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Evidence that the efficacy of the nitrification inhibitor dicyandiamide (DCD) is affected by soil properties in UK soils

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ABSTRACT

A laboratory incubation study was conducted with nine UK soils to determine the effect of soil physical and chemical properties, and temperature, on the efficacy the nitrification inhibitor dicyandiamide (DCD). Nitrogen was applied to soil as ammonium chloride at a rate of 100 μg N g^{-1} dry soil, and incubated at 60% water-filled-pore-space at either 5, 15 or 25 °C. The ammonium (NH_4^+) pool was enriched with ^{15}N to 60 atom% excess and DCD was applied at a rate of 10 μg g^{-1} dry soil. The concentrations and enrichments of the NH_4^+ and nitrate (NO_3^-) pools, along with nitrous oxide (N_2O) flux measurements, were determined regularly for 60 days after N application. Gross soil N transformation rates were quantified with a ^{15}N tracing model. The persistence of DCD was strongly related ($P < 0.001$) to temperature with the measured half-life across all soils of 89, 37 and 18 days at 5, 15, and 25 °C, respectively. There was wide variation in the half-life of DCD among soils; which was predominantly associated with the soil oxalate extractable Fe concentration. Greater ($P < 0.001$) inhibition in autotrophic nitrification by DCD occurred at 5 and 15 °C compared to 25 °C. Across all soils and temperatures DCD increased the rate of mineralisation of recalcitrant organic-N and the rate of adsorption of free NH_4^+, however, effects varied between soils. DCD did not have a significant effect on the rate of oxidation of recalcitrant organic-N to NO_3^- or on any of the immobilisation processes or mineralisation of labile N to NH_4^+. The efficacy of DCD in inhibiting net NO_3^- production best correlated with soil Cu ($r = -0.82$), % clay ($r = -0.71$), total N ($r = -0.66$) and LOI ($r = -0.61$). Stepwise multiple regression showed that Cu, oxalate extractable Fe and oxalate extractable Al explained 85.0% of the variation in the percentage inhibition of net NO_3^- production by DCD. The inhibitor also reduced cumulative N_2O emissions, with reductions negatively correlated with a range of soil properties associated with organic matter. We provide evidence that the interaction between temperature, soil clay content and soil organic matter governs the efficacy of DCD. The grassland soils had higher native total N concentrations than the arable soils, hence the inhibition of net NO_3^- production by DCD was lower and this resulted in an overall inhibition in N_2O emissions of 58% and 81% for grassland and arable soils respectively.

1. Introduction

Build-up of nitrate (NO_3^-) in agricultural systems, formed by nitrification of ammonium (NH_4^+), is a precursor to nitrogen (N) loss processes such as leaching, and denitrification into gaseous nitrous oxide (N_2O) and dinitrogen (N_2) (Cameron and Moir, 2013). Chemical N fertiliser constitutes approximately 75% of the total EU input of reactive N (van Grinsven et al., 2014) and between 40 and 70% of the fertiliser N applied is lost to the atmosphere or the hydrosphere (Sutton et al., 2011). Not only do these loss processes decrease the economic efficiency of fertiliser-N, they also have a detrimental impact on the environment. Hence there are many legislative requirements (EU Water Framework (2000/60/EC), EU Nitrates Directives (91/676/EEC), and UK Climate Change Act reduction targets) aimed at reducing N losses which necessitate an increase in N use efficiency.

Nitrification inhibitors, such as dicyandiamide (DCD), inhibit the conversion of NH_4^+ to NO_3^-. It is suggested that DCD binds to the

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active sites of the copper-containing ammonia monooxygenase (AMO) metalloenzyme required by ammonia oxidising bacteria (AOB) (Amberger, 1989), and therefore can be used as a tool to reduce NO_3^- production and subsequent leaching (Dennis et al., 2012), and N_2O emissions (Di and Cameron, 2003). DCD has recently been shown to also inhibit ammonia oxidising archea (AOA) in acidic soils in soil microcosm studies (Zhang et al., 2012; Lehtovirta-Morley et al., 2013) and is therefore not a selective inhibitor targeting AOB alone.

Although many studies have shown the efficacy of DCD in reducing N_2O emissions and NO_3^- leaching in the field (Williamson et al., 1998; Di and Cameron, 2002, 2003, 2005, 2006; Smith et al., 2008; Monaghan et al., 2009; Cameron et al., 2014), this is not always the case and nitrification inhibitors have at times had nil or variable effects on N losses. If DCD is to be more widely adopted as a mitigation strategy to reduce N losses, it is important to understand the reasons for the variability in its efficacy and its potential effect on other soil gross N transformations.

It has been well established that the effectiveness of DCD is strongly related to temperature (Zerulla et al., 2001; Di and Cameron, 2004; Kelliher et al., 2008). Its persistence is also known to be influenced by pH (Keeney, 1986), moisture content (Hendrickson and Keeney, 1979; Puttanna et al., 1999), soil organic matter content (Prasad and Power, 1995; Singh et al., 2008), soil aeration (Balaine et al., 2015), and DCD application rate (Hauser and Haselwandter, 1990). Besides environmental and edaphic factors, DCD longevity in the field may also be affected by additional factors such as leaching (Shepherd et al., 2014). Few studies have been conducted to elucidate the extent to which specific soil physical and chemical properties may affect the performance of DCD. In a study using three soils, Ernfors et al. (2014) suggested that the efficacy of DCD in reducing the rate of NH_4^+ oxidation was related to its rate of degradation which was found to be soil specific. In contrast, Wakelin et al. (2014) found that the % efficacy of DCD in reducing the potential nitrification rate was not related to the soil type, but was strongly related to the abundance of AOB. While there is evidence in several studies on the effect of single factors on the effect of DCD on nitrification, what is missing is a comprehensive study that takes into account not only abiotic factors but also the range of soil properties across agricultural soils.

The aim of the current study was to examine the effect of both temperature and soil physical and chemical properties on the inhibitory effect of DCD on a range of soil gross N transformations and N_2O emissions in a laboratory study with nine soils from the UK Agricultural Greenhouse Gas (GHG) Platform sites. These soils were selected because they represent the key UK soil/climatic zones to test the mitigation potential of DCD. The current study aimed to explain some of the variability in the efficacy of DCD that was observed at the associated field sites.

2. Materials and methods

2.1. Soil collection and preparation

Soil was collected from nine sites across the UK in the period February to early March 2011. These locations were the experimental sites for the UK Agricultural Greenhouse Gas Research Platform. There were five grassland soils (Crichton, Drayton, Hillsborough, North Wyke, Pwllpeiran) and four arable soils (Boxworth, Gilchristen, Rosemaund and Woburn). The soils did not receive any N inputs from the end of the 2010 growing season to collection. From each location 75 kg of soil was collected from the upper 10 cm, partially air-dried to a target gravimetric moisture content of 20%, passed through 6 mm and 2 mm sieves to remove large stones and roots, and then stored at a temperature of 4 °C (±1 °C) for up to 180 days before use. Site locations and soil characteristics are presented in Table 1.

2.2. Experimental design

An aerobic soil incubation study was conducted over a 60 day period at three temperatures (5, 15, 25 °C) with destructive sampling initiated on eight occasions. The partially air-dried soil (equivalent to 100 g oven-dry soil) was placed into 300 ml clear polystyrene incubation jars, pre-wetted with deionised water to approximately 40% water-filled pore space (WFPS) and equilibrated at the designated experimental temperature for three days, prior to the addition of labelled ^{15}N. Ammonium chloride (NH_4Cl) was applied with and without DCD at a N rate of 100 μg N g^{-1} oven-dry soil, and the NH_4^+ pool was enriched with ^{15}N at 60 atom% excess. A rate of 100 μg N g^{-1} oven-dry soil was selected as Watson et al. (1995) found that at higher application rates of a NH_4^+ the rate of net nitrification was inhibited. DCD was applied at the rate of 10 μg g^{-1} oven-dry soil. Immediately prior to application the $^{15}NH_4Cl$ and DCD solutions were mixed and uniformly applied with a syringe over the soil surface. The DCD solution was replaced with deionised water in the treatment without the inhibitor. Post treatment the soil in the incubation jars was at a WFPS of 60%, which is the optimum WFPS for nitrification to occur, with the relative activity of anaerobic denitrification being negligible at this WFPS (Linn and Dornan, 1984). The jars were covered with Parafilm® (Bemis Company Incorporated, US) to prevent moisture loss but allow gaseous exchange. The soil in each jar was checked periodically for water loss and if required adjusted to maintain a WFPS of 60% over the 60 day incubation period. Jars for each of the eight extraction times (0, 5, 10, 15, 20, 30, 40 and 60 days) were randomly allocated to a shelf (of differing heights) within the 5, 15, 25 °C incubators; the sample jars for each time period were fully randomised within each shelf. A previous soil incubation study showed that there was no significant positional effect within the incubators on any of the soil processes measured (Watson et al., 2008). Due to the limitation in incubator space, it was only possible to carry out one replicate at a time. A single replicate run comprised 432 jars (i.e. 3 temperatures, 2 DCD treatments, 9 soils, 8 extractions times), so with 3 replicates this resulted in a total of 1296 jars. As the replicates were staggered in time soil was stored at 4 °C (±1 °C) for up to 180 days before use. There was good agreement between replicates for $NH_4^+ - N$, $NO_3^- - N$, DCD soil concentrations over time, indicating that there was little effect of cold storage on the soil N and DCD dynamics.

2.3. Soil extraction and determination of mineral N concentration, ^{15}N enrichment and DCD concentration

The soil in the jars was extracted with 2 M KCl at 0, 5, 10, 15, 20, 30, 40 and 60 days after treatment addition and mineral N (NH_4^+ and NO_3^-) in the extract was determined by the procedure of Stevens and Laughlin (1995). The soil in the jars was transferred to a food homogeniser with 200 ml 2 M KCl. Portions of the suspension were centrifuged immediately at 2000 × *g* for 5 min and the supernatant filtered sequentially through GF/D and GF/F glass-fibre papers (Whatman International Ltd, Kent, UK). Filtrates were stored at 4 °C prior to analyses for inorganic N concentrations and ^{15}N enrichments of NH_4^+ and NO_3^-. Concentrations of mineral N in the KCl extracts were determined using a Skalar San Plus automated wet chemistry analyser (Skalar Analytical B.V., Breda, Netherlands). The ^{15}N contents of the NH_4^+ and NO_3^- pools were determined by the generation of N_2O (Stevens and Laughlin, 1994; Laughlin et al., 1997) with subsequent ^{15}N