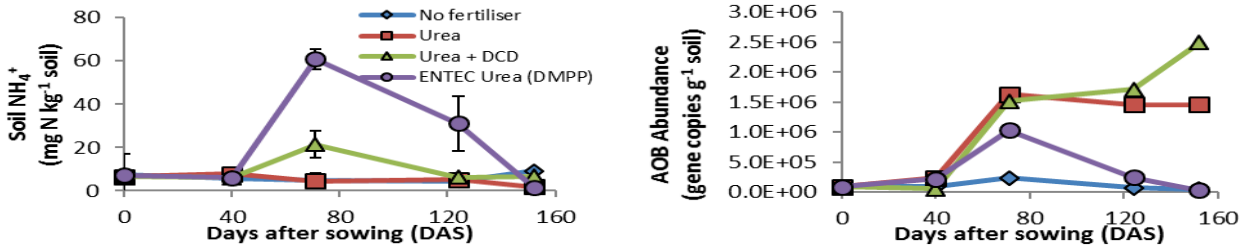

Figure 2. Soil NH_4^+ concentrations (left) and Ammonia Oxidising Bacteria (AOB) abundances (right) over time in glasshouse trial (tenosol from Wongan Hills) containing wheat cv. Mace fertilized at a concentration of 150 mg N kg^{-1}soil. Error bars represent the standard error of the mean (*n*=3).

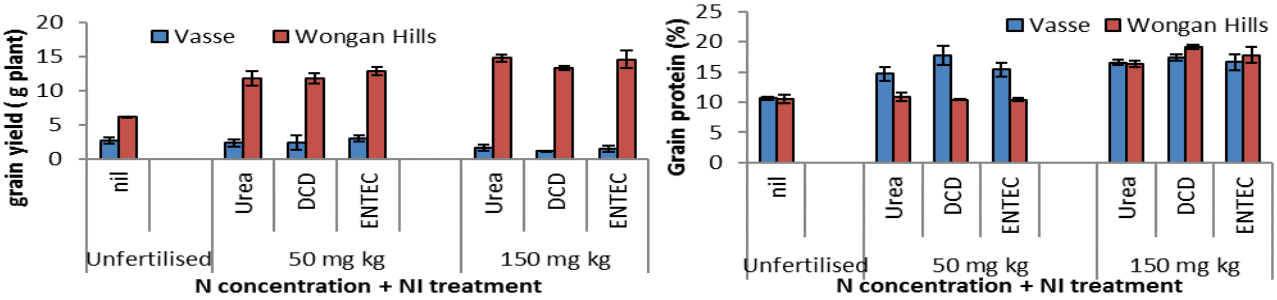

Figure 3. Grain yields (g^{-1} plant) (left) and grain protein (%) (right) of cv. Mace when grown in glasshouse trial in Vasse hydrosol (blue) and Wongan Hills tenosol (red). Error bars represent the standard error of the mean (*n*=3).

Table 1. Mean grain yield (kg ha^{-1}) data from field trials. Values presented are means ± SE (*n*=3). (# = statistically significant P<0.05; * = fertilizer applied entirely at sowing; $^{A, B}$ indicate groups of treatments with different LSD's (B>A); WH= Wongan Hills; CUN = Cunderdin; MER = Merredin; SH PK = Shenton Park).

Trial	Unfertilised	30 kg N ha^{-1}	60 kg N ha^{-1}	30 kg N ha^{-1}	60 kg N ha^{-1}	60 kg N ha^{-1} *
		Urea		ENTEC^{TM} Urea		
WH 1	3892 ± 24	3807 ± 129	3971 ± 88	3906 ± 79	3971 ± 29	3779 ± 79
WH 2#	2623 ± 88 ^{A}	2609 ± 196 ^{A}	2970 ± 61 ^{B}	2975 ± 254 ^{B}	3276 ± 191 ^{B}	2735 ± 79 ^{A}
MER 1	1950 ± 30	2230 ± 116	2491 ± 169	2097 ± 91	2356 ± 177	2008 ± 116
MER 2	1813 ± 25	2004 ± 78	2228 ± 144	2005 ± 120	2159 ± 207	1946 ± 100
CUN 1#	909 ± 33 ^{A}	1323 ± 126 ^{B}	1292 ± 140 ^{B}	1422 ± 201 ^{B}	1325 ± 105 ^{B}	1366 ± 165 ^{B}
CUN 2#	1994 ± 223 ^{A}	2527 ± 294 ^{B}	2644 ± 83 ^{B}	2856 ± 263 ^{B}	2648 ± 160 ^{B}	2843 ± 186 ^{B}
		180 kg N ha^{-1}	180 kg N ha^{-1}	180 kg N ha^{-1} *	180 kg N ha^{-1}	180 kg N ha^{-1} *
		Urea	ENTEC^{TM} Urea		Urea + DCD	
SH PK		814 ± 86	509 ± 112	742 ± 95	635 ± 122	615 ± 63

From the laboratory incubations and glasshouse pot trials it is clear that DMPP is highly effective at slowing nitrification under 'ideal conditions' (i.e. constant moisture & temperature) and did so for ≈100 days which exceeded that of other inhibitors (DCD & nitrapyrin) (Figs 1-2). In addition, abundances of AOB were lower when DMPP was applied relative to other treatments (Fig 1-2). All soils used in laboratory incubation experiments and glasshouse pot trials were low in total Cu (<1.5 mg Cu kg^{-1} soil) which is a possible

explanation as to why DMPP was so effective in these soils over a long period of time, given that DMPP is proposed to inhibit nitrification via chelating-Cu (an essential cofactor in the AMO enzyme) (McCarty 1999). Despite being able to inhibit nitrification and inhibit the growth of AOB, neither DMPP nor DCD regularly increased grain yield or grain quality of wheat cv. Mace under 'ideal' conditions in both the glasshouse (Fig 3) and the field (Table 1). Cereals such as wheat have been proposed to preferentially take up NO_3^- rather than NH_4^+ (Recous, Machet et al. 1988) and thus the increased persistence of NH_4^+ in the soil may actually have little benefit to the crop. In two of the seven field trials, DMPP (applied as ENTEC™ urea) improved crop yields by up to 300 kg ha^{-1} (Table 1), but this only occurred on soils with a shallow coarse-grained layer (<30cm) over a heavy clay pan. It is likely that the clay pan slowed the movement of water (and NO_3^-) into the subsoil thus allowing the crop longer access to the fertiliser N. If DMPP was able to slow nitrification it is possible that the wheat crop would have had even longer access to the fertiliser N which facilitated yield increases. These soil types are found extensively in Western Australia (McArthur 1991) and thus future research should include a cost-benefit analysis of whether NI treated fertilisers should be used with more regularity in those regions. Although they were successful in slowing nitrification and limiting AOB population growth under controlled conditions, DMPP and DCD were less effective under field conditions. It is likely that a combination of soil wetting and drying cycles favoured nitrification (Abalos, Sanz-Cobena et al. 2016) and high temperatures (often >15 ^oC) likely enhanced DMPP degradation (Weiske, Benckiser et al. 2001) thus reducing its long-term effectiveness. Even under 'ideal' conditions DMPP and DCD had little or no influence on AOA abundances (data not shown). However, AOA abundances were also not stimulated by urea applications which suggests that these organisms typically play a minimal role in nitrification in these systems. Overall, it is clear that both NIs tested here have the ability to inhibit nitrification and limit the growth of AOB in the soil. In addition, under some conditions NIs are able to facilitate yield and NUE increases, however, the mechanism behind this is uncertain. Future research should focus on the economics of NI use to ascertain whether they could become an alternate N management practice in the region.

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