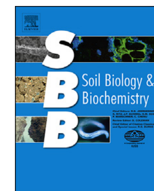
Soil Biology & Biochemistry 109 (2017) 23–34

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



# Gross nitrogen transformations in grassland soil react differently to urea stabilisers under laboratory and field conditions

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ARTICLE INFO

*Article history:*
Received 21 August 2016
Received in revised form
6 January 2017
Accepted 31 January 2017
Available online 10 February 2017

*Keywords:*
Gross N transformations
DCD
NBPT
Combined inhibitor
Laboratory and field study

ABSTRACT

A laboratory and a field study were conducted on a permanent grassland soil in Northern Ireland to investigate the effects of urea in combination with N process inhibitors such as the urease inhibitor N-(butyl) thiophosphoric triamide (NBPT) and/or the nitrification inhibitor dicyandiamide (DCD) on soil N dynamics. Urea enriched with n-butyl $^{15}N$ to 60 atom % was applied to soil at a rate of 100 μg N $g^{-1}$ dry soil in the laboratory and 100 kg N $ha^{-1}$ in the field. A numerical $^{15}N$ tracing model was used to quantify several simultaneously occurring gross N transformation rates in both studies. The changes in soil nitrate ($NO_3^-$) and ammonium ($NH_4^+$) concentrations and $^{15}N$ enrichment over a 25-day period as well as the concentration and $^{15}N$ enrichment of plant N at harvest were used to model soil gross N transformations. The results showed that the effect of N process inhibitors varied firstly between laboratory and field studies and secondly whether the inhibitors were applied individually or in combination. Overall DCD had a greater effect on the major soil N transformations than NBPT; reducing oxidation of $NH_4^+$, total nitrification, net $NO_3^-$ produced, total mineralisation and the net adsorption of $NH_4^+$ at both laboratory and field scale. The effect of DCD was similar for these transformations whether applied alone or co-applied with NBPT. In contrast NBPT had no significant effect on oxidation of $NH_4^+$, total nitrification, total mineralisation or total immobilisation compared to urea in the field, while the effect on these transformations in the laboratory was significant. The contrasting effects of inhibitors on gross N transformations between laboratory and field may relate to the differences in experimental conditions, e.g. soil preparation, environmental conditions and the contribution of plant biomass. To obtain a more realistic assessment of gross soil N transformations *in situ*, it is essential that laboratory experiments are supplemented with field studies.

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## 1. Introduction

By 2050 food production must increase by 70% to meet the demands of the projected world population of 9.1 billion (FAO, 2014). Current agricultural production requires the addition of substantial quantities of mineral nitrogen (N) which is inefficiently assimilated into plant biomass resulting in large N losses to the environment. Estimates of direct N losses of the applied N from conventional agricultural systems vary, ranging from 10 to 78% (Allison, 1955; Steinheimer et al., 1998; David and Gentry, 2000; Watson and Foy, 2001; Li et al., 2013). There is also a sizeable proportion of indirect N loss; projected to be 200 Tg N $yr^{-1}$ by 2050 (Galloway et al., 2008; Jahangir et al., 2013). As well as the financial cost, N loss results in detrimental effects to human health, such as nitrate poisoning increasing the risks of cancer and birth defects, and the environment. There is a focus in the agriculture sector on controlling emissions of the potent greenhouse gas (GHG) nitrous oxide ($N_2O$), with 60% of all anthropogenic $N_2O$ emissions estimated to come from food production in 2006 (Syakila and Kroeze, 2011). One strategy proposed to reduce N loss in the form of $N_2O$

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http://dx.doi.org/10.1016/j.soilbio.2017.01.025
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emission is to switch fertiliser formulation from the very dynamic form ($NO_3^-$) to the relative less mobile form ($NH_4^+$) (Harty et al., 2016). Accumulation of $NO_3^-$ in agricultural soils either directly applied or from nitrification of ammonium ($NH_4^+$) has been identified as the source of $N_2O$ (Cameron et al., 2013). Results from an associated study examining the effect of fertiliser form on $N_2O$ emissions show that calcium ammonium nitrate (CAN) applications in grassland systems in Ireland, especially those with impeded drainage and high rainfall, are at risk of N loss as $N_2O$ (Harty et al., 2016). In this study, they also determined that switching to urea has the potential to reduce $N_2O$ emissions and the risk of pollution swapping of $N_2O$ for $NH_3$ can be mitigated by stabilising urea with NBPT. This urea stabiliser works by impeding the enzyme urease, thereby slowing the rate of transformation of urea to $NH_4{}^+$ and reducing the associated pH rise and the potential toxic effects of $NH_4^+$on $NO_2^-$ oxidising microbes (Maharjan and Venterea, 2013). The urease inhibitory activity of NBPT is associated with the conversion to its oxygen analogue, N-(n-butyl) phosphoric triamide (NBPTO), which generally occurs within minutes or hours in aerobic soils (Byrnes and Freney, 1995). Use of NBPT can reduce $NH_3$ emissions by up to 80% (Watson et al., 2008; Zaman et al., 2008). NBPT used in combination with strong plant growth can help maximise $NH_4^+$ uptake, minimise N loss and increase N use efficiency (Akiyama et al., 2010). The nitrification inhibitor dicyandiamide (DCD) can reduce $N_2O$ losses (Selbie et al., 2014), $NO_3^-$ leaching (Dennis et al., 2012; Zaman and Nguyen, 2012), $N_2$ emissions (Selbie et al., 2015) and increase soil mineralisation-immobilisation turnover (Ernfors et al., 2014). It is difficult to distinguish between the large native soil organic N pools and the fertiliser N applied without the use of $^{15}N$ tracers (Fillery and Recous, 2001). Various $^{15}N$ tracing approaches have been developed since the original dilution approach was published by Kirkham and Bartholomew (1954). While the early approaches were based solely on the $^{15}N$ dilution theory, later approaches also included simultaneous calculation of rates using of the FLUAZ model (Mary et al., 1998) which could for instance be used to separate combined gross N transformations such as nitrification in autotrophic and heterotrophic pathways (Barraclough and Puri, 1995). In the Ntrace model, used in this study, this simultaneous dilution and enrichment of $^{15}N$ in various pools has further been extended to include separation into labile and recalcitrant pathways. Many studies have used $^{15}N$ tracing models (Chen et al., 1998; Watson and Mills, 1998; Accoe et al., 2005; McGeough et al., 2014; Ernfors et al., 2014) to quantify soil gross N transformations, however, most have used ammonium chloride or ammonium nitrate. To date, no $^{15}N$ isotope studies have examined the N dynamics of urea amended with the urease inhibitor NBPT alone and/or with DCD. The aim of the current study was to examine the effect of NBPT and/or DCD on gross N transformations of urea in a permanent grassland soil under both laboratory and field conditions.

## 2. Materials and methods

### 2.1. Laboratory study

#### 2.1.1. Soil collection and preparation

Soil was collected from the upper 7.5 cm, as most of the pasture feeding roots lie in this active zone, from a permanent grassland site at the Agri-Food and Biosciences Institute, Hillsborough, Co. Down, Northern Ireland (54 °27′827 N, 6 °04′57,873 W) (HB) in Sept 2013. The soil (Dystric Umbric Stagnosol, (FAO, 2015)) had a clay loam texture with a pH of 5.7, total N was 0.6%, total carbon was 6.0%, and organic matter was 14.3%. The experimental baseline mineral N concentrations and changes in soil mineral N concentration over the two experiments is presented in Supplemental Data (Table S1). Soil was sieved sequentially through a 25 mm sieve and a 6 mm sieve and partially air dried to a target gravimetric moisture content of 20% and stored at 4 °C for one week. The fresh weight equivalent of 100 g oven dry soil was packed into 300mL clear polystyrene incubation jars (Medfor Products Farnborough, England) to an average packing density of 0.71 g $cm^3$ (s.e. 0.0082, n = 5). The total volume of liquid added to each jar was calculated to give a water filled pore space (WFPS) of 65% as this is the optimum WFPS for nitrification and denitrification processes to occur (Linn and Doran, 1984). The treatment volume was subtracted and jars were pre-wetted with the remaining volume of deionised (DI) water and stored at 4 °C for 7 days This was done as pre-incubation to avoid the flush of N which occurs as a result of soil disturbance. The jars were then incubated at 15 °C for 3 days prior to treatment application.

#### 2.1.2. Experimental design

An aerobic soil laboratory study was conducted over a 25 day period with destructive sampling initiated on eight occasions (0, 1, 2, 4, 7, 10, 15 and 25 days). There were four fertiliser treatments with four replicates: urea, urea + NBPT, urea + DCD and urea + NBPT + DCD. Urea was applied at a rate of 100 μg N $g^{-1}$ oven-dry (OD) soil, enriched to 60 atom % $^{15}N$. DCD was applied at a rate of 10 μg $g^{-1}$ OD soil (10% of urea-N weight) and NBPT was applied at a rate of 10.7 μg $g^{-1}$ OD soil (0.05% of urea weight). A rate of 100 μg N $g^{-1}$ OD soil was selected as Watson et al. (1995) found that at higher N application rates the rate of net nitrification was inhibited. At treatment application, the aliquots of urea/DCD/NBPT were mixed together and brought up to 15 mL with DI water as required. Each combined treatment solution was applied evenly to the soil surface in the sample jars using a syringe and a wide-bore needle. Jars for each of the eight extraction times were each randomly allocated to a separate shelf within an incubator (Model LT601, LEEC, Nottingham England) and the sample jars on each shelf were fully randomised. Sample jars (128 jars in total) were covered in Parafilm® (Bemis Company Incorporated, US) to prevent moisture loss but allow gaseous exchange and incubated at 15 °C. All jars were maintained at 65% WFPS with DI water supporting maximum microbial activity (Linn and Doran, 1984). Moisture loss was minimal. The T0 jars were destructively sampled immediately after the fertiliser was applied.

#### 2.1.3. Soil extraction

The entire contents of the soil jars were extracted with 2M potassium chloride (KCl), pH adjusted to 8.0 with potassium hydroxide (KOH) to prevent $NO_2^-$ oxidising to $NO_3^-$ or exchanging with an organic form (Stevens and Laughlin, 1995). Extractions took place on eight occasions (0, 1, 2, 4, 7, 10, 15 and 25 days) after the addition of treatments. The soil in the jars was transferred to a food homogeniser (Waring, New Hartford, Conn.) with 200 mL 2M KCl, plus KOH aliquot, and blended for 1 min at 2000 rpm. The suspension was 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) and filtrates were stored at 4 °C prior to analyses. Sub-samples of the soil extracts were analysed immediately via manual colorimetric methods to quantify the concentrations of urea (Mulvaney and Bremner, 1979) and nitrite ($NO_2^-$) (Keeney and Nelson, 1982). $NO_3^-$ and $NH_4^+$ concentrations were determined using an automated continuous flow wet chemistry analyser (Skalar Analytical B.V., Breda, Netherlands). $^{15}N$ enrichment of the $NH_4^+$ and $NO_3^-$ pools were determined by conversion to $N_2O$ (Laughlin et al., 1997; Stevens and Laughlin, 1994) and the $^{15}N$ enrichment of the evolved $N_2O$ quantified using an Isotope-Ratio Mass Spectrometry (IRMS) system (Europa Scientific 20-20 Stable Isotope Analyser) interfaced to a Europa

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