Science of the Total Environment 603–604 (2017) 370–380

Contents lists available at ScienceDirect

Science of the Total Environment

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

Do glucosinolate hydrolysis products reduce nitrous oxide emissions from urine affected soil?

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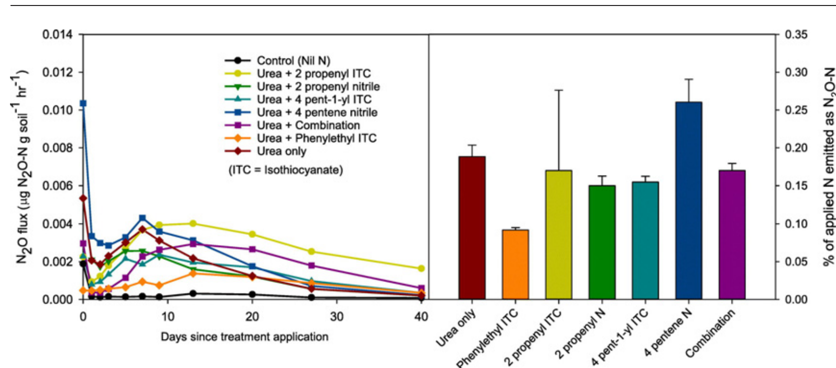
HIGHLIGHTS

- The effectiveness of glucosinolate hydrolysis products at inhibiting N_2O emissions in a high N environment was tested.
- Laboratory and field trials measuring N_2O production and soil mineral N cycling were conducted.
- Some glucosinolate hydrolysis products reduced nitrification rates.
- Where inhibition occurred it was short lived.
- Glucosinolate hydrolysis products with different R groups did not inhibit soil nitrogen processes to the same degree.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 April 2017
Received in revised form 9 June 2017
Accepted 10 June 2017
Available online 23 June 2017

Editor: D. Barcelo

Keywords:
Nitrogen
Urine
Nitrous oxide
Nitrification inhibitor
Glucosinolate

ABSTRACT

New Zealand agriculture is predominantly comprised of pastoral grazing systems and deposition of animal excreta during grazing has been identified as a major source of nitrous oxide (N_2O) emissions. Nitrification inhibitors have been shown to significantly reduce nitrous oxide emissions from grazing pastoral systems, and some plants have been identified as having nitrification inhibiting properties. Brassica crops are one such example as they contain the secondary metabolite glucosinolate (GLS) whose hydrolysis products are thought to slow soil nitrogen cycling. Forage brassicas have been increasingly used to supplement the diet of grazing animals. The aim of this study was to determine if GLS hydrolysis products (phenylethyl isothiocyanate, 4-pent-1-yl isothiocyanate, 2-propenyl nitrile, 2 propenyl isothiocyanate, 4-pentene nitrile) produced in brassica crops reduced N_2O emissions from soil amended with urea or animal urine. In the laboratory, some GLS hydrolysis products added with urea to soil were found to decrease N_2O emissions and the most effective product (phenylethyl isothiocyante) reduced N_2O emissions by 51% during the study. There was some evidence that the reduction in N_2O emissions found in the lab could be attributed to inhibition of nitrification. Results suggest that the inhibition by GLS hydrolysis products was short-lived and, if considered for use, multiple applications may be necessary to achieve effective inhibition of N_2O emissions. This reduction, however, was not observed under field conditions. Further investigation is required to test more GLS hydrolysis products to fully understand their impact on N_2O emissions from urine affected soil.

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http://dx.doi.org/10.1016/j.scitotenv.2017.06.089
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1. Introduction

Global food production needs to increase because the population is expected to reach 9 billion by the middle of the century (Godfray et al., 2010). At the same time, it is important that we consider the environmental effects of increased production. New Zealand agriculture is predominantly comprised of pastoral grazing systems where high levels of nitrogen (N) are lost to potentially sensitive environments (e.g. Ledgard et al., 2000). This is due to the inefficient use of dietary N by ruminants where 70 to 95% of ingested N is excreted in urine and dung (Oenema et al., 2005). N loading rates from urine can be as high as 1000 kg N ha^{-1} (average 613 kg N ha^{-1}; Selbie et al., 2015), depending on plant and dietary content. This loading exceeds the N requirements of the pasture or forage in the vicinity (Haynes & Williams, 1993) and so the excess N is available for loss via nitrate (NO_3^-) leaching, or emissions of gaseous N such as ammonia (NH_3), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) (Luo et al., 2010). N_2O is produced during the biological transformation of urinary N in soil, particularly, the microbial processes of nitrification and denitrification (Firestone & Davidson, 1989). Losses of N_2O are of particular concern as it is a potent greenhouse gas with a global warming potential 298 times that of CO_2 (Myhre et al., 2013), and because they contribute significantly to the depletion of stratospheric ozone (Ravishankara et al., 2009). In New Zealand, about 11% of the national greenhouse gas production can be attributed to N_2O emissions derived from deposited urine patches (Ministry for the Environment, 2015). Identifying approaches that decrease N_2O production is a key strategy for reducing greenhouse gas emissions in New Zealand and in other countries with grazed pastures.

Over the last decade, there has been a significant amount of research on the use of the nitrification inhibitors to reduce N_2O emissions from urine patches. Much of this work was carried out using the inhibitor dicyandiamide (DCD) which proved to be effective at reducing N_2O emissions from urine patches by an average of 57% (de Klein et al., 2011; Di & Cameron, 2011; Li et al., 2015; Luo et al., 2013). However, use of DCD was temporarily halted in 2013, waiting for the establishment of standards by the Codex Committee for Food. Inhibition of nitrification remains a promising tool for mitigation of both N_2O emissions and nitrate leaching and so alternative 'biological' inhibitors have been investigated. Biological nitrification inhibitors (BNIs) are secondary metabolites that are produced in, and are released to the soil by plants, and inhibit nitrification. The term BNIs is used to distinguish them from synthetic nitrification inhibitors (Subbarao et al., 2007). A range of plants have been shown to release compounds that inhibit nitrification e.g. *Sorghum*, tropical grass *Brachiaria* (Gopalakrishnan et al., 2007; Ishikawa et al., 2003) and some pines (Lodhi & Killingbeck, 1980). Research suggests that the grazing of certain pasture and forage species may reduce N_2O emissions because secondary metabolites contained in these plants are excreted in urine patches and deposited onto soil, thus inhibiting N cycling and microbial processes (e.g. Prasad & Power, 1995; Ryan et al., 2006).

Brassica crops, in particular, may reduce N_2O emissions as they contain secondary metabolites called glucosinolates (GLS), whose hydrolysis products (isothiocyanates (ITCs), thiocyanates and nitriles) have been shown to inhibit nitrification processes in soil incubated with GLS hydrolysis products (Bending & Lincoln, 2000) and soil incubated with brassica seed meals that also contain GLSs (Reardon et al., 2013). Brassica plants have also been shown to inhibit soil N cycling in situ (Kirkegaard et al., 1999; Ryan et al., 2006), which has been attributed to GLS hydrolysis products. In soil incubation studies, Bending and Lincoln (2000) demonstrated a reduction in nitrifying bacteria population following application of several different GLS hydrolysis products. The mode by which GLS hydrolysis products such as ITCs might inhibit nitrification is not entirely clear, however, it appears that ITCs in particular are toxic to a range of soil organisms (Bending & Lincoln, 2000; Choesin & Boerner, 1991; Kawakishi & Kaneko, 1987; Subbarao et al., 2013). These studies measured the impact of GLS hydrolysis products on N cycling in soils with relatively low N inputs. However, in grazed pasture the majority of N_2O emissions arise from high N inputs such as those from a urine patch. Utilising the potential of forage crops to reduce N loss from pastoral grazing systems requires understanding how GLSs might function in the presence of high N inputs. Additionally, none of these previous studies examined the impact of GLS hydrolysis products on N_2O production. The objective of this study was to test the impact of brassica derived GLS hydrolysis products on N_2O production from soil. We hypothesise that GLS hydrolysis products will inhibit nitrification and N_2O production from urine patches. We initially tested the efficacy of GLS hydrolysis products in a laboratory screening trial and subsequently promising candidate products were tested in a field trial.

2. Materials and methods

2.1. Experiment 1 – laboratory incubation

2.1.1. Trial set-up

A Bruntwood silt loam soil (Typic Impeded Allophanic Soil; Hewitt, 1998) of 0–7.5 cm depth, from under a mixed perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture, was collected for an incubation study. General soil properties are presented in Table 1.

The soil was sieved to 4 mm, homogenised and allowed to pre-incubate for 24 h to allow for the effects of soil disturbance to subside. Moist soil (140 g oven dry equivalent) was weighed into preserving jars (1 L) and amended with N (as urea 600 μg N g^{-1} soil) plus one of five GLS hydrolysis products (phenylethyl ITC, 4 pent-1-yl ITC, 2 propenyl nitrile, 2 propenyl ITC, 4 pentene nitrile) at 2 rates. Other treatments included: A combination of all hydrolysis products, Urea Only, and a nil N control (Table 2). Each treatment was replicated 6 times. The GLS hydrolysis products were applied at either 30 or 60 μg g^{-1} soil. The addition rates of hydrolysis products was determined based on Kirkegaard and Sarwar (1998) who estimated that upon breakdown of brassica green manures incorporated into soil GLS was released at 135 μg g^{-1} soil. This equated to about 20 μg g^{-1} soil of ITC cation, and 30 and 60 μg g^{-1} soil of hydrolysis products contain about 10 and 20 μg g^{-1} soil of ITC or nitrile cation respectively. Urea was applied as a solution and the GLS hydrolysis products were dispersed in water by sonicating for 30 min. The soil was placed in a randomised block design in a constant temperature room where temperature was maintained at 20 °C. Jars were covered in perforated parafilm™ to allow for normal gas exchange yet minimising evaporation loss. During subsequent incubation, soil moisture content was maintained at 70% of water holding capacity with distilled water following weighing to determine water loss.

A matching set of soils and treatments was established to allow for measurement of mineral N transformations. Soil (140 g) was weighed into plastic bags and the same treatments applied. These bags were also stored in a constant temperature room at 20 °C. The bags were stored closed but opened regularly to allow for gas exchange as per the jars and soil moisture monitored and adjusted through time.

2.1.2. Gas flux measurement

Nitrous oxide and CO_2 fluxes were measured 11 times over the 40 days of incubation (0, 1, 2, 3, 5, 7, 9, 13, 20, 27 and 40 days following treatment application). This time length was chosen because N_2O flux had generally returned to background by this time. Sampling was

Table 1
General physical and chemical properties of the soil (Bruntwood silt loam, 0–7.5 cm) used for the incubation trial.

Soil texture (%)			Total-N (%)	Total-C (%)	Olsen P (mg kg^{-1})	pH
Sand	Silt	Clay				
17	61	22	0.61	6.0	34	5.9

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