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ARLETE SIMÕES BARNEZE

N_2O emission from soil due to urine deposition by grazing cattle and potential mitigation

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“All our dreams can come true, if we have the courage to pursue them”
Walt Elias Disney

ABSTRACT

BARNEZE, A. S. **N_2O emission from soil due to urine deposition by grazing cattle and potential mitigation**. 2013. 87 p. Dissertation (M.S.) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2013.

Grazing pasture is a major system of livestock production in many countries and it has been identified as an important source of N_2O from urine deposition on soils. The aim of this study was to determinate the N_2O emissions from soil after urine deposition and the emission factor, in addition, determine how temperature and water content of the soil influence these emissions. We also intended to study a potential of mitigation using nitrification inhibitors. Soil and gas samples were collected in traditional livestock areas in Brazil and UK to evaluate the N_2O emission dynamics under field conditions. In addition, incubation experiments were conducted to evaluate how temperature and water content affect N_2O emissions in the soil and to study the potential mitigation on N_2O emission from the soil after urine application, using two distinct nitrification inhibitors. In the field experiment, the N_2O emission factor for cattle urine was 0.20% of the applied urine N in Brazil and 0.66% for the UK conditions. The incubation experiments showed the N_2O emissions after urine application are higher in soils with high moisture and high temperature. The nitrification inhibitor effectiveness was not statistically significant, however had shown some N_2O emission absolute reductions among 6% to 33% comparing with urine only application on the soil. Various physical and biological factors can be influence the effectiveness of the products. It confirmed that urine deposition can contribute to N_2O emission from the soil and the temperature and water content can markedly increase these emissions. The nitrification inhibitors have a potential mitigation effect since some decreased emissions of almost 40%. The results in this study are pioneers and can be used as a basis for more complex evaluations and to help with determining the carbon footprint of beef production worldwide.

Keywords: Liquid manure. Nitrous oxide emission. Nitrification inhibitors. Livestock. Temperature. Water-filled pore space. Soil mineral nitrogen.

RESUMO

BARNEZE, A. S. **Emissão de N_2O do solo devido à aplicação de urina e o potencial de mitigação**. 2013. 87 p. Dissertação (Mestrado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2013.

Considerado o maior sistema de produção animal em muitos países, as pastagens tem sido identificadas como uma importante fonte de emissão de N_2O, devido à deposição de urina ao solo. O objetivo deste estudo foi determinar as emissões de N_2O do solo após a deposição de urina e seu fator de emissão, além disso, determinar como temperatura e teor de água do solo influenciam as emissões. Pretendeu-se também estudar o potencial de mitigação das emissões de N_2O usando inibidores de nitrificação. Amostras de solo e de gás foram coletadas em áreas tradicionais de pastagens do Brasil e do Reino Unido para avaliar a dinâmica das emissões de N_2O. Experimentos de incubação também foram realizados para avaliar a influência de fatores como temperatura e teor de água no solo nas emissões, além de avaliar o potencial de redução das emissões de N_2O do solo após a aplicação da urina, utilizando dois inibidores de nitrificação. Nos experimentos de campo realizados no Brasil e no Reino Unido, o fator de emissão do N_2O para a urina foi de 0,20% e 0,66% do nitrogênio na forma de urina bovina aplicada, respectivamente. Nos experimentos de incubação, as emissões de N_2O após a aplicação de urina foram maiores em solos com alta umidade e alta temperatura. A eficácia no uso dos inibidores de nitrificação não foi estatisticamente significativa, no entanto mostrou uma redução absoluta entre 6% a 33% nas emissões de N_2O comparado com a aplicação de apenas urina ao solo. Vários fatores físicos e biológicos podem ter influenciado a eficácia dos produtos. Dessa forma, confirma-se que a deposição de urina pode contribuir para a emissão de N_2O do solo e que a temperatura e o teor de água no solo podem aumentar consideravelmente essas emissões. Os inibidores de nitrificação podem ser usados como um potencial de mitigação, já que houve redução em termos absolutos de quase 40% nas emissões. Os resultados encontrados neste estudo são pioneiros e poderão ser utilizados como base para avaliações mais complexas e contribuir para a determinação da pegada de carbono na produção de carne mundial.

Palavras-chave: Dejeto líquido. Óxido nitroso. Inibidores de nitrificação. Pecuária. Temperatura. Teor de água no solo. Nitrogênio mineral no solo.

SUMMARY

1 INTRODUCTION

Recently one of world´s most concern is related to environmental impacts. Greenhouse gas emissions (GHG) are on the top of studied problems and their mitigation is a major challenge to modern society. Countries with reduction targets have developed studies to understand the processes and reduce emissions. These issues will become more important as nations align themselves with international agreements and policies to reduce environmental impacts.

Life cycle inventories are derived from indicators of environmental impact dealing with the potential effects on humans, environmental health and resources (SAUR, 1997). The carbon footprint has become the most important environmental protection indicator over the last few years (WIEDMANN; MINX, 2008; LAM et al., 2010). It usually stands for the amount of carbon dioxide (CO_2) and other greenhouse gases (converted to CO_2-equivalent) emitted over the full life cycle of a process or product. In that way, it is important to know the effect of each gas on the whole life cycle.

Agriculture releases to the atmosphere significant amounts of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (COLE et al., 1997; IPCC, 2001; PAUSTIAN et al., 2004). The most important practices include cropland management, grazing land management/pasture improvement, management of agricultural organic soils, livestock management and manure/bio-solid management. Their contribution is about 58% of total anthropogenic N_2O emissions, with a wide range of uncertainty in the estimates of both the agricultural contribution and the anthropogenic total (IPCC, 2007).

Grazing pasture is the main system of livestock production in many countries and it has been identified as an important source of N_2O from urine deposition on soils. Extensive management cause a substantial impact due to the large number of animals that such systems support, although it is characterized with low grazing intensity (1 $head^{-1}$ ha^{-1} $year^{-1}$ – Brazilian average) (FERRAZ; FELICIO, 2010) distributed in an abundant grazing land in the *Cerrados* region.

Nitrous oxide is the third most important anthropogenic GHG with a global warming potential 298 times that of CO_2 (IPCC, 2007). Besides that, the continuous increase in N_2O emissions to the atmosphere constitutes a major environmental

concern. An increase in N_2O emission by 35-60% up to 2030 is expected due to increases in nitrogen (N) fertilizer use and animal manure production (FAO, 2003).

Adequate mitigation of these emissions is only possible if we understand the processes of N_2O production. In view of this, the goal of this study was to determine the specific emission factor for tropical conditions, understand the process and the factors that affect it, including the investigation of strategies to mitigate the emissions.

Initially, one pasture area in Southeast region of Brazil was chosen to provide some of the first data relating N_2O emissions to urine deposition by grazing cattle in Brazil (*Chapter 3*). After that, an experiment under controlled conditions was set up to determinate the effects of temperature and water content on N_2O emissions and on mineral N dynamics in the soil (*Chapter 4*). Field and incubation experiments were conducted in United Kingdom to evaluate the N_2O emissions after urine application with or without nitrification inhibitor, a potential mitigation option (*Chapter 5*).

This insight about N_2O emission after urine deposition provides valuable information to improve the knowledge about the emission from grazing system. This information can be used to calculate the carbon footprint of beef production and to incentive the research on mitigation strategies.

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2. LITERATURE REVIEW

2.1 Greenhouse effect and climate change

The atmosphere and land surface are kept heated by energy from the sun in the form of ultra-violet, visible and infrared radiation. The ultra-violet radiation, in the stratosphere is intercepted by gas molecules such as diatomic oxygen and ozone and only a fraction of this energy reaches the earth. Fifty percent of incident energy is absorbed by the Earth´s surface; other 20% are absorbed by the gas phase and water droplets in the air. The remaining 30% are reflected back into space by reflective bodies. To keep the earth temperature constant, the amount of energy that the planet absorbs and releases must be the same. Some gases present in the air can temporarily absorb thermal infrared light, hence that not all energy released from the earth escapes into space. The light absorbed by these gas molecules is re-emitted in all directions randomly, resulting in energy reabsorption and causing an additional warming of the earth's surface and the air. This phenomenon is known as the greenhouse effect and is responsible for the average surface temperature at the middle ground to be between 15°C and 18°C (BAIRD, 2002).

Over the last century, the increase of trace gases concentration in the atmosphere is intensifying the natural greenhouse effect in about 0.74°C (IPCC, 2007). The main greenhouse gases are CO_2, CH_4, N_2O, water vapour (H_2O), ozone (O_3) and chlorofluorocarbons (CFCs). They reach the atmosphere mainly due to anthropogenic activities, either directly by increased use of fossil fuels, industrial pollution and fires, or indirectly by irrational use of natural resources and also by agriculture, in many cases, practiced in an unsustainable way.

2.2 The nitrous oxide

Nitrous oxide is well known as “laughing gas”, despite it is becoming a highly dangerous gas. N_2O emissions have been and still are steadily rising since the start of the industrial era. This gas become the prime ozone depleting emission while breakdown stratospheric ozone (CRUTZEN, 1981; DUXBURY et al., 1993) and the third most important anthropogenic greenhouse gas (IPCC, 2007), with a global warming potential, approximately, 300 times higher than CO_2.

Nitrous oxide is produced through several processes in the N cycle, related to the cycling of reactive N. It mainly may be emitted by nitrification and subsequent denitrification of the formed NO_3^-.

Microorganisms can break down organic N to (inorganic) ammonium (NH_4^+) through mineralization. In this step the organic N become available for plants and microorganisms. Microorganisms can take up NH_4^+ and convert it to nitrite (NO_2^-) and nitrate (NO_3^-) by nitrification. Through denitrification, microorganism turns NO_3^- again to (gaseous) N_2 (KNOWLES, 1982; ZUMFT, 1997).

These processes may occur simultaneously in different microsites of the same soil (STEVENS et al., 1997), but there is often uncertainty associated with which process is predominantly contributing to emissions.

N_2O production from urine occurs only under specific conditions combining aerobic and anaerobic processes, in other words, nitrification and denitrification, respectively. Recently it is increasingly been suggested that nitrifer denitrification (denitrification by autotrophic nitrifiers) may constitute a considerable contribution to N_2O production in soil (WRAGE et al., 2004; MA et al., 2007). Although the N_2O production by nitrification is possible, the N_2O emission peaks in soils are generally attributed to the denitrification process (WRAGE et al., 2001; LEE et al., 2006; LIU et al., 2007).

According with Koops et al. (1997) N_2O emission by urine applied in very dry soil is mainly produced by nitrification, as an aerobic process. The maximum nitrification occurs with soil at 35-60% water content (KHALIL et al., 2004; BATEMAN; BAGGS, 2005) and NH_4^+ is available in the soil.

In moist soil with 60% of water filled pore space (WFPS), denitrification is the predominant source of nitrous oxide emissions, due to N mineralization and hindered diffusion of O_2 into the soil, favouring the formation of anaerobic environments (MONAGHAN; BARRACLOUGH, 1993; PARTON et al., 1996; MERINO et al., 2001; BATEMAN; BAGGS, 2005). However, ambient with low O_2 may occur naturally in aerated soil like within an aggregate, where the diffusion of O_2 is low. In the two processes described, N_2O is an intermediate product of soil microorganism's metabolism being released to the atmosphere.

In addition to the factors mentioned above, temperature is another important factor that can affect microbial activity. The temperature effect is normally expressed as Q_{10} value, which has the advantage to standardize the temperature effect. This

value corresponds to the increase of reaction rate due to the increase in temperature of 10°C. For N_2O emission, values reported in the literature vary between 2 to 10 (DOBBIE et al., 1999; SKIBA; SMITH, 2000).

Globally, livestock grazing is estimated to contribute with 1.5 Tg of N_2O–N release per year, which is more than 10% to the global annual N_2O budget (KHALIL; RASMUSSENR, 1992; OENEMA et al., 1997; 2005; IPCC, 2007). In a literature review, Oenema et al. (1997) estimated that between 0.1 and 3.8% of urine-N is emitted to the atmosphere as N_2O.

2.3 The importance of livestock on greenhouse gas emission

In grazing system, carbon and nitrogen can be exchanged in many forms among atmosphere, plant, soil and animal. Sustainable pasture depends of nutrients balance to produce dry matter of sufficient nutritional quality for livestock (JARVIS, 2000). Recently, studies are focused on the impact of nutrient management on environmental quality and effects on air, soil and water composition.

The principle for livestock production is the conversion of plant protein to animal protein. Unfortunately, the conversion is inefficient, as for every 1 kg of high-quality animal protein produced, livestock consumed about 6 kg of plant protein is consumed (PIMENTEL; PIMENTEL, 2003). The inefficient conversion is a natural limitation, not dependent only of the grass management and quality but also animal genetic.

The production of high-quality animal protein depends of the availability of nitrogen, which is determined by the balance among inputs of biological N_2 fixation, anthropogenic sources and atmospheric N deposition, the recycling of plant residues and losses in gaseous (N_2O and N_2), inorganic (NO_3^- and NH_4^+) and dissolved organic matter forms (VITOUSEK et al., 2002).

Losses of carbon and nitrogen in gaseous form are the most important contributor for greenhouse gases emissions from grazing system, and consequently to climate change through CH_4 and N_2O emissions. The emission rates are related to the management adopted in the production system as a whole. The most important gas is CH_4 derived from cattle´s enteric fermentation, which represent about 22% of total global emissions generated by anthropogenic sources, while animal waste is estimated to be around 7% (IPCC, 2007).

The emission of N_2O is mainly related to the deposition of urine on the soil by animals, and also to fertilization with mineral N of pastures, thereby stimulating bacterial activity that produces nitrous oxide (WILLIAMS et al., 1999).

Uneven deposition of excretal N by grazing animals can result in ´hotspots' equivalent to an application of 400-2000 kg N ha^{-1} $year^{-1}$ in the small affected area (WATSON; FOY, 2001), leading to wide spatial and magnitude variations in N_2O emissions. These ´hotspots´ correspond to 14.5% of 1 ha during one year, considering a patch area of 0.4 m^2 per urine deposition (HAYNES; WILLIAMS, 1993). In general with cattle, the proportion of the excretal N occurring in the urine increases from about 45% when the diet contains 1.5% N on dry matter basis to about 80% when the diet contains 4.0% N. Urea generally accounts for between 60 and 90% of the total N in the urine (BRISTOW et al., 1992; LANTINGA et al., 1987). Urine includes, in addition to urea, a number of other nitrogenous compounds, mainly hippuric acid, allantoin, uric acid, xanthine, hypoxanthine, creatine and creatinine. The proportion of the total urinary N accounted for by these compounds varies considerable.

Extensive cattle breeding occupy approximately 48% of arable land in Brazil. It represents the largest commercial herd in the world, accounting in 2010 for 14% of global beef production, according to the United Nations Food and Agriculture Organization (FAO, 2013). The latest agricultural census released by the Brazilian Institute of Geography and Statistics (IBGE/PPM, 2011), indicates that Brazil has 212.8 millions of beef cattle head, or more than one head per person. The main beef production system (96%) is characterized by the use of a large territory with pasture management performed in a continuous manner (ANUALPEC, 2011). Most of the slaughtered animals (60%) for beef production are 4.5 years old steers, with an average weight of 450 kg (FERRAZ; FELICIO, 2010).

Agriculture and livestock are the major contribute for greenhouse gas emission in Brazil, promoting 476 Gg N_2O emissions, representing 87% of the total N_2O emission. The urine deposition in soil by animals during grazing is the most important source of N_2O emissions from agricultural soils in Brazil. These emissions contribute with 48% of N_2O on agriculture; it means 41% of total CO_2-equivalent emissions (BRASIL, 2010; CERRI et al., 2009).

In by comparison, the managed grassland (< 5 years old and permanent grassland) occupies c. 7.0 M ha in UK. The animals for beef production consist of

steers (castrated bulls), heifers (young females) and young bulls. The calves can be from beef herds or dairy herds, depending of the rearing and finishing system. The complex mix accounts for the wide diversity in the quality and prices of beef products in the UK. This sector is recognized in the national inventory as being the largest single source of N_2O (JACKSON et al., 2009) corresponding to more than 50% of GHG emissions in CO_2-equivalent basis. The N_2O emissions represent 80% of total emission, originated by fertilizer N applications, grazing (urine) returns and manure applications to land on agriculture according with UK GHG Emission Inventory 2007 (JACKSON et al., 2009). This inventory also assumes that grazing returns contribute with 4.3 Mt CO_2-equivalent or 17% of total N_2O emissions from UK agriculture. These values had shown the importance to study the environmental impacts of this livestock activity in the world and build a GHG Emissions National Inventory.

2.4 Potential mitigation: the nitrification inhibitors

In grazing production systems, most of the NO_3^- leached and N_2O-N emitted are derived from the N deposited particularly by animal urine (DI; CAMERON, 2002; DI et al., 2007).

One of the new technologies that has been shown to be effective to decrease both NO_3^- leaching and N_2O emissions is the use of a nitrification inhibitor (NI) to treat grazed pasture soils (e.g. ABBASI; ADAMS, 2000; DI et al., 2007; ZAMAN et al., 2009; SAGGAR et al., 2009; AKIYAMA et al., 2010). In recent years, numerous compounds, chemical, agents or materials have been identified and used as NIs. The application to the soil temporarily delays the bacterial oxidation of the NH_4^+ to nitrite by inhibiting Nitrosomonas spp. (ZERULLA et al., 2001). The prerequisite for denitrification is the presence of a source of nitrate; in view of this, the application of nitrification inhibitor can minimize the N_2O emission from denitrification as well as from the nitrification process. Both processes are main responsible for N_2O emission on the soil.

DCD (dicyandiamide) is a very popular NI in European countries, and has been shown to be an effective nitrification inhibitor (DI; CAMERON, 2003; 2006; DI et al., 2007; DE KLEIN; ECKARD, 2008; SMITH et al., 2008). The DCD can retain the N in the ammonium form for a longer period, reducing nitrate leaching losses, increasing N retention in soil and pasture production (DI et al. 2007). It has potential to improve

environmental sustainability and advance the efficiency of mineral N cycles within soil, plant and animal systems (DI; CAMERON, 2003, 2005).

DCD is naturally broken down in the soil into nontoxic products, with no traces of residue left beyond the cropping year (AMBERGER, 1989). Also, it is easy to blend with fertilizers due to minimally volatile nature (CAMERON; DI, 2002).

It has also some disadvantages as a slightly more expensive for large-scale use in agriculture. Its action is comparatively weak, so high application rates are needed for effective nitrification inhibition. Additionally, under certain agroclimatological conditions, DCD use may cause phytotoxic problems like visible plant damage (MACADAM et al., 2003), which, although not leading to reduced yields, affects marketability of leaf vegetables.

Many experiments have been testing the DCD application directly on urine patch in pastures. These studies shows high efficacy of DCD in reducing N leaching and N_2O emissions (DI; CAMERON 2007; MONAGHAN, 2009). However, it is difficult to apply DCD on commercial pastoral farms because there is no technology available to precisely apply only on urine patch and the application over the whole farm has not been economically efficient.

In New Zealand they currently spray the DCD onto the pasture, but this would not be cost-effective in the other countries with large extensive grazing. In view of this, studies led by New Zealand are looking at feeding DCD to the cattle. Then the oral administration of DCD to ruminant animals will be subsequent excreted in the urine (LEDGARD et al., 2008). According with Ledgard et al. (2008) there are a number of potential methods of administering DCD to ruminants including daily oral drenching, delivery in feed supplements and controlled release delivery systems in the rumen. Welten et al. (2013) found that prolonged daily administration of DCD to dairy heifers resulted in sustained excretion of DCD in the urine that effectively inhibited nitrification of urinary-N. So, this could be a solution, but may have some ethical/food safety issues to discuss later on.

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3 NITROUS OXIDE EMISSIONS FROM SOIL DUE TO URINE DEPOSITION BY GRAZING CATTLE IN BRAZIL

Abstract

Urine deposition to the soil can result in nitrous oxide emissions through the microbial processes of nitrification and denitrification. The objective of this experiment was to estimate N_2O emissions from urine depositions in summer to grassland in Southeast Brazil. In order to achieve the objective, a field experiment was conducted in which N_2O emission from known volumes of urine applied to the soil were measured using the static chamber method. Measurements continued for one month after application. Application of urine to soil increased N_2O fluxes compared to those from the control site. There were two significant N_2O emission peaks for the urine treatment, between days 2 and 9. The N_2O emissions accounted for 0.2% of the urine N, lower than the current IPCC default emission factor of 2% of N excretion for grazing cattle. The information obtained from this research will be useful as a contribution to the scientific basis for developing inventories of GHG emissions with different levels of complexity (Business, Regional or National) for extensive cattle production of cattle.

Keywords: Livestock. Greenhouse gas emissions. Liquid manure. Pasture. Emission factor. Nitrogen.

3.1 Introduction

One of the most important current environmental issues is the increase in global warming caused by greenhouse gas (GHG) emissions. Nitrous oxide (N_2O) is a potent greenhouse gas for which agriculture is a major source, with a global warming potential 298 times that of carbon dioxide (FORSTER et al., 2007).

In grazing livestock systems, urine patches contribute significantly to anthropogenic emissions, with nitrogen (N) deposition rates of between 500-1000 kg ha^{-1} to the soil (HAYNES; WILLIAMS, 1993). The urea content of cattle urine will readily hydrolyze to form ammonium after deposition to the soil. Nitrous oxide may then be emitted through the microbiological processes of nitrification and denitrification, which are affected by soil and climatic factors such as soil enzyme activities, nitrate concentrations, pH, available carbon, rainfall/irrigation, water-filled pore space and temperature (BOLAN et al., 2004; LUO et al., 2008).

The Intergovernmental Panel on Climate Change (IPCC, 2006) gives a default N_2O emission factor from cattle urine of 2%, i.e. 2% of the urine nitrogen is assumed to be emitted as N_2O-N. However, there is large uncertainty in this emission factor and different countries and regions must have different specific N_2O emission factors due to different soil, temperature, rainfall and grazing systems, among others. In addition, the emission factor may be quite different for urine and faecal depositions (IPCC doesn't differentiate); it is generally assumed that emissions are greater from the urine N which is in a more readily available form than the faecal N (e.g. YAMULKI et al., 1998; LUO et al., 2009). To improve national inventories of GHG emissions, and as a first step to developing mitigation strategies for this source, it is necessary to develop specific emission factors for the systems and conditions relative to the specific country.

In order to estimate total N_2O emissions from urine deposition by grazing cattle, it is necessary to know the numbers of animals, the specific N_2O emission factor as described above, and the quantity of urine nitrogen being deposited. This latter would require an estimate of total urine volume and nitrogen concentration. However, total urine sampling is difficult in grazing animals due to the presence of catheters or funnels, causing discomfort in the animals. As an alternative to total urine collection, several authors, Valadares et al., 1999; Oliveira et al. 2001; Silva et al, 2001, have proposed the use of a spot sampling technique measuring urine creatinine

concentration to estimate total urine volume. According with Borsook and Dubnoff (1947), creatinine is synthesized in the muscles and is excreted in the urine steadily in relation to body weight. This urine volume estimate obtained from spot sample is based on a constant creatinine excretion for a given body mass and it is not influenced with animal dietary, as described by Palmer et al. (1914), cited by ørskov e Macleod (1982). Thus, once it is possible to estimate the daily creatinine excretion from animal's body weight, the daily urine volume can be estimated from the creatinine concentration in a urine sample collected from spot sample, and then it will be possible to estimate the urinary volume.

The aim of this experiment was to provide the first data for Brazil relating to N_2O emissions from urine deposition by grazing cattle. Specifically, to measure N_2O emissions from urine applied to pasture in the summer in the Southeast region of Brazil.

3.2 Material and Methods

3.2.1 Experimental site

The experiment was carried out on a permanent grassland, from 31 January to 29 February of 2012 (summer) at Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ), Piracicaba, São Paulo state, Brazil (22º 42´07´´S; 47º 37´17´´W, 530 m above sea level) under tropical climatic conditions (Kö ppen climatic classification). The soil was a sandy loam classified as Nitisol (FAO, WRB). Soil properties (upper 10 cm) at the start of the experiment were: total N of 0.29%, total C of 3.03%, organic matter of 35 g dm^{-3}, pH of 5.6 and bulk density of 1.13 Mg m^{-3}. The pasture was not grazed by livestock before or during the experiment and had not received any N fertilizer for five months prior to the experiment.

3.2.2 Urine characteristics

Urine was collected from a group of 10 steers (*Nelore)* about three years old with an average live weight of 350 kg. The steers were grazing pasture (*Brachiaria decumbens*) supplemented with mineral salts.

In order to estimate the daily urine production, a spot sampling technique was used to assess the excretion of creatinine. Spot samples were collected (totalling 20 L) and sub-samples of 10 mL of urine were taken and diluted with 40 mL of a solution of 0.036 mmol L^{-1} H_2SO_4 and stored at −20°C for later analysis of creatinine. This compound of the purine derivates was determined by a colorimetric system with end point reaction, using picrate and an acidifier, using commercial kits (555-A Sigma Chemical Co., St. Louis, MO).

From the measured creatinine concentration in the spot sample and the estimated daily average creatinine excretion according with equation from Chizzotti et al. (2004), the daily urine volume can be estimated divided measured creatinine concentration by estimated creatinine. From analysis of the spot samples, total N and ammonium N concentrations of the urine were 5.5 g L^{-1} and 12 mg L^{-1}, respectively. Urine total volume was estimated using a creatinine analysis as 10.5 L $steer^{-1}$ day^{-1}, agreeing well with estimates of 10.9 - 11.1 L day^{-1} given by Silva (2001). From observations of the animal behaviour over a period of 15 days, an average of 10 urination events per day was estimated, agreeing well with observations by Orr et al. (2012) and Whitehead (1995). A typical urination event for a given steer would therefore constitute a volume of approximately 1L urine. This was the volume of urine used in this experiment to simulate a single urine patch.

3.2.3 Nitrous oxide measurement

Nitrous oxide fluxes were determined using the static chamber method (BOWDEN et al., 1990). Chambers (0.064 m^2) were randomly distributed across the field with a minimum distance of 5 m from each other. Chambers either received 1L of urine (urine treatment) or no urine (control treatment), with 5 replicates for each treatment. The application of urine was carried out on the first day of the experiment, less than half-hour before the beginning of sampling.

For each flux measurement, the static chambers were closed and a first gas sample (t_0) was taken immediately. After 5, 10 and 20 minutes additional samples were taken (t_5, t_10 and t_20). Gas samples were taken using a 20 mL polypropylene syringe and stored in pre-evacuated vials. Nitrous oxide concentrations were measured using a Gas Chromatograph (Shimadzu) fitted with an electron capture device with packed columns and oven temperature between 75

and 85°C. The N_2O concentration was determined using an electron capture detector (ECD). Gas fluxes were calculated by fitting linear regressions through the data collected at t_0, t_5, t_10 and t_20 and were corrected for temperature and barometric pressure according to ideal gas law from *Eq. (1):*

$$F = \rho \frac{V}{A} \frac{\Delta c}{\Delta t} \frac{273}{(T+273)} \qquad \textit{Eq. (1)}$$

Where: F= N_2O flux (mg m^{-2} h^{-1}); ρ = density of N_2O (mg m^{-3}); V = volume of chamber (m^3); A = base area of chamber (m^2); $\Delta c/\Delta t$ = average rate of change of concentration wit time (ppmv h^{-1}) and T = temperature in the chamber (°C).

Gas sampling was conducted every three hours after the treatment application on the first day and then daily during the first week of the experiment, and twice a week until the last day of the experiment. Cumulative N_2O flux was calculated by linear interpolation of the average N_2O emissions between the measurements and summing the results over the total time period.

Meteorological data (rainfall and air temperature) were recorded at the nearest meteorological station, which was within 1 km of the field site. The air temperature inside and outside the chamber was measured at each site and used to correct the concentrations of N_2O inside the chamber as described above.

3.2.4 Derivation of an emission factor

An emission factor (EF) was calculated according to *Eq. (2)*, by using the arithmetic mean per treatment of the accumulated N_2O emissions over the experimental time.

$$\mathrm{EF}_{\%} = \left[\frac{(\mathrm{N_2O} - \mathrm{N}_{urine} - \mathrm{N_2O} - \mathrm{N}_{control})}{\mathrm{N}_{applied}}\right] \times 100 \qquad \textit{Eq. (2)}$$

3.2.5 Statistical analysis

Data were verified for normal distribution and treatment means for daily N_2O fluxes and cumulative flux over the period of the experiment were compared using one-way analysis of variance (GenStat, VSN International). To determine the statistical significance of the mean differences, Tukey tests were carried out at 0.05 probability level.

3.3 Results and discussion

3.3.1 Climatic conditions and nitrous oxide emissions

The air temperature ranged from 22 to 28°C (Figure 1a). The daily soil temperature ranged from 15 to 26°C (Figure 1b), although it was above 21°C for more than 75% of the time. There were two peak rainfall events between 10-22 days after urine application, with a maximum daily rainfall of 49.8 mm on day 11 (Figure 1c).

There was an immediate step increase of N_2O emission from the soil after urine application compared to the control treatment (Figure 1d). There were two significant N_2O emission peaks for the urine treatment, between days 0 and 9 with a maximum emission rate of 1250.25 (± 336.7) μg N_2O-N m^{-2} h^{-1}, and between days 10 and 18 with a maximum emission rate of 863.32 (± 414.4) μg N_2O-N m^{-2} h^{-1}. After that, emission from the urine treatment was not significantly different from the control (P>0.05) (Figure 1d). The highest peak N_2O emission was on the 3rd day, very shortly after the urine application when the soil temperature was 24ºC. The second peak coincided with rainfall events at 10-15 days after urine application. The higher N_2O emissions are related with higher standard error. It often occurs when we study the gases emissions; the emissions variability is common as found in many studies (UCHIDA et al., 2011; DOBBIE; SMITH, 2001; LUO et al., 2008; ZAMAN; NGUYEN, 2012). However, it can be observed that these peaks corresponding with statistical difference between control and urine treatments.

The measured N_2O emissions had a temporal variation between around 100 to 900 μg N_2O-N m^{-2} h^{-1}, dependent especially on climatic conditions. Previous studies also have shown this interaction (HYDE et al., 2006; RAFIQUE et al., 2012).

The first peak in our study is comparable with other studies. Maljanen et al. (2007) reported a maximum emission rate of 1200 μg N_2O–N m^{-2} h^{-1}, while de Klein et al. (2003) recorded maximum emission rate of 300 to 4900 μg N_2O–N m^{-2} h^{-1} from cattle urine applied to grass. Ma et al. (2006) and Lin et al. (2009) reported peak rates of 1426 and 1707 μg N_2O–N m^{-2} h^{-1} from sheep and yak urine, respectively. In New Zealand, in studies on silt loam soil with clover-based pasture, Luo et al. (2008) reported higher N_2O emissions from the soil after cow urine application compared with those from our study, with emission peaks between days 1 and 21. The emission observed immediately after urine application may be as a result of nitrification, due to the increase in ammonium nitrogen levels in the soil.

The first peak was about one and half times higher than the second. For the second peak, we can assume that denitrification was the predominant process leading to N_2O emissions due to the rainfall increasing the soil water content. Soil water content is an important factor influencing denitrification, with increasing rates under more anaerobic conditions (DE KLEIN; VAN LOGTESTIJN, 1994; JARVIS et al., 1991). This suggests that nitrification conditions (from a water-filled pore space (WFPS) perspective) were more frequently experienced at this site than denitrification conditions.

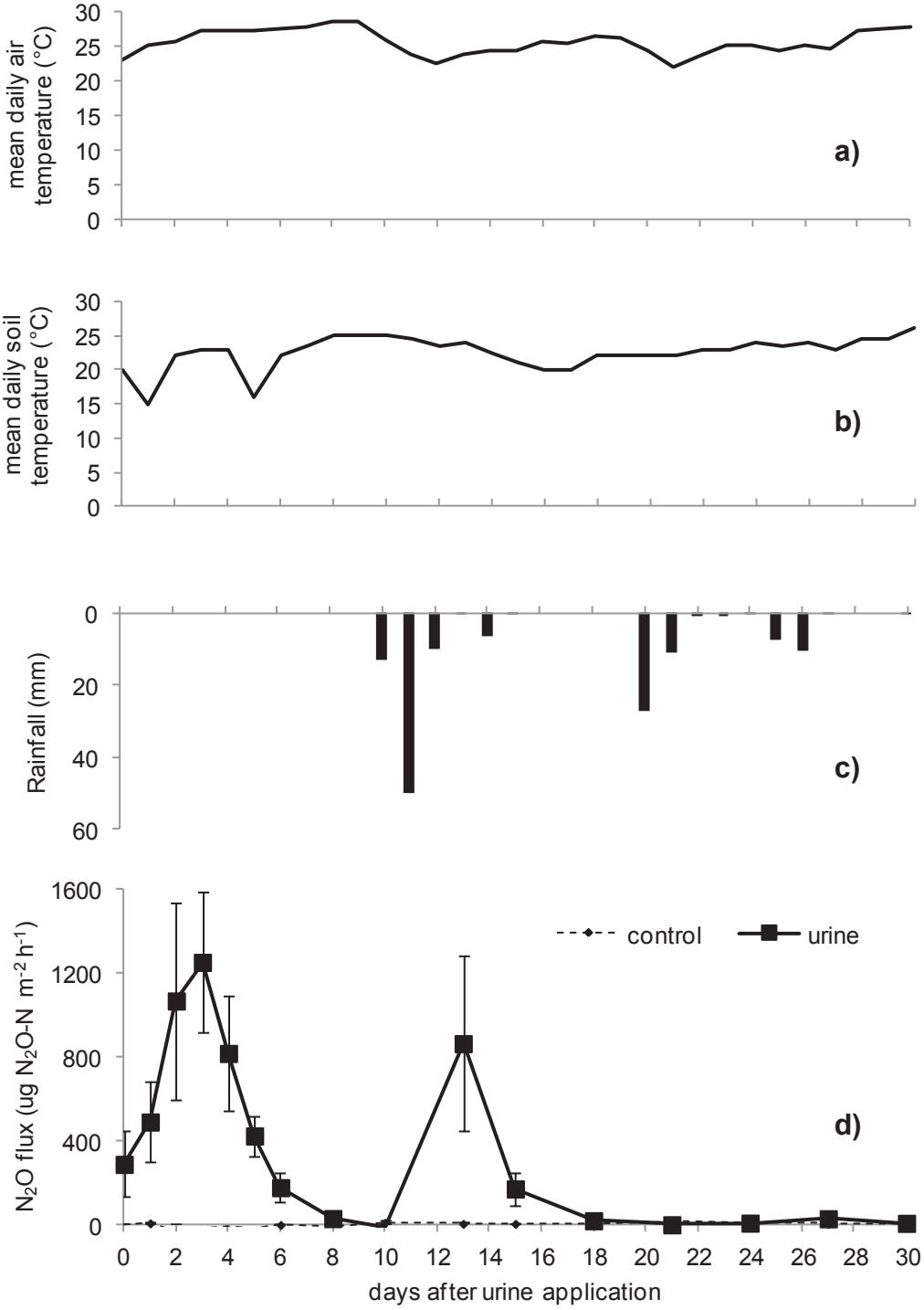

Figure 1 - (a) mean daily air temperature in °C; (b) mean daily soil temperature measured at 5 cm in °C; (c) daily rainfall in mm; (d) N_2O emission from the soil following urine application on summer season. Vertical bars show ±1 standard error (n=5). Piracicaba-SP, Brazil. 2012.

3.3.2 Nitrous oxide emission factor

The cumulative N_2O-N emission from urine application was higher than the control, with a net emission of 0.17 g N_2O-N m^{-2} after 30 days of the experiment. Following the *Eq. (2)*, with 85 g N-input m^{-2} was found 0.20% of N_2O EF from the summer experiment in Brazil.

Using this EF, an emission per animal can be derived using typical country values for urination rates and urine nitrogen content, which can be expressed as CO_2 equivalents. Using collected information (typical values for steers from grazing system in Southeast Brazil) as: urination per day = 10.5 L day^{-1}; nitrogen in urine = 5.5 g L^{-1}, was found 0.11 g N_2O-N $animal^{-1}$ day^{-1}; that can be expressed as 51.5 g CO_2 equivalent $animal^{-1}$ day^{-1}.

The current IPCC default inventory EF for N deposition by grazing cattle is 2% (IPCC, 2006). In our study we found a much lower EF (0.2%). Luo et al. (2008) reported EFs for urine applications in New Zealand of between 0.02 and 1.59%, depending on the season.

This variability in reported EFs highlights the importance to determine specific EFs for each country or climatic region. The results from this study represent some of the first data for Brazil, but are limited in terms of duration (one month only), spatial representation (one site only) and seasonal representation (summer only). Extrapolating these results to regional or national emissions is therefore not appropriate. In addition, when expressing the EF per animal, the diet and level of productivity of the animal are also important factors to consider. To develop regional or national EF, many other studies are necessary to taking into account the range of soil, climate and management conditions within a country.

3.4 Conclusions

Application of steer urine to the soil increased N_2O emissions during the summer season in Southeast Brazil. Emission rates fell to background within one month after application. The N_2O emission factor for cattle urine was 0.20% of the applied urine N, much lower than the current IPCC default emission factor for grazing

cattle. This was equivalent to 0.0515 kg CO_2-eq $animal^{-1}$ day^{-1}. More research is needed in order to determine robust emission factors for the different regions of Brazil.

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4 NITROUS OXIDE EMISSIONS AND SOIL NITROGEN DYNAMICS DUE TO SOIL MOISTURE CONTENTS AND TEMPERATURES

Abstract

In urine patches on grazed pasture systems, nitrous oxide emissions can increase markedly due to biological processes such as nitrification and denitrification. Important factors can influence on these processes and consequently in nitrous oxide emissions, as soil water content and temperature. Soil incubation experiments were conducted in laboratory conditions at three levels of water-filled pore space (40%, 60% and 80% WFPS) and two temperatures (25ºC and 35ºC) with and without urine, with five replicates each. The mineral nitrogen dynamics and the N_2O emissions were measured over a period of 55 days. Urea hydrolysis, nitrogen mineralization and nitrification were higher at the higher temperature and higher soil water content. Significant effects of urine application, temperature and moisture were found ($P<0.05$) on the cumulative N_2O emissions. Mineral nitrogen transformations in the soil were faster at higher temperature and soil moisture content. Nitrous oxide emissions were greater from the urine treatments, with greatest emission reaching of 36 mg m^{-2} from the highest moisture content and at the higher temperature. The values on experimental conditions may not exactly represent field situations, but the trends relating to temperature and soil moisture content are relevant. Understanding the influence of these factors is important in predicting and seeking to minimise N_2O emissions from grassland soils.

Keywords: N_2O emissions. Temperature. Water-filled pore space. Cattle urine. Mineralization. Nitrification. Denitrification.

4.1 Introduction

The microbiological processes of nitrification and denitrification responsible for N_2O formation and emission in soils, and these processes are affected by several soil and climatic factors, such as water-filled pore space (WFPS), temperature (BOLAN et al., 2004; LUO et al., 2008; DAVIDSON, 1991; KEENEY et al., 1979) and soil nitrate concentrations (RYDEN; LUND, 1980).

Soil temperature is an important factor that affects the processes responsible for both N_2O and CO_2 production from soils (MCKENNEY et al., 1980; TIEDJE, 1988). It affects denitrification directly, considering that microbial activity generally increases with higher temperature, reaching its maximum at 30ºC (BOLAN et al., 2004). It is also expected that increases in soil temperature will lead to enhanced soil nitrogen (N) mineralization (KLADIVKO; KEENEY, 1987; STANFORD et al., 1973), and this potentially has implications for N_2O emissions.

In addition to soil temperature, the N_2O-forming processes are affected by soil water content. The soil moisture controls the intensity of aeration and the oxygen concentration in the soil. The interaction between moisture and aeration (oxygen status) in the soil matrix greatly influences nitrification (GOODROAD; KEENEY, 1984), nitrate formation and its stability (FOCHT; VERSTRAETE, 1977). Denitrification increases when WFPS increases above 60% for a wide range of intensive agricultural systems (LINN; DORAN, 1984; CONEN et al., 2000).

It is unclear which process is the major contributor to N_2O emissions. Koops et al. (1997) and Klemedtsson et al. (1988) have suggested that nitrification may be the major source of N_2O production from urine patches and soils receiving urea or ammonium fertilizers. The large amounts of NH_4^+ typically nitrified in the urine patch make it likely that nitrification would contribute significantly to N_2O losses from grazed pastures. However, Saggar et al. (2002) cite nitrification as much as denitrification as the major contributor to N_2O loss from dairy grazed pastures.

An improved understanding of the N_2O emissions dynamics and influencing factors is a key to proposing good practices for grassland livestock production systems leading to the reduction of such emissions. In the present study, our goal was to evaluate the effects of temperature and water-filled pore space on N_2O emissions and on the mineral N dynamics after urine application to the soil under controlled experimental conditions.

4.2 Materials and methods

4.2.1 Soil and urine samples

The soil used in this study was collected from the 0-10 cm layer of a grassland field in Piracicaba-SP, Brazil (22º 42´07´´S; 47º 37´17´´W). The soil texture is sandy loam (37.06% sand, 39.32% silt and 23.62% clay), classified as Nitisols (FAO, WRB). The soil was manually sieved at 2 mm, mechanically homogenized and then stored at 4ºC during two weeks prior to the beginning of the experiment. The main physico-chemical properties of the soil are presented in Table 1.

Table 1 - Soil characteristics in the incubation experiment. Piracicaba-SP, Brazil. 2012

Bulk density g cm^{-3}	pH	Total C	Total N	$[NH_4^+-N]$	$[NO_3^--N]$
		------- g kg^{-1} -------		------- mg kg^{-1} -------	
1.0	5.6	32.4	2.7	12.4	8.9

Urine was obtained from a randomly selected group of typical Brazilian steers (*Nelore*) in Southeast Brazil fed a fresh forage diet supplemented with mineral salts. Over a period of 72 hours before the start of the experiment urine was collected directly from under the urethra of the animals using plastic containers embedded in ice. The collected urine was bulked to form a composite sample and kept refrigerated (2ºC) to avoid urea hydrolysis. Before the beginning of the experiment, urine subsamples were analysed using standard laboratory methods to assess the following physico-chemical properties: pH: 8.3; total [N]: 5.5 g L^{-1}.

4.2.2 Experimental design

The experiment was conducted under controlled laboratory conditions, incubating soils in 1.2 L Kilner jars. The treatments comprised three levels of WFPS (40%, 60% and 80%) and two temperatures (25ºC and 35ºC) with and without urine addition, with five replicates of each for N_2O emission measurement and 1 replication of each for soil sampling.

Before the beginning of the experiment, soil was mixed with deionised water to bring the soil moisture content to 40%, 60% and 80% WFPS. Soil was packed

in each Kilner jar equivalent to 0.6 kg dry soil, leaving a headspace of about 0.6 L of air. For the amended treatments, 0.15 mL of urine was applied to the soil surface of each jar.

The lids of Kilner jars were removed between sampling dates to ensure aerobic conditions and the development of a uniform headspace above the soil surface. During the 55 days of incubation, the soil moisture content was kept constant by regularly weighing and adding deionised water whenever necessary.

4.2.3 Soil mineral N content

To follow the soil mineral N dynamics, a second series of Kilner jars (one per treatment) were packed with soil, receiving the same treatments as the Kilner jars for the N_2O flux measurements. Three soil cores (100 mm depth) were taken per Kilner jar, once per week and analysed for mineral N and water content. Soil mineral N was determined by extraction with 2 M KCl at a 1:2 proportion of soil and extractor (ZAMMAN et al., 1999). Soil extracts were filtered after 24 hours and stored at 4°C. NH_4^+ and NO_3^- were determined by automated flow injection analysis (FIA). Gravimetric moisture content was determined after drying soil sample at 105°C for 48 hours.

4.2.4 Measurement of N_2O emissions

Headspace gas N_2O concentration measurements were conducted daily during the first week of the experiment, then three times per week for the next two weeks and then twice per week until 55 days after treatment application. For each measurement, the Kilner jar was hermetically sealed by replacing the lid and an initial gas sample taken immediately (time-zero, t_0 sample) using a syringe and stored in a pre-evacuated glass vial. After 30 minutes the headspace of each jar was sampled again (time-thirty, t_30 sample). The jar lids were then removed and remained off until the next sampling occasion. Nitrous oxide concentrations in the sampled air were measured using a SRI Gas Chromatograph (Model 8610C). Gas fluxes were calculated by fitting linear regressions through the data collected at t_0 and t_30 and were corrected for temperature and amount of soil incubated according to ideal gas law from Eq. *(1)*:

$$F = \rho \frac{V}{A} \frac{\Delta c}{\Delta t} \frac{273}{(T+273)} \qquad \textit{Eq. (1)}$$

Where: F= N_2O flux (mg m^{-2} h^{-1}); ρ = density of N_2O (mg m^{-3}); V = volume of Kilner jar (m^3); A = base area of Kilner jar (m^2); $\Delta c/\Delta t$ = average rate of change of concentration with time (ppmv h^{-1}) and T = temperature in the Kilner jar (°C).

Cumulative emissions were calculated as the product of the mean flux rate between two successive sampling dates and the time interval between those dates.

4.2.5 Statistical analysis

Following verification that data were normally distributed, treatment means for daily N_2O fluxes, cumulative flux and soil mineral N content over the period of the experiment were compared using one-way analysis of variance (GenStat, VSN International). To determine the statistical significance of the mean differences, Tukey tests were carried out at 0.05 probability level.

4.3 Results

4.3.1 Soil mineral nitrogen content

Figure 1 and Figure 2 show the NO_3^--N and NH_4^+-N concentrations in the soil after urine application at 25°C and 35°C, respectively. The soil mineral concentrations showed similar temporal dynamics at both temperatures, but at different levels.

The NH_4^+ concentration was higher than NO_3^- concentration for the first day after urine application in both temperature experiments. However, the conversion of NH_4^+ to NO_3^- had no effect on 40% WFPS in both temperature experiments.

At 60% and 80% WFPS, at 25°C, urea hydrolysis occurred readily, giving increased contents of NH_4^+ in the first 12 and 9 days after urine application, respectively. At 35°C the peaks were found after 9 days of urine application with 230 mg NH_4^+-N kg^{-1} of dry soil.

The NO_3^- concentration had a higher peak at 80% WFPS in comparison with 40% and 60% WFPS in both temperature experiments.

4.3.2 N_2O emissions from the soil

Figure 1 and Figure 2 show the N_2O emissions from the soil following urine application over the 55 days of the incubation experiment at 25°C and 35°C, respectively. Nitrous oxide fluxes can be seen to begin as soon as the NH_4^+ concentration increases in the soil.

At 25°C N_2O emissions were recorded from all moisture contents until 27 days after the urine application, after which emissions became not significantly ($P>0.05$) different from the control treatments.

At 35°C the N_2O emission increased within 24 hours, fluxes increased to a maximum and then decreased after 16 days, except of one large peak at 41 days (80% WFPS). The higher temperature increased the N degradation rate in the soil and all emission occurred during the first 15 days. Peak N_2O emission rate was 2.4-fold at 35°C (after 14 days) compared to 25°C (after 3-4 days) for the 80% WFPS.

In both temperature experiments, N_2O emission was lower at 40% WFPS. The small significant emission was observed after 7 and 4 days after urine application at 25°C and 35°C, respectively. After that emissions were not significantly different from the control treatment at 40% WFPS.

Urine addition, across all treatments, showed significantly higher emissions than the control treatments until 43 days after application.

Analysis of variance ($P<0.05$) showed that there to be significant interactions between the moisture, temperature and urine. After the first day, there were significant effects of moisture, urine, temperature and significant interactions between moisture and urine until 14 days after urine application.

4.3.3 Cumulative emissions

Figure 3 shows the cumulative N_2O emission for each treatment. Overall, there were significant effects of urine, temperature and moisture on the cumulative emissions measured, except for 40% WFPS at 25°C and 35°C where there were no significant differences between the control and urine treatments. Generally,

emissions were greater from the urine treatments, when highest moisture content and higher temperature were applied, with maximum cumulative emissions of 10,223 and 36,205 µg N_2O-N m^{-2} at 80% WFPS for 25 and 35°C, respectively. Also, overall there were significant interactions of urine, temperature and moisture on the treatments, particularly for moisture content effects.

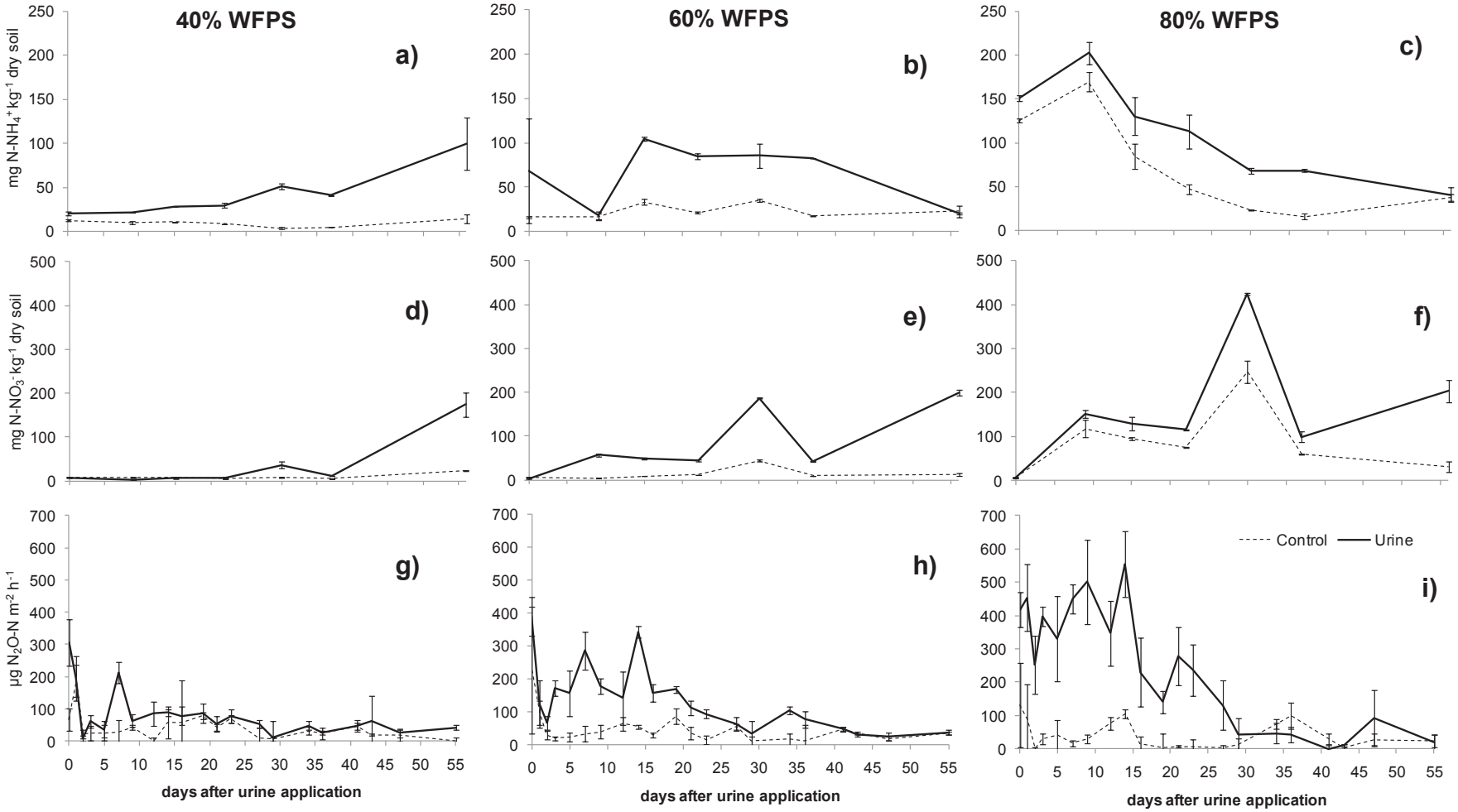

Figure 1 - Soil mineral NH_4^+-N, NO_3^--N concentrations and N_2O emission from the soil following urine application with 40%, 60% and 80% WFPS at 25°C. Mean values are shown (*n*=5) ± 1standard error. Piracicaba-SP, Brazil. 2012

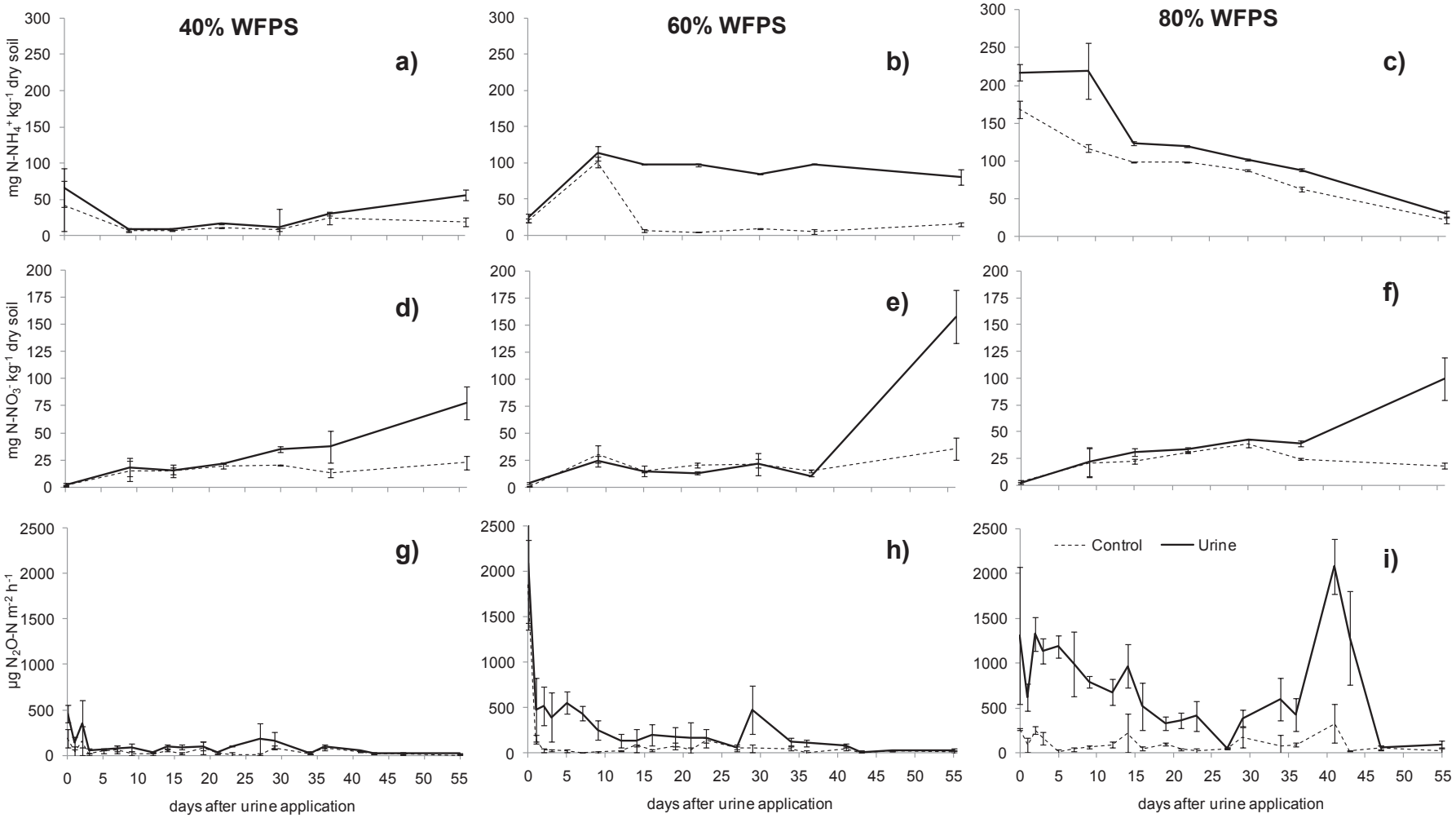

Figure 2 - Soil mineral NH_4^+-N, NO_3^--N concentrations and N_2O emission from the soil following urine application with 40%, 60% and 80% WFPS at 35°C. Mean values are shown (n=5) ± 1 standard error. Piracicaba-SP, Brazil. 2012

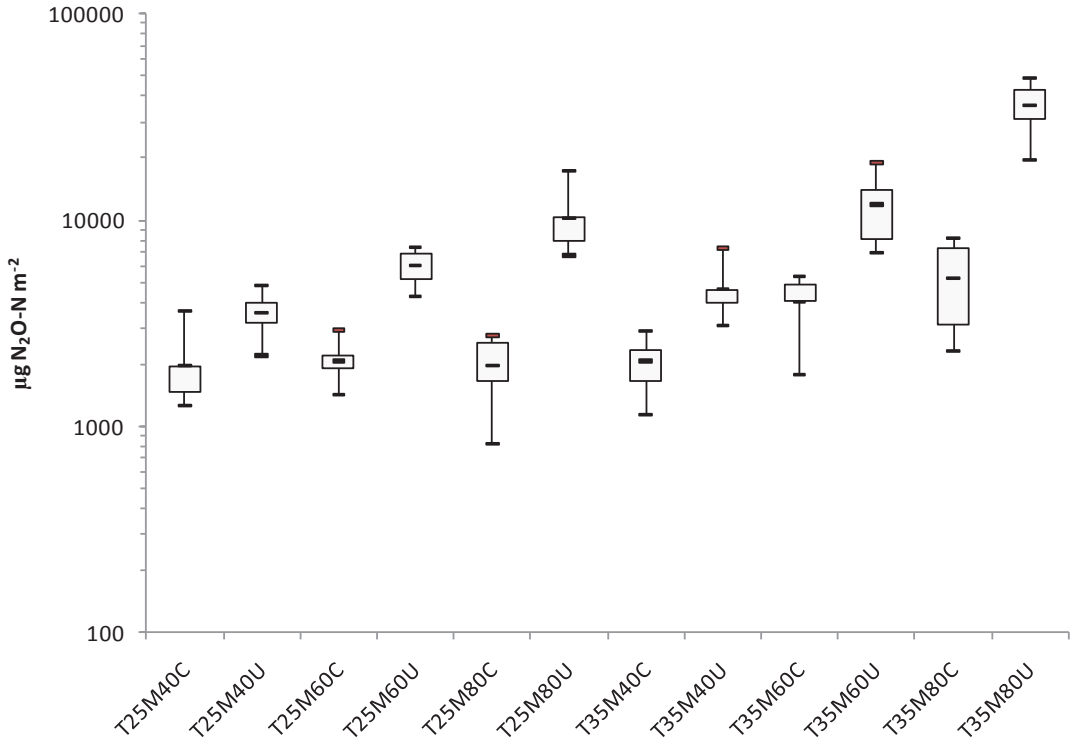

Figure 3 - Cumulative N_2O emission from each treatment (T25 and T35=temperature of 25°C and 35°C, respectively; M40, M60 and M80=moisture of 40%, 60% and 80% WFPS, respectively, C=control and U=urine). Vertical bars indicate standard errors. Piracicaba-SP, Brazil. 2012.

4.4 Discussion

4.4.1 Soil mineral N content

The observed dynamics (Fig. 1 and 2) were as expected according to the processes of urea hydrolysis and nitrification, as observed by other (ZAMAN et al., 2007; VLEK; CARTER, 1983). At 40% WFPS, the lack of conversion of NH_4^+ to NO_3^- was probably due to reduced soil moisture (EMMETT et al., 2004; LARSEN et al., 2011).

The peak events occurred at 8 days after application reaching ca. 220 mg NH_4^+ kg^{-1} dry soil, which declined by day 35 as nitrification occurred, similar to that reported by Zaman et al., 2009; Pereira et al., 2013. According with mineral N dynamics in the soil, when the NO_3^- concentration started low and increased as NH_4^+ concentration decreased over 30 days agreeing with Kool et al. (2006) and Zaman et al. (2009). The decrease of NH_4^+ and the increase of NO_3^- occurred at 15 days after urine application on 60% WFPS and later at 10 days at 80% WFPS at 25°C. The

WFPS influence the rate of O_2 diffusion into the soil profile and thus also determines if soil is predominantly aerobic or anaerobic.

The NO_3^- concentrations were higher at 35ºC than 25ºC, confirming that the temperature influences the N transformations in the soil (SAGGAR et al., 2007), affecting enzyme activities.

4.4.2 N_2O emissions from the soil

The magnitude of N_2O emissions increased with increasing WFPS, in agreement with other studies (DOBBIE et al., 1999; ABBASI; ADAMS, 2000; SKIBA; BALL, 2002). Relatively high N_2O emissions were found to occur only when soil WFPS, temperature, and NO_3^- concentration values were higher than 65%, 4.5°C and 5 µg N g^{-1} soil, respectively (DOBBIE; SMITH, 2003). Dobbie and Smith (2001) also found that water application resulted in an immediate increase in emissions.

Other studies have reported this initial emission within 24 hours (DOBBIE; SMITH, 2001; UCHIDA, et al., 2011) and increased emissions with temperature (DOBBIE; SMITH, 2001) and increased emissions with temperature (DOBBIE; SMITH, 2001). The effect of soil temperature on N_2O emissions is generally immediate. Temperature influences enzyme kinetics and metabolic turnover rates of nitrifiers and denitrifiers with the optimum temperature at approximately 30°C to 35°C (USSIRI; LAL, 2013).

The peaks were lower in relation to other similar experiments (DE KLEIN; VAN LOGTESTIJN, 1994; ALLEN et al., 1996; LOVELL; JARVIS, 1996) that found N_2O emission rates following urine application about 18-fold higher. Dobbie and Smith (2001) also found N_2O emissions increased about 12-fold from pasture soils when WFPS was increased from 60% to 80%, whereas Dobbie et al. (1999) found exponential increases in N_2O flux with increasing WFPS with the highest fluxes at 70% to 90% WFPS (CLOUGH et al., 2003).

Soil moisture effect is mostly indirect, since it affects the rate of O_2 diffusion into the soil profile and thus also determines if soil is predominantly aerobic or anaerobic (SMITH, 1980). The nitrification process occurs with WFPS up to 50%, while denitrification occurs at levels higher than 75% of the WPFS (MOSIER et al., 1996).

The addition of urine showed immediate higher emissions reflecting the addition of readily available mineral N source for nitrification and denitrification processes to occur (CLOUGH et al., 2009).

The NO_3^- levels remain elevated in the soil probably due to no plant uptake or leaching. Consequently are expected some N_2O losses by denitrification process via nitrate reduction (CARTER, 2007). Altering soil NH_4^+ and NO_3^- contents can influence the N_2O production on the soil (JONES et al., 2007).

4.4.3 Cumulative emissions

N_2O emission was higher for the urine treatment at 35°C with 80% of WFPS on the soil. It showed that the temperature, moisture and N available in the soil contribute to the N_2O emission. The information is very important for Brazilian conditions to improve the mitigation strategies of N_2O emission, considering the large beef cattle constituted in Brazil. Obviously, the results are from laboratory experiments however they have important regard of Brazilian grazing systems. Also, field experiments are needed to develop robust system specific emission factor of cattle urine.

4.5 Conclusions

This study demonstrated that N_2O emissions after urine application are higher in soils with high water content and temperature. The cumulative emissions were related to interactions among moisture, temperature and urine. Soil N mineralization rates also reached maximum values at the higher moisture and temperature treatments.

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5 NITROUS OXIDE EMISSIONS FROM THE SOIL FOLLOWING CATTLE URINE APPLICATION: THE EFFECT OF NITRIFICATION INHIBITORS

Abstract

Nitrous oxide has become the prime ozone depleting emission and the third most important anthropogenic greenhouse gas with a global warming potential approximately 300 times higher than CO_2. Nitrification and denitrification are processes responsible for N_2O emission from the soil after nitrogen input. The application of a nitrification inhibitor can reduce N_2O emissions from these processes. The objective of this study was to assess the effect of two different nitrification inhibitors (dicyandiamide (DCD) and a commercial formulation, Piadin®, containing two pyrazole derivatives) on N_2O emissions from cattle urine applications for summer grazing conditions in the UK: 1) under controlled conditions in a laboratory incubation experiment; and 2) in a field experiment on a grassland soil. The N_2O emissions showed similar temporal dynamics in both experiments, but at different levels. DCD concentration in the soil showed an exponential degradation during the experiment, with a half-life of the order of only 10 days. DCD and Piadin® at the highest inclusion rate reduced N_2O emissions by 13 and 29% in the incubation experiment and by 33 and 6% in the field experiment, respectively, although these reductions were not statistically significant ($P>0.05$). Under UK summer grazing conditions, these nitrification inhibitors appear to be less effective at reducing N_2O emissions than reported for other conditions elsewhere in the literature, presumably due to the higher soil temperature.

Key-words: Cattle urine. Nitrous oxide emissions. Mitigation potential. Dicyandiamide. Pyrazole derivatives.

5.1 Introduction

Nitrous oxide (N_2O) contributes about 6% to the anthropogenic greenhouse effect and it has approximately 300 times the global warning potential of carbon dioxide (CO_2) on a mass basis (IPCC, 2007). Agriculture is a major source of N_2O emission from agriculture and in the UK accounts for 79% of the total (MACCARTHY et al., 2011). It is mainly produced as a by-product of the nitrification and denitrification pathways. Many organisms are involved in these processes. The simple way to describe the process of nitrification is the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-). The prerequisite for denitrification is the presence of a source of NO_3^-; in view of this, the application of a nitrification inhibitor can minimise the N_2O emission from denitrification as well as from the nitrification process (Figure 1).

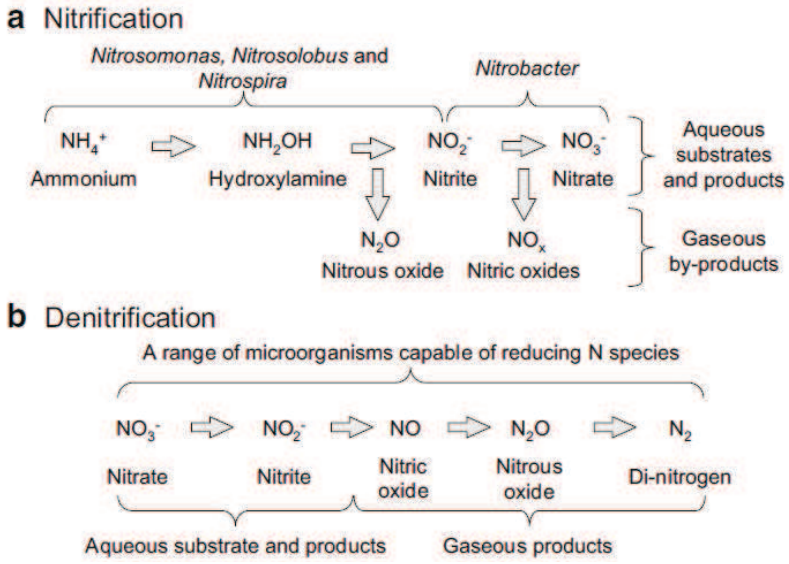

Figure 1 - Transformations of soil nitrogen species involved in the processes of a) nitrification and b) denitrification. Source: Farquharson, R.; Baldock, J. (2008)

The use of nitrification inhibitors (NI) has been shown to be a useful technique to reduce N_2O emissions and to promote better nitrogen utilization in the soil. The application of a nitrification inhibitor to the soil temporarily delays the bacterial oxidation of the NH_4^+ to nitrite by inhibiting Nitrosomonas spp. in the soil (ZERULLA et al., 2001). A number of studies have been conducted, particularly in New Zealand, showing that NI can reduce the N_2O emission by 30-80% (ABBASI; ADAMS, 2000;

DI et al., 2007; ZAMAN et al., 2009; SAGGAR et al., 2009; AKIYAMA et al., 2010). Also, NI can increase the efficiency of applied fertilizers by reducing N losses due to denitrification (BRONSON et al., 1992) and preventing leaching (AULAKH; RENNIE, 1984).

In particular, dicyandiamide (DCD) has been shown to be an effective nitrification inhibitor (DI; CAMERON, 2003, 2006; DI et al., 2007; DE KLEIN; ECKARD, 2008; SMITH et al., 2008a). According to Zaman and Blennerassett (2010) the effect of DCD is through inhibition of the nitrification process, indirectly reducing the NO_3^- availability for denitrification, consequently reducing the N_2O emission from the soil. The efficacy of DCD depends on factors such as soil moisture, temperature and DCD application rate, among others (CHAVES et al., 2006; GUIRAUD; MAROL, 1992, KUMAR et al., 2000). A large reduction effect of DCD (60–85%) on N_2O emissions from soils treated with animal urine has been reported (KELLIHER et al., 2007; DI; CAMERON, 2002; 2003; 2006; DI et al., 2007; DE KLEIN; ECKARD, 2008; SMITH et al., 2008a, 2008b).

Pyrazole derivatives have also been reported to act as effective NI (e.g. MCCARTY; BREMNER, 1990; AULAKH et al., 2001). Piadin® (SKW, Piesteritc, Germany) is a commercial formulation incorporating two active compounds: 1,2,4-triazole and 3-methylpyrazole, which is marketed as a product to improve N use efficiency and improve crop yields when used with nitrogen (N) amendments to soils.

In the present study, our goal was to evaluate the effects of these two different NI (DCD and Piadin®) on N_2O emissions from the soil after application of cattle urine under typical UK summer grazing conditions. Significant quantities of urine are returned to the soil by grazing cattle during the summer months (cattle are predominantly housed throughout the winter) when soil temperatures are higher than those under which many previously reported assessments of NI have been conducted. Our evaluation consisted of two experiments: 1) a controlled laboratory incubation experiment; and 2) a replicated small-plot field experiment.

5.2 Material and Methods

5.2.1 Laboratory experiment

Soil was obtained from the site of field experiment (see section 5.2.2.1) and collected from the 0-10 cm layer. Then it was dried and manually sieved at 2 mm, mechanically homogenized and stored at 4ºC until required.

Before the beginning of the experiment, all soil was mixed with deionised water to bring the soil moisture content to 60% WFPS to promote the nitrification process, since the diffusion of substrates and O_2 is not restricted at this WFPS level (PARTON et al., 1996).

5.2.1.1 Experimental design

The experiment consisted of a control plus five treatments with five replicates each: soil only (C), soil-urine (U), soil-urine-DCD (U+DCD) and soil-urine-Piadin® with Piadin® applied at three levels: 5, 20 and 80 L ha^{-1} namely urine low Piadin® (U+LP), urine medium Piadin® (U+MP) and urine high Piadin® (U+HP), respectively. The DCD was applied at a rate of 10 kg ha^{-1}, in accordance with current commercial guidelines (MOIR et al., 2007), being dissolved into the urine immediately prior to application. The commercial recommended rate for Piadin® is 5 L ha^{-1} (300 g L^{-1} of active ingredients; including urea, ammonium and nitrate)

The urine was obtained from a group of Holstein/Friesian dairy cows on a grass and maize silage diet supplemented with concentrates. A composite sample of all urine was made and immediately frozen until required to avoid urea hydrolysis. Immediately before the beginning of the experiment, the composite urine was sampled and analysed by standard laboratory methods to assess the physico-chemical properties of each treatment. Total urine-N content was: 9.05, 8.90, 8.91, 9.13 and 9.40 g N L^{-1} in U; U+DCD; U+LP; U+MP and U+HP, respectively. Urine was applied at a constant rate of 5 L m^{-2} for each treatment, although the N content in the urine had some variations due to N compounds in nitrification inhibitor products.

5.2.1.2 Experimental set up

The experiment was carried out in a controlled temperature laboratory at a constant temperature of 20°C. Soil was packed into 0.8 L Kilner jars (5 jars per treatment for N_2O sampling and 1 jar per treatment for soil sampling) with the equivalent of 0.27 kg dry soil per jar to achieve a bulk density of 0.9 g cm^{-3}. The soil moisture content at packing was such that the soil water-filled pore space (WFPS) after treatment addition would be 60%. The treatments were then applied at a rate of urine equivalent to 5 L m^{-2}; the control treatment received an equivalent amount of deionised water instead of urine. The septa from the lids of the Kilner jars were removed between sampling dates to ensure aerobic conditions and development of a uniform headspace above the soil surfaces. During the incubation, the soils were maintained at 60% WFPS for 7 days following urine application, by spraying deionised water onto the soil surface as required (checked through daily weighing of the jars). After 7 days, the WFPS was increased to 80%, representing a significant rainfall event, and maintained at this WFPS until the end of the experiment at 43 days.

5.2.1.3 Nitrous oxide measurement

For each measurement, the Kilner jar was hermetically sealed by replacing the lid. The initial headspace concentration was assumed to be the same as the average ambient air value for which 6 samples were taken at Kilner jar height on each sampling occasion (3 at the beginning of sampling and 3 at the end) (time-zero, t_0 sample) using a syringe and stored in a pre-evacuated gas vial. After 30 minutes the headspace of each jar was sampled again (time-thirty, t_30 sample). The lids were then removed and kept off until the next sampling day. Nitrous oxide concentrations in the sampled air were measured using a Perkin Elmer Clarus 580 Gas Chromatograph and TurboMatrix 110 auto headspace sampler with an electron capture detector (ECD). The separation column employed was a Perkin Elmer EliteQ PLOT megabore capillary (30m _ 0.53 mm i.d.) which was operated at 50ºC. The ECD detector was set at 300ºC and the carrier gas was N_2. Gas fluxes were calculated from the increase in headspace concentration between t_0 and t_30, assuming linear increase, and were corrected for temperature according to Eq. *(1)*:

$$F = \rho \frac{V}{A} \frac{\Delta c}{\Delta t} \frac{273}{(T+273)} \qquad \textit{Eq. (1)}$$

Where: F= N_2O flux (mg m^{-2} h^{-1}); ρ = density of N_2O (mg m^{-3}); V = volume of Kilner jar (m^3); A = base area of Kilner jar (m^2); $\Delta c/\Delta t$ = average rate of change of concentration with time (ppmv h^{-1}) and T = temperature in the Kilner jar (°C).

Gas measurements were carried out daily during the first week of the experiment, then three times per week for the next two weeks, then twice a week up to day 43, when the N_2O emissions had fallen to background levels. Cumulative gas losses were calculated assuming a mean flux rate between two successive sampling dates and multiplying that flux by the time interval between the measurements.

5.2.1.4 Soil mineral N

To follow the soil N dynamics, narrow core samples were taken from three points inside the soil sampling jar for each treatment for analysis for NH_4^+-N and NO_3^--N. Samples were taken at 4, 12, 32, 36 and 43 days after the start of incubation. Soil mineral N was determined by extraction with 2 M KCl in a 1:2 (soil:extractant) ratio (BREMMER; KEENEY, 1966), shaking for 1 hour in the laboratory. Soil extracts were filtered and stored at 4°C. NH_4^+ and NO_3^- were determined by automated colorimetry. Gravimetric moisture content was determined after drying at 105°C for 48 hours.

Soil extracts were also used to determine DCD concentration in the soil by HPLC analysis. The HPLC instrument was calibrated using four points of known DCD concentration (in 2 M KCl): 2.5, 5, 10 and 25 mg L^{-1}, and the derived calibration curve used to determine DCD concentration in the soil sample KCl extracts. The DCD concentrations in the soil, C_{DCD} (mg g^{-1} dry soil), were then calculated according to *Eq. (2)*:

$$C_{DCD} = \frac{(C_i - C_b) \times V}{DS} \qquad \textit{Eq. (2)}$$

Where: C_{DCD} = DCD concentration (mg g^{-1} dry soil); C_i = initial concentration in the sample (mg L^{-1}); C_b = concentration in blank sample (mg L^{-1}); V = water volume of (fresh soil weight – dry soil weight) + extractant volume (mL); DS = dry soil weight (g).

5.2.1.5 Statistical analyses

Following verification that data were normally distributed, treatment means for daily N_2O fluxes and cumulative flux over the period of each experiment were compared using one-way analysis of variance using GenStat (VSN International). Tukey tests were used to make pair wise comparisons between treatment means and were carried out at 0.05 probability level. A first order exponential decay function (Genstat, VSN International)) was fitted to the relationship between DCD concentration in the soil and time after application for the U+DCD treatment.

5.2.2 Field experiment

5.2.2.1 Site description

Our experiment was conducted on permanent grassland at North Wyke Research, Rothamsted Research, Devon, UK (50.8°N, 3.9°W, 180 m a.s.l) under maritime temperate climatic conditions (Kö ppen climatic classification), typical of the South-West of England. Temperature and rainfall rate for the period of the field experiment are shown in Figure 2.

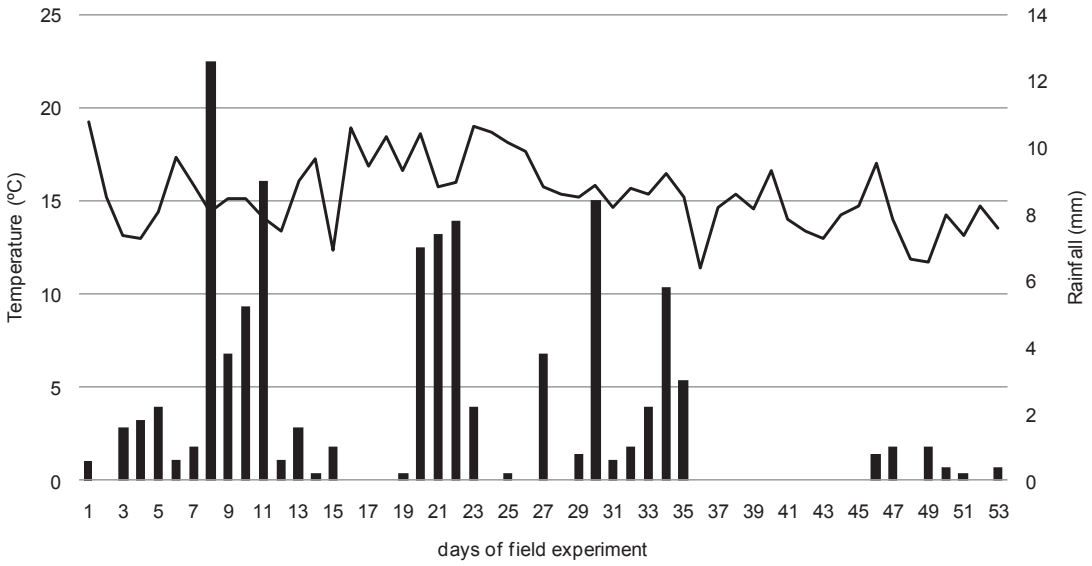

Figure 2 – Daily air temperature in °C and precipitation rate in mm during the field experiment between July and September 2012. North Wyke, UK.

The selected area in permanent grassland had not been grazed by livestock and had not received any N fertilizer for five months prior to the experiment.

The soil was clayey typical non-calcareus pelosoil of the Halstow series (AVERY, 1980), classified as stagni-vertic cambisol and as aeric haplaquept according to the FAO and USDA taxonomies, respectively. The soil texture was a silty clay loam (HARROD, 1981). Initial analyses of the properties of the upper 10 cm of the soil profile were: total N content 0.44%, total C content 3.94%, C/N ratio of 8.9, pH of 5.9 and bulk density of 0.88 Mg m^{-3}.

The selected site was divided in 30 plots. Plots consisted of an area for N_2O sampling (0.16 m^2) and another area for soil sampling (0.36 m^2) such that soil sampling did not interfere with the N_2O measurements.

5.2.2.2 Experimental design

The field experiment consisted of the same five treatments plus control as the incubation experiment, except that in the control treatment no water was applied. Five replicates of each treatment and control were arranged across the site in a randomised block design. Total urine-N for each treatment was 7.98, 8.26, 8.14, 8.04 and 8.52 g N L^{-1} in U; U+DCD; U+LP; U+MP and U+HP, respectively. Urine and the NI were applied in July 2012 at the same rates as in the incubation experiment.

5.2.2.3 Nitrous oxide measurement

The static chamber technique (MOSIER, 1989) was used for N_2O flux measurements. For each gas measurement the chambers were sealed and after 40 minutes headspace samples were taken using a syringe and stored in pre-evacuated gas vials. Initial chamber concentration was assumed to be the same as the average ambient air value, for which 10 samples were taken at chamber height on each sampling occasion (5 at the beginning of sampling and 5 at the end). For one chamber, namely the “linearity box”, samples were also taken after 15 and 30 minutes after the closure of the chamber to check the linearity of headspace N_2O concentration accumulation. The lids were then removed and kept off until the next sampling day. Nitrous oxide concentrations in the sampled air were measured and calculated as described for the laboratory incubation experiment.

N_2O sampling was carried out daily during the first week of the experiment, and then twice a week for three weeks, and then once a week up to day 54. Cumulative gas losses were calculated assuming a mean flux rate between two successive sampling dates and multiplying that flux by the time interval between the measurements.

5.2.2.4 Soil mineral N

Three soil cores (10 cm depth) were taken from the soil sampling area of each plot and bulked for analysis. Soil samples were taken once per week and analyzed for mineral N and water content. Soil mineral N, moisture content and DCD concentration were determined as described for the laboratory incubation experiment.

5.2.2.5 Yields and N analysis from pasture

At the end of the field experiment, the grass inside each chamber was cut and the dry matter yield determined by drying at 105°C for 48 hours. Samples were then ground and analyzed for total N content. Total N content was determined using an elemental analyser (NA2000, Carlo Erba Instruments, Milan, Italy) linked to a Sercon

20-22 isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK). Wheat flour (IA-R001, Iso-Analytical, Crewe, UK) was used as the reference.

5.2.2.6 Statistical analyses

Following verification that data were normally distributed, treatment means for daily N_2O fluxes, cumulative flux, soil mineral N content, herbage yield and herbage N offtake were compared using one-way analysis of variance using GenStat (VSN International). Tukey tests were used to make pairwise comparisons between treatment means and were carried out at 0.05 probability level. A first order exponential decay function (Genstat, VSN International) was fitted to the relationship between DCD concentration in the soil and time after application for the U+DCD treatment.

5.3 Results

5.3.1 Laboratory incubation

5.3.1.1 Nitrous oxide emissions

On the first day following urine application there was a significant difference in N_2O emission rate between urine and the NI treatments (Figure 3). However, emission rates then declined rapidly to background values by day 3.

Emission rates increased again following addition of water to bring soil WFPS to 80%, and N_2O fluxes from all treatments remained above those of the control until the end of the experiment at 43 days after application. After that, only U+MP and U+HP were higher than urine treatments. None of the NI treatments significantly reduced N_2O flux compared with the urine treatment (although U+DCD and U+HP were numerically lower for some of that period). From day 17 to the end of measurement, N_2O emissions were significantly greater from U+LP compared with the urine treatment.

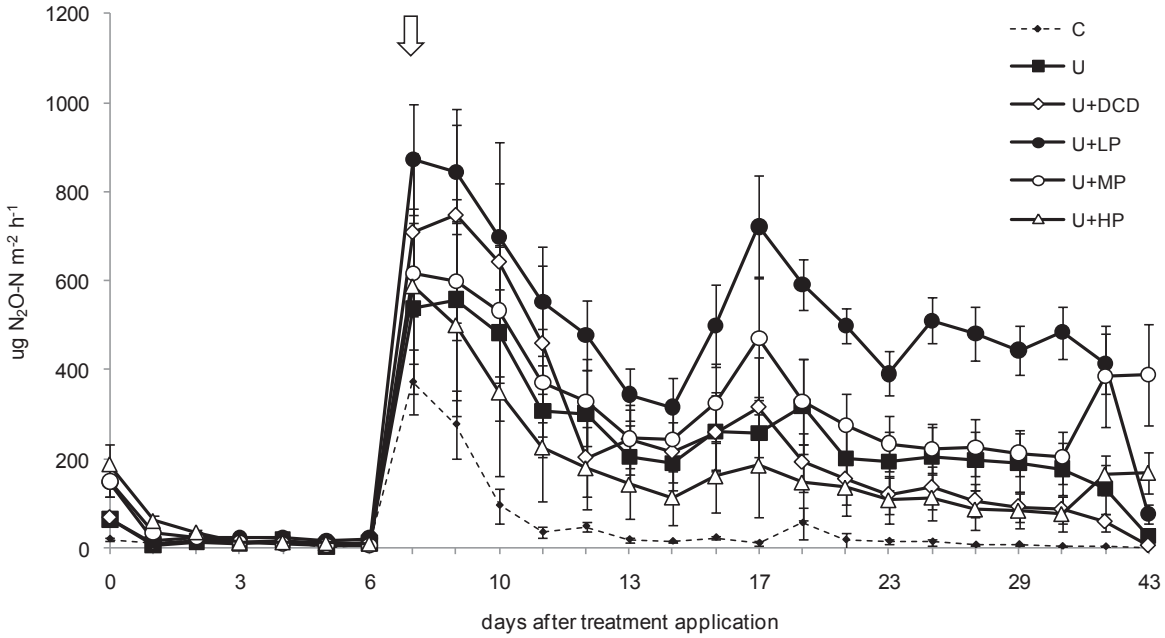

Figure 3 - N_2O emission from the soil following treatment application in the laboratory incubation experiment. Arrow indicates addition of water to increase soil WFPS to 80%. Vertical bars show ±1 standard error.

5.3.1.2 Cumulative emissions

Cumulative N_2O emissions from the urine treatment were significantly greater than from the control (Table 1). However, there was no significant effect of NIs (P>0.05), although some showed numerical reductions in cumulative emission i.e. of 13% and 29% for U+DCD and U+HP, respectively.

Table 1 - Cumulative and net cumulative emissions for each treatment in the incubation experiment 43 days after treatment application

Treatments	Cumulative emissions	Net cumulative emissions
	g N_2O-N ha^{-1}	
C	14 ± 2.5	--
U	80 ± 27	66
U+DCD	70 ± 21	56
U+LP	173 ± 20	160
U+MP	124 ± 32	111
U+HP	56 ± 24	42
Grand mean	86.36	--
LSD (P<0.05)	101.9	--

Values are means (*n*=5) ± standard error.

5.3.1.3 Soil mineral N

Figure 4 shows soil mineral N dynamics following treatment application. Values for the control treatment were very low throughout the measurement period. For all other treatments, NH_4^+-N content was initially high, then declining with time. There is some evidence of delayed nitrification for U+DCD, U+HP and U+MP as the soil NH_4^+-N content stays above that of the other treatments.

Nitrate content showed the greatest increase in U and U+LP, with concentrations remaining high at the end of the experiment. We noticed that from 12 days after treatments application, NH_4^+-N concentrations were declining while NO_3^--N concentrations began increasing in the soil.

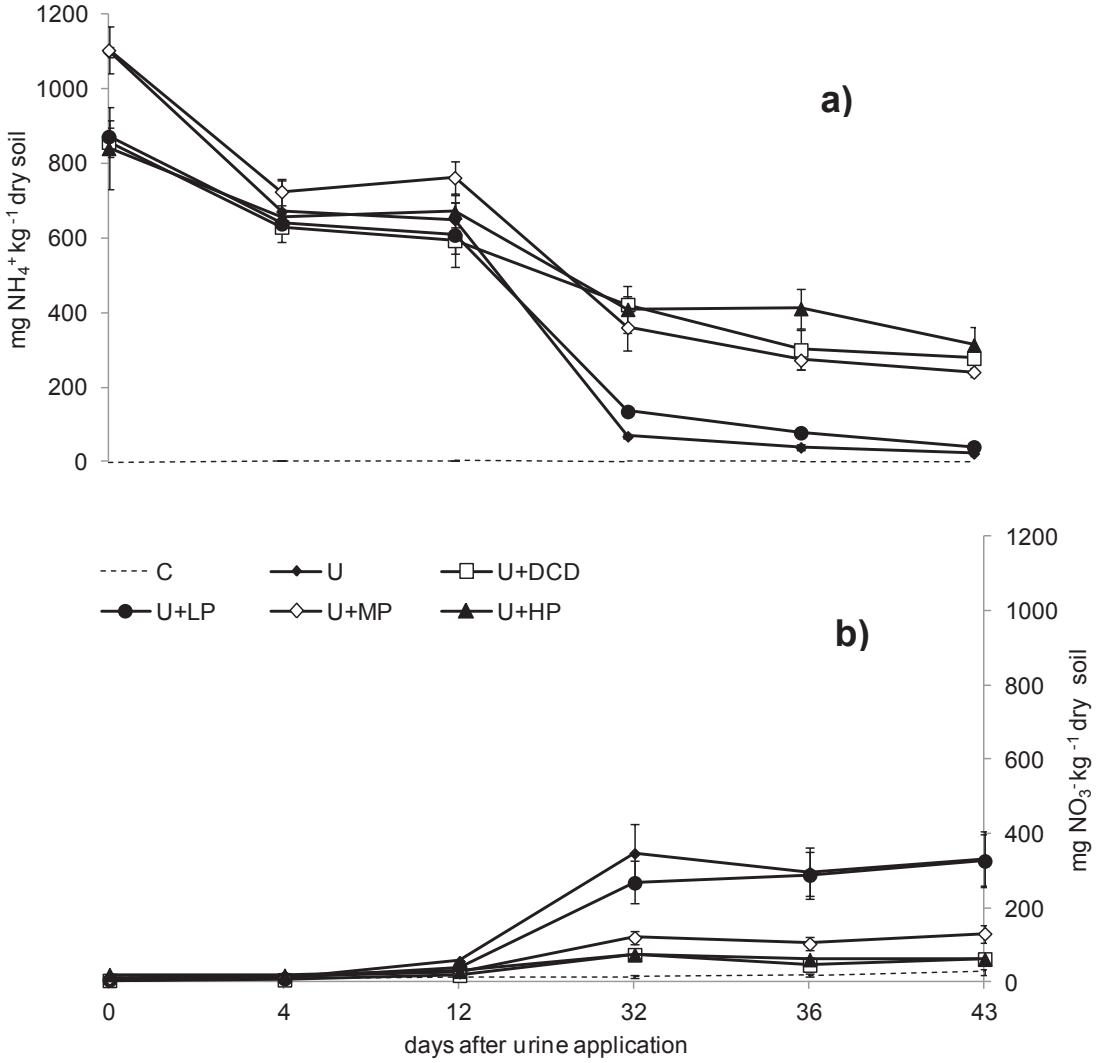

Figure 4 – Soil mineral N dynamics: a) NH_4^+-N and b) NO_3^--N in the incubation experiment as influenced by application of different N inhibitors to pasture soil receiving cattle urine. Error bars show ±1 standard error.

5.3.1.4 DCD degradation

Figure 5 shows the DCD concentration in the soil over the 43 days following treatment application. The fitted first-order exponential regression shows the decrease of DCD concentration with time, with a half-life approximately of 10 days.

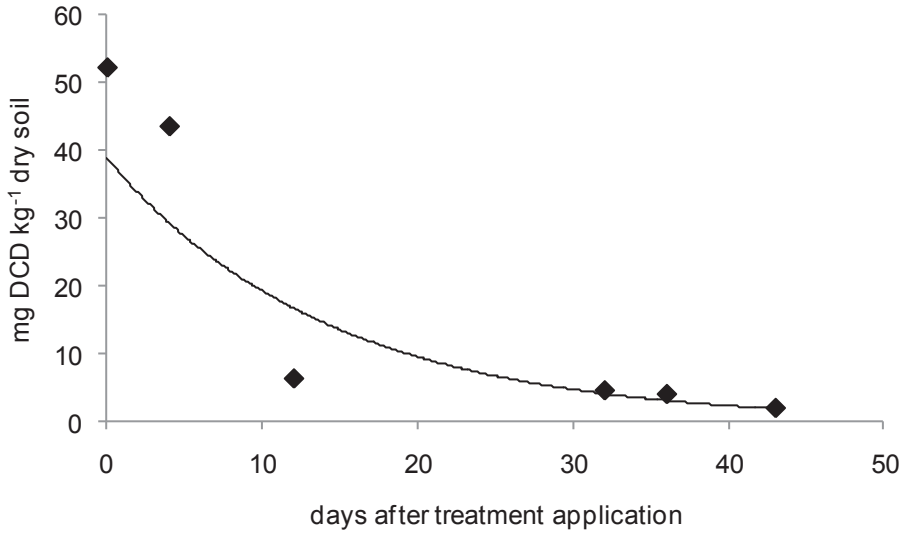

Figure 5 - Relation between the DCD concentration (DCD) and time (t) since treatment application in the incubation experiment. Regression analysis $DCD_t = 38.8\ e^{-0,07t}$ that accounted for 86% of the variance.

5.3.2 Field experiment

5.3.2.1 Nitrous oxide emissions

Throughout the 53 days after treatment application in the field experiment, N_2O emission from the control was very low (<14.5 g $N\text{-}N_2O$ ha^{-1} day^{-1}, Figure 6) compared with the other treatments. There were two clear emission peaks during the experiment, at 13 and 25 days after application. There was no effect of the NIs on N_2O emissions for the first 20 days after the treatment application when compared with the urine treatment. After 21 days there was some reduction in emission from the U+HP and U+DCD treatments, with the DCD showing the greater effect.

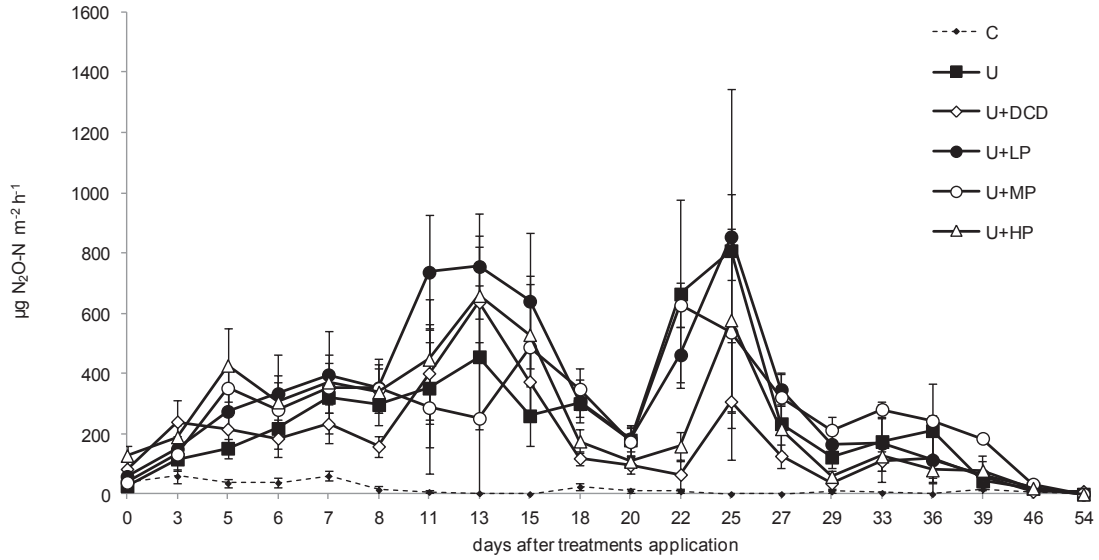

Figure 6 - N_2O emission from the soil following treatment application to the field experiment. Vertical bars show ±1 standard error.

5.3.2.2 Cumulative emissions

As for the incubation experiment, the NIs had no significant effect ($P>0.05$) on cumulative N_2O emissions in the field experiment compared with the urine treatment, although some showed numerical reductions. Table 2 shows the cumulative emissions and the emission factor for each treatment (i.e. the loss of N_2O-N as a % of the total N applied), although these are only short-term measurements and only for one set of conditions, so cannot be extrapolated for annual/national conditions.

The U+DCD and U+HP treatments showed reductions in net emission of 33 and 6%, respectively.

Table 2 - Cumulative emission and emission factor for each treatment for the field experiment 54 days after treatments application.

Treatments	Cumulative emissions	Emission Factor
	g N_2O-N ha^{-1}	*%*
C	147.4 ± 34	--
U	2770.4 ± 701	0.66
U+DCD	1894.7 ± 398	0.42
U+LP	3154.3 ± 589	0.74
U+MP	3516.0 ± 500	0.84
U+HP	2609.0 ± 470	0.58
Grand mean	2348.6	--
LSD (P<0.05)	2162.4	--

Values are means (*n*=5) ± standard error.

5.3.2.3 Soil mineral N

The soil NH_4^+-N content in the control was very low (< 6 mg N kg^{-1} dry soil) throughout the 53 days of the field experiment (Figure 7). The addition of urine, with or without NI, resulted in very elevated contents of NH_4^+-N on the first day, as with incubation experiment, showing that urea hydrolysis occurred readily caused by soil urease (VLEK; CARTER, 1983), declining with time. Over the duration of the experiment the concentration declined to background levels, with urine not being significantly (P>0.05) different from NI treatments. Although not significant, there is some evidence that the DCD delayed the nitrification process between days 12 and 32, resulting in slightly higher NH_4^+-N concentrations for DCD treatment.

Nitrate concentrations showed delayed nitrification in the soil, with NO_3^--N concentrations increasing slowly initially with peaks 19 days after treatment application and falling to background concentration by day 54 (Figure 7). There were no significant differences (P>0.05), but show some evidence of lower nitrate concentrations after addition of DCD and in Piadin® at the high rate relative to other treatments (Figure 7).

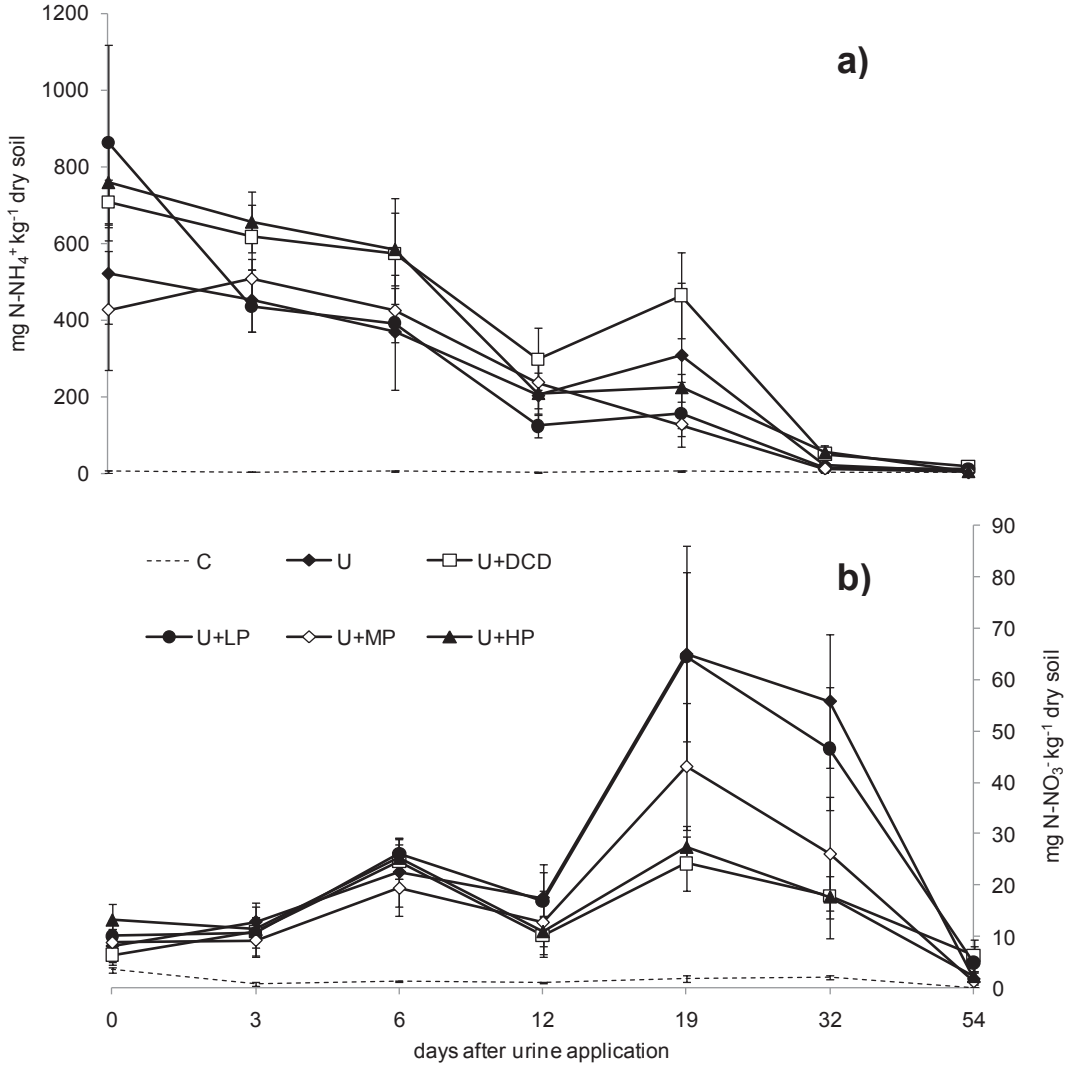

Figure 7 – Soil mineral N dynamics: a) NH_4^+-N and b) NO_3^--N for the field experiment as influenced by application of different N inhibitors to pasture soil receiving cattle urine. Error bars show ±1 standard error.

5.3.2.4 DCD degradation

Figure 8 shows DCD concentration in the soil during the field experiment over the 54 days following treatment application.

The fitted first-order exponential regression shows the decrease of DCD concentration with time, with a half-life approximately of 7 days.

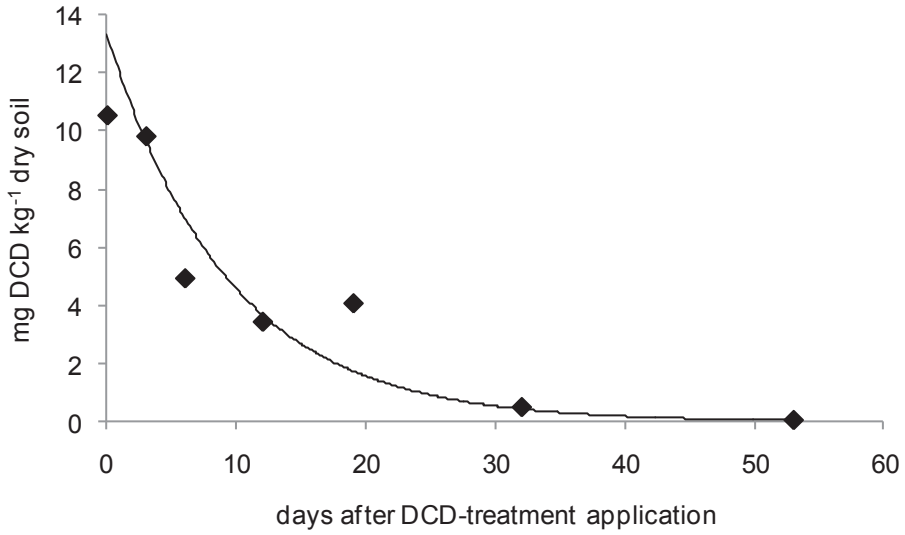

Figure 8 – Relation between the DCD concentration (*DCD*) and time (*t*) since treatment application in the field experiment. Regression analysis $DCD_t = 13.3\ e^{-0,107t}$ that accounted for 96% of the variance.

5.3.2.5 Herbage yield and N offtake

Urine addition increased pasture dry matter (PDM) production, with net PDM production (treatment minus control) across all treatments ranging from 196 g m^{-2} (urine treatment) to 255 g m^{-2} (U+DCD) (Table 3).

Table 3 – Pasture dry matter and N uptake following application of cattle urine and N inhibitors to pasture soil.

Treatments	PDM (g m^{-2})	N uptake (g N m^{-2})
C	233.63 ± 35.7	5.42 ± 0.9
U	430.38 ± 44.3	11.90 ± 1.1
U+DCD	488.63 ± 30.2	15.78 ± 0.6
U+LP	450.13 ± 24.0	14.77 ± 1.1
U+MP	440.50 ±36.5	13.99 ± 1.4
U+HP	433.38 ± 29.0	13.45 ± 1.3
Grand mean	412.80	12.55
LSD (P<0.05)	62.50	2.39

Values are means (*n*=5) ± standard error.

There was a large increase in the pasture N uptake in the treatments with urine compared with the control (Table 3). The maximum pasture N uptake, net of the control plot, was measured as 10.36 g N m^{-2} (U+DCD). The addition of the NI DCD to urine significantly increased N uptake, by 60% net of control ($P<0.05$). The addition of Piadin® at all levels numerically increased N uptake, although part of this would have been due to the additional urea, ammonium and nitrate applied with the Piadin® solution, but these increases above the urine treatment were not statistically significant ($P>0.05$), except for the U+LP treatment.

5.4 Discussion

5.4.1 N_2O flux dynamics

Nitrogen flux dynamics may change due to many factors including temperature, water content and soil mineral N content in the soil, among others.

For the incubation experiment, initial water content in the soil was not sufficient to promote the denitrification process which occurs at higher values of WFPS; significant emissions were not observed until day 6 when the additional water was applied.

In both the incubation and the field experiment there were two distinct peaks in N_2O emission. This pattern of two emission peaks following urine application to soil has been observed before (e.g. ZAMAN; NGUYEN, 2012). These may be associated with the two processes contributing to N_2O emission (nitrification and denitrification), it may reflect denitrification from two different pools of added nitrogen with different mineralisation rates, or for the field experiment it could reflect the pattern of rainfall events.

The nitrification process in the soil is the oxidation of NH_4^+ to NO_3^-, with N_2O being produced directly from this process and also subsequently from denitrification of the NO_3^-. In this way, the soil mineral N content is related to N_2O emission (JONES et al., 2007).

In these experiments, high initial NH_4^+ concentrations in the soil occurred from rapid urea hydrolysis after urine application. Then this decreased as nitrification occurred. In the field experiment, NO_3^- concentrations increased from day 12 onwards, so then denitrification occurred, and after that concentrations decreased

with time. In the incubation experiment, no plant uptake or leaching of NO_3^- occurred, so concentration remained higher at the end of the experiment.

In the field experiment, between day 13 and 25 after treatment application there was a steady increase in a soil NO_3^- concentration and rainfall during days 20-23 led to soil WFPS > 60%, resulting in the observed peak increase and peak in N_2O emissions at around day 25. A similar situation was reported by Zamam and Nguyen (2012) for a spring urine application experiment. In the incubation experiment, the second peak happened on day 17 corresponding to nitrification process due to the high NO_3^- concentration and/or denitrification process due to increase of WFPS in the soil.

Moir et al. (2012) also found in patches treated with DCD a large proportion of urine-N was retained in the soil as NH_4^+-N; in contrast, urine patches not treated with DCD contained high levels of NO_3^--N and overall had more rapid rates of reduction in soil NH_4^+-N compared to the DCD treatments. DCD application in the soil also delayed nitrification process according to Zamam and Nguyen (2012), who reported that from day 1 after NI application and therefore produced comparatively lower concentrations of NO_3^--N. This is consistent with the lower N_2O emissions from that treatment for that same period.

In relation with N_2O emission, DCD treatment showed some evidence of effect in comparison with urine alone by day 21 after treatment application, practically the same as found by Zamam and Nguyen (2012) which showed some effect of NI treatments by day 15 after application. There was some evidence of an effect with Piadin® at the medium rate, but Piadin® at the low rate (commercially-recommended rate) showed no effect at all.

The current default IPCC emission factor, expressing N_2O emissions from cattle urine applied to soil as a proportion of the urine N, is 2%. In our field experiment, the value for urine alone (without NI) was much lower than this at c. 0.7%. However, as mentioned before, our study was over a relatively short period (although emissions had fallen to background values). Zamam and Nguyen (2012) reported an emission factor for spring and autumn season urine applications of 0.6 and 2.3%, respectively.

This variation in emission factors, and the dependence on soil and weather conditions, highlights the importance to determine specific emission factors applicable to each country and region.

5.4.2 Effectiveness of NI

After the treatment application to the soil, microbial degradation of DCD will occur. This process is temperature dependent, with maximum nitrification inhibition occurring at soil temperatures ≤ 10°C (AMBERGER, 1986; DI and CAMERON, 2004; SMITH et al., 1989). According to Schwarzer and Haselwandter (1991), DCD degradation occurs over a temperature range of 10-33°C with the fastest degradation rate at 25°C. The temperature in both of our experiments, approximately 15°C (but 25°C on the first day in the field experiment), resulted in rapid DCD degradation in soil and the relatively limited effectiveness of DCD under these conditions.

Other limitations to the effectiveness of DCD, which may have applied in this study, are leaching of DCD under very wet soil conditions and/or interception of DCD by the plant canopy (SMITH, 2009; KIM et al., 2012). Another potential reason for the lack of NI effectiveness was the high organic matter content of the soil (REDDY, 1964; AMBERGER; VILSMEIER, 1979; KUTZOVA et al., 1993).

Further research is needed to assess the effectiveness of the pyrazole derivative compounds as present in Piadin®, but these first data show that it is not an effective inhibitor of N_2O emissions when used with urine applications to grassland soil under summer conditions in the UK.

5.4.3 PDM and N uptake

Pasture dry matter yields from the field study were similar to those reported by Moir et al. (2012), who reported PDM ranging from 187.8 to 369.7 g m^{-2}.

The addition of NI as DCD or Piadin® to urine gave a small, but non-significant increase in PDM ($P>0.05$) compared with the urine treatment. Zaman and Nguyen (2012) also found a non-significant increase in PDM for spring and autumn experiments. However, according to a number of studies treating urine patches with NI (DCD) improves the bioavailability of nitrogen (MOIR et al., 2012; ZAMAM et al., 2008; ZAMAN; BELNNERHASSETT, 2010).

The NI application also resulted in an increase in N uptake by the pasture. Moir et al (2012), applying DCD at the same rate in August (spring) in New Zealand found a 5-fold N uptake compared with control plots and 2.5-fold compared with urine alone.

5.5 Conclusions

Nitrous oxide emission from cattle urine applied to grassland soil under UK summer conditions represented 0.7% of the applied urine N, much lower than the current IPCC default emission factor of 2% for cattle excreta. DCD and the pyrazole derivatives in the commercial formulation Piadin® had only limited effectiveness in reducing N_2O emissions and improving herbage N uptake from summer urine applications to a grassland soil in the UK. Various physical and biological factors are important to the effectiveness of NI in the soil. Under the conditions of these experiments, there was a very fast degradation of DCD in the soil, probably due to high temperature, and DCD had a half-life of only approximated 10 days.

Further research is required to assess the effectiveness of these NI under different conditions (early and late grazing season) and for different soil types.

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6 FINAL CONSIDERATIONS

Experiments were selected in order to study the nutrients cycles of grazing system and to evaluate nitrogen losses by the process. This information will contribute to understand probable factors that influence them and select ways of mitigation.

The experiment described on *Chapter 3* was located at an extensive system in Southeast Brazil where the climate is characterized by constant mean temperature and a considerable amount of rainfall throughout the year. The average temperatures range from 30 to 32°C (summer) and 6 to 20°C (winter); rainfall is usually maximum in January and the minimum in July (while the dry period is usually concentrated in the winter). The experiment described on *Chapter 5* was performed on grazing system in South-West of England under maritime temperate climatic conditions. Typically it experiences cool winters with warmer summers and precipitation all year round. Nitrous oxide emission factor on these grazing systems were evaluated.

The variability of emission factor can be explained in several aspects listed below:

1) Climate difference, which noticed temperature and water content as a major factor interfering on nitrous oxide emissions (*Chapter 4*);

2) Soil characteristics, which directly affect the porous and microorganisms in the soil and consequently the gaseous emission;

3) Livestock production, when it is expressed per animal, the diet, level of productivity of the animal and production system are also important factors to consider;

4) Physiological differences between steers raised for beef production and cows raised for milk production. The nitrogen requirement for milk is higher than for beef production; it implies in different diets for each one. The nitrogen increases in the diet promote an excess of crude protein that will be excreted proportionally more in the urine and consequently contribute to susceptible losses, as N_2O emission.

Besides provide the emission factor for each different climate conditions situations, also can improve the knowledge about the gaseous losses of N cycle. These experiments will help to understand most of the variables that influence the emissions by the grazing system.

Finally, field and incubation experiments showed that nitrification inhibitors had only limited effectiveness in reduce nitrous oxide emissions from summer urine applications to a grassland soil in UK. Some physical and biological conditions probably interfere on the degradation rate of the soil, inhibiting their effectiveness.

The knowledge of all this factors is very important especially nowadays. Numerous protocols of emission reductions are being proposed by different countries. Concerns about climate change and the increase of greenhouse gas emission promote many environmental reports and policies of the countries.

Further research is required to develop more accurate emission factor for greenhouse gas emission inventories that reflect the soil, climate and management conditions for specific countries. And also in a near future, promote policies maker specific for each region dependent of the specific emission factor for the livestock production on grazing system.