UNIVERSIDADE FEDERAL DO PARANÁ

PRISCILA LUZIA SIMON

USO DE PLANTAS ALTERNATIVAS NA MITIGAÇÃO DA EMISSÃO DE ÓXIDO NITROSO A PARTIR DE URINA BOVINA EM SISTEMAS SUBTROPICAL E TEMPERADO

PLANT USE AS ALTERNATIVE TO CURB NITROUS OXIDE EMISSIONS FROM CATTLE URINE PATCHES IN SUBTROPICAL AND TEMPERATE SYSTEMS

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Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Ciência do Solo.

Orientador: Prof. Dr. Jeferson Dieckow

Coorientadoras: Dra. Josiléia Acordi Zanatta

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **PRISCILA LUZIA SIMON** intitulada: **Uso de plantas alternativas na mitigação da emissão de óxido nitroso a partir de urina bovina em sistemas subtropical e temperado** , após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 20 de Fevereiro de 2019.

JEFERSON DIECKOW
Presidente da Banca Examinadora (UFPR)

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Avaliador Interno (UFPR)

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JOSILÉIA ACORDI ZANATTA
Avaliador Externo (EMBRAPA)

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“Ouse saber!”

Immanuel Kant

RESUMO

A maior parte do nitrogênio (N) consumido pelos animais em sistemas à base de pastos é excretada como urina, e essas manchas de urina são a principal fonte na produção óxido nitroso (N_2O), um poderoso gás de efeito estufa com um potencial de aquecimento global 298 vezes maior que dióxido de carbono (CO_2) .Algumas espécies de plantas têm a capacidade de produzir metabólitos secundários que atuam na eficiência do uso do N, através da inibição da nitrificação no solo ou no processo de digestão animal, melhorando a absorção e liberando menos N nas excretas. O objetivo deste estudo foi avaliar o papel de diferentes espécies de plantas nas emissões de N_2O a partir de urina bovina, em um Cambissolo subtropical do Brasil (2017) e em um Cambissolo temperado da Nova Zelândia (2018). No Brasil, o objetivo do experimento foi avaliar a eficiência da brachiaria (*Brachiaria humidicola)* em reduzir as emissões de N_2O a partir de manchas de urina em comparação com o Campim áries (*Panicum maximmum cv. Aries*). Na Nova Zelândia, o experimento teve como objetivo avaliar o efeito de diferentes proporções de Plantain (*Plantago lanceolata*) misturado ao azevém/ trevo branco (*Lolium multiflorum/ Trifolium repens*) na redução das emissões de N_2O em manchas de urina. As medidas de N_2O foram realizadas pelo método da câmara estática fechada e as análises foram realizadas via cromatografia gasosa. O N inorgânico do solo foi determinado por espectrofotometria em sistema de análise por injeção em fluxo. A emissão de N_2O foi 20% menor quando urina foi aplicada sobre braquiária comparado a aplicação sobre capim áries. Esses resultados coincidiram com menores concentrações e intensidades de nitrato no solo, sugerindo a inibição biológica da nitrificação como mecanismo de redução das emissões de N_2O. DCD misturado com urina aplicada sobre a braquiária e capim áries diminuiu em 40-50% das emissões de N_2O em comparação com a aplicação somente de urina sobre as pastagens. No clima temperado da Nova Zelândia, o fator de emissão de N_2O de urina bovina reduziu linearmente com o aumento da proporção de plantain, variando de 0,60 a 0,36% (r^2= 0,9743). O uso de plantain na dieta de animais levou à diminuição das taxas de liberação de N na urina, mas não há evidências do efeito do plantain na urina sob o fator de emissão de N_2O comparado ao pasto de azevém/ trevo branco.

Palavras-chave: Gases de efeito estufa. Espécies de plantas. Manchas de urina.

ABSTRACT

Most of the nitrogen (N) consumed by animals in pasture-based systems is excreted as urine, and these animals urine patches are the key source of Nitrous oxide (N_2O) production, a powerful greenhouse gas with a global warming potential of 298 times that of carbon dioxide (CO_2). Some plant species have the capacity to produce secondary metabolites that act in soil N efficiency use, through the soil nitrification inhibition or in the process of animal digestion improving the N absorption and releasing less in excreta. The aim of this study was to assess the role of different plant species on N_2O emissions from cattle urine. Experiments were conducted in a subtropical Cambisol of Brazil (2017) and in a temperate Dystrudept soil of New Zealand (2018). In Brazil the experiment aimed to evaluate the efficacy of brachiaria (*Brachiaria humidicola)* in to curb N_2O emissions from urine patches compared to Guinea grass (*Panicum maximum cv. Aries*). In New Zealand the experiment aimed to evaluate the effect of different proportions of Plantain (*Plantago Lanceolata*) mixed to the standard ryegrass/ white clover (*Lolium perenne/Trifolium repens*) in reducing N_2O emissions from urine patches. N_2O measurements were performed using the closed static chamber method and the analyses were carried out via gas chromatography. Soil inorganic N was determined by spectrophotometry with flow injection analysis system. The emission of N_2O was 20% lower when urine was applied onto brachiaria compared with urine applied onto guinea grass. These results coincided with lower soil nitrate concentrations and intensities where brachiaria was grown, thus suggesting a biological nitrification inhibition as the mechanism of N_2O emissions reduction. DCD mixed with urine applied onto brachiaria and guinea grass decreased by 40-50% of N_2O emissions compared with urine only application onto these pastures. In temperate climate of New Zealand the emission factor of N_2O from cattle urine reduced linearly with the increase of plantain proportion swards, ranging from 0.60 to 0.36% (r^2= 0.9743). The use of plantain in animal diet led to decreasing on urine-N loading rates, but no evidences of plantain effect in urine over N_2O emission factor compared to ryegrass/white clover pasture.

Keywords: Greenhouse gases. Plant species. Inorganic N. Urine patches.

FIGURE CAPTIONS

CHAPTER I

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TABLE CAPTIONS

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SUMMARY

1 GENERAL INTRODUCTION

Grazing animals consume more N than they need for grown and production, and much of the N ingested as feed (80-95%) is returned to soil as urine or dung (Haynes and Williams, 1993). The animal urine patches are the key source of nitrous oxide (N_2O) emissions in grazed systems considering the high concentrations (500-1000 kg ha^{-1}) of readily available N. N_2O is a potent greenhouse gas with a global warm potential 298 times greater than carbon dioxide (CO_2) (Forster *et al.*, 2007), and has been estimated to be responsible for 7% of the total anthropogenic radiative forcing, besides of to contribute to the ozone layer depletion (IPCC, 2006). In Brazil 64% of N_2O emissions derive from agricultural soils, within this 43% come from grazing animal, with a heard near of 220 million of cows (MCTIC, 2016; USDA, 2017). The New Zealand herd is approximately 11 million of cows and 95% of the emitted N_2O is derived from agricultural soils via urine patches, considering the high relevance of grazed pasture systems in the country (Ministry for the Environment, 2017).

N_2O emission is a product of microbial nitrification and denitrification processes and abiotic chemodenitrification reactions (Zhu *et al.*, 2013; Stanton *et al.*, 2018). When cattle urine is excreted, urea [$(NH_2)_2CO$], the main N form in this excreta, is hydrolysed to ammonia (NH_3) catalysed by the enzyme urease (Vertregt and Rutgers, 1987). In aerobic conditions the NH_3 will be oxidized to hydroxylamine (NH_2OH) catalysed by the ammonia monooxigenase enzyme (AMO). Successively catalysed by the enzyme hydroxylamine oxidoreductase (HAO), NH_2OH is oxidized to NO_2^-. This biotic process, carried out under aerobic conditions by chemoautotrophs using NH_3 as an energy source, can produce N_2O and NO by several pathways, namely nitrifier nitrification, nitrifier denitrification, and nitrification-coupled denitrification (Hollocher *et al.*, 1981). NO_2^- is oxidized further by Nitrobacter, catalysed by nitrite oxidoreductase in a one-step reaction to nitrate (NO_3^-). Heterotrophic denitrification is performed by heterotrophic bacteria (mainly Pseudomonas) using NO_3^- as alternate electron acceptors to oxygen (O_2) reducing this anion to NO_2, NO, N_2O and N_2 (Haynes and Williams, 1993; Zumft, 1997; Heil *et al.*, 2016).

Mitigation technologies of N_2O emissions that have been developed over the past few years include the use of nitrification inhibitors (NI), as the dicyandiamide (DCD), which blocks the AMO enzyme activity in soil, stopping the nitrification and maintaining the NH_4 as N source (Di and Cameron, 2002; Di *et al.*, 2007; de Klein *et al.*, 2014; Luo *et al.*, 2016). DCD reduces by 70% of N_2O emissions and by 30% of NO_3 leaching (Monaghan *et al.*, 2013), and was commercially available to New Zealand farmers until 2014 (Luo *et al.*, 2016). In Brazil the efficacy of DCD is still questioned, once in tropical brazilian pastures no results were found in N_2O emissions reduction with DCD application (Mazzetto *et al.*, 2015), while in subtropical brazilian pasture the DCD application has shown to be effective (Simon *et al.*, 2018). It is known that nitrification inhibitors decrease direct N_2O emission and indirect N_2O emission due to NO_3 leaching from agroecosystems. However, this beneficial effect can be weakened by the increase in indirect N_2O emission though the increase of volatilization and deposition of NH_3 after DCD application onto pasture (Lam *et al.*, 2017). According to IPCC (2006) approximately 20% of the N in cattle excreta is lost by NH_3 volatilization and 1% (uncertainty range 0.2-5%) returns to soil by atmospheric deposition and may be re-emitted as N_2O (N_2O-N= kg NH_3-N + NO_X-N volatilised). Thus, appropriate NH_3 mitigation measures should be taken where nitrification inhibitors are being used to decrease direct N_2O emissions, to enable effective and viable climate change mitigation.

Plants are strong modifiers of N cycling processes in soil, therefore can be expected to influence N_2O emission (Haichar *et al.*, 2008) mostly by four mechanisms: i) reducing N concentration in urine reducing the N loading rate in individual urine patches, ii) exudates from plant roots that impact on soil N processes, iii) compounds excreted in animal urine that impact on soil N processes, and iv) effect of plant shoot and root morphology on soil N processes through changes in the soil microclimate (Badri and Vivanco, 2009; Cheng *et al.*, 2014).

The suppression of nitrification has been observed to occurs naturally in some ecosystems where certain plant species release organic molecules from their roots that suppress the function and growth of nitrifying bacteria, a phenomenon termed biological nitrification inhibition (BNI) (Subbarao *et al.*, 2007a; Subbarao *et al.*, 2015). The BNI brachialactone, found in root exudates of tropical forage *Brachiaria humidicola,* appears to block both ammonia mono-oxygenase (AMO) and hydroxylamine oxidoredutase (HAO) enzymatic pathways involved in ammonia

oxidation in Nitrossomonas (Subbarao *et al.*, 2009). This is in contrast to most synthetic nitrification inhibitors such as DCD and nitrapyrin that suppress Nitrossomonas function mostly by blocking the AMO pathway (McCarty and Bremner, 1989).

In temperate system Luo *et al.* (2018) found lower N_2O emissions from cattle urine applied onto plantain (*Plantago lanceolata*) compared with urine applied to ryegrass (*Lolium perenne*) pasture. The authors suggest that the biological nitrification inhibition potential was the dominant mechanism by which plantain reduced N_2O emissions. Soil incubation experiments with plantain leaf materials and extract confirmed they significantly inhibited N mineralisation and nitrification (Dietz *et al.*, 2013). Plantain can produce secondary metabolites including the iridoid glucosides aucubin and catalpol, and, the phenylethanoid glucoside, acteoside, which are known for their diuretic effect and antimicrobial activity (Gardiner *et al.*, 2016). These characteristics place plantain as a potential strategy for mitigating N_2O emissions in pasture-based livestock systems.

Our study aimed to assess the capacity of different plant and forage species to reduce N_2O emissions from cattle urine patches in subtropical and temperate systems.

REFERENCES

BADRI, D.V.; VIVANCO, J.M., 2009. Regulation and function of root exudates. **Plant, Cell & Environment** 32, 666-681.

CHENG, W., PARTON, W.J., GONZALEZ-MELER, M.A., PHILLIPS, R., ASAO, S., MCNICKLE, G.G., BRZOSTEK, E., JASTROW, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. **New Phytologist** 201, 31-44.

DE KLEIN, C.A.M., LETICA, S.A., MACFIE, P.I., 2014. Evaluating the effects of dicyandiamide (DCD) on nitrogen cycling and dry matter production in a 3-year trial on a dairy pasture in South Otago, New Zealand. **New Zealand Journal of Agricultural Research** 57, 316-331.

DI, H.J., CAMERON, K.C., 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. **Soil Use and Management** 18, 395-403.

DI, H.J., CAMERON, K.C., SHERLOCK, R.R., 2007. Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. **Soil Use and Management** 23, 1-9.

DIETZ, M., MACHILL, S., HOFFMANN, H.C., SCHMIDTKE, K., 2013. Inhibitory effects of Plantago lanceolata L. on soil N mineralization. **Plant and Soil** 368, 445-458.

FORSTER, P., RAMASWAMY, V., ARTAXO, P., BERNTSEN, T., BETTS, R., FAHEY, D.W., HAYWOOD, J., LEAN, J., LOWE, D.C., MYHRE, G., NGANGA, J., PRINN, R., RAGA, G., SCHULTZ, M., VAN DORLAND, R., 2007. Changes in atmospheric constituents and in radiative forcing. In: SOLOMON, S., QIN, D., MANNING, M., CHEN, Z., MARQUIS, M., AVERYT, K.B., TIGNOR, M., MILLER, H.L. (Eds.). **Cambridge University Press**, Cambridge, United Kingdom, pp. 129-234.

GARDINER, C.A., CLOUGH, T.J., CAMERON, K.C., DI, H.J., EDWARDS, G.R., DE KLEIN, C.A.M., 2016. Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N_2O emissions: a review. **New Zealand Journal of Agricultural Research** 59, 301-317.

HAICHAR, F.E.Z., MAROL, C., BERGE, O., RANGEL-CASTRO, J.I., PROSSER, J.I., BALESDENT, J., HEULIN, T., ACHOUAK, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. **The Isme Journal** 2, 1221.

HAYNES, R.J., WILLIAMS, P.H., 1993. Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. **Advances in Agronomy** 49, 119-199.

HEIL, J., VEREECKEN, H., BRÜGGEMANN, N., 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. **European Journal of Soil Science** 67, 23-39.

HOLLOCHER, T.C., TATE, M.E., NICHOLAS, D.J., 1981. Oxidation of ammonia by Nitrosomonas europaea. Definite 18O-tracer evidence that hydroxylamine formation involves a monooxygenase. **The Journal of biological chemistry** 256, 10834-10836.

IPCC, 2006. 2006 IPCC guidelines for National Greenhouse Gas Inventories, prepared by the National Greenhouse Gas Inventories Programme. In: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (Eds.), **2006 IPCC Guidelines for National Greenhouse Gas Inventories**. Published: IGES, Japan.

LAM, S.K., SUTER, H., MOSIER, A.R., CHEN, D., 2017. Using nitrification inhibitors to mitigate agricultural N_2O emission: a double-edged sword? **Glob. Change Biol.** 23, 485-489.

LUO, J., BALVERT, S.F., WISE, B., WELTEN, B., LEDGARD, S.F., DE KLEIN, C.A.M., LINDSEY, S., JUDGE, A., 2018. Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. **Science of the Total Environment** 610-611, 1271-1280.

LUO, J., LEDGARD, S., WISE, B., LINDSEY, S., 2016. Effect of dicyandiamide (DCD) on nitrous oxide emissions from cow urine deposited on a pasture soil, as

influenced by DCD application method and rate. **Animal Production Science** 56, 350-354.
MAZZETTO, A.M., BARNEZE, A.S., FEIGL, B.J., VAN GROENIGEN, J.W., OENEMA, O., DE KLEIN, C.A.M., CERRI, C.C., 2015. Use of the nitrification inhibitor dicyandiamide (DCD) does not mitigate N_2O emission from bovine urine patches under Oxisol in Northwest Brazil. **Nutr. Cycl. Agroecosyst**. 101, 83-92.

MCCARTY, G.W., BREMNER, J.M., 1989. Laboratory evaluation of dicyandiamide as a soil nitrification inhibitor. **Communications in Soil Science and Plant Analysis** 20, 2049-2065.

MCTIC, 2016. **Ministério da Ciência Tencologia Inovações e Comunicações**. Estimativas anuais de emissões de gases de efeito estufa no Brasil. Brasil-Governo Federal.

MINISTRY FOR THE ENVIRONMENT, 2017. **New Zealand's Environmental Reporting Series**: Our atmosphere and climate 2017. Retrieved from www.mfe.govt.nz and www.stats.govt.nz.

MONAGHAN, R.M., SMITH, L.C., DE KLEIN, C.A.M., 2013. The effectiveness of the nitrification inhibitor dicyandiamide (DCD) in reducing nitrate leaching and nitrous oxide emissions from a grazed winter forage crop in southern New Zealand. **Agriculture Ecosystems & Environment** 175, 29-38.

SIMON, P.L., DIECKOW, J., DE KLEIN, C.A.M., ZANATTA, J.A., VAN DER WEERDEN, T.J., RAMALHO, B., BAYER, C., 2018. Nitrous oxide emission factors from cattle urine and dung, and dicyandiamide (DCD) as a mitigation strategy in subtropical pastures. **Agriculture, Ecosystems & Environment** 267, 74-82.

STANTON, C., REINHARD, C., F. KASTING, J., E. OSTROM, N., HASLUN, J., LYONS, T., GLASS, J., 2018. Nitrous oxide from chemodenitrification: A possible missing link in the Proterozoic greenhouse and the evolution of aerobic respiration. **Geobiology** 16.

SUBBARAO, G.V., NAKAHARA, K., HURTADO, M.P., ONO, H., MORETA, D.E., SALCEDO, A.F., YOSHIHASHI, A.T., ISHIKAWA, T., ISHITANI, M., OHNISHI-KAMEYAMA, M., YOSHIDA, M., RONDON, M., RAO, I.M., LASCANO, C.E., BERRY, W.L., ITO, O., 2009. Evidence for biological nitrification inhibition in Brachiaria pastures. **Proc. Natl. Acad. Sci**. U. S. A. 106, 17302-17307.

SUBBARAO, G.V., RONDON, M., ITO, O., ISHIKAWA, T., RAO, I.M., NAKAHARA, K., LASCANO, C., BERRY, W.L., 2007. Biological nitrification inhibition (BNI)—is it a widespread phenomenon? **Plant and Soil** 294, 5-18.

SUBBARAO, G.V., YOSHIHASHI, T., WORTHINGTON, M., NAKAHARA, K., ANDO, Y., SAHRAWAT, K.L., RAO, I.M., LATA, J.-C., KISHII, M., BRAUN, H.-J., 2015. Suppression of soil nitrification by plants. **Plant Science** 233, 155-164.

USDA, 2017. Livestock and Poultry: World Markets and Trade. **United States Department of Agriculture.** Foreign Agricultural Service/USDA.

VERTREGT, N., RUTGERS, B., 1987. Ammonia volatilization from urine patches in grassland. In: VAN DER MEER, H.G., UNWIN, R.J., VAN DIJK, T.A., ENNIK, G.C. (Eds.), Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Proceedings of an International Symposium of the European Grassland Federation, Wageningen, The Netherlands, 31 August-3 September 1987. **Springer Netherlands, Dordrecht**, pp. 361-363.

ZHU, X., BURGER, M., DOANE, T.A., HORWATH, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2O and NO under low oxygen availability. **Proc Natl Acad Sci U S A** 110, 6328-6333.

ZUMFT, W.G., 1997. Cell biology and molecular basis of denitrification. **Microbiology and molecular biology reviews : MMBR** 61, 533-616.

CHAPTER I: POTENTIAL OF BRACHIARIA HUMIDICOLA AND DICYANDIAMIDE IN REDUCING NITROUS OXIDE EMISSIONS FROM CATTLE URINE PATCHES IN BRAZILIAN SUBTROPICAL CLIMATE

1.1 ABSTRACT

Pasture-based livestock system in Brazil contributes to 34% of nitrous oxide (N_2O) emissions, a powerful greenhouse gas and ozone depletor. A field and a greenhouse experiment were conducted in a Cambisol under subtropical climate, aiming: (i) to evaluate the potential of brachiaria humidicola, a known forage specie for its ability to inhibit biologically soil nitrification, in reducing N_2O emissions from urine patches; (ii) to determine the efficacy of the synthetic nitrification inhibitor, dicyandiamide (DCD) to decrease N_2O emissions; and (iii) to determine the effect of two forage species and DCD application on ammonia (NH_3) volatilization. The field experiment was carried out in a split-plot experimental design, with forage species (Brachiaria and Guinea grass) in the main plots and cattle urine and DCD treatments (No urine, Urine and Urine+DCD) in the subplots. Over the 67-day measurement period, N_2O emissions were 20% lower from urine patches applied to brachiaria (1138 mg N m^{-2}, Emission factor= 1.06%) compared to emissions from urine on guinea grass (1436 mg N m^{-2}, Emission factor= 1.33%) ($p<0.10$). The results of a greenhouse experiment, using pots with either brachiaria or guinea grass plants that received the same treatments as in the field experiment, suggested that this could be a result of the lower soil nitrate (NO_3^-) concentration and intensities under brachiaria forage than in guinea grass, indicating a potential of biological nitrification inhibition (BNI) from brachiaria. The DCD application was effective in both forage species, decreasing N_2O emissions from urine+DCD by 40-50% ($p<0.10$) compared with Urine only. Approximately 25% of urine applied N was lost via NH_3 volatilization; NH_3 loss was not affected by forage species or DCD application ($p>0.10$). Overall, the results demonstrated that brachiaria and DCD use can be efficient strategies to reduce N_2O emissions from urine patches.

Keywords: BNI. Forage species. NH_3 volatilization. Ammonium. Nitrate.

1.2 RESUMO

O sistema pecuário baseado em pasto no Brasil contribui com 34% das emissões de óxido nitroso (N_2O), um poderoso gás de efeito estufa e depletor do ozônio. Um experimento de campo e casa de vegetação foram conduzidos em um Cambissolo sob clima subtropical visando: (i) avaliar o potencial da brachiaria humidicola, uma espécie forrageira conhecida por sua capacidade de inibir biologicamente a nitrificação do solo, em reduzir as emissões de N_2O de em manchas de urina; (ii) determinar a eficácia do inibidor sintético da nitrificação, dicianodiamida (DCD) em diminuir as emissões de N_2O; e (iii) determinar o efeito de duas espécies forrageiras e aplicação de DCD na volatilização de amônia (NH_3). O experimento de campo foi conduzido em delineamento experimental em parcelas subdivididas, com as espécies forrageiras (Brachiaria e Panicum) nas parcelas principais e com urina de bovinos e tratamentos com DCD (Sem urina, Urina e Urina + DCD) nas subparcelas. Durante o período de medição de 67 dias, as emissões de

N_2O foram 20% mais baixas a partir de manchas de urina aplicadas sobre braquiária (1138 mg N m^{-2}, fator de emissão = 1,06%) em comparação com as emissões em urina sobre panicum (1436 mg N m^{-2}, Fator de emissão = 1,33%) ($p < 0,10$). Os resultados de um experimento em casa de vegetação, utilizando vasos com capim-braquiária ou panicum que receberam os mesmos tratamentos do experimento de campo, sugeriram que isso poderia ser resultado da menor concentração e intensidades de nitrato (NO_3^-) na forragem de braquiária comparado com panicum, indicando um potencial de inibição da nitrificação biológica (BNI) pela brachiaria. A aplicação do DCD foi efetiva em ambas as espécies forrageiras, diminuindo as emissões de N_2O do tratamento Urina + DCD em 40-50% ($p < 0,10$) em comparação apenas com Urina. Aproximadamente 25% do N aplicada na urina foi perdido através da volatilização de NH_3; A perda de NH_3 não foi afetada pela espécie forrageira ou aplicação de DCD ($p > 0,10$). No geral, os resultados demonstraram que o uso de brachiaria e DCD pode ser uma estratégia eficiente para reduzir as emissões de N_2O a partir de manchas de urina.

Palavras-chave: IBN. Espécies forrageiras. Volatilização de NH_3. Amônio. Nitrato.

2.3 INTRODUCTION

Nitrous oxide is a long-lasting greenhouse gas (lifetime 150 years), and an ozone depletion substance, counting on 7% of the global greenhouse effect (Duxbury, 1994; Forster *et al.*, 2007). The human activity is responsible for 6.7 Tg N_2O-N emitted annually, and the agricultural sector contributed with 84% of N_2O emissions in 2010, being 60% from agricultural soils via direct and indirect emissions (IPCC, 2014; Brasil, 2016; Broucek, 2017). The Brazilian livestock activity accounts with 34% of direct N_2O emissions, with a herd of approximately 220 million cattle head (Hungate *et al.*, 2003; USDA, 2017).The contribution of this sector to N_2O emissions increased from 31% in 2010 to 34% in 2014 (2016), and it is possibly related to the intensification of the production system. Alternatives to reduce the N_2O emissions and increasing the nitrogen efficiency use (NEU) have been sought by researchers, through the attempt to decrease the urinary N output, and/or reducing the proportion of the urinary-N that is emitted as N_2O.

Some plant species have the ability to produce and release roots exudates that can supress the N oxidizing microorganism activity and keep the NH_4^+ for longer in soil, in a phenomenon termed biological nitrification inhibition (BNI) (Subbarao *et al.*, 2007a; Subbarao *et al.*, 2009; Meena *et al.*, 2014; Subbarao *et al.*, 2015; Byrnes *et al.*, 2017; Gardiner *et al.*, 2017; O'Sullivan *et al.*, 2017). A cyclic diterpene, named brachialactone, was identified in roots exudates of *Brachiaria humidicola* after a

laboratory assay conducted by Subbarao *et al.* (2007a) with recombinant luminescent *Nitrossomonas europaea*.

The brachialactone structure is composed of a unique dicyclopenta [a, d] cyclooctane skeleton (5-8-5 ring system) with a lactone ring bridging one of the five-membered rings and the eight-membered ring (Subbarao *et al.*, 2009). The mechanism of this BNI acting on nitrifiers bacteria in the soil are not well elucidated yet, however, it may be related to the effect of brachialactone on enzymatic pathways in the soil blocking the activity of ammonia mono-oxygenase (AMO) and hydroxylamine oxidoreductase (HAO) during the ammonia oxidation in Nitrossomonas, as observed for the BNI sorgoleone identified in Sorghum (*Sorghum bicolor*) roots (Zakir *et al.*, 2008). Byrnes *et al.* (2017) found lower N_2O emissions after cattle urine application onto *Brachiaria humidicola* compared to emissions from *Brachiaria mulato* in a study carried out at the International Centre for Tropical Agriculture (CIAT), in Colombia. In a temperate climate, recent studies undertaken in New Zealand have found reductions in N_2O emissions from urine patches deposited onto alternative plant species such as Plantain (*Plantago lanceolate*) (Gardiner *et al.*, 2017; Luo *et al.*, 2018), Forage rape (*Brassica napus L.*) (Luo *et al.*, 2014; Hoogendoorn *et al.*, 2016), Lucerne (*Medicago sativa L.*) (Di *et al.*, 2016) Luo *et al.*, 2018) and Fodder beet (*Beta vulgaris L.*) (Di *et al.*, 2016). The authors suggest that the results are related to BNI capacity of some plants after animal ingestion in partitioning the excreted-N, or decreasing the N output and also to ability of some exudates released by plants in inhibit the nitrification activity in soil (Dietz *et al.*, 2013; Cheng *et al.*, 2017; Gardiner *et al.*, 2017).

Another alternative for reducing N_2O emissions is through the use of synthetic nitrification inhibitors, as reported for some studies using: 3,4-dimethylpyrazole phosphate (DMPP) (Marsden *et al.*, 2016), nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] (Ward *et al.*, 2018) and dicyandiamide (DCD) (de Klein *et al.*, 2014). DCD is a synthetic nitrification inhibitor and acts in the AMO enzymatic pathways in soil, reaching an efficacy level by 70% on N_2O emissions reduction under temperate climate (de Klein and Ledgard, 2005; Smith *et al.*, 2008; O'Callaghan *et al.*, 2010; Monaghan *et al.*, 2013; Cardenas *et al.*, 2016). In tropical Brazilian pastures there was no efficacy of DCD application (Mazzetto *et al.*, 2015). However, under subtropical Brazilian conditions, the application of DCD appears to

be a good alternative to reduce N_2O emissions from urine patches, as reported in a recent study by Simon *et al.* (2018), where the authors suggest that the effectiveness of DCD in subtropical climate may be related to the lower temperatures in these regions compared to the tropical Brazilian climate, once that DCD degradation in soil is evidenced and conditioned to soil temperature increasing (Kelliher *et al.*, 2008). Although DCD has been showed as a good alternative to mitigate N_2O emission in some livestock systems, researchers have been questioning its real effect on N_2O emissions, once DCD can increase NH_3 volatilization due to the soil pH increasing and longer period of NH_4^+ in soil, being the NH_3 volatilized an indirect source of N_2O emissions after its deposition onto soil (Singh *et al.*, 2008; Zaman *et al.*, 2009; Zaman and Nguyen, 2012; Lam *et al.*, 2017).

The current study aimed to evaluate the potential of *Brachiaria humidicola* and the synthetic nitrification inhibitor, DCD, in reducing N_2O emissions from cattle urine patches, and the effect of them on NH_3 volatilization in Brazilian subtropical climate.

2.4 MATERIAL E METHODOS

2.4.1 Field experiment

2.4.1.1 Site description

The study was carried out at the experimental farm of the Federal University of Parana, Pinhais-Brazil (25º23'55'' S and 49º07'29'' W) in an area of 525 m^2 (35 x 15 m), at 912 m altitude, under a free drained Cambisol (Sugamosto, 2002), with clay-loam textured (28.2% sand, 57.1% clay) topsoils (0-20 cm), a pH of 4.7, and a bulk density of 1.2 g cm^{-3} (Table 1). The climate is subtropical humid with mean annual precipitation of 1408 mm and mean annual temperature of 16.1 ºC (INMET, 2009). The experimental area was cultivated with black oats (*Avena strigosa*) in the winter and corn (*Zea mays*) in the summer for 15 years in a conventional management until the moment of the forage experiment establishment.

The experiment was constituted of two forage species, brachiaria (*Brachiaria humidicola cv. humidicola*) and guinea grass (*Panicum maximum cv. aries*) combined with urine and DCD treatments (No urine, urine and urine + DCD). In October 2016 black oats was laid down simulating a no-tillage system and in November 2016 brachiaria and guinea grass were manually sown in rows at spacing of 15 cm in plots of 5 x 4 m, at rates equivalents to 25 and 20 kg seed ha^{-1}, respectively. At 30 and 60

days after sowing the forages were clipped at 15 cm height and the biomass removed from the plots, due to simulating the animal grazing. The experiment was arranged in a split-plot randomized block design with the 2 forage species in the main plots, and the urine and DCD treatments in the subplots, with 4 replicates. Each subplot was constituted of 4 delimited spots of 0.179 m^2 where the urine treatments were applied: one for N_2O measurement, one for NH_3 volatilization, one for soil parameters (inorganic N and moisture), and one for plant attributes. Urine treatments were constituted of manual application of dairy cattle urine with volume equivalent to one urination, in a previously area delimited by metallic rectangular bases of 0.179 m^2, inserted into 5 cm soil layer. For DCD treatments, the product was mixed into urine previously to application at a rate equivalent to 10 kg ha^{-1}, amount commonly utilized in commercial scale (Di and Cameron, 2003).

TABLE 1 - SOIL PROPERTIES AT THE 0-20 CM SOIL LAYER AFTER FERTILIZATION WITH 20-80-100 kg ha^{-1} OF N, P_2O_5 AND K_2O, RESPECTIVELY.

Soil test	Unit	Result
pH	$CaCl_2$	4.7
Ca	$cmol_c\ dm^{-3}$	6.0
Mg	$cmol_c\ dm^{-3}$	3.2
K	$cmol_c\ dm^{-3}$	0.05
Na	$cmol_c\ dm^{-3}$	0.003
Al	$cmol_c\ dm^{-3}$	0.5
Al+H	$cmol_c\ dm^{-3}$	13.4
P	$mg\ dm^{-3}$	14.3
Fe	$mg\ dm^{-3}$	102.2
Mn	$mg\ dm^{-3}$	19.1
Cu	$mg\ dm^{-3}$	0.7
Zn	$mg\ dm^{-3}$	0.2
OM	%	4
Sand	%	31.7
Silt	%	51.7
Clay	%	16.6
$T_{efective}$	$cmol_c\ dm^{-3}$	9.7
$T_{pH7.0}$	$cmol_c\ dm^{-3}$	22.7
V	%	40.8
NH_4^+	$mg\ N\ kg^{-1}$	7.2
NO_3^-	$mg\ N\ kg^{-1}$	4.5

Source: The author (2017).

2.4.1.2 Urine characteristics

Urine was collected at 4:30 am from a group of 30 Friesian dairy cows, seven years old aged and approximately 450 kg live weight. The volume urinated by each animal was measured and stored at 5ºC for 48h until completing the required amount to the experiment. After finishing the samplings, it was determined the mean volume of urine excreted per animal. The mean volume of urination was 2 L, and this amount was applied into the delimitated rectangular bases of 0.179 m^2 being equivalent to an application rate of 11 L urine m^{-2}.

Previously to application onto the soil, all the urine volume was homogenised in barrel of 150 L. Two subsamples were taken and forwarded to total urine-N determination by Kjeldahl method (Bremner, 1996). The urine-N content was 9.3 g N L^{-1}, and the amount applied into each base was equivalent to 103.9 g N m^{-2}.

2.4.1.3 Gas measurements

2.4.1.3.1 N_2O emissions

A static closed chamber method was used to measure N_2O emissions (Parkin and Venterea, 2010). During measurement the edge of the chamber was inserted into a water channel built around the top edge of the base casing in order to ensure a gas-tight seal. At the beginning of each measurement period the chamber was placed slowly on top of each base and headspace gas samples were taken through a septum fitted into the top of the chamber initially (t0), after 15 minutes (t15), 30 minutes (t30) and 45 (t45) minutes. Samples were transferred from the chamber to a pre-evacuated 12 ml glass vial using a plastic syringe. Headspace samples were taken between 09:00 and 10:00 hours. Samples were taken every 3 days in the first 2 weeks and then every 7 days (n= 14) for 67 days post urine application.

The N_2O concentration was measured in a gas chromatograph (GC trace - 1310) belonging to Embrapa Forest, in Brazil, and the daily N_2O fluxes (mg N m^{-2} h^{-1}) was determined by the linear regression that describes the increasing of the fluxes over 45 minutes of evaluation, air temperature inside the chamber, pressure (1 atm), internal volume of the chamber and area of the rectangular bases.

2.4.1.3.2 Volatilized NH_3

In a second delimited area of 0.179 m^2 in the subplot semi-open static collectors made of 2L polyethylene terephthalate (PET) transparent plastic bottles (35 cm high, 10 cm diameter) and 80 cm^2 area according to Araújo *et al.* (2009),

were kept at 5 cm from the soil surface by an iron cable 10 mm, so that air could circulate inside simulating natural field conditions. Two collectors were used per plot totalizing 48 samplers. A polyurethane sponge strip 2.5 cm wide and 30 cm long was fixed inside the collector with a stainless steel wire. The sponge was kept with the lower end in contact with an acid solution of 0.05 M H_2SO_4 + 2% (v/v) glycerol in a 50 ml graduated pot for NH_3 absorption. Sponge and the sulphuric acid solution were replaced for each new sampling.

Volatilized gas samples were taken at 1, 2, 3, 4, 6, 9, 12 and 18 days after cattle urine application onto soil. Samples were extracted directly after collecting and frozen-stored until NH_3 determination by flow injection analysis (FIA).

2.4.1.4 Soil inorganic N and moisture

In a third delimited area three soil samples were taken per subplot in the 0-5 cm soil layer obtaining a composite sample per sampling, which were stored in plastic bags until laboratory determinations. Approximately 30 g of moist soil was weighed and dried in oven 105 ºC for 48 hours to determination of gravimetric moisture content. Water-filled pore space (WFPS) was calculated from gravimetric soil moisture content, bulk density and an assumed particle density of 2.65 tonne m^{-3}. For inorganic N (ammonium and nitrate) determinations a 10 g of moist soil was extracted in 1M KCl solution and then stored at -5 ºC until determination by flux injection analysis (FIA).

2.4.1.5 Plant attributes

Aboveground samples were harvested in all 0.179 m^2 of a fourth delimited area per subplot at 27 and 67 days after application (daa) of the urine and DCD treatments. The biomass was stored into paper bags and oven-dried at 60 ºC during 48 hours until constant weight. The forage was ground and passed through a 0.25 mm sieve to the total N content determination using a Perkin Elmer CHNS-O elemental analyser.

Roots were sampled after the second aboveground harvesting at 67 daa, taking soil cores of 5.88 cm diameter and 9.83 cm depth (volume of 266.9 cm^3) by using an auger collector (Böhm, 1979; Bengough *et al.*, 2000). The samples were taken in duplicates, stored in individual plastic bags and forwarded to freezing at -4 ºC. During processing, each soil core was immersed in water and the roots washed

free dispersant use for 10 times, and then stored in flasks with ethanol 40% for preserving the roots integrity. Afterwards the roots were scanned by the software WinRhizo (Regent, 2015) to determinate length and superficial area of roots. Subsequently the material was oven-dried at 40 ºC and weighed to determination of dry matter of roots.

2.4.2 Greenhouse experiment

2.4.2.1 Site description

Pots of 10 L were filled up with 0-0.20 m soil layer from the field experiment site and placed in a greenhouse from January to March 2017. The soil was air-dried for 15 days and passed throw a 4 mm sieve. It was calculated the soil gravimetric moisture and added 1.4 kg of moist soil per pot, achieving a bulk density of 1 tonne m^{-3}. The experiment was organized as a completely randomized block design with the same treatments utilized for the field experiment (two forage species combined with cattle urine and DCD treatments). Two days after the pot settled up it was sown 30 seeds of brachiaria and guinea grass at 0.02 m soil depth. The soil moisture was kept at 70% of the field capacity throughout the trial. At 3 and 5 days after sowing guinea grass and brachiaria, respectively, seedlings were carefully removed so that 10 plants remained per pot. The mean temperature in the greenhouse was 30ºC reaching a maximum of 45ºC in January and minimum of 12º in February.

The forages were clipped at 20 days after sowing for standard homogenization at 15 cm height. At 35 days after sowing brachiaria and guinea grass were clipped again followed by urine and DCD treatments application.

2.4.2.2 Soil inorganic N and moisture

Soil samples were taken at 0-0.15 m soil layer at -3, 1, 3, 6, 12 and 29 days after urine and DCD treatments application. The soil was stored in plastic bags and frozen until determination of ammonium and nitrate by flow injection analysis. 10 g of moist soil was weighed and oven-dried at 105ºC for 48h. The dry soil was weighed and calculated the gravimetric moisture. Water-filled pore space (WFPS) was calculated from gravimetric soil moisture content, bulk density and an assumed particle density of 2.75 tonne m^{-3}.

2.4.3 Statistical analysis

The results were tested for error normality (Shapiro wilk) and variance homogeneity (Bartlett), no needing data transformation. When significant the means were compared by the Tukey's test at 10% significance level for gas emissions and at 5% significance for plant parameters. Pearson's correlation analyses were carried out as to compare N_2O cumulative emissions and soil inorganic N.

2.5 RESULTS

2.5.1 N_2O emissions

N_2O fluxes were affected by forage specie ($p<0.10$) with the lowest emissions from brachiaria, reaching a peak of 5.59 mg N m^{-2} h^{-1} at 3 daa (Figure 1a). Emissions from guinea grass presented a peak of 7.26 mg N m^{-2} h^{-1}, also at 3 daa (Figure 1a). Cumulative N_2O emissions over the 67-day period were 1138 mg N m^{-2} for brachiaria and 1436 mg N m^{-2} from guinea grass, representing a reduction of 20% on N_2O emissions from brachiaria use as forage ($p<0.10$) (Table 2). The background (no urine) cumulative N_2O emissions were significantly lower than for urine and DCD treatments, with 42 and 57 mg N m^{-2} for brachiaria and guinea grass, respectively ($p<0.10$) (Table 2). The emission factor (EF; percentage N_2O-N of urine-N applied) of N_2O was also influenced by the forage specie, with lower EF for brachiaria (1.06 %) than for guinea grass (1.33 %) ($p<0.1$) (Table 2).

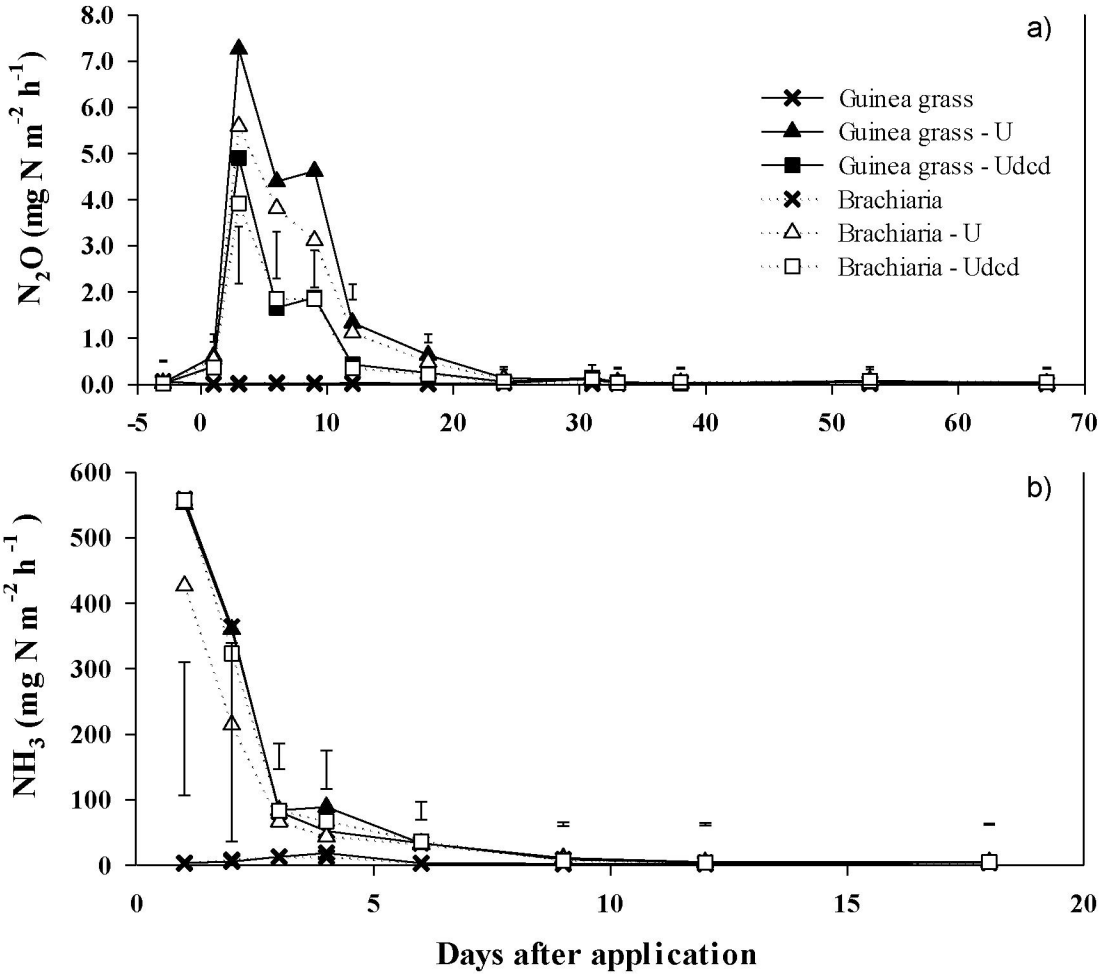
The DCD application decreased the N_2O fluxes in both forage species, with considerably lower emission peaks than those obtained from the urine treatments: 3.92 and 4.91 mg m^{-2} h^{-1} at 3 daa for brachiaria and guinea grass, respectively. The N_2O cumulative emission reduced 41% (666 mg N m^{-2}) and 49% (726 mg N m^{-2}) when DCD was applied onto brachiaria and guinea grass, respectively, compared with the urine-only treatments ($p<0.10$). Furthermore, the EF values were also reduced by the application of DCD when compared with urine-only, and were 0.60% and 0.64% for brachiaria and guinea grass, respectively ($p<0.10$) (Table 2).

2.5.2 NH_3 volatilization

Ammonia volatilization responded as quickly to urine application as N_2O emissions did, reaching to 427 and 551mg N m^{-2} h^{-1} after urine application onto brachiaria and guinea grass, respectively. When DCD was applied, NH_3 volatilization rates were 557 and 559 mg N m^{-2} h^{-1} for brachiaria and guinea grass, respectively

(Figure 1b). The volatilization rapidly decreased over the time with background levels reached at 9 daa.

FIGURE 1 – FLUXES OF NITROUS OXIDE (a) AND AMMONIA VOLATILIZATION (b) OVER 67 AND 18 DAYS, RESPECTIVELY, AFTER APPLICATION OF CATTLE URINE (U) AND URINE WITH DICYANDIAMIDE (UDCD) ONTO FIELD PLOTS GUINEA GRASS AND BRACHIARIA. VERTICAL BARS ARE THE LSD ACCORDING TO TUKEY'S TEST (P < 0.10). PINHAIS-PR, BRAZIL, 2017.



Source: The author (2017).

Total NH_3 volatilization losses were equivalent to 18% and 26% of applied N onto brachiaria and guinea grass for urine application, respectively, and 25% of applied N onto both forage species after DCD application (Table 2). Neither forage specie nor DCD application affected NH_3 volatilization ($p>0.10$).

TABLE 2 – CUMULATIVE EMISSIONS (mg N m^{-2}) AND EMISSION FACTOR (%) OF NITROUS OXIDE AFTER APPLICATION OF CATTLE URINE AND DICYANDIAMIDE (DCD) ONTO FIELD PLOTS OF GUINEA GRASS AND BRACHIARIA. PINHAIS-PR, BRAZIL, 2017.

	Guinea grass			Brachiaria		
	No urine	Urine	Urine+DCD	No urine	Urine	Urine+DCD
Nitrous oxide						
Cumulative emissions (mg N m^{-2})	57 cA*	1436 aA	726 bA	42 cA	1138 aB	666 bA
Emission factor (%)		1.33 aA	0.64 bA		1.06 aB	0.60 bA
Ammonia						
Cumulative volatilization (mg N m^{-2})	1224 bA	27288 aA	26592 aA	1128 bA	19176 aA	25968 aA
Loss equivalent to applied N (%)	1 bA	26 aA	25 aA	1 bA	18 aA	25 aA

Probability (Cumulative N_2O emission) = Forage specie: 0.008; Treatments: <0.001; Intercation: 0.02; SED: 35.70
Probability (Total EF) = Forage specie: 0.024; Treatments: <0.001; Interaction: 0.07; SED: 0.052
(*)Uppercase letter compares differences between forage species and lowercase letter compare differences between urine treatments within the same forage specie according to Tukey's test (P < 0.10).

Source: The author (2017).

2.5.3 Soil inorganic N

2.5.3.1 Ammonium

In the field experiment, ammonium concentration in soil increased markedly after urine application in the field, reaching 550 and 510 mg N kg^{-1} one day after urine application onto brachiaria and guinea grass, respectively (Figure 2b). When DCD was applied the ammonium concentrations were even higher than for urine, with 611 and 587 mg N kg^{-1} one day after application onto brachiaria and guinea grass, respectively. These values decreased over the time until the background level at approximately 18 daa for the urine-only treatment and at 24 daa for the DCD treatment. The intensity of ammonium (mg N kg^{-1} d) in the field experiment was not influenced by the forage specie ($p>0.10$). However, the DCD application increased these values ($p<0.10$): to 9544 compared with 6981 when urine was applied onto brachiaria, and to 8875 compared with 7498 when urine was applied onto guinea grass (Table 3). Both, urine and DCD application onto soil led to an increase in ammonium intensity compared to no-urine treatments in brachiaria (418) and guinea grass (555) ($p<0.10$) (Table 3).

In the greenhouse experiment, the ammonium concentration (mg N kg^{-1}) was highest at 6 daa, with 849 mg N kg^{-1} for the DCD treatment applied onto guinea grass and 819 mg N kg^{-1} for the urine-only treatment applied onto brachiaria (Figure 3b). As for the field experiment, the ammonium intensity (mg N kg^{-1} d) in greenhouse experiment was not affected by forage specie ($p>0.10$), although the intensities were significantly higher with DCD application onto guinea grass (15847) compared to urine-only treatment (12068) ($p<0.10$). For brachiaria ammonium intensity was apparently higher with DCD application (14821) compared to urine-only treatment (13743), but this difference was non-significant ($p>0.10$) (Table 3).

2.5.3.2 Nitrate

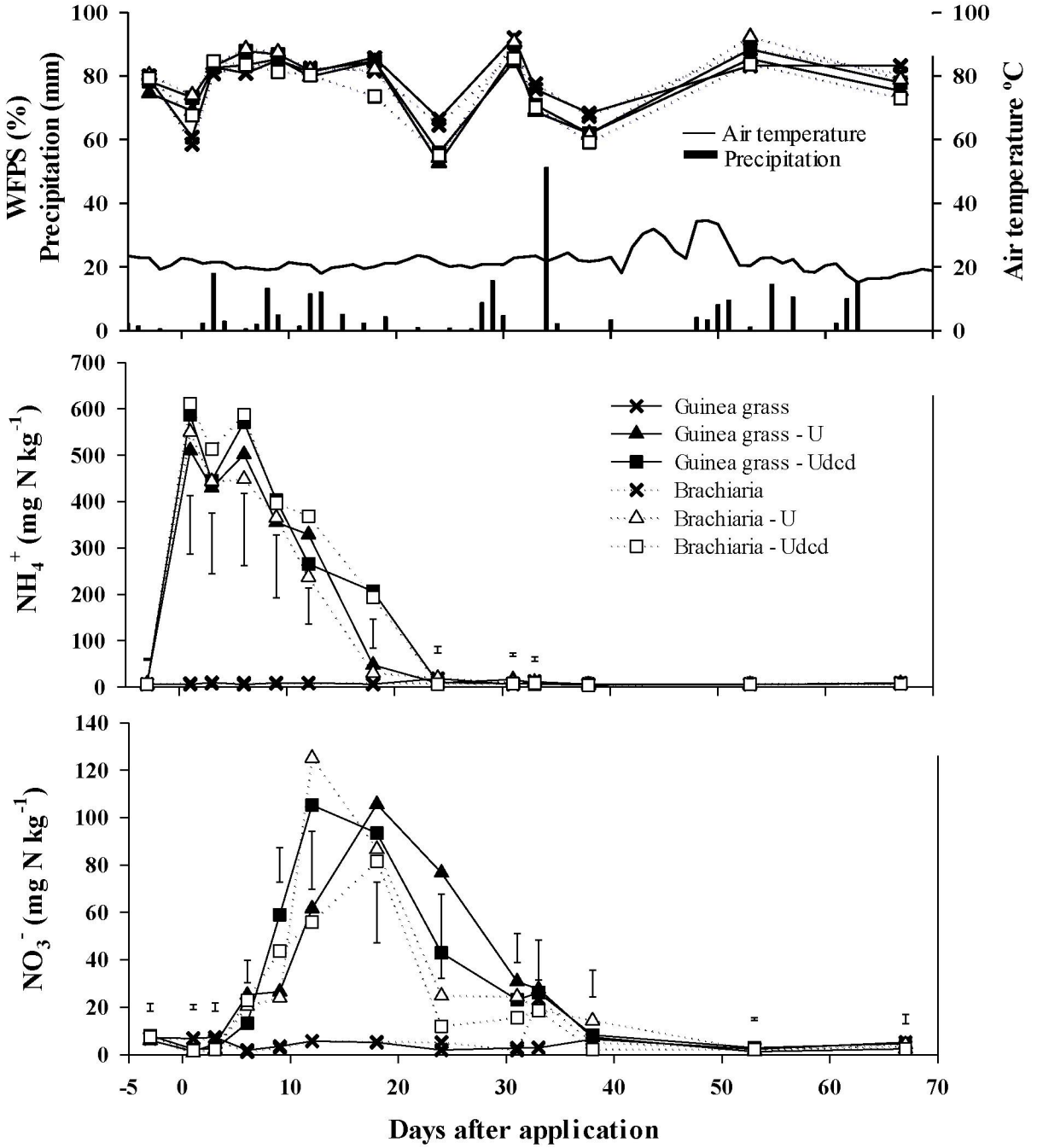
In the field experiment, the nitrate concentration increased as the ammonium reduced in the soil (Figure 2c), reaching to 125 and 105 mg N kg^{-1} for urine application onto brachiaria and guinea grass, respectively. The highest concentrations were usually obtained for guinea grass treatments throughout the measurement period (Figure 2c). The intensity of nitrate was significantly higher when DCD was applied onto guinea grass (2074 mg N kg^{-1} d) than when it was applied onto brachiaria (1360 mg N kg^{-1} d) ($p<0.10$) (Table 3). While for guinea grass

the nitrate intensity was not affected by DCD use compared with urine-only application, in brachiaria the nitrate intensity was significantly lower when DCD was applied onto the forage ($p<0.10$) (Table 3).

In the greenhouse experiment the highest nitrate concentrations were obtained when urine was applied onto guinea grass, with values reaching to 335 mg N kg^{-1} of nitrate compared with 80 mg N kg^{-1} for brachiaria, at 12 daa ($p<0.10$) (Figure 3c). Nitrate intensity in the soil was also affected by forage specie, with lower intensities when urine was applied onto brachiaria (1661 mg N kg^{-1} d) compared to guinea grass (5219 mg N kg^{-1} d), respectively ($p<0.10$) (Table 3).

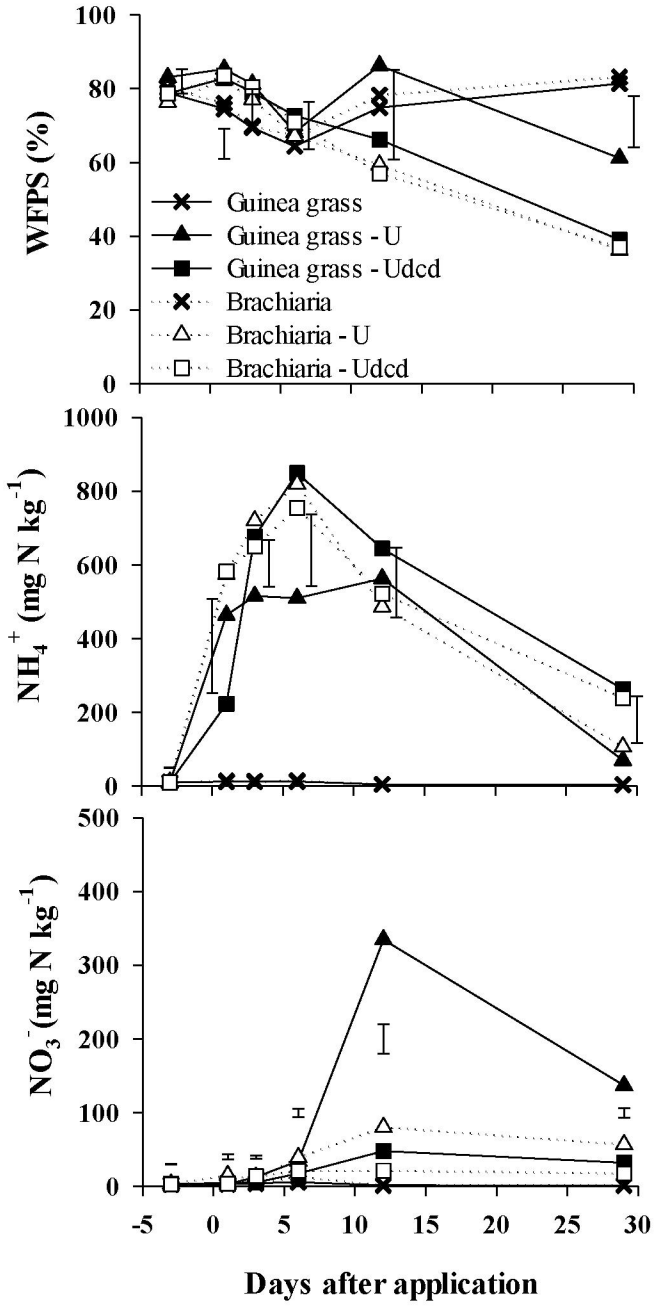
The nitrate intensities in the DCD treatments applied to brachiaria and guinea grass were not significantly different ($p>0.1$) (Table 3). However, compared with the urine-only treatment, DCD application reduced the nitrate intensity in both forage species from 1661 to 544 in brachiaria and from 5291 to 939 in guinea grass ($p<0.1$) (Table 3).

FIGURE 2 – WATER-FILLED PORE SPACE (WFPS) IN 0-5 CM SOIL LAYER, MEAN DAILY TEMPERATURE (°C, LINES) AND DAILY PRECIPITATION (mm, BARS) (a) AND CONCENTRATION OF AMMONIUM (NH_4^+) (b) AND NITRATE (NO_3^-) (c) IN 0-5 CM SOIL LAYER, OVER 67 DAYS AFTER APPLICATION OF CATTLE URINE (U) AND URINE WITH DICYANDIAMIDE (UDCD) ONTO FIELD PLOTS OF GUINEA GRASS AND BRACHIARIA. VERTICAL BARS ARE THE LSD ACCORDING TO TUKEY'S TEST (P < 0.10). PINHAIS-PR, BRAZIL, 2017.



Source: The author (2017).

FIGURE 3 – WATER-FILLED PORE SPACE (WFPS) (a), CONCENTRATION OF AMMONIUM (b) AND NITRATE (c) IN 0-20 cm SOIL LAYER AFTER APPLICATION OF URINE (U) AND URINE WITH DICYANDIAMIDE (UDCD) ONTO POTS WITH GUINEA GRASS AND BRACHIARIA KEPT IN A GREENHOUSE. VERTICAL BARS ARE THE LSD ACCORDING TO TUKEY'S TEST (P < 0.10). PINHAIS-PR, BRAZIL, 2017.



Source: The author (2017).

TABLE 3 – INTENSITY OF AMMONIUM AND NITRATE OVER 70 DAYS OF EVALUATION IN A FIELD EXPERIMENT, AND 29 DAYS OF EVALUATION IN A GREENHOUSE EXPERIMENT, AFTER CATTLE URINE AND DICYADIAMIDE (DCD) APPLICATION ONTO PASTURES OF GUINEA GRASS AND BRACHIARIA. PINHAIS-PR, BRAZIL, 2017.

(mg N kg^{-1} d)	Guinea grass			Brachiaria		
	No urine	Urine	Urine+DCD	No urine	Urine	Urine+DCD
Field experiment						
Ammonium intensity	555 cA	7498 bA	8875 aA	418 cA	6981 bA	9544 aA
Nitrate intensity	297 bA	2110 aA	2074 aA	360 cA	1938 aA	1360 bB
Greenhouse experiment						
Ammonium intensity	239 cA	12068 bA	15847 aA	254 bA	13743 aA	14821 aA
Nitrate intensity	85 cA	5219 aA	939 bA	116 bA	1661 aB	544 bA

(*)Uppercase letter compares differences between forage species and lowercase letter compare differences between urine treatments within the same forage specie according to Tukey's test ($P < 0.10$).

Source: The author (2017).

2.5.4 Weather and soil moisture

The total precipitation over the evaluation period was 249 mm with a rainfall concentrated period between 2 and 20 daa (73 mm) and a large single rainfall event on 34 daa (51 mm) (Figure 2a). The average air temperature obtained throughout the season was 22 ºC, with a minimum of 15 ºC in March and maximum of 32 ºC in February (Figure 2a).

The mean WFPS throughout the field experiment was 77% and no significant difference was obtained between treatments. In the greenhouse experiment, the WFPS throughout 29 days of evaluation presented an average WFPS of 72%, and also was not influenced by forage specie nor DCD application ($p>0.10$) (Figure 3a).

2.5.5 Plant attributes

The length of roots was higher when urine and urine+DCD were applied onto brachiaria (4452 cm and 3974 cm, respectively) than when these treatments were applied onto guinea grass (3586 cm and 3466 cm, respectively) ($p<0.05$) (Table 4). For brachiaria, root length increased with treatment in the following order: No urine<Urine+DCD<Urine ($p<0.05$) (Table 4). For Guinea grass no root-length differences were observed between the No urine, Urine and Urine+DCD treatments. The specific density of roots was also influenced by forage specie, with greater values for Urine (1422 cm g^{-1}) and DCD (9462 cm g^{-1}) applied onto brachiaria than when Urine (8403 cm g^{-1}) and DCD (6949 cm g^{-1}) were applied onto guinea grass ($p<0.05$) (Table 4). The specific density of roots was significantly lower when DCD was applied onto the forage species compared to urine-only application for both species ($p<0.05$) (Table 4). For the other evaluated root parameters [superficial area (cm^2), dry matter (kg ha^{-1}) and density (g m^{-3})] no differences were observed between forage species nor urine and urine+DCD treatments ($p>0.05$) (Table 4).

Aboveground dry matter (DM) sampled at 27 daa did not present any difference between the evaluated treatments. However, when aboveground DM was harvested at 67 daa, brachiaria had a greater mass (5722 kg DM ha^{-1}) than guinea grass (4154 kg DM ha^{-1}) after Urine+DCD application ($p<0.05$) (Table 4). No differences in DM were obtained between the Urine and Urine+DCD application for either forage species ($p>0.05$). Total N in aboveground samples was not affected by forage species nor by DCD application ($p>0.05$) (Table 4).

TABLE 4 – ROOT ATTRIBUTES IN 0-15 cm SOIL LAYER AT 67 DAYS AFTER APPLICATION OF CATTLE URINE (U) AND DICYANDIAMIDE (DCD), AND ABOVEGROUND DRY MATTER, AMMONIUM AND NITRATE AT 27 AND 67 DAYS AFTER APPLICATION OF URINE AND DCD ONTO FIELD PLOTS OF GUINEA GRASS AND BRACHIARIA. PINHAIS-PR, BRAZIL, 2017.

	Guinea grass						Brachiaria					
	No urine		Urine		Urine+DCD		No urine		Urine		Urine+DCD	
Root												
Dry matter (kg ha^{-1})	1437	bA	1584	abA	1842	aA	1179	aA	1437	aA	1547	aA
Density (g m^{-3})	1475	bA	1599	abA	1869	aA	1205	aA	1460	aA	1574	aA
Lenght (cm)	3181	aA	3586	aB	3466	aB	2853	cA	4452	aA	3974	bA
Specific density (cm g^{-1})	8082	aA	8403	aB	6949	bB	8869	bA	11422	aA	9462	bA
Superficial area (cm^{2})	327	ns	336	ns	339	ns	257	ns	345	ns	330	ns
Aboveground 27 daa												
Dry matter (kg ha^{-1})	2195	bA	4854	aA	5542	aA	2041	bA	4880	aA	5581	aA
Total N (g N kg^{-1})	25	bA	35	aA	33	aA	17	bB	33	aA	33	aA
Aboveground 67 daa												
Dry matter (kg ha^{-1})	3165	aA	3953	aA	4154	aB	3315	bA	4555	abA	5722	aA
Total N (g N kg^{-1})	11	bA	12	abA	13	aA	11	bA	11	bA	13	aA
Shoot: root ratio 67 daa (kg ha^{-1})	2.2	aA	2.5	aA	2.3	aA	2.8	bA	3.2	abA	3.7	aA

Source: The author

(*)Uppercase letter compares differences between forage species and lowercase letter compare differences between urine treatments within the same forage specie according to Tukey's test ($P < 0.05$).

Source: The author (2017).

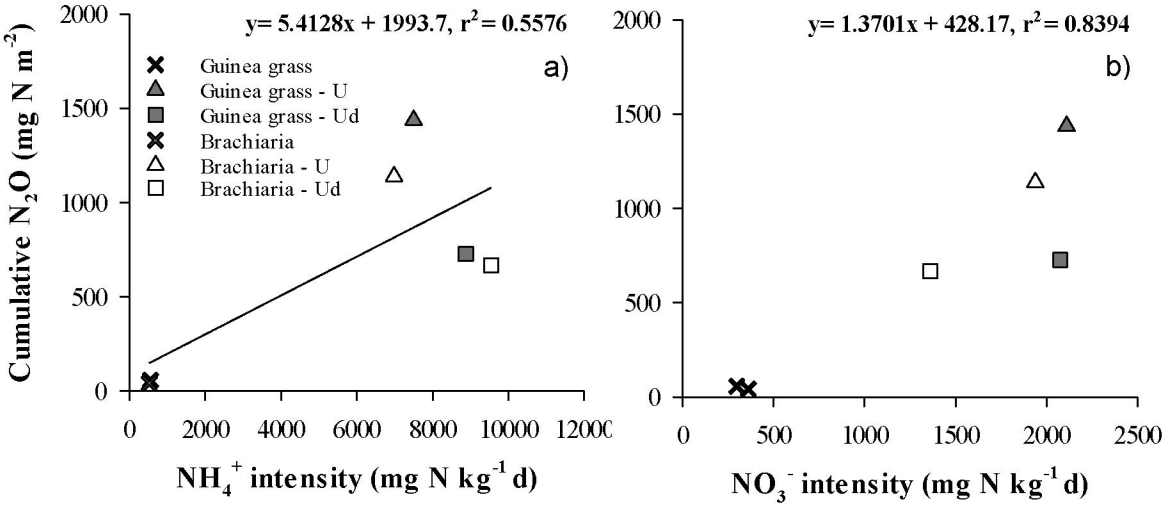
2.6 DISCUSSION

2.6.1 N_2O emissions suppression by brachiaria humidicola

The reduction of 20% on N_2O emissions from brachiaria in relation to guinea grass evidenced the potential of brachiaria to mitigate N_2O emissions from cattle urine patches. It coincided with lower soil nitrate concentration and intensities, suggesting an inhibitory effect of this forage specie on nitrification activity in soil. Similar result was found by Byrnes *et al.* (2017) in a field study evaluating the forage effect on N_2O emissions with a reduction of 60% after cattle urine application onto *brachiaria humidicola* (forage with high BNI capacity) compared to *brachiaria mulato* (forage with low BNI capacity) . In a 3-year field experiment in Colombia, researchers found a decrease in soil ammonium oxidation rates of up to 90% and a low population of nitrifiers bacteria in soil grown with *brachiaria humidicola*, and they suggest that it is possibly due to an inhibitory effect of brachiaria on nitrification process in soil (Subbarao *et al.*, 2009; Subbarao *et al.*, 2015; Coskun *et al.*, 2017). There is still no elucidation of the brachialactone pathways suppressing nitrification activity in soil, however it is suggested that this molecule may act as the sorgoleone, a BNI found out in sorghum crop (Subbarao *et al.*, 2013; Zeng *et al.*, 2016). This molecule competes with soil ammonium through binding to ammonia monooxigenase (AMO) and hydroxylamine oxidoredutase (HAO) thus keeping ammonium in the soil as an N source in the system and reducing nitrification activity (Gopalakrishnan *et al.*, 2007; Subbarao *et al.*, 2015).

In our study, the potential of brachiaria to inhibit nitrification, as suggested by the lower cumulative emission and EF of N_2O found in brachiaria compared with guinea grass after cattle urine application, was also supported by the strong relationship obtained between soil nitrate intensity and cumulative N_2O oxide emission (r^2=0.84) (Figure 4b). Soil nitrate concentration was also highly correlated with N_2O cumulative emission in brachiaria ($p<0.001$), but not for guinea grass ($p>0.05$). In an incubation experiment undertaken by Meena *et al.* (2014) the authors obtained a nitrification inhibition rate of 60-90% after root exudates extracted from *brachiaria humidicola* was applied onto the soil, and this effect was even higher than with DCD application, supporting the BNI capacity of this forage.

FIGURE 4 – RELATIONSHIP BETWEEN AMMONIUM INTENSITY (a) OR NITRATE (b) INTENSITY AND CUMULATIVE N2O EMISSION AFTER APPLICATION OF URINE (U) AND URINE WITH DCD (UDCD) ONTO FIELD PLOTS OF GUINEA GRASS AND BRACHIARIA. PINHAIS-PR, BRAZIL, 2017.



Source: The author (2017).

In our study, the effect of brachiaria was also observed in the field experiment, where root length (cm) and specific density of roots (cm g^{-1}) were higher compared with guinea grass ($p<0.05$). These root parameters were negatively correlated with soil nitrate intensity in brachiaria ($p<0.05$), which suggests that the higher length from brachiaria may have led to a higher distribution of root exudates released in the soil and to a lower nitrate formation. However, no differences were obtained for soil nitrate intensities between the forage species ($p>0.10$). According to Gopalakrishnan *et al.* (2007) about 30% of the roots mass is turned over annually being equivalent to 1 Mg of roots ha^{-1} (DM basis), causing an additive effect over time in decreasing the nitrification rates in soils where brachiaria is grown, which may be attributed to the root effects observed in our study.

The greenhouse experiment suggested a forage specie effect on soil inorganic N transformations and soil moisture content ($p<0.05$), as evidenced by the lower soil nitrate concentrations under brachiaria and by the lower soil moisture in these plots from 12 daa. The soil moisture content for brachiaria in greenhouse was negatively correlated to the root length for this forage ($p<0.05$), suggesting that the larger root length of brachiaria, allowed an increase in water uptake. According to Cantarel *et al.* (2015) the maximum nitrification rate (NEA)

in soil was strongly correlated to specific root length, suggesting that a reduction in soil water may have be led to a decrease in the anaerobic microsites and, consequently, in a reduction in N_2O production in soil. Although brachiaria had the largest roots length, there was no significant difference in total N uptake from the soil between the two forage species ($p>0.05$).

The average emission factor of N_2O for cattle urine application of 1.2% obtained in our study was still below of the N_2O emission factor (EF_3) default 2% suggested by IPCC for urine cattle in pastures (IPCC, 2006). Other studies undertaken in different Brazilian climates found EF values below 2% (Barneze *et al.*, 2014; Sordi *et al.*, 2014; Mazzetto *et al.*, 2015; Simon *et al.*, 2018), which demonstrates the need of determining a specific EF for Brazilian conditions. It is well-known that not only climate influences N_2O emissions, but also other 'country-specific' characteristics such as animal breed, diet and forage type (Lessa *et al.*, 2014; Pelster *et al.*, 2016).

2.6.2 DCD application as mitigation strategy of N_2O emission

The application of DCD resulted in an average reduction of 45% on N_2O emissions after cattle urine application onto both forages, which suggests that this product has promise as a N_2O mitigation option in Brazilian subtropical climate. DCD is a bacteriostatic product, acting over AMO enzyme, responsible for ammonia oxidation and maintenance of the ammonium for longer in soil (Amberger, 1989; O'Callaghan *et al.*, 2010). Previous researchers have shown a reduction potential of DCD application onto pastures up to 78% and 45% of N_2O emissions and soil nitrate leaching, respectively, for temperate climates (Di and Cameron, 2002; Di *et al.*, 2007; de Klein *et al.*, 2014; Ledgard *et al.*, 2014; Cardenas *et al.*, 2016). In a tropical climate, Mazzetto *et al.*, (2015) found that DCD application did not have any effect on N_2O emission reduction (Mazzetto *et al.*, 2015), and these authors suggest that the lack of efficacy of DCD was due to the high soil temperature effect, which led to the quick degradation of the product reducing its potential in soil. As reported by Di and Cameron (2004), DCD has a half-life of 18-25 days at 20 ºC while at 8 ºC its half-life is of 111-116 days. The same effect of DCD degradation in soil was observed in a data analysis by Kelliher *et al.* (2008) with an exponential decrease in the half-life of DCD as the soil temperature increased.

The DCD efficacy in reducing N_2O emissions in our study was supported by soil nitrate intensities in both the field and greenhouse studies for brachiaria. The lower soil nitrate concentrations when DCD was applied onto this forage compared to urine only application ($p<0.10$), is potentially related to the additive effect of DCD, which complemented the BNI activity from brachiaria and further decreased of the soil nitrate production.

2.6.3 Forage specie and DCD influence on NH_3 volatilization

The NH_3 volatilization was not affected by forage specie or DCD application onto soil, although it totalled about 25% of the N applied. Although NH_3 is not a greenhouse gas, it is a secondary source of N_2O production from soil, once the volatilized NH_3 is redeposited onto soil and emitted as N_2O contributing to the global warming (Denier van der Gon and Bleeker, 2005). According to IPCC guidelines, about 1% (0.2-5% uncertainty range) of the volatilized NH_3 is converted to N_2O (IPCC, 2006). Lam *et al.* (2017) showed in a review study that DCD application in agricultural systems caused an increase in NH_3 volatilization of 3-65%, thus decreasing the net effect of the nitrification inhibitor on N_2O emission when taken into account the estimated indirect N_2O emission from deposited NH_3.

2.7 CONCLUSIONS

Cattle urine applied to brachiaria had 20% lower N_2O emissions compared with cattle urine applied to guinea grass. This coincided with lower nitrate concentration and intensities in soil where brachiaria was grown, which may be related to ability of brachiaria to biologically inhibit nitrification activity. The addition of DCD to the urine was also efficient in reducing direct N_2O emission from both brachiaria and guinea grass plots, decreasing emissions by 40-50% compared with an urine-only treatment. Although NH_3 volatilization was not affected by either forage specie or DCD use, it totalled about 25% of the urine N applied. Further research into options for mitigating NH_3 volatilization from livestock systems in Brazil seems therefore warranted. The N_2O EF values found at this study were still lower than default 2% suggested by the IPCC for national greenhouse gas inventories, which suggests that this default value may need to be revised for subtropical condition.

REFERENCES

AMBERGER, A., 1989. Research on dicyandiamide as a nitrification inhibitor and future outlook. **Communications in Soil Science and Plant Analysis** 20, 1933-1955.

ARAÚJO, E.D.S., MARSOLA, T., MIYAZAWA, M., SOARES, L.H.D.B., URQUIAGA, S., BODDEY, R.M., ALVES, B.J.R., 2009. Calibração de câmara semiaberta estática para quantificação de amônia volatilizada do solo. **Pesquisa Agropecuária Brasileira** 44, 769-776.

BARNEZE, A.S., MAZZETTO, A.M., ZANI, C.F., MISSELBROOK, T., CERRI, C.C., 2014. Nitrous oxide emissions from soil due to urine deposition by grazing cattle in Brazil. **Atmospheric Environment** 92, 394-397.

BENGOUGH, A.G., CASTRIGNANO, A., PAGÈS, L., VAN NOORDWIJK, M., 2000. Sampling strategies, scaling, and statistics. In: SMIT, A.L.; BENGOUGH, A.G.; ENGELS, C.; VAN NOORDWIJK, M.; PELLERIN, S.; VAN DE GEIJN, S.C. (Ed.). Root methods: a handbook. **Springer**.

BÖHM, W., 1979. Methods of studying root systems. Ecological Studies: Analysis and Synthesis. **Springer**, Berlin Heidelberg New York.

BRASIL, 2016. Ministério da Ciência Tecnologia e Inovação Secretaria de Políticas e Programas de Pesquisa e desenvolvimento Coordenação Geral de Mudanças Globais de Clima Brasil. Terceira Comunicação Nacional do Brasil à Convenção-Quadro das Nações Unidas sobre Mudança do Clima – Volume III. Brasília: **Ministério da Ciência, Tecnologia e Inovação**, p. 336.

BREMNER, J.M., 1996. NITROGEN - TOTAL. IN: SPARKS, D.L., PAGE, A.L., HELMKE, P.A., LOEPPERT, R.H., SOLTANPOUR, P.N., TABATABAI, M.A., JOHNSTON, C.T., SUMNER, M.E. (Eds.), **Methods of Soil Analysis Part 3 Chemical Methods. SSSA**, Madison, pp. 1085-1121.

BYRNES, R.C., NUNEZ, J., ARENAS, L., RAO, I., TRUJILLO, C., ALVAREZ, C., ARANGO, J., RASCHE, F., CHIRINDA, N., 2017. Biological nitrification inhibition by Brachiaria grasses mitigates soil nitrous oxide emissions from bovine urine patches. **Soil Biology & Biochemistry** 107, 156-163.

CANTAREL, A.A.M., POMMIER, T., DESCLOS-THEVENIAU, M., DIQUÉLOU, S., DUMONT, M., GRASSEIN, F., KASTL, E.-M., GRIGULIS, K., LAÎNÉ, P., LAVOREL, S., LEMAUVIEL-LAVENANT, S., PERSONENI, E., SCHLOTER, M., POLY, F., 2015. Using plant traits to explain plant–microbe relationships involved in nitrogen acquisition. **Ecology** 96, 788-799.

CARDENAS, L.M., MISSELBROOK, T.M., HODGSON, C., DONOVAN, N., GILHESPY, S., SMITH, K.A., DHANOA, M.S., CHADWICK, D., 2016. Effect of the application of cattle urine with or without the nitrification inhibitor DCD, and dung on greenhouse gas emissions from a UK grassland soil. **Agriculture Ecosystems & Environment** 235, 229-241.

CHENG, L., G. JUDSON, H., BRYANT, R., MOWAT, H., GUINOT, L., HAGUE, H., TAYLOR, S., R. EDWARDS, G., 2017. The effects of feeding cut plantain and perennial ryegrass-white clover pasture on dairy heifer feed and water intake, apparent nutrient digestibility and nitrogen excretion in urine.

COSKUN, D., BRITTO, D.T., SHI, W., KRONZUCKER, H.J., 2017. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. **Nature plants** 3, 17074.

DE KLEIN, C.A.M., LEDGARD, S.F., 2005. Nitrous oxide emissions from New Zealand agriculture - key sources and mitigation strategies. **Nutr. Cycl. Agroecosyst.** 72, 77-85.

DE KLEIN, C.A.M., LETICA, S.A., MACFIE, P.I., 2014. Evaluating the effects of dicyandiamide (DCD) on nitrogen cycling and dry matter production in a 3-year trial on a dairy pasture in South Otago, New Zealand. **New Zealand Journal of Agricultural Research** 57, 316-331.

DENIER VAN DER GON, H., BLEEKER, A., 2005. Indirect N2O emission due to atmospheric N deposition for the Netherlands. **Netherlands**, p. 13.

DI, H.J., CAMERON, K.C., 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. **Soil Use and Management** 18, 395-403.

DI, H.J., CAMERON, K.C., 2003. Mitigation of nitrous oxide emissions in spray-irrigated grazed grassland by treating the soil with dicyandiamide, a nitrification inhibitor. **Soil Use and Management** 19, 284-290.

DI, H.J., CAMERON, K.C., 2004. Effects of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial biomass in a grazed pasture soil. **Soil Res**. 42, 927-932.

DI, H.J., CAMERON, K.C., PODOLYAN, A., EDWARDS, G.R., DE KLEIN, C.A.M., DYNES, R., WOODS, R., 2016. The potential of using alternative pastures, forage crops and gibberellic acid to mitigate nitrous oxide emissions. **J. Soils Sediments** 16, 2252-2262.

DI, H.J., CAMERON, K.C., SHERLOCK, R.R., 2007. Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. **Soil Use and Management** 23, 1-9.

DIETZ, M., MACHILL, S., HOFFMANN, H.C., SCHMIDTKE, K., 2013. Inhibitory effects of Plantago lanceolata L. on soil N mineralization. **Plant and Soil** 368, 445-458.

GARDINER, C.A., CLOUGH, T.J., CAMERON, K.C., DI, H.J., EDWARDS, G.R., DE KLEIN, C.A.M., 2017. Potential inhibition of urine patch nitrous oxide

emissions by Plantago lanceolata and its metabolite aucubin. **New Zealand Journal of Agricultural Research**, 1-9.

GOPALAKRISHNAN, S., SUBBARAO, G.V., NAKAHARA, K., YOSHIHASHI, T., ITO, O., MAEDA, I., ONO, H., YOSHIDA, M., 2007. Nitrification Inhibitors from the Root Tissues of Brachiaria humidicola, a Tropical Grass. **Journal of Agricultural and Food Chemistry** 55, 1385-1388.

HOOGENDOORN, C.J., LUO, J., LLOYD-WEST, C.M., DEVANTIER, B.P., LINDSEY, S.B., SUN, S., PACHECO, D., LI, Y., THEOBALD, P.W., JUDGE, A., 2016. Nitrous oxide emission factors for urine from sheep and cattle fed forage rape (Brassica napus L.) or perennial ryegrass/white clover pasture (Lolium perenne L./Trifolium repens). **Agriculture, Ecosystems & Environment** 227, 11-23.

INMET, I.N.D.M., 2009. Normais Climatológicas do Brasil 1961-1990. **INMET**, Brasília.

IPCC, 2006. 2006 IPCC guidelines for National Greenhouse Gas Inventories, prepared by the National Greenhouse Gas Inventories Programme. In: EGGLESTON, H.S., BUENDIA, L., MIWA, K., NGARA, T., TANABE, K. (Eds.), **2006 IPCC Guidelines for National Greenhouse Gas Inventories**. Published: IGES, Japan.

KELLIHER, F.M., CLOUGH, T.J., CLARK, H., RYS, G., SEDCOLE, J.R., 2008. The temperature dependence of dicyandiamide (DCD) degradation in soils: A data synthesis. **Soil Biology and Biochemistry** 40, 1878-1882.

LAM, S.K., SUTER, H., MOSIER, A.R., CHEN, D., 2017. Using nitrification inhibitors to mitigate agricultural N2O emission: a double-edged sword? **Glob. Change Biol**. 23, 485-489.

LEDGARD, S.F., LUO, J., SPROSEN, M.S., WYATT, J.B., BALVERT, S.F., LINDSEY, S.B., 2014. Effects of the nitrification inhibitor dicyandiamide (DCD) on pasture production, nitrous oxide emissions and nitrate leaching in Waikato, **New Zealand. New Zealand Journal of Agricultural** Research 57, 294-315.

LESSA, A.C.R., MADARI, B.E., PAREDES, D.S., BODDEY, R.M., URQUIAGA, S., JANTALIA, C.P., ALVES, B.J.R., 2014. Bovine urine and dung deposited on Brazilian savannah pastures contribute differently to direct and indirect soil nitrous oxide emissions. **Agriculture, Ecosystems & Environment** 190, 104-111.

LUO, J., BALVERT, S.F., WISE, B., WELTEN, B., LEDGARD, S.F., DE KLEIN, C.A.M., LINDSEY, S., JUDGE, A., 2018. Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. **Science of the Total Environment** 610-611, 1271-1280.

LUO, J., SUN, X.Z., PACHECO, D., LEDGARD, S.F., LINDSEY, S.B., HOOGENDOORN, C.J., WISE, B., WATKINS, N.L., 2014. Nitrous oxide

emission factors for urine and dung from sheep fed either fresh forage rape (Brassica napus L.) or fresh perennial ryegrass (Lolium perenne L.). **Animal** 9, 534-543.

MARSDEN, K.A., MARÍN-MARTÍNEZ, A.J., VALLEJO, A., HILL, P.W., JONES, D.L., CHADWICK, D.R., 2016. The mobility of nitrification inhibitors under simulated ruminant urine deposition and rainfall: a comparison between DCD and DMPP. **Biology and Fertility of Soils** 52, 491-503.

MAZZETTO, A.M., BARNEZE, A.S., FEIGL, B.J., VAN GROENIGEN, J.W., OENEMA, O., DE KLEIN, C.A.M., CERRI, C.C., 2015. Use of the nitrification inhibitor dicyandiamide (DCD) does not mitigate N2O emission from bovine urine patches under Oxisol in Northwest Brazil. **Nutr. Cycl. Agroecosyst**. 101, 83-92.

MEENA, H.M., SACHDEV, M.S., MANJAIAH, K.M., DOTANIYA, M.L., 2014. Nitrification Inhibition Potential of Brachiaria humidicola. **National Academy Science Letters** 37, 113-116.

MONAGHAN, R.M., SMITH, L.C., DE KLEIN, C.A.M., 2013. The effectiveness of the nitrification inhibitor dicyandiamide (DCD) in reducing nitrate leaching and nitrous oxide emissions from a grazed winter forage crop in southern New Zealand. **Agriculture Ecosystems & Environment** 175, 29-38.

O'SULLIVAN, C.A., DUNCAN, E.G., WHISSON, K., TREBLE, K., WARD, P.R., ROPER, M.M., 2017. A colourimetric microplate assay for simple, high throughput assessment of synthetic and biological nitrification inhibitors. **Plant and Soil** 413, 275-287.

O'CALLAGHAN, M., GERARD, E.M., CARTER, P.E., LARDNER, R., SARATHCHANDRA, U., BURCH, G., GHANI, A., BELL, N., 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. **Soil Biology and Biochemistry** 42, 1425-1436.

PARKIN, T., VENTEREA, R., 2010. **USDA-ARS GRACEnet. Project Protocols Chapter 3**. Chamber-Based Trace Gas Flux Measurements.

PELSTER, D.E., GISORE, B., GOOPY, J., KORIR, D., KOSKE, J.K., RUFINO, M.C., BUTTERBACH-BAHL, K., 2016. Methane and Nitrous Oxide Emissions from Cattle Excreta on an East African Grassland. **Journal of environmental quality** 45, 1531-1539.

SIMON, P.L., DIECKOW, J., DE KLEIN, C.A.M., ZANATTA, J.A., VAN DER WEERDEN, T.J., RAMALHO, B., BAYER, C., 2018. Nitrous oxide emission factors from cattle urine and dung, and dicyandiamide (DCD) as a mitigation strategy in subtropical pastures. **Agriculture, Ecosystems & Environment** 267, 74-82.

SINGH, J., SAGGAR, S., GILTRAP, D.L., BOLAN, N.S., 2008. Decomposition of dicyandiamide (DCD) in three contrasting soils and its effect on nitrous oxide emission, soil respiratory activity, and microbial biomassan incubation study. **Soil Res**. 46, 517-525.

SMITH, L.C., DE KLEIN, C.A.M., MONAGHAN, R.M., CATTO, W.D., 2008. The effectiveness of dicyandiamide in reducing nitrous oxide emissions from a cattle-grazed, winter forage crop in Southland, New Zealand. **Aust. J. Exp. Agric**. 48, 160-164.

SORDI, A., DIECKOW, J., BAYER, C., ALBURQUERQUE, M.A., PIVA, J.T., ZANATTA, J.A., TOMAZI, M., DA ROSA, C.M., DE MORAES, A., 2014. Nitrous oxide emission factors for urine and dung patches in a subtropical Brazilian pastureland. **Agriculture, Ecosystems & Environment** 190, 94-103.

SUBBARAO, G.V., NAKAHARA, K., HURTADO, M.P., ONO, H., MORETA, D.E., SALCEDO, A.F., YOSHIHASHI, A.T., ISHIKAWA, T., ISHITANI, M., OHNISHI-KAMEYAMA, M., YOSHIDA, M., RONDON, M., RAO, I.M., LASCANO, C.E., BERRY, W.L., ITO, O., 2009. Evidence for biological nitrification inhibition in Brachiaria pastures. **Proc. Natl. Acad. Sci. U. S. A**. 106, 17302-17307.

SUBBARAO, G.V., RONDON, M., ITO, O., ISHIKAWA, T., RAO, I.M., NAKAHARA, K., LASCANO, C., BERRY, W.L., 2007. Biological nitrification inhibition (BNI)—is it a widespread phenomenon? **Plant and Soil** 294, 5-18.

SUBBARAO, G.V., SAHRAWAT, K.L., NAKAHARA, K., RAO, I.M., ISHITANI, M., HASH, C.T., KISHII, M., BONNETT, D.G., BERRY, W.L., LATA, J.C., 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). **Annals of Botany** 112, 297-316.

SUBBARAO, G.V., YOSHIHASHI, T., WORTHINGTON, M., NAKAHARA, K., ANDO, Y., SAHRAWAT, K.L., RAO, I.M., LATA, J.-C., KISHII, M., BRAUN, H.J., 2015. Suppression of soil nitrification by plants. **Plant Science** 233, 155-164.

SUGAMOSTO, M.L., 2002. Uso de técnicas de geoprocessamento para elaboração do mapa de aptidão agrícola da adequação de uso do centro de estações experimentais do Canguiri, Pinhais–PR. **Universidade Federal do Paraná**, Curitiba, p. 133.

WARD, G.N., KELLY, K.B., HOLLIER, J.W., 2018. Greenhouse gas emissions from dung, urine and dairy pond sludge applied to pasture. 1. Nitrous oxide emissions. **Animal Production Science** 58, 1087-1093.

ZAKIR, H.A.K.M., SUBBARAO, G.V., PEARSE, S.J., GOPALAKRISHNAN, S., ITO, O., ISHIKAWA, T., KAWANO, N., NAKAHARA, K., YOSHIHASHI, T., ONO, H., YOSHIDA, M., 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (Sorghum bicolor). **New Phytologist** 180, 442-451.

ZAMAN, M., NGUYEN, M.L., 2012. How application timings of urease and nitrification inhibitors affect N losses from urine patches in pastoral system. **Agriculture, Ecosystems & Environment** 156, 37-48.

ZAMAN, M., SAGGAR, S., BLENNERHASSETT, J.D., SINGH, J., 2009. Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. **Soil Biology and Biochemistry** 41, 1270-1280.

ZENG, H.Q., DI, T.J., ZHU, Y.Y., SUBBARAO, G.V., 2016. Transcriptional response of plasma membrane H^+-ATPase genes to ammonium nutrition and its functional link to the release of biological nitrification inhibitors from sorghum roots. **Plant and Soil** 398, 301-312.

3 CAPÍTULO II: *Plantago lanceolata* EFFICACY IN MITIGATING NITROUS OXIDE EMISSIONS FROM CATTLE URINE PATCHES

3.1 ABSTRACT

Nitrous oxide (N_2O) emissions contribute to 7% of total greenhouse gases emissions in the world. The global warming potential of N_2O is 298 fold greater than carbon dioxide (CO_2) over a 100-year time frame. Some plants secondary metabolites have the capacity of inhibit the soil nitrification in a process termed Biological nitrification inhibition (BNI), which may reduce N_2O emissions. The aims of this study were: i) To compare the effect of a pasture with high BNI capacity (Plantain - *Plantago lanceolata*) with a pasture with low BNI capacity (Ryegrass/white clover - *Lolium perenne* + *Trifolium repens*) on N_2O emissions in cattle urine patches; and, ii) to evaluate whether the effect of plantain on N_2O emissions reduction is mostly related to the urine-N loading rates and composition from animals fed on plantain, or to the plantain effect in soil (e.g. through the releasing of root exudates and inhibiting biologically soil nitrification and N_2O production). A closed static chamber method was used to measure the N_2O fluxes in delimited circular areas of 0.049 m^2 and an adjacent area was utilized for inorganic N analysis. The treatments included filed plots with six proportions of plantain in the sward (0, 15, 30, 45, 60 and 100%) and urine collected from animals fed with four levels of plantain in the diet (0, 15, 30 and 45%). The N_2O emissions reduced linearly with the increase of plantain proportion in the sward after application of standard cattle urine (from animals fed on ryegrass/white clover) (r^2= 0.9743), but no statistic difference was observed for soil inorganic N ($p>0.10$). Animals fed on plantain had a urine-N loading rate significantly lower than urine-N loading rates from animals fed on ryegrass/white clover (r^2= 0.9873). There was a clear trend of lower N_2O emissions with increasing plantain in the diet, but the emission factor was not statistically different between the different urine sources ($p>0.10$). The results suggest that the efficacy of plantain as a N_2O mitigation option is due to both a reduction in urinary N, as well as a plant effect. The latter is possibly a result of biological nitrification inhibition through root exudates released from plantain.

Key words: BNI. Gas emissions. Dairy cattle. Ammonium. Nitrate.

3.2 RESUMO

As emissões de óxido nitroso (N_2O) contribuem com 7% das emissões totais de gases de efeito estufa no mundo. O potencial de aquecimento global do N_2O é 298 vezes maior do que o dióxido de carbono (CO_2) em um período de 100 anos. Alguns metabólitos secundários de plantas têm a capacidade de inibir a nitrificação do solo em um processo denominado inibição biológica da nitrificação (IBN), levando a redução das emissões de N_2O. Os objetivos deste estudo foram: i) Comparar o efeito de um pasto com alta capacidade de IBN (Plantain - *Plantago lanceolata*) com uma pastagem com baixa capacidade de IBN (azevém/trevo branco - *Lolium perenne/ Trifolium repens*) nas emissões de

N_2O em manchas de urina bovina; e, ii) avaliar se o efeito do plantain na redução das emissões de N_2O está principalmente relacionado às taxas de liberação de N e/ou composição da urina de animais alimentados com plantain, ou ao efeito do plantain no solo (por exemplo, através da liberação de exsudatos radiculares inibindo biologicamente a nitrificação do solo e a produção de N_2O). O método de câmara estática fechada foi utilizado para medir os fluxos de N_2O em áreas circulares delimitadas de 0,049 m^2 e uma área adjacente foi utilizada para análise de N inorgânico. Os tratamentos incluíram parcelas preenchidas com seis proporções de plantain no pasto (0, 15, 30, 45, 60 e 100%) e urina coletada de animais alimentados com quatro níveis de plantain na dieta (0, 15, 30 e 45%). As emissões de N_2O reduziram linearmente com o aumento da proporção de plantain no pasto após a aplicação de uma urina bovina padrão (coletada de animais alimentados com azevém/trevo branco) (r^2= 0,9743), mas nenhuma diferença estatística foi observada para o N inorgânico do solo (p> 0,10). Os animais alimentados com plantain tiveram uma taxa de liberação de N na urina significativamente menor do que as taxas de liberação de N na urina de animais alimentados com azevém/ trevo branco (r^2= 0,9873). Houve uma clara tendência de menores emissões de N_2O com o aumento de plantain na dieta, mas o fator de emissão não foi estatisticamente diferente entre as diferentes fontes de urina (p> 0,10). Os resultados sugerem que a eficácia do plantain como opção de mitigação de N_2O se deve tanto à redução do N urinário quanto ao efeito da planta. Este último é possivelmente um resultado da inibição biológica da nitrificação através de exsudatos radiculares liberados pelo plantain.

Palavras-chave: IBN. Emissão de gases. Gado leiteiro. Amônio, Nitrato.

3.3 INTRODUCTION

Agricultural activity is the main source of global nitrous oxide (N_2O) emissions, and the N_2O represents 44% of the emission from this sector, with 22% from soil in pastoral dairy farming systems (de Klein *et al.*, 2001). In New Zealand, 95% of the emitted N_2O comes from agriculture, largely as a results of direct emissions from urine and dung deposited to soils in the predominantly grass-based livestock system in the country (Ministry for the Environment, 2017). The urine deposited onto grazed pasture is the main driver for N_2O production, considering that 80-90% of the consumed N by the animal is released in excreta (Kelliher *et al.*, 2014; Selbie *et al.*, 2015). The N excreted via urine, mainly in urea form, is readily available in soils, and equivalent to 200-2000 kg N ha^{-1} per cow urine patch (Selbie *et al.*, 2015). On average, cows excrete about 25 kg of dung and 21 L of urine over 12.8 dung patches and 10.2 urine patches, respectively, per day (Haynes and Williams, 1993; Saggar *et al.*, 2004; Luo *et al.*, 2018). This is far in excess of the capacity of plants to utilise

the N and so it is potentially lost to the environment, including as N_2O emissions to atmosphere (Luo *et al.*, 2008; Cardenas *et al.*, 2016). Due to these environmental concerns, there is ongoing research to look for alternatives to mitigate N_2O emissions.

Recent studies evaluating the plant capacity to curb N_2O emission from livestock systems have shown some hypothesis for the mechanisms of action in these plants: through roots exudates released in the rhizosphere area via N immobilization by the increase of C in the rhizosphere (Williams and Haynes, 1994; Bowatte *et al.*, 2018) and/or through the biological inhibition of the nitrification activity in the soil (Subbarao *et al.*, 2006; Byrnes *et al.*, 2017); reduction of urine-N output via diuretic effect (O'Connell *et al.*, 2016) an/or partitioning the N into dung rather than urine (Carulla *et al.*, 2005; Luo and Kelliher, 2014).

It is already known that some plants produce secondary metabolites that are released by roots exudation and can inhibit the soil nitrification activity, in a process termed biological nitrification inhibition (BNI) (Subbarao *et al.*, 2009; Gardiner *et al.*, 2017). Further studies found these BNIs composites present in the excreta from animals fed with plants containing BNI in its composition, which is possibly involved in some process of excreta-N formation and output (Cheng *et al.*, 2017). In tropical pastures, some N_2O studies show the inhibition capacity of brachiaria humidicola to curb nitrification activities in soil, in a BNI composite identified as brachialactone (Subbarao *et al.*, 2009), from roots exudates released in the soil. Brachialactone has a dicyclopenta [a,d]cyclooctane skeleton (5-8-5 ring system) with a γ-lactone ring bridging one of the five-membered rings and the eight membered ring and act over the ammonia monooxigenase (AMO) enzyme in soil. The biochemical mechanism of action of brachialactone is still in elucidation phase, but it is known that the releasing of this compound in soil is stimulated by the presence of NH_4^+ in the rhizosphere, once no exudates were detected in soil when brachiaria was submitted to the N in NO_3^- form (Subbarao *et al.*, 2007b), and studies have shown a decreasing up to 90% on soil ammonium oxidation rates and N_2O emissions (Subbarao *et al.*, 2009; Byrnes *et al.*, 2017).

In New Zealand, researchers have been exploring temperate plant species with capacity to produce and release BNI compounds, as plantain

(*Plantago lanceolata*) (Dietz *et al.*, 2013; Gardiner *et al.*, 2017), fodder beet (*Beta vulgaris*) (Di *et al.*, 2016), Lucerne (*Medicago sativa*) (Luo *et al.*, 2018) and Forage rape (*Brassica napus*) (Luo *et al.*, 2014; Hoogendoorn *et al.*, 2016). Plantain has some bioactive compounds as the iridoid glycosides aucubin and catalpol and the polyphenol verbascoside/ acteoside with potential BNI effects (Figure 1) (Wichtl, 2004; Gardiner *et al.*, 2016). Aucubin is a chemical composite known for its antimicrobial activity, and it is quickly converted on its active form aglycone aucubigenin (Dietz *et al.*, 2013). The aucubigenin is a lactone structure and can irreversibly inhibit enzymes by the reaction of its dialdehyde tautomer with free amino groups (Kim *et al.*, 2000). The aucubigenin is also known by its action inhibiting cytochrome P-450, which can be related to its ability of inhibit ammonia oxidation by the inhibition of AMO activity in soil (Davini *et al.*, 1986; Bartholomaeus and Ahokas, 1995).

FIGURE 1. CHEMICAL STRUCTURE OF THE SECONDARY METABOLITES PRODUCED IN PLANTAIN, AUCUBIN, CATALPOL AND ACTEOSIDE.

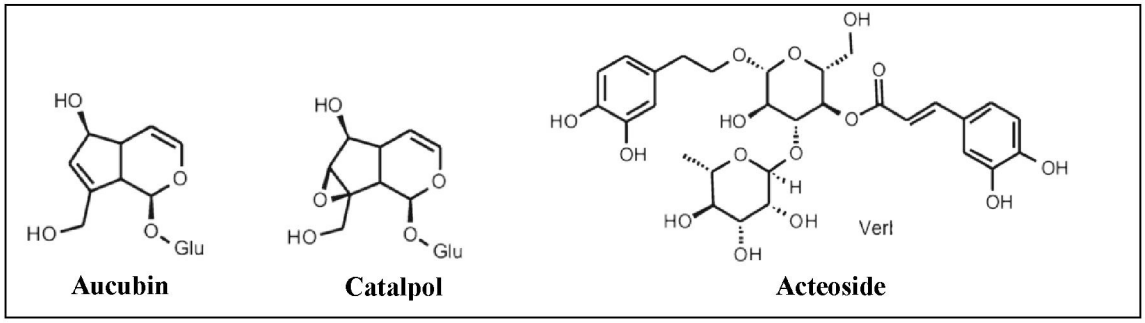


IMAGE EXTRACTED FROM Dietz *et al.* (2013).

The introduction of plantain mixed to the standard ryegrass/ white clover pasture in the farms of New Zealand appears to be a useful alternative to mitigate N_2O emissions in livestock systems. At this study we evaluated i) the potential of plantain for reducing N_2O emissions from urine patches, ii) the effect of plantain in animal diet on urine-N loading and its influence on N_2O emissions, and iii) whether the effects on N_2O emissions reduction are due to the urine effect (e.g. urine N loading or plantain metabolites in urine influencing soil process and N_2O production) or to the plantain sward effect (e.g. root exudates releasing from plantain with BNI activity).

3.4 MATERIAL AND METHODS

3.4.1 Site description and trial establishment

The field site was located at the AgResearch Limited, Invermay Agricultural Centre in Mosgiel, New Zealand (45.8° S 170.3° E). The soil at the site is classified as a moderately well-drained Wingatui silt loam (NZ Taxonomy, weathered Fluvial Recent soil; USDA Taxonomy, Dystrudepts (Hewitt, 2010) (Table 1). The trial area had previously contained a ryegrass/white clover pasture, which was previously rotationally grazed by sheep. In November 2017 the trial was established by sowing 3m x 10m plots with six rates of plantain mixed with the standard pasture ryegrass/white clover in a randomised block design (five replicates per sowing rate; Table 2). The plantain proportions were based in sowing rates equivalent to 0, 15, 30, 45, 60 and 100% of plantain. Before sowing the trial area was fenced off, sprayed, ploughed, disked, harrowed and levelled before seeding by direct drill.

Following seeding the trial site was sprayed out with glyphosate based round-up as a pre-emergent 4L ha^{-1} and dressed with urea 50kg ha^{-1} to help with establishment. The trial area was periodically mown with hand mowers and a ride on mower to maintain an average 5 cm pasture height. Before urine treatment application, the plants on each plot were cut to 5 cm above ground level and the herbage removed.

Daily rainfall and soil temperatures (at 10 cm depth) were logged for the entire trial period, beginning at least 1 week before treatment application at a site as close as possible to the trial site. A manual rain gauge was installed within the site to determine total rainfall between sampling days.

A 15-week N_2O measurement trial was conducted in autumn of 2018 (8^{th} March-18^{th} June), to measure emissions from urine, collected from animals on varying rates of plantain in the diet, that was applied to plots with varying rates of plantain in the sward. Due to the complexity of the treatments the experiment was run in three components (trial I, trial II and trial III).

TABLE 1: PHYSICAL AND CHEMICAL PROPERTIES OF WELL-DRAINED WINGATUI SILT LOAM SOIL (0-10 CM SOIL LAYER). DUNEDIN, NEW ZEALAND.

Property	Wingatui silt loam
*Physical**	
Fine sand (%) (60-200 µm)	7
Silt (%) (2-60 µm)	54
Clay (%) (<2 µm)	37
Bulk density (Mg m^{-3})	0.99
Particle density (Mg m^{-3})	2.6
Chemical	
pH ($CaCl_2$)	5.9
Olsen P, extractable (mg L^{-1})	23
K extractable ($cmol_c$ dm^{-3})	0.5
Mg extractable ($cmol_c$ dm^{-3})	1.3
Ca extractable ($cmol_c$ dm^{-3})	14.1
Na extractable ($cmol_c$ dm^{-3})	0.1
Total base saturation (%)	75.7
Cation exchange capacity ($cmol_c$ dm^{-3})	21.1

Source: The author (2018).
*Value of physical properties extracted from van der Weerden *et al.* (2012)

3.4.2 Specific urine trials

3.4.2.1 Trial I

Trial I aimed to assess the effect of plantain swards proportions (0, 15, 30 and 45%) mixed with the standard ryegrass/white clover, on N_2O emissions from urine collected from animals on diets with the same proportions of plantain (Table 3). In metabolism stalls, animals were fed ryegrass/white clover mixed with the respective proportions of plantain on a dry matter basis during 48 hours. Thus, ensured that the excreta released by the animals were derived of the diet offered to them.

Fresh cow urine was collected on 4 March 2018 by Dairy NZ. At collection, a subsample was taken for immediate total N analysis. All urine was collected over a 24 hour period, bulked, and stored at 4 ºC, after which the urine was transported to Invermay in chilly bins packed with ice. On 8 March 2018, the urine was applied within each plantain plot to the designated areas for N_2O emission and soil mineral N measurements. For the N_2O measurements, 0.49 L of urine was applied to each gas chamber area (0.049m²) to achieve a typical

dairy cow urination rate of 10 L m² (Haynes and Williams, 1993). The urine was applied slowly and evenly to the whole area using watering cans, at a speed that avoided runoff and excessive ponding, to the inside of the receiving gas chamber area. Directly adjacent to the N_2O measurement area, separate 0.7m² soil sampling areas were marked out within each plot for soil Mineral N analysis. These areas received the same urine at the equivalent rate of N as its corresponding gas chamber area. The control treatments received no urine.

3.4.2.2 Trial II

A second experiment was carried out to assess the effect of differences in urine composition due to dietary plantain on N_2O emissions. Urine from animals fed on 0% and 45% plantain were applied onto the standard ryegrass/white clover sward (Table 3).

Urine collection from the metabolisms stall at Diary NZ and the procedures of establishment and gas sampling were similarly performed as described for trial I.

3.4.2.3 Trial III

The trial III aimed to evaluate the effect of the proportion of plantain in the sward (0, 30, 60 and 100% plantain) on N_2O emissions. For this trial, fresh urine was collected from animals fed on standard ryegrass/white clover (i.e. 0% plantain). This urine was then applied onto plots with different proportions of plantain in the sward (Table 3).

Fresh cow urine was collected on 14 March 2018 from animals fed on standard ryegrass/white clover pasture (0% plantain) and a subsample was taken for immediate total N analysis. The urine was thoroughly mixed and held at 4 ºC overnight prior to application. On 15 March 2018, the urine was applied within each plantain plots to the designated areas for N_2O emission and soil mineral N measurements. The procedures for N_2O and soil core samplings were the same as described for trial I.

3.4.3 N_2O measurements

N_2O emission rates were measured using a non-vented closed chamber technique (de Klein *et al.*, 2003), with stainless steel chamber base rings of

25cm diameter containing a 'water trough' flange, inserted approximately 10 cm into the soil 3 days prior to urine treatment application. On each sampling occasion, stainless steel chambers were inserted into the water-filled trough to provide an airtight seal around the chamber. Chambers were 27cm in diameter and insulted with polystyrene foam and covered with self-adhesive aluminium foil to minimise temperature and pressure fluctuations within the enclosed space. The chambers were also fitted with sub-seal sampling ports that were removed at the time of chamber placement within the water trough and replaced when sampling commenced. Chamber base heights were measured after installation and volume calculated.

On each sampling day and following rainfall, N_2O measurements were carried out between 10 am and 1 pm. Headspace gas samples were taken during a cover period of 60 minutes at times t0, t30 and t60 (or similar), twice weekly, for the first 15 gas samplings and during a cover period of 60 minutes (or similar) at times t0 and t60, once a week for the remainder of the sampling occasions. On each sampling day, two background atmosphere samples were taken. The hourly N_2O fluxes (mg N m^{-2} h^{-1}) were calculated from the increase in head space N_2O over the sampling time (de Klein *et al.*, 2003).

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A} \quad (1)$$

Where δN_2O is the increase in head space N_2O over time (µL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of gas at the sampling temperature (L mol^{-1}); V is the headspace volume (m^3); and A is the area covered (m^2).

These hourly emissions were integrated over time, for each chamber, to estimate the total emission over the measurement period. The N_2O emission factors (EF_3, N_2O-N emitted as % of N applied) for the measurement periods were then calculated for the urine treatments using Eq. (2).

$$EF_3 = \frac{N_2O\text{-N total(urine)} - N_2O\text{-N total (control)}}{\text{Urine N applied}} \quad (2)$$

Where N_2O-N total (urine) and N_2O-N total (control) are the cumulative N_2O-N emissions for the measurement periods from the urine and control plots respectively (kg N ha^{-1}), and Urine N applied is the rate of urine N applied (kg N ha^{-1}).

3.4.4 Soil inorganic N and soil water measurement

Soil samples (7.5 cm deep, 25 mm diameter) were taken for the determination of soil nitrate, ammonium and water content. 2 soil cores were taken once a week at sampling time, each core was taken from a different area of the soil plots on each sampling day. Immediately after sampling, the hole was back-filled with sealed PVC tubes to minimise any effects on soil aeration. Back in the laboratory on the following day, the samples were thoroughly mixed and 15 g fresh soil (about 10 g dry soil equivalent) was extracted for 1 hour in 100 mL 2 M KCl (the ratio of soil and 2 M KCl is 1:10). The filtered solutions were frozen until analysed for nitrate N (plus nitrite N), and ammonium N. The remainder of the mixed soil was dried at 105ºC for 24 hours, to determine gravimetric soil water content.

Direct measurements of the Volumetric Soil Water Content (VSW %) for further determination of water-filled pore space (WFPS) of the soil plots were conducted each gas sampling occasion, three separate reading were taken on each plot using a MPM160 Moisture Probe Meter.

TABLE 2: CULTIVARS AND SOWING RATES OF PERENNIAL RYEGRASS (*Lolium perenne CV. One50 AR37*), WHITE CLOVER (*Trifolium repens CV. Tribute*) AND PLANTAIN (*Plantago lanceolata CV.* Agritonic) SEEDED IN THE PLOTS.

Cultivar	**Plantain (%)**	**Sowing rate (kg ha^{-1})**
Ryegrass	0	20
White clover	0	4
Plantain	15	1
Ryegrass	15	18
White clover	15	4
Plantain	30	4
Ryegrass	30	16
White clover	30	4
Plantain	45	6
Ryegrass	45	12
White clover	45	4
Plantain	60	10
White clover	60	2
Ryegrass	60	8
Plantain	100	12

Source: The author (2018).

TABLE 3. DISTRIBUTION OF TREATMENTS WITH URINE FROM ANIMALS FED ON DIFFERENT PROPORTIONS OF PLANTAIN APPLIED ONTO THE RESPECTIVE PROPORTIONS OF PLANTAIN SWARD (TRIAL I), URINE FROM ANIMALS FED ON 0% AND 45% PLANTAIN (TRIAL II), AND STANDARD FRESH URINE FROM ANIMALS WITH ONLY RYEGRASS/WHITE CLOVER IN DIET (TRIAL III).

Experiment	**Plantain swards**	**No urine**	**Urine - Dairy NZ feed trial**	**Urine - N**
1	0%	Control	0% plantain in diet	10 g N L^{-1}
	15%	Control	15% plantain in diet	8.7 g N L^{-1}
	30%	Control	30% plantain in diet	7.5 g N L^{-1}
	45%	Control	45% plantain in diet	5.6 g N L^{-1}
2	0%	Control	0% plantain in diet	10 g N L^{-1}
	0%	Control	45% plantain in diet	5.6 g N L^{-1}
	Plantain swards	**No urine**	**Urine - local standard**	**Urine - N**
3	0%	Control	0% plantain in diet	6.1 g N L^{-1}
	30%	Control	0% plantain in diet	6.1 g N L^{-1}
	60%	Control	0% plantain in diet	6.1 g N L^{-1}
	100%	Control	0% plantain in diet	6.1 g N L^{-1}

Source: The author (2018).

3.4.5 Statistical analysis

Statistical analyses were conducted using GenStat 18th Edition to examine analysis of variance. Log transformed data was used in a factorial analysis to explore interaction of sward-plantain and urine-plantain effects on EF%. Regression analyses were realized for experiments I and III to compare the N_2O cumulative emissions and emission factor over different plantain proportions in swards and in animal feeding. In experiment II comparing two treatments variance analysis was undertaken and means compared by Tukey's test ($p<0.10$).

3.5 RESULTS

3.5.1 N_2O emissions

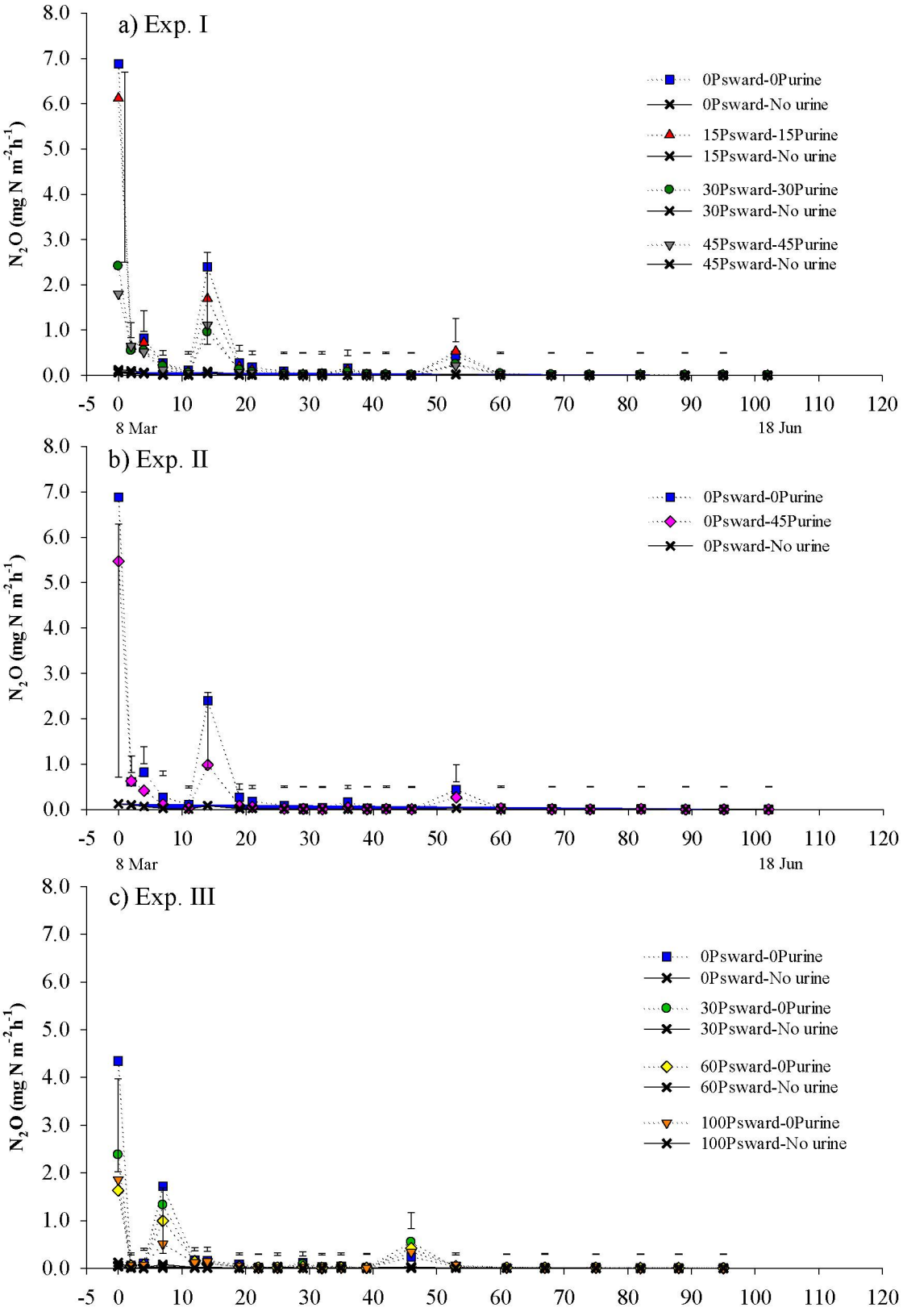
Daily N_2O fluxes in experiment I presented a peak at the day of urine application with the greatest emission from 0Psward-0Purine (6.88 mg N m^{-2} h^{-1}), followed by 15Psward-15Purine (6.12 mg N m^{-2} h^{-1}) , 30Psward-30Purine (2.41 mg N m^{-2} h^{-1}) and 45Psward-45Purine (1.80 mg N m^{-2} h^{-1}), with the lowest and highest plantain proportions being statistically different ($p<0.10$) (Figure 2a). A second and lower peak was obtained at 14 daa with the same trends of the first peak, although there was no difference between treatments (Figure 2a). The fluxes dropped down at 21 daa and presented another low peak at 53 daa, with coincided with a major rainfall event of 47 mm (Figure 6a). Cumulative N_2O emissions over the 15-week period decreased linearly with increasing proportions of plantain in the diet and sward, from 6.55 kg N ha^{-1} in 0Psward-0Purine to 2.86 kg N ha^{-1} in 45Psward-45Purine (r^2= 0.9127) (Figure 3b). N_2O emissions from the control treatments also reduced as the proportion of plantain in the diet and sward increased (r^2= 0.8296, Figure 3a). The emission factor of N_2O tended to reduce with increasing proportions of plantain in the diet and sward proportions (r^2= 0.5598) (Figure 3c).

In the second trial, applying urine from animal fed on ryegrass/white clover (0% plantain) and 45% plantain onto the standard pasture ryegrass/white clover, we observed similar peaks as in trial I, with a higher first peak on the day of urine application reaching 6.88 mg N m^{-2} h^{-1} in 0Psward-0Purine (Figure 2b). This peak was not significantly different to the peak obtained in 0Psward-45Purine of 5.47 mg N m^{-2} h^{-1} ($p>0.10$). A second peak was also obtained at 14 daa, with the same tendency in treatments obtained in the first peak. Cumulative N_2O emission in 0Psward-0Purine was significantly higher (6.55 kg N ha^{-1}) than in 0Psward-45Purine (3.65 kg N ha^{-1}) ($p<0.10$) (Table 4). The emission factor values were not statistically different between the two rates of plantain urine, being 0.62% and 0.58% in 0Purine and 45Purine, respectively (Table 4).

The third trial was undertaken to determine the effect of plantain plants on N_2O emissions by application of the same type and rate of cattle urine (animals fed on ryegrass/white clover) to swards with different proportions of

plantain. Daily N_2O emissions presented a first peak at the day of urine application with significant differences between the 0 and 60% and between 0 and 100% plantain swards ($p<0.10$) (Figure 2c). Cumulative N_2O emissions decreased linearly with increasing proportion of plantain in the sward, from 4.03 kg N ha^{-1} to 2.27 kg N ha^{-1}, in 0 and 100% of plantain sward, respectively (r^2= 0.9691) (Figure 3e). The differences were observed even for control treatments, with a linear decrease in emissions from 0Psward (0.41 kg N ha^{-1}) to 100Psward (0.09 kg N ha^{-1}) (r^2= 0.9576) (Figure 3d). The emission factor values also decreased with increasing proportions of plantain in the sward, with values of 0.60, 0.51, 0.42 and 0.36% for plantain swards proportions of 0, 30, 60 and 100%, respectively (r^2= 0.9743) (Figure 3f).

FIGURE 2 - NITROUS OXIDE FLUX (mg N m^{-2} h^{-1}) AFTER APPLICATION OF URINE FROM CATTLE FED WITH DIFFERENT RATES OF PLANTAIN ONTO THE SAME PLANTAIN PROPORTIONS SWARD (a), CATTLE FED WITH DIFFERENT RATES OF PLANTAIN ONTO 0% PLANTAIN SWARD (b), AND FROM CATTLE STANDARD URINE (RYEGRASS+WHITE CLOVER FED) APPLIED ONTO DIFFERENT PLANTAIN PROPORTIONS SWARD (c). ERROR BARS DENOTE LSD ACCORDING TO TUKEY'S TEST ($p<0.10$).



3.5.2 Soil inorganic nitrogen

3.5.2.1 Ammonium

The soil ammonium concentration over 118 days of evaluation in experiment I presented a peak at 4 daa, reaching 603.46 mg N kg^{-1} in the treatment 15Psward-15Purine, and 527.23 mg N kg^{-1} in 0Psward-0Purine. These treatments maintained statistically higher soil ammonium concentrations than 45Psward-45Purine (210.42 mg N kg^{-1}) throughout the experiment ($p<0.10$) (Figure 4a). After peaking, ammonium decreased constantly until 40 daa, when the concentrations became similar to the control treatments (Figure 4a). The treatments showed a similar trend to the N_2O emissions evaluations, with the highest plantain proportions presenting the lowest soil ammonium concentrations.

In the trial II, the peak soil ammonium concentration was obtained 4 daa of urine and the concentrations were higher for 0Psward-0Purine (527.23 mg N ha^{-1}) than for 0Psward-45Purine (270.99 mg N ha^{-1}) ($p<0.10$) (Figure 4b). The ammonium concentrations decreased over time until 40 daa, with values coming back to the background level (Figure 4b).

Soil ammonium concentration in swards with different proportions of plantain receiving the same standard cattle urine (trial III) were not significantly different ($p>0.10$) (Figure 4c). Peak emissions occurred on the day of urine application and the highest soil ammonium concentrations were found in 30Psward-0Purine (616.68 mg N ha^{-1}). Soil ammonium concentrations then decreased over time until the background level at 32 daa (Figure 4c).

TABLE 4: CUMULATIVE AND EMISSION FACTOR OF N_2O FROM CATTLE URINE FROM ANIMALS FED ON 45% PLANTAIN AND WITH STANDARD RYEGRASS/WHITE CLOVER (0% PLANTAIN) ONTO A STANDARD RYEGRASS/WHITE CLOVER SWARD.

Treatment	Cumulative emission (kg N ha^{-1})	Emission factor (%)
No urine	0.41 c[a]	-
0% plantain urine	6.55 a	0.62 a
45% plantain urine	3.65 bc	0.58 a

[a]Means followed by the same lowercase letter within columns are not statistically different, according to Tukey's test ($p<0.10$).

Source: The author (2018).

FIGURE 3 - RELATIONSHIP BETWEEN CUMULATIVE N2O EMISSIONS (KG N HA-1) AND DIFFERENT PROPORTIONS OF PLANTAIN SWARD (%) FOR CONTROL TREATMENTS (a, d); RELATIONSHIP BETWEEN CUMULATIVE N_2O EMISSIONS (kg N ha^{-1}) (URINE-N LOADING OF 10, 8.8, 7.5 AND 5.6 g N L^{-1} FOR 0, 15, 30 AND 45% PLANTAIN IN DIET, RESPECTIVELY) (b, e) OR EMISSIONS FACTOR (%) (SAME URINE-N LOADING OF 6.1%) (c, f) AND DIFFERENT PROPORTIONS OF PLANTAIN SWARD (%) FOR URINE TREATMENTS.

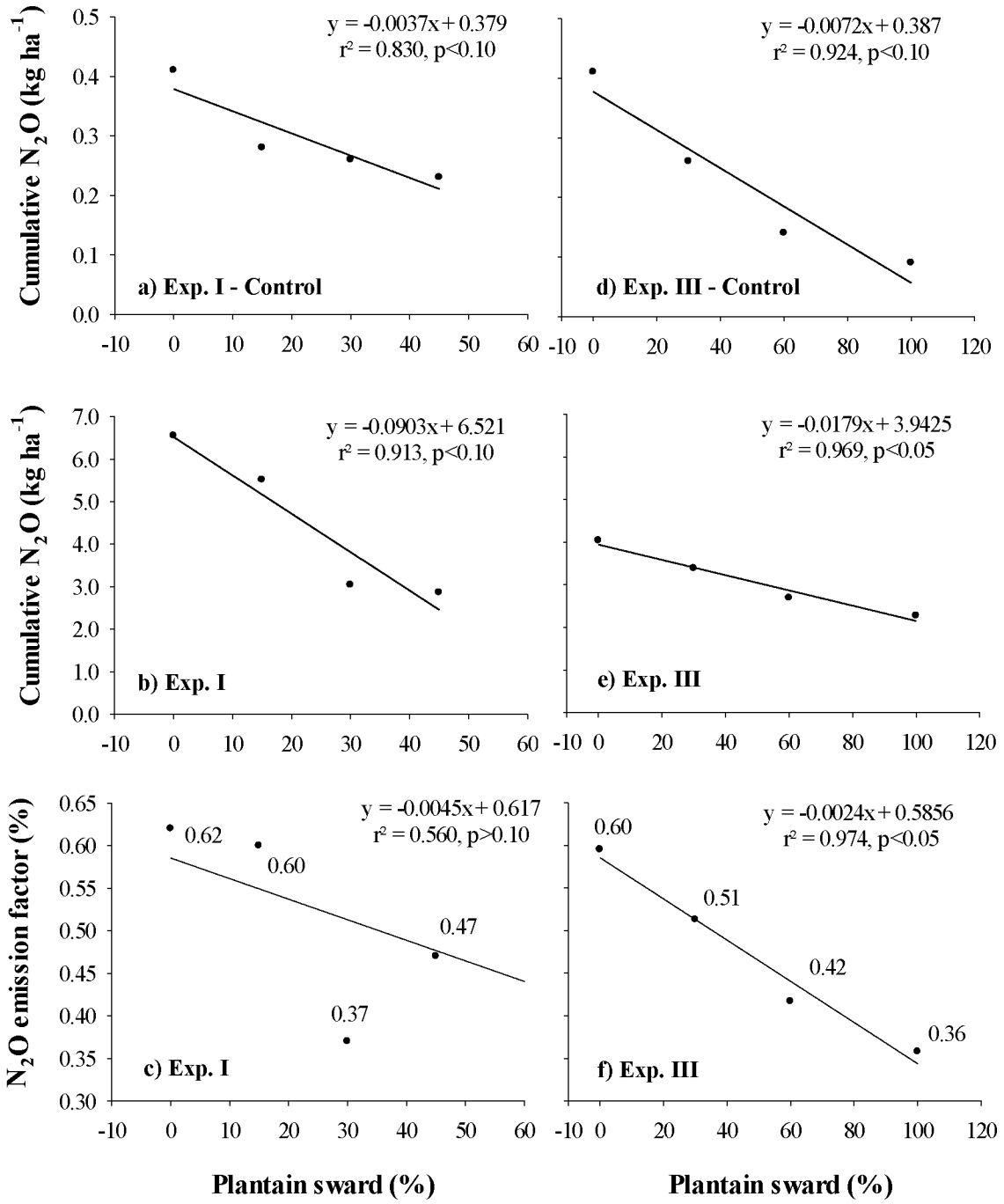
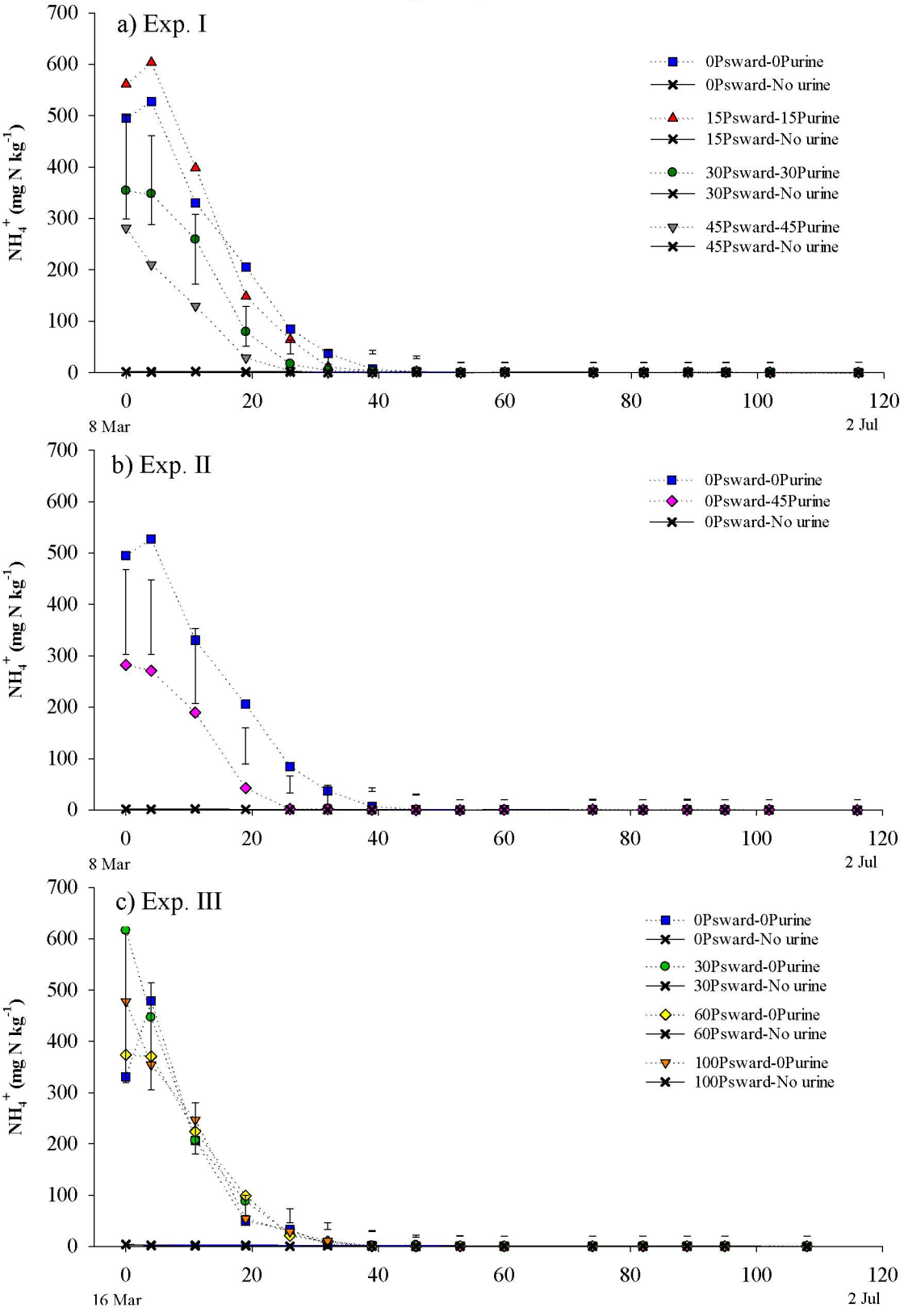


FIGURE 4 - SOIL AMMONIUM N CONCENTRATION (mg N kg^{-1}) AT 0-5 CM SOIL LAYER AFTER APPLICATION OF URINE FROM CATTLE FED WITH DIFFERENT RATES OF PLANTAIN ONTO THE SAME PLANTAIN PROPORTIONS SWARD (a), CATTLE FED WITH DIFFERENT RATES OF PLANTAIN ONTO 0% PLANTAIN SWARD (b), AND FROM CATTLE STANDARD URINE (RYEGRASS+WHITE CLOVER FED) APPLIED ONTO DIFFERENT PLANTAIN PROPORTIONS SWARD (c). ERROR BARS DENOTE LSD ACCORDING TO TUKEY'S TEST
($p<0.10$).



3.5.2.2 Nitrate

The soil nitrate concentrations were initially low after cattle urine application with values similar to the control levels in the three trials (Figures 5a, b, c). In the trial I, the nitrate concentrations increased over time peaking at 26 daa of urine with a significant higher value for 0Psward-0Purine (330.78 mg N ha^{-1}) compared to 45Psward-45Purine (192.06 mg N ha^{-1}) ($p<0.10$) (Figure 5a). This tendency between treatments maintained throughout the experiment, with soil nitrate concentrations returning to the background level at 53 daa (Figure 5a).

In trial II, soil nitrate concentration showed a similar pattern to soil ammonium concentration, with levels being significantly higher in 0Psward-0Purine (330.78 mg N ha^{-1}) compared with 0Psward-45Purine (183.47 mg N ha^{-1}) ($p<0.10$) (Figure 5b) in a peak obtained at 26 daa and returning to the background level at 53 daa (Figure 5b). The soil nitrate concentration in the trial III did not present any difference between treatments, and its peak occurred at 19 daa of urine, dropping down to background levels at 46 daa ($p>0.10$) (Figure 5c).

3.5.3 Weather and soil moisture conditions

The average air and soil temperature during the first 4 weeks following urine application, a period with significant N_2O fluxes, were 14 and 16 ºC, respectively (Figure 6b), and the cumulative precipitation during this period was 56 mm (Figure 6a). The temperatures were higher during the beginning of the experiment, however these values decreased over time. The average air and soil temperature over the whole period of experiment conduction (120 days) were 8.3 and 9.5 ºC, respectively (Figure 6b), and the total cumulative precipitation was 302 mm (Figure 6a).

The WFPS% was not different between treatments and presented the lower values during the first 4 weeks of evaluation, with a mean of 67, 70 and 71% for trials I, II and III, respectively (Figures 7a, b, c). However, the mean WFPS throughout the experiments were of 80, 82 and 81%, respectively (Figures 7a, b, c).

FIGURE 5 - SOIL NITRATE N CONCENTRATION (MG N KG-1) AT 0-5 CM SOIL LAYER AFTER APPLICATION OF URINE FROM CATTLE FED WITH DIFFERENT RATES OF PLANTAIN ONTO THE SAME PLANTAIN PROPORTIONS SWARD (A), CATTLE FED ON DIFFERENT RATES OF PLANTAIN ONTO 0% PLANTAIN SWARD (B), AND FROM CATTLE STANDARD URINE (RYEGRASS+WHITE CLOVER FED) APPLIED ONTO DIFFERENT PLANTAIN PROPORTIONS SWARD (C). ERROR BARS DENOTE LSD ACCORDING TO TUKEY'S TEST (p<0.10).

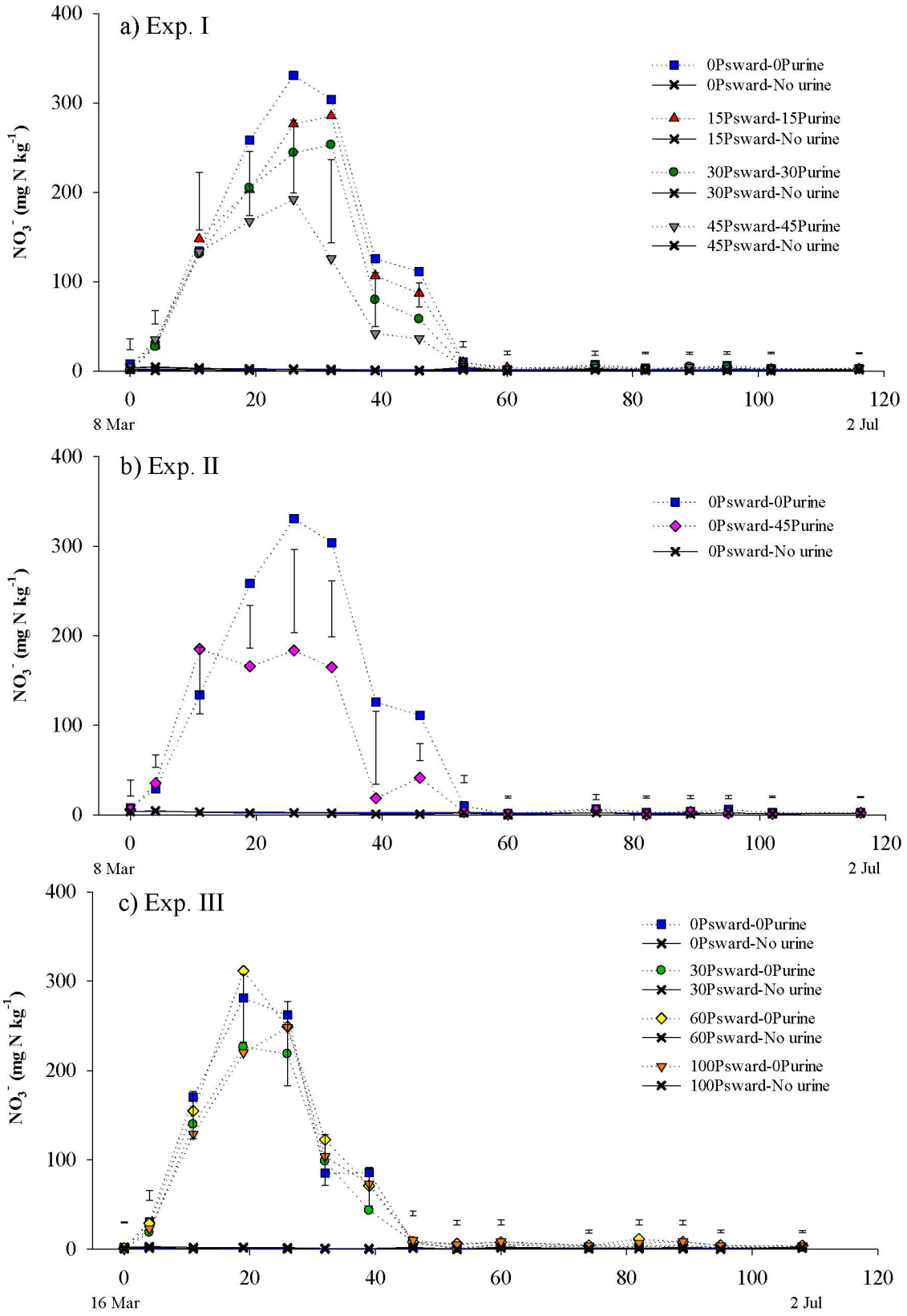


FIGURE 6 - DAILY PRECIPITATION (mm) (a) AND DAILY AVERAGE SOIL AND AIR TEMPERATURE (ºC) (b) OVER 120 DAYS AT THE FIELD TRIAL.

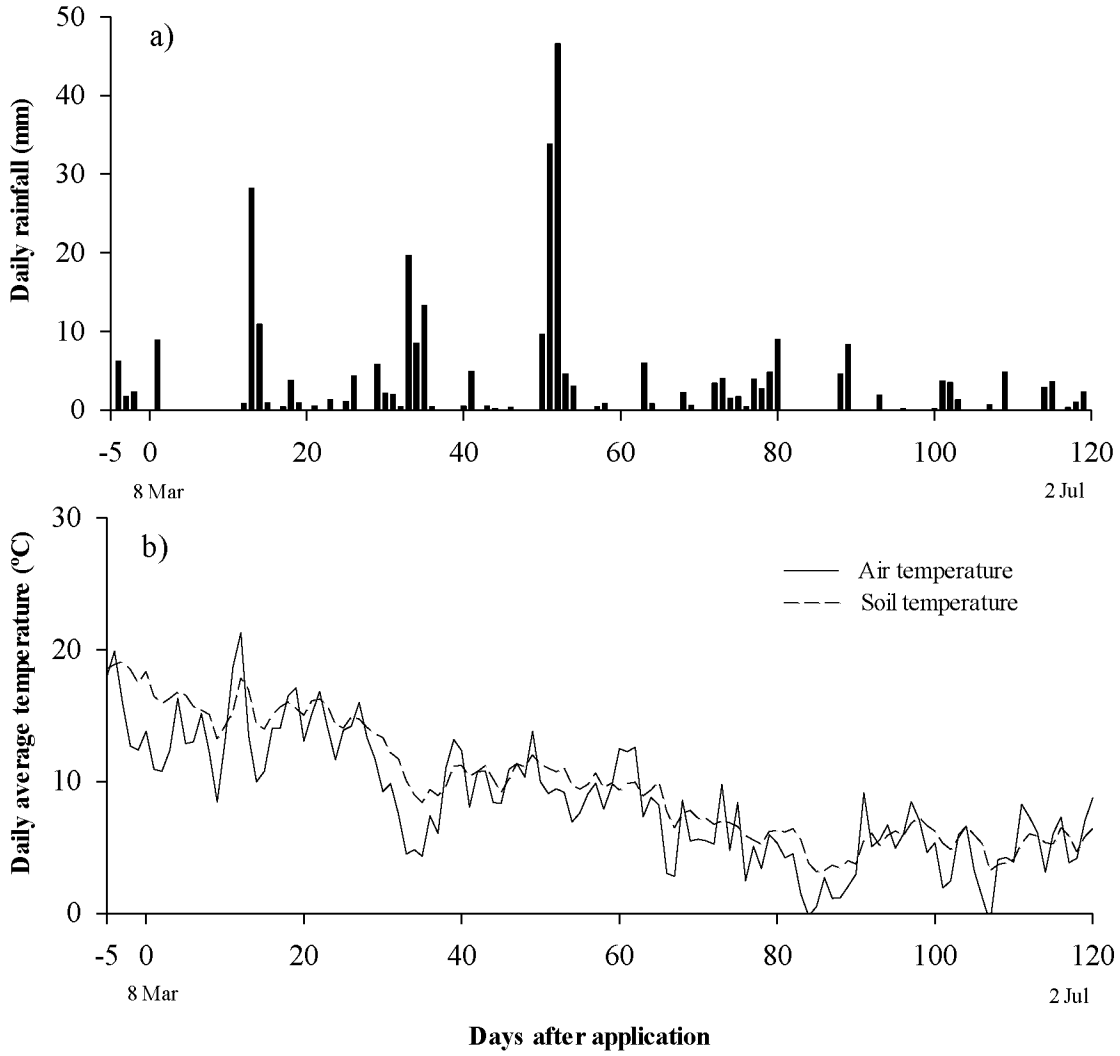
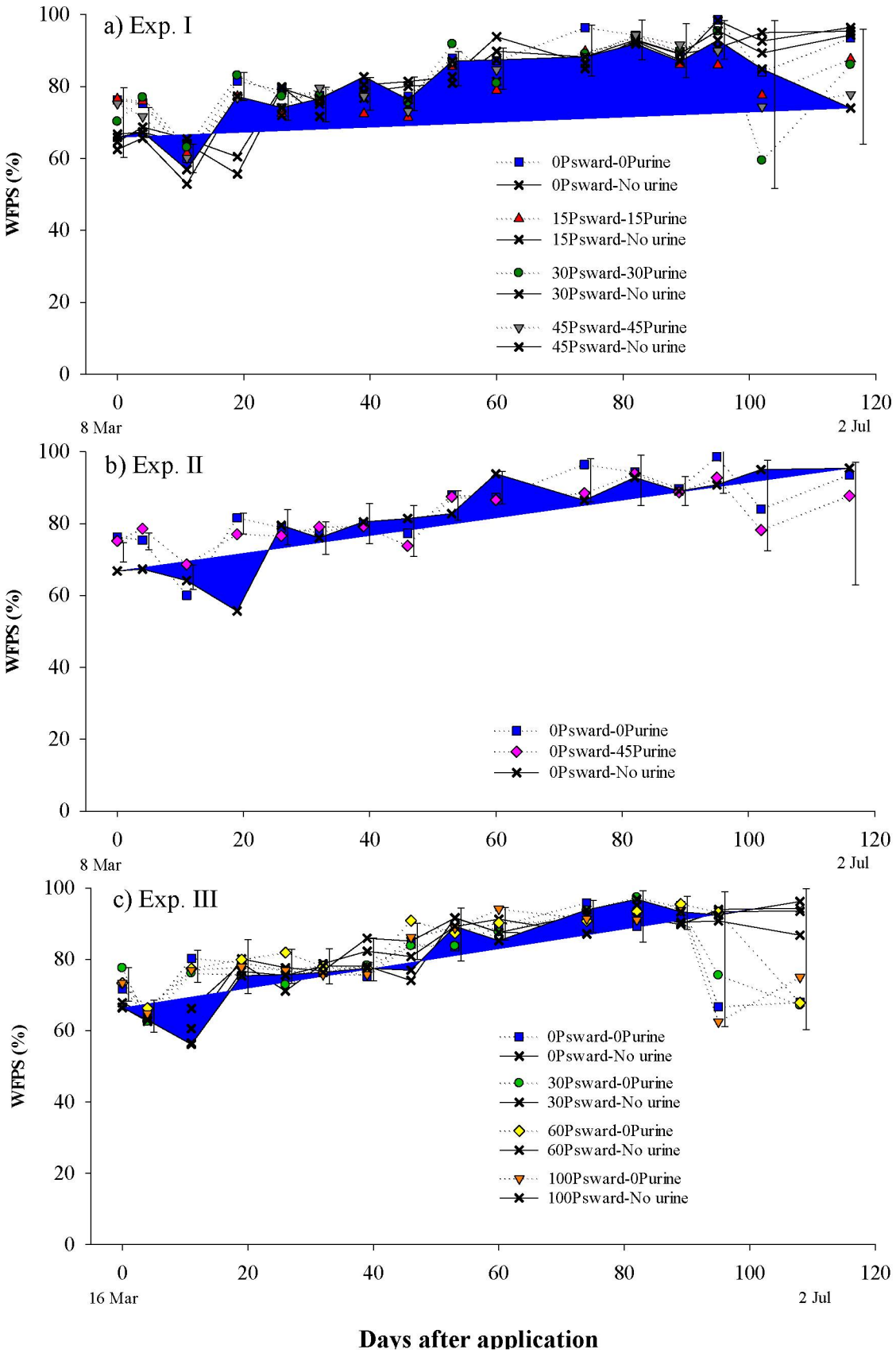


FIGURE 7 - SOIL WATER FILLED-PORE SPACE (%) AFTER APPLICATION OF URINE FROM CATTLE FED WITH DIFFERENT RATES OF PLANTAIN ONTO THE SAME PLANTAIN PROPORTIONS SWARD (a), CATTLE FED ON DIFFERENT RATES OF PLANTAIN ONTO 0% PLANTAIN SWARD (b), AND FROM CATTLE STANDARD URINE (RYEGRASS+WHITE CLOVER FED) APPLIED ONTO DIFFERENT PLANTAIN PROPORTIONS SWARD (C). ERROR BARS DENOTE LSD ACCORDING TO TUKEY'S TEST (p<0.10).



3.6 DISCUSSION

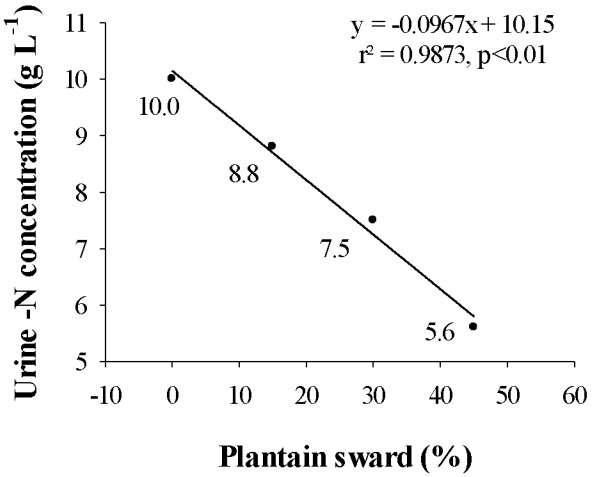
3.6.1 Effect of plantain on N_2O emissions

Cumulative emissions of N_2O decreased with increasing plantain proportions in animal diet and swards (r^2= 0.9127) (Figure 3b), while N_2O emission factors also showed a reduction trend (r^2= 0.5598) (Figure 3c). There are two explanations that may be associated to the reduction of N_2O emissions by plantain. The first is associated with the effect of plantain on soil processes, e.g. through exudates released by plantain roots, inducing a biological nitrification inhibition effect, thus reducing nitrification rates and consequently, N_2O emissions (Dietz *et al.*, 2013; Gardiner *et al.*, 2017; Luo *et al.*, 2018). The iridoid glycosides aucubin and catalpol and the polyphenol acteoside, the main secondary metabolites found in leaves and roots of plantain, are known for their antimicrobial activity. These can affect the soil nitrification pathways through two main mechanisms: Binding and interacting with AMO enzyme via competitive/ non-competitive process against NH_3 and metal chelators and/or inactivating AMO enzymatic pathways (suicide mechanism), through the inhibition of NH_3 oxidation by chemical interaction of aucubigenin and acteoside with cytochrome P450 (Herbert *et al.*, 1991; Bartholomaeus and Ahokas, 1995; Subbarao *et al.*, 2007a; Dietz *et al.*, 2013).

Another explanation for the reduction observed in N_2O emissions with plantain is associated with the urine properties. The urine-N loading rates from animals fed on plantain reduced from 1000 kg N ha^{-1} for animals on 0% plantain, to 560 kg N ha^{-1} for animals on 45% plantain (r^2= 0.9873) (Figure 8). It is also possible that secondary metabolites present in plantain were excreted with the urine (Gardiner *et al.*, 2016). The effect of plantain on urine-N loading rates could be associated with a diuretic effect due to the reduction of water reabsorption in the animal kidneys, thus increasing the volume of urine released (Di *et al.*, 2016; O'Connell *et al.*, 2016; Cheng *et al.*, 2017). Furthermore, plantain could also have the capacity to partition more of the excreted N into dung, rather than urine. The partitioning the N into dung occurs via increasing of the incorporated urea in the microbial protein of the rumen (Raab *et al.*, 1983; Russell *et al.*, 1992; Tamura and Nishibe, 2002; Wilman and Derrick, 2009; Navarrete *et al.*, 2016). Some condensed tannins present in plantain composition may complex with proteins in the rumen and protect them from

microbial digestion, improving the digestion efficiency and complex tannin-protein being excreted in the dung (Carulla *et al.*, 2005; Ramírez-Restrepo and Barry, 2005; de Klein and Eckard, 2008). However, as less N was excreted in urine with higher proportions plantain, cumulative emissions are likely to be associated with the lower urine-N loading, but not with the presence of composites in urine from plantain treatments.

FIGURE 8. RELATIONSHIP BETWEEN URINE-N LOADING RATES AND PLANTAIN PROPORTIONS IN ANIMAL DIET.



The possibility of the higher N partitioning into dung rather than urine will also be contributing to the reduction of N_2O emissions in a field scale, considering that the emission factor from dung patch is approximately four times lower than emissions factor of urine patches (van der Weerden *et al.*, 2011). The lower soil ammonium and nitrate concentrations in treatments with plantain (Figures 4a and 5a), due to the lower urine-N loading rates on these treatments leads consequently to the lower possibility of N losses in soil as N_2O emissions and/or via nitrate leaching (Monaghan and de Klein, 2014).

In the trial III we obtained information about the 'plant' effect of plantain on N_2O emissions, with urine at the same N loading rate (610 kg N ha^{-1}) being applied onto swards with different proportions plantain (0, 30, 60 and 100%). The results showed a linear decrease in cumulative N_2O emissions and emission factors with increasing proportion of plantain in the sward, r^2= 0.9691 (Figure 3e), and r^2= 0.9743 (Figure 3f), respectively. The reduction in N_2O emissions reduction in urine patches with increasing proportions of plantain in

the sward supports the hypothesis that plantain has the ability to inhibit nitrification, possibly via BNI compounds released to soil through root-exudates, as suggested in previous studies (Dietz *et al.*, 2013; Di *et al.*, 2016; Gardiner *et al.*, 2017; Luo *et al.*, 2018). In the long term, the effects of plantain can be enhanced by higher concentration of aucubin, which could be related to the age of the sward. Navarrete *et al.* (2016) found a variation in the maximum aucubin concentration in leaves of plantain from 3.80 mg g^{-1} DM in the first year of establishment to 6.87 mg g^{-1} DM in the second year, and for acteoside the range was from 35.4 to 41.7 mg g^{-1} DM in the first and second year, respectively.

The effect of plantain sward was observed even in control, where no urine was applied (r^2= 0.9576) (Figure 3d), suggesting that plantain can also reduce N_2O emissions under low soil N concentrations. This is again suggesting that BNI could be the main mechanism leading to reductions in nitrification rates and N_2O emissions (Luo *et al.*, 2018). However, the ammonium and nitrate concentrations in experiment III did not differ when standard urine was applied onto different proportions of plantain sward (Figures 4c and 5c), which does not support the BNI hypothesis. The lack of effect of proportion plantain on soil mineral N concentrations may be related to a methodological issue such as a mismatch of the soil sampling depth and the soil depth where root exudation occurs, or because other mechanisms could be involved with inhibiting N_2O production in soil throughout the evaluation period. Gardiner *et al.* (2017) also did not find differences in soil inorganic N between cattle urine, urine + aucubin extracted by leaves of plantain or urine + aucubin synthetic solution. However, the authors found lower N_2O emissions in plantain treatments, and they suggested the lack of effect on soil inorganic N concentration was due to the low aucubin concentration applied.

The linear reduction of N_2O emissions after application of standard cattle urine to different proportions of plantain swards reinforces the efficacy of plantain as a component of pasture mixes with ryegrass/white clover in temperate livestock systems. However, future studies are needed to confirm the mechanisms involved in N_2O emissions from urine patches applied onto plantain, as well as the effect on of plantain on soil N immobilization via C

released by root-exudates and/or fixed on clays and soil microclimate (e.g. soil pH and moisture).

3.7 CONCLUSIONS

The use of plantain mixed to the standard ryegrass/white clover pasture has shown to be an effective strategy to reduce N_2O emissions from cattle urine, as observed through the linear decrease in emissions with increasing proportions of plantain in the diet and/or the sward. This reduction capacity is related to the direct effect of the plantain plant on soil, possibly due to the biological nitrification inhibition via root-exudates released in soil from plantain pasture. In addition, N_2O emissions are also likely to be reduced due to lower urinary N excretion by animals on a plantain-rich diet. However, the results did not show the effect of proportion plantain in the animal diet on the N_2O emission factors, suggesting that more research is needed to fully understand the mechanisms involved at inhibiting process by plantain in soil.

REFERENCES

BARTHOLOMAEUS, A., AHOKAS, J., 1995. Inhibition of P-450 by aucubin: is the biological activity of aucubin due to its glutaraldehyde-like aglycone? **Toxicology letters** 80, 75-83.

BOWATTE, S., HOOGENDOORN, C.J., NEWTON, P.C.D., LIU, Y., BROCK, S.C., THEOBALD, P.W., 2018. Grassland plant species and cultivar effects on nitrous oxide emissions after urine application. **Geoderma** 323, 74-82.

BYRNES, R.C., NUNEZ, J., ARENAS, L., RAO, I., TRUJILLO, C., ALVAREZ, C., ARANGO, J., RASCHE, F., CHIRINDA, N., 2017. Biological nitrification inhibition by Brachiaria grasses mitigates soil nitrous oxide emissions from bovine urine patches. **Soil Biology & Biochemistry** 107, 156-163.

CARDENAS, L.M., MISSELBROOK, T.M., HODGSON, C., DONOVAN, N., GILHESPY, S., SMITH, K.A., DHANOA, M.S., CHADWICK, D., 2016. Effect of the application of cattle urine with or without the nitrification inhibitor DCD, and dung on greenhouse gas emissions from a UK grassland soil. **Agriculture Ecosystems & Environment** 235, 229-241.

CARULLA, J.E., KREUZER, M., MACHMÜLLER, A., HESS, H.D., 2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. **Australian Journal of Agricultural Research** 56, 961-970.

CHENG, L., G. JUDSON, H., BRYANT, R., MOWAT, H., GUINOT, L., HAGUE, H., TAYLOR, S., R. EDWARDS, G., 2017. The effects of feeding cut plantain and perennial ryegrass-white clover pasture on dairy heifer feed and water intake, apparent nutrient digestibility and nitrogen excretion in urine.

DAVINI, E., JAVARONE, C., TROGOLO, C., AURELI, P., PASOLINI, B., 1986. The quantitative isolation and antimicrobial activity of the aglycone of aucubin. **Phytochemistry** 25, 2420–2422.

DE KLEIN, C.A.M., BARTON, L., SHERLOCK, R.R., LI, Z., LITTLEJOHN, R.P., 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. **Aust. J. Soil Res**. 41, 381-399.

DE KLEIN, C.A.M., ECKARD, R.J., 2008. Targeted technologies for nitrous oxide abatement from animal agriculture. **Aust. J. Exp. Agric**. 48, 14-20.

DE KLEIN, C.A.M., SHERLOCK, R.R., CAMERON, K.C., VAN DER WEERDEN, T.J., 2001. Nitrous oxide emissions from agricultural soils in New Zealand - a review of current knowledge and directions for future research. **J. R. Soc. N.Z.** 31, 543-574.

DI, H.J., CAMERON, K.C., PODOLYAN, A., EDWARDS, G.R., DE KLEIN, C.A.M., Dynes, R., Woods, R., 2016. The potential of using alternative pastures, forage crops and gibberellic acid to mitigate nitrous oxide emissions. J. **Soils Sediments** 16, 2252-2262.

DIETZ, M., MACHILL, S., HOFFMANN, H.C., SCHMIDTKE, K., 2013. Inhibitory effects of Plantago lanceolata L. on soil N mineralization. **Plant and Soil** 368, 445-458.

GARDINER, C.A., CLOUGH, T.J., CAMERON, K.C., DI, H.J., EDWARDS, G.R., DE KLEIN, C.A.M., 2016. Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N2O emissions: a review. **New Zealand Journal of Agricultural Research** 59, 301-317.

GARDINER, C.A., CLOUGH, T.J., CAMERON, K.C., DI, H.J., EDWARDS, G.R., DE KLEIN, C.A.M., 2017. Potential inhibition of urine patch nitrous oxide emissions by Plantago lanceolata and its metabolite aucubin. **New Zealand Journal of Agricultural Research**, 1-9.

HAYNES, R.J., WILLIAMS, P.H., 1993. Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. **Advances in Agronomy** 49, 119-199.

HERBERT, J.M., MAFFRAND, J.P., TAOUBI, K., AUGEREAU, J.M., FOURASTE, I., GLEYE, J., 1991. Verbascoside Isolated from Lantana camara, an Inhibitor of Protein Kinase C. **Journal of Natural Products** 54, 1595-1600.

HEWITT, A., 2010. New Zealand Soil Classification. Landcare Research Science Series, **Manaaki Whenua Press**.

HOOGENDOORN, C.J., LUO, J., LLOYD-WEST, C.M., DEVANTIER, B.P., LINDSEY, S.B., SUN, S., PACHECO, D., LI, Y., THEOBALD, P.W., JUDGE, A., 2016. Nitrous oxide emission factors for urine from sheep and cattle fed forage rape (Brassica napus L.) or perennial ryegrass/white clover pasture (Lolium perenne L./Trifolium repens). **Agriculture, Ecosystems & Environment** 227, 11-23.

KELLIHER, F.M., COX, N., VAN DER WEERDEN, T.J., DE KLEIN, C.A.M., LUO, J., CAMERON, K.C., DI, H.J., GILTRAP, D., RYS, G., 2014. Statistical analysis of nitrous oxide emission factors from pastoral agriculture field trials conducted in New Zealand. **Environmental Pollution** 186, 63-66.

KIM, D.H., KIM, B.R., KIM, J.Y., JEONG, Y.C., 2000. Mechanism of covalent adduct formation of aucubin to proteins. **Toxicology letters** 114, 181-188.

LUO, J., BALVERT, S.F., WISE, B., WELTEN, B., LEDGARD, S.F., DE KLEIN, C.A.M., LINDSEY, S., JUDGE, A., 2018. Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. **Science of the Total Environment** 610-611, 1271-1280.

LUO, J., KELLIHER, F., 2014. Partitioning of Animal Excreta N Into Urine and Dung and Developing the N_2O Inventory. **Ministry for Primary Industries.**

LUO, J., LEDGARD, S.F., DE KLEIN, C.A.M., LINDSEY, S.B., KEAR, M., 2008. Effects of dairy farming intensification on nitrous oxide emissions. **Plant and Soil** 309, 227-237.

LUO, J., SUN, X.Z., PACHECO, D., LEDGARD, S.F., LINDSEY, S.B., HOOGENDOORN, C.J., WISE, B., WATKINS, N.L., 2014. Nitrous oxide emission factors for urine and dung from sheep fed either fresh forage rape (Brassica napus L.) or fresh perennial ryegrass (Lolium perenne L.). **Animal** 9, 534-543.

MINISTRY FOR THE ENVIRONMENT, 2017. New Zealand's Environmental Reporting Series: **Our atmosphere and climate 2017**. Retrieved from www.mfe.govt.nz and www.stats.govt.nz.

MONAGHAN, R.M., DE KLEIN, C.A.M., 2014. Integration of measures to mitigate reactive nitrogen losses to the environment from grazed pastoral dairy systems. **Journal of Agricultural Science** 152, S45-S56.

NAVARRETE, S., KEMP, P.D., PAIN, S.J., BACK, P.J., 2016. Bioactive compounds, aucubin and acteoside, in plantain (Plantago lanceolata L.) and their effect on in vitro rumen fermentation. **Anim. Feed Sci. Technol**. 222, 158-167.

O'CONNELL, C., JUDSON HG, GK., B., 2016. Sustained diuretic effect of plantain when ingested by sheep. **Proceedings of the New Zealand Society of Animal Production,** Adelaide, pp. 14-17.

RAAB, L., CAFANTARIS, B., JILG, T., MENKE, K.H., 1983. Rumen protein degradation and biosynthesis. I. A new method for determination of protein degradation in rumen fluid in vitro. **The British journal of nutrition** 50, 569-582.

RAMÍREZ-RESTREPO, C.A., BARRY, T.N., 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. **Anim. Feed Sci. Technol**. 120, 179-201.

RUSSELL, J.B., O'CONNOR, J.D., FOX, D.G., VAN SOEST, P.J., SNIFFEN, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. **Journal of animal science** 70, 3551-3561.

SAGGAR, S., ANDREW, R.M., TATE, K.R., HEDLEY, C.B., RODDA, N.J., TOWNSEND, J.A., 2004. Modelling nitrous oxide emissions from dairy-grazed pastures. **Nutr. Cycl. Agroecosyst.** 68, 243-255.

SELBIE, D.R., BUCKTHOUGHT, L.E., SHEPHERD, M.A., 2015. The Challenge of the Urine Patch for Managing Nitrogen in Grazed Pasture Systems. **Advances in Agronomy** 129, 229-292.

SUBBARAO, G.V., ISHIKAWA, T., ITO, O., NAKAHARA, K., WANG, H.Y., BERRY, W.L., 2006. A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with Brachiaria humidicola. **Plant and Soil** 288, 101-112.

SUBBARAO, G.V., NAKAHARA, K., HURTADO, M.P., ONO, H., MORETA, D.E., SALCEDO, A.F., YOSHIHASHI, A.T., ISHIKAWA, T., ISHITANI, M., OHNISHI-KAMEYAMA, M., YOSHIDA, M., RONDON, M., RAO, I.M., LASCANO, C.E., BERRY, W.L., ITO, O., 2009. Evidence for biological nitrification inhibition in Brachiaria pastures. **Proc. Natl. Acad. Sci. U. S. A**. 106, 17302-17307.

SUBBARAO, G.V., RONDON, M., ITO, O., ISHIKAWA, T., RAO, I.M., NAKAHARA, K., LASCANO, C., BERRY, W.L., 2007a. Biological nitrification inhibition (BNI)—is it a widespread phenomenon? **Plant and Soil** 294, 5-18.

SUBBARAO, G.V., WANG, H.Y., ITO, O., NAKAHARA, K., BERRY, W.L., 2007b. NH_4^+ triggers the synthesis and release of biological nitrification inhibition compounds in Brachiaria humidicola roots. **Plant and Soi**l 290, 245-257.

TAMURA, Y., NISHIBE, S., 2002. Changes in the Concentrations of Bioactive Compounds in Plantain Leaves. **Journal of Agricultural and Food Chemistry** 50, 2514-2518.

VAN DER WEERDEN, T.J., LUO, J.F., DE KLEIN, C.A.M., HOOGENDOORN, C.J., LITTLEJOHN, R.P., RYS, G.J., 2011. Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral soils. **Agriculture Ecosystems & Environment** 141, 426-436.

VAN DER WEERDEN, T.J., KELLIHER, F.M., DE KLEIN, C.A.M., 2012. Influence of pore size distribution and soil water content on nitrous oxide emissions. **Soil Res**. 50, 125-135.

WICHTL, M., 2004. Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis. **Medpharm GmbH Scientific Publishers**, Stuttgart.

WILLIAMS, P.H., HAYNES, R.J., 1994. Comparison of initial wetting pattern, nutrient concentrations in soil solution and the fate of15N-labelled urine in sheep and cattle urine patch areas of pasture soil. **Plant and Soil** 162, 49-59.

WILMAN, D., DERRICk, R.W., 2009. Concentration and availability to sheep of N, P, K, Ca, Mg and Na in chickweed, dandelion, dock, ribwort and spurrey, compared with perennial ryegrass. **The Journal of Agricultural Science** 122, 217-223.

4 GENERAL CONCLUSIONS

The use of plant species as a mitigation strategy for N_2O emissions from cattle urine patches showed to be an effective alternative in subtropical and temperate climates.

Brachiaria humidicola, known for its capacity in to inhibit biologically soil nitrification through releasing of compound via root exudates in tropical systems reduced 20% of N_2O emissions after cattle urine applied to the soil. The BNI effect is suggested as the main mechanism responsible for this reduction, supported by lower soil nitrate concentrations in treatments with brachiaria compared with guinea grass.

Cattle urine applied to *Plantago lanceolata* had up to 40% lower N_2O emissions compared with cattle urine applied to ryegrass/white clover in temperate climate. Decreasing on N_2O emissions was possibly due to both a urine effect (through the reduction of N-loading and releasing of secondary metabolites in excreta) as well as a plant effect (biological nitrification inhibition via root exudates released in soil).

A wider view is required and a greater emphasis on the influence of plants and plant/microbe interactions in soil to better understanding of N cycling and N_2O production, and thus a way in which better mitigation strategies might be identified. The possibility of providing ruminant animals with pastures that may alleviate N losses is likely to be of considerable interest to pasture-based livestock producers.

REFERENCES

AMBERGER, A., 1989. Research on dicyandiamide as a nitrification inhibitor and future outlook. **Communications in Soil Science and Plant Analysis** 20, 1933-1955.

ARAÚJO, E.D.S., MARSOLA, T., MIYAZAWA, M., SOARES, L.H.D.B., URQUIAGA, S., BODDEY, R.M., ALVES, B.J.R., 2009. Calibração de câmara semiaberta estática para quantificação de amônia volatilizada do solo. **Pesquisa Agropecuária Brasileira** 44, 769-776.

BADRI, D.V., VIVANCO, J.M., 2009. Regulation and function of root exudates. **Plant, Cell & Environment** 32, 666-681.

BARNEZE, A.S., MAZZETTO, A.M., ZANI, C.F., MISSELBROOK, T., CERRI, C.C., 2014. Nitrous oxide emissions from soil due to urine deposition by grazing cattle in Brazil. **Atmospheric Environment** 92, 394-397.

BARTHOLOMAEUS, A., AHOKAS, J., 1995. Inhibition of P-450 by aucubin: is the biological activity of aucubin due to its glutaraldehyde-like aglycone? **Toxicology letters** 80, 75-83.

BENGOUGH, A.G., CASTRIGNANO, A., PAGÈS, L., VAN NOORDWIJk, M., 2000. Sampling strategies, scaling, and statistics. In: SMIT, A.L.; BENGOUGH, A.G.; ENGELS, C.; VAN NOORDWIJK, M.; PELLERIN, S.; VAN DE GEIJN, S.C. (Ed.). **Root methods: a handbook. Springer.**

BÖHM, W., 1979. Methods of studying root systems. **Ecological Studies: Analysis and Synthesis. Springer**, Berlin Heidelberg New York.

BOWATTE, S., HOOGENDOORN, C.J., NEWTON, P.C.D., LIU, Y., BROCK, S.C., THEOBALD, P.W., 2018. Grassland plant species and cultivar effects on nitrous oxide emissions after urine application. **Geoderma** 323, 74-82.

BRASIL, 2016. Ministério da Ciência Tecnologia e Inovação Secretaria de Políticas e Programas de Pesquisa e desenvolvimento Coordenação Geral de Mudanças Globais de Clima Brasil. Terceira Comunicação Nacional do Brasil à Convenção-Quadro das Nações Unidas sobre Mudança do Clima – Volume III. Brasília: **Ministério da Ciência, Tecnologia e Inovação**, p. 336.

BREMNER, J.M., 1996. NITROGEN - TOTAL. IN: SPARKS, D.L., PAGE, A.L., HELMKE, P.A., LOEPPERT, R.H., SOLTANPOUR, P.N., TABATABAI, M.A., JOHNSTON, C.T., SUMNER, M.E. (Eds.), Methods of Soil Analysis Part 3 **Chemical Methods. SSSA**, Madison, pp. 1085-1121.

BYRNES, R.C., NUNEZ, J., ARENAS, L., RAO, I., TRUJILLO, C., ALVAREZ, C., ARANGO, J., RASCHE, F., CHIRINDA, N., 2017. Biological nitrification inhibition by Brachiaria grasses mitigates soil nitrous oxide emissions from bovine urine patches. **Soil Biology & Biochemistry** 107, 156-163.

CANTAREL, A.A.M., POMMIER, T., DESCLOS-THEVENIAU, M., DIQUÉLOU, S., DUMONT, M., GRASSEIN, F., KASTL, E.-M., GRIGULIS, K., LAÎNÉ, P., LAVOREL, S., LEMAUVIEL-LAVENANT, S., PERSONENI, E., SCHLOTER, M., POLY, F., 2015. Using plant traits to explain plant–microbe relationships involved in nitrogen acquisition. **Ecology** 96, 788-799.

CARDENAS, L.M., MISSELBROOK, T.M., HODGSON, C., DONOVAN, N., GILHESPY, S., SMITH, K.A., DHANOA, M.S., CHADWICK, D., 2016. Effect of the application of cattle urine with or without the nitrification inhibitor DCD, and dung on greenhouse gas emissions from a UK grassland soil. **Agriculture Ecosystems & Environment** 235, 229-241.

CARULLA, J.E., KREUZER, M., MACHMÜLLER, A., HESS, H.D., 2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. **Australian Journal of Agricultural Research** 56, 961-970.

CHENG, L., G. JUDSON, H., BRYANT, R., MOWAT, H., GUINOT, L., HAGUE, H., TAYLOR, S., R. EDWARDS, G., 2017. The effects of feeding cut plantain and perennial ryegrass-white clover pasture on dairy heifer feed and water intake, apparent nutrient digestibility and nitrogen excretion in urine.

CHENG, W., PARTON, W.J., GONZALEZ-MELER, M.A., PHILLIPS, R., ASAO, S., MCNICKLE, G.G., BRZOSTEK, E., JASTROW, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. **New Phytologist** 201, 31-44.

COSKUN, D., BRITTO, D.T., SHI, W., KRONZUCKER, H.J., 2017. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. **Nature plants** 3, 17074.

DAVINI, E., JAVARONE, C., TROGOLO, C., AURELI, P., PASOLINI, B., 1986. The quantitative isolation and antimicrobial activity of the aglycone of aucubin. **Phytochemistry** 25, 2420–2422.

DE KLEIN, C.A.M., BARTON, L., SHERLOCK, R.R., LI, Z., LITTLEJOHN, R.P., 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. **Aust. J. Soil Res**. 41, 381-399.

DE KLEIN, C.A.M., ECKARD, R.J., 2008. Targeted technologies for nitrous oxide abatement from animal agriculture. **Aust. J. Exp. Agric**. 48, 14-20.

DE KLEIN, C.A.M., LEDGARD, S.F., 2005. Nitrous oxide emissions from New Zealand agriculture - key sources and mitigation strategies. **Nutr. Cycl. Agroecosyst.** 72, 77-85.

DE KLEIN, C.A.M., LETICA, S.A., MACFIE, P.I., 2014. Evaluating the effects of dicyandiamide (DCD) on nitrogen cycling and dry matter production in a 3-year trial on a dairy pasture in South Otago, New Zealand. **New Zealand Journal of Agricultural Research** 57, 316-331.

DE KLEIN, C.A.M., SHERLOCK, R.R., CAMERON, K.C., VAN DER WEERDEN, T.J., 2001. Nitrous oxide emissions from agricultural soils in New Zealand - a review of current knowledge and directions for future research. **J. R. Soc. N.Z**. 31, 543-574.

DENIER VAN DER GON, H., BLEEKER, A., 2005. Indirect N2O emission due to atmospheric N deposition for the Netherlands. **Netherlands**, p. 13.

DI, H.J., CAMERON, K.C., 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. **Soil Use and Management** 18, 395-403.

DI, H.J., CAMERON, K.C., 2003. Mitigation of nitrous oxide emissions in spray-irrigated grazed grassland by treating the soil with dicyandiamide, a nitrification inhibitor. **Soil Use and Management** 19, 284-290.

DI, H.J., CAMERON, K.C., 2004. Effects of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial biomass in a grazed pasture soil. **Soil Res**. 42, 927-932.

DI, H.J., CAMERON, K.C., PODOLYAN, A., EDWARDS, G.R., DE KLEIN, C.A.M., DYNES, R., WOODS, R., 2016. The potential of using alternative pastures, forage crops and gibberellic acid to mitigate nitrous oxide emissions. **J. Soils Sediments** 16, 2252-2262.

DI, H.J., CAMERON, K.C., SHERLOCK, R.R., 2007. Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. **Soil Use and Management** 23, 1-9.

DIETZ, M., MACHILL, S., HOFFMANN, H.C., SCHMIDTKE, K., 2013. Inhibitory effects of Plantago lanceolata L. on soil N mineralization. **Plant and Soil** 368, 445-458.

FORSTER, P., RAMASWAMY, V., ARTAXO, P., BERNTSEN, T., BETTS, R., FAHEY, D.W., HAYWOOD, J., LEAN, J., LOWE, D.C., MYHRE, G., NGANGA, J., PRINN, R., RAGA, G., SCHULTZ, M., VAN DORLAND, R., 2007. Changes in atmospheric constituents and in radiative forcing. In: SOLOMON, S., QIN, D., MANNING, M., CHEN, Z., MARQUIS, M., AVERYT, K.B., TIGNOR, M., MILLER, H.L. (Eds.). **Cambridge University Press**, Cambridge, United Kingdom, pp. 129-234.

GARDINER, C.A., CLOUGH, T.J., CAMERON, K.C., DI, H.J., EDWARDS, G.R., DE KLEIN, C.A.M., 2016. Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N_2O emissions: a review. **New Zealand Journal of Agricultural Research** 59, 301-317.

GARDINER, C.A., CLOUGH, T.J., CAMERON, K.C., DI, H.J., EDWARDS, G.R., DE KLEIN, C.A.M., 2017. Potential inhibition of urine patch nitrous oxide emissions by Plantago lanceolata and its metabolite aucubin. **New Zealand Journal of Agricultural Research**, 1-9.

GOPALAKRISHNAN, S., SUBBARAO, G.V., NAKAHARA, K., YOSHIHASHI, T., ITO, O., MAEDA, I., ONO, H., YOSHIDA, M., 2007. Nitrification Inhibitors from the Root Tissues of Brachiaria humidicola, a Tropical Grass. **Journal of Agricultural and Food Chemistry** 55, 1385-1388.

HAICHAR, F.E.Z., MAROL, C., BERGE, O., RANGEL-CASTRO, J.I., PROSSER, J.I., BALESDENT, J., HEULIN, T., ACHOUAK, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. **The Isme Journal** 2, 1221.

HAYNES, R.J., WILLIAMS, P.H., 1993. Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. **Advances in Agronomy** 49, 119-199.

HEIL, J., VEREECKEN, H., BRÜGGEMANN, N., 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. **European Journal of Soil Science** 67, 23-39.

HERBERT, J.M., MAFFRAND, J.P., TAOUBI, K., AUGEREAU, J.M., FOURASTE, I., GLEYE, J., 1991. Verbascoside Isolated from Lantana camara, an Inhibitor of Protein Kinase C. **Journal of Natural Products** 54, 1595-1600.

HEWITT, A., 2010. New Zealand Soil Classification. Landcare Research Science Series, **Manaaki Whenua Press**.

HOLLOCHER, T.C., TATE, M.E., NICHOLAS, D.J., 1981. Oxidation of ammonia by Nitrosomonas europaea. Definite 18O-tracer evidence that hydroxylamine formation involves a monooxygenase. **The Journal of biological chemistry** 256, 10834-10836.

HOOGENDOORN, C.J., LUO, J., LLOYD-WEST, C.M., DEVANTIER, B.P., LINDSEY, S.B., SUN, S., PACHECO, D., LI, Y., THEOBALD, P.W., JUDGE, A., 2016. Nitrous oxide emission factors for urine from sheep and cattle fed forage rape (Brassica napus L.) or perennial ryegrass/white clover pasture (Lolium perenne L./Trifolium repens). **Agriculture, Ecosystems & Environment** 227, 11-23.

INMET, I.N.D.M., 2009. Normais Climatológicas do Brasil 1961-1990. **INMET**, Brasília.

IPCC, 2006. 2006 IPCC guidelines for National Greenhouse Gas Inventories, prepared by the National Greenhouse Gas Inventories Programme. In: EGGLESTON, H.S., BUENDIA, L., MIWA, K., NGARA, T., TANABE, K. (EDS.), 2006 **IPCC Guidelines for National Greenhouse Gas Inventories**. Published: IGES, Japan.

KELLIHER, F.M., CLOUGH, T.J., CLARK, H., RYS, G., SEDCOLE, J.R., 2008. The temperature dependence of dicyandiamide (DCD) degradation in soils: A data synthesis. **Soil Biology and Biochemistry** 40, 1878-1882.

KELLIHER, F.M., COX, N., VAN DER WEERDEN, T.J., DE KLEIN, C.A.M., LUO, J., CAMERON, K.C., DI, H.J., GILTRAP, D., RYS, G., 2014. Statistical analysis of nitrous oxide emission factors from pastoral agriculture field trials conducted in New Zealand. **Environmental Pollution** 186, 63-66.

KIM, D.H., KIM, B.R., KIM, J.Y., JEONG, Y.C., 2000. Mechanism of covalent adduct formation of aucubin to proteins. **Toxicology letters** 114, 181-188.

LAM, S.K., SUTER, H., MOSIER, A.R., CHEN, D., 2017. Using nitrification inhibitors to mitigate agricultural N_2O emission: a double-edged sword? **Glob. Change Biol.** 23, 485-489.

LEDGARD, S.F., LUO, J., SPROSEN, M.S., WYATT, J.B., BALVERT, S.F., LINDSEY, S.B., 2014. Effects of the nitrification inhibitor dicyandiamide (DCD) on pasture production, nitrous oxide emissions and nitrate leaching in Waikato, New Zealand. **New Zealand Journal of Agricultural Research** 57, 294-315.

LESSA, A.C.R., MADARI, B.E., PAREDES, D.S., BODDEY, R.M., URQUIAGA, S., JANTALIA, C.P., ALVES, B.J.R., 2014. Bovine urine and dung deposited on Brazilian

savannah pastures contribute differently to direct and indirect soil nitrous oxide emissions. **Agriculture, Ecosystems & Environment** 190, 104-111.

LUO, J., BALVERT, S.F., WISE, B., WELTEN, B., LEDGARD, S.F., DE KLEIN, C.A.M., LINDSEY, S., JUDGE, A., 2018. Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. **Science of the Total Environment** 610-611, 1271-1280.

LUO, J., KELLIHER, F., 2014. Partitioning of Animal Excreta N Into Urine and Dung and Developing the N_2O Inventory. **Ministry for Primary Industries**.

LUO, J., LEDGARD, S., WISE, B., LINDSEY, S., 2016. Effect of dicyandiamide (DCD) on nitrous oxide emissions from cow urine deposited on a pasture soil, as influenced by DCD application method and rate. **Animal Production Science** 56, 350-354.

LUO, J., LEDGARD, S.F., DE KLEIN, C.A.M., LINDSEY, S.B., KEAR, M., 2008. Effects of dairy farming intensification on nitrous oxide emissions. **Plant and Soil** 309, 227-237.

LUO, J., SUN, X.Z., PACHECO, D., LEDGARD, S.F., LINDSEY, S.B., HOOGENDOORN, C.J., WISE, B., WATKINS, N.L., 2014. Nitrous oxide emission factors for urine and dung from sheep fed either fresh forage rape (Brassica napus L.) or fresh perennial ryegrass (Lolium perenne L.). **Animal** 9, 534-543.

MARSDEN, K.A., MARÍN-MARTÍNEZ, A.J., VALLEJO, A., HILL, P.W., JONES, D.L., CHADWICK, D.R., 2016. The mobility of nitrification inhibitors under simulated ruminant urine deposition and rainfall: a comparison between DCD and DMPP. **Biology and Fertility of Soils** 52, 491-503.

MAZZETTO, A.M., BARNEZE, A.S., FEIGL, B.J., VAN GROENIGEN, J.W., OENEMA, O., DE KLEIN, C.A.M., CERRI, C.C., 2015. Use of the nitrification inhibitor dicyandiamide (DCD) does not mitigate N_2O emission from bovine urine patches under Oxisol in Northwest Brazil. **Nutr. Cycl. Agroecosyst.** 101, 83-92.

MCCARTY, G.W., BREMNER, J.M., 1989. Laboratory evaluation of dicyandiamide as a soil nitrification inhibitor. **Communications in Soil Science and Plant Analysis** 20, 2049-2065.

MCTIC, 2016. **Ministério da Ciência Tencologia Inovações e Comunicações.** Estimativas anuais de emissões de gases de efeito estufa no Brasil. Brasil-Governo Federal.

MEENA, H.M., SACHDEV, M.S., MANJAIAH, K.M., DOTANIYA, M.L., 2014. Nitrification Inhibition Potential of Brachiaria humidicola. **National Academy Science Letters** 37, 113-116.

MINISTRY FOR THE ENVIRONMENT, 2017. **New Zealand's Environmental Reporting Series:** Our atmosphere and climate 2017. Retrieved from www.mfe.govt.nz and www.stats.govt.nz.

MONAGHAN, R.M., DE KLEIN, C.A.M., 2014. Integration of measures to mitigate reactive nitrogen losses to the environment from grazed pastoral dairy systems. **Journal of Agricultural Science** 152, S45-S56.

MONAGHAN, R.M., SMITH, L.C., DE KLEIN, C.A.M., 2013. The effectiveness of the nitrification inhibitor dicyandiamide (DCD) in reducing nitrate leaching and nitrous oxide emissions from a grazed winter forage crop in southern New Zealand. **Agriculture Ecosystems & Environment** 175, 29-38.

NAVARRETE, S., KEMP, P.D., PAIN, S.J., BACK, P.J., 2016. Bioactive compounds, aucubin and acteoside, in plantain (Plantago lanceolata L.) and their effect on in vitro rumen fermentation. **Anim. Feed Sci. Technol**. 222, 158-167.

O'CONNELL, C., JUDSON HG, GK., B., 2016. Sustained diuretic effect of plantain when ingested by sheep. **Proceedings of the New Zealand Society of Animal Production**, Adelaide, pp. 14-17.

O'SULLIVAN, C.A., DUNCAN, E.G., WHISSON, K., TREBLE, K., WARD, P.R., ROPER, M.M., 2017. A colourimetric microplate assay for simple, high throughput assessment of synthetic and biological nitrification inhibitors. **Plant and Soil** 413, 275-287.

O'CALLAGHAN, M., GERARD, E.M., CARTER, P.E., LARDNER, R., SARATHCHANDRA, U., BURCH, G., GHANI, A., BELL, N., 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. **Soil Biology and Biochemistry** 42, 1425-1436.

PARKIN, T., VENTEREA, R., 2010. **USDA-ARS GRACEnet. Project Protocols Chapter 3**. Chamber-Based Trace Gas Flux Measurements.

PELSTER, D.E., GISORE, B., GOOPY, J., KORIR, D., KOSKE, J.K., RUFINO, M.C., BUTTERBACH-BAHL, K., 2016. Methane and Nitrous Oxide Emissions from Cattle Excreta on an East African Grassland. **Journal of environmental quality** 45, 1531-1539.

RAAB, L., CAFANTARIS, B., JILG, T., MENKE, K.H., 1983. Rumen protein degradation and biosynthesis. I. A new method for determination of protein degradation in rumen fluid in vitro. **The British journal of nutrition** 50, 569-582.

RAMÍREZ-RESTREPO, C.A., BARRY, T.N., 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. **Anim. Feed Sci. Technol**. 120, 179-201.

RUSSELL, J.B., O'CONNOR, J.D., FOX, D.G., VAN SOEST, P.J., SNIFFEN, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. **Journal of animal science** 70, 3551-3561.

SAGGAR, S., ANDREW, R.M., TATE, K.R., HEDLEY, C.B., RODDA, N.J., TOWNSEND, J.A., 2004. Modelling nitrous oxide emissions from dairy-grazed pastures. **Nutr. Cycl. Agroecosyst**. 68, 243-255.

SELBIE, D.R., BUCKTHOUGHT, L.E., SHEPHERD, M.A., 2015. The Challenge of the Urine Patch for Managing Nitrogen in Grazed Pasture Systems. **Advances in Agronomy** 129, 229-292.

SIMON, P.L., DIECKOW, J., DE KLEIN, C.A.M., ZANATTA, J.A., VAN DER WEERDEN, T.J., RAMALHO, B., BAYER, C., 2018. Nitrous oxide emission factors from cattle urine and dung, and dicyandiamide (DCD) as a mitigation strategy in subtropical pastures. **Agriculture, Ecosystems & Environment** 267, 74-82.

SINGH, J., SAGGAR, S., GILTRAP, D.L., BOLAN, N.S., 2008. Decomposition of dicyandiamide (DCD) in three contrasting soils and its effect on nitrous oxide emission, soil respiratory activity, and microbial biomassan incubation study. **Soil Res**. 46, 517-525.

SMITH, L.C., DE KLEIN, C.A.M., MONAGHAN, R.M., CATTO, W.D., 2008. The effectiveness of dicyandiamide in reducing nitrous oxide emissions from a cattle-grazed, winter forage crop in Southland, New Zealand. **Aust. J. Exp. Agric.** 48, 160-164.

SORDI, A., DIECKOW, J., BAYER, C., ALBURQUERQUE, M.A., PIVA, J.T., ZANATTA, J.A., TOMAZI, M., DA ROSA, C.M., DE MORAES, A., 2014. Nitrous oxide emission factors for urine and dung patches in a subtropical Brazilian pastureland. **Agriculture, Ecosystems & Environment** 190, 94-103.

STANTON, C., REINHARD, C., F. KASTING, J., E. OSTROM, N., HASLUN, J., LYONS, T., GLASS, J., 2018. Nitrous oxide from chemodenitrification: A possible missing link in the Proterozoic greenhouse and the evolution of aerobic respiration. **Geobiology** 16.

SUBBARAO, G.V., ISHIKAWA, T., ITO, O., NAKAHARA, K., WANG, H.Y., BERRY, W.L., 2006. A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with Brachiaria humidicola. **Plant and Soil** 288, 101-112.

SUBBARAO, G.V., NAKAHARA, K., HURTADO, M.P., ONO, H., MORETA, D.E., SALCEDO, A.F., YOSHIHASHI, A.T., ISHIKAWA, T., ISHITANI, M., OHNISHI-KAMEYAMA, M., YOSHIDA, M., RONDON, M., RAO, I.M., LASCANO, C.E., BERRY, W.L., ITO, O., 2009. Evidence for biological nitrification inhibition in Brachiaria pastures. **Proc. Natl. Acad. Sci. U. S. A**. 106, 17302-17307.

SUBBARAO, G.V., RONDON, M., ITO, O., ISHIKAWA, T., RAO, I.M., NAKAHARA, K., LASCANO, C., BERRY, W.L., 2007a. Biological nitrification inhibition (BNI)—is it a widespread phenomenon? **Plant and Soil** 294, 5-18.

SUBBARAO, G.V., SAHRAWAT, K.L., NAKAHARA, K., RAO, I.M., ISHITANI, M., HASH, C.T., KISHII, M., BONNETT, D.G., BERRY, W.L., LATA, J.C., 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). **Annals of Botany** 112, 297-316.

SUBBARAO, G.V., WANG, H.Y., ITO, O., NAKAHARA, K., BERRY, W.L., 2007b. NH4+triggers the synthesis and release of biological nitrification inhibition compounds in Brachiaria humidicola roots. **Plant and Soil** 290, 245-257.

SUBBARAO, G.V., YOSHIHASHI, T., WORTHINGTON, M., NAKAHARA, K., ANDO, Y., SAHRAWAT, K.L., RAO, I.M., LATA, J.-C., KISHII, M., BRAUN, H.-J., 2015. Suppression of soil nitrification by plants. **Plant Science** 233, 155-164.

SUGAMOSTO, M.L., 2002. Uso de técnicas de geoprocessamento para elaboração do mapa de aptidão agrícola da adequação de uso do centro de estações experimentais do Canguiri, Pinhais–PR. **Universidade Federal do Paraná**, Curitiba, p. 133.

TAMURA, Y., NISHIBE, S., 2002. Changes in the Concentrations of Bioactive Compounds in Plantain Leaves. **Journal of Agricultural and Food Chemistry** 50, 2514-2518.

USDA, 2017. Livestock and Poultry: World Markets and Trade. United States Department of Agriculture. **Foreign Agricultural Service/USDA**.

VAN DER WEERDEN, T.J., KELLIHER, F.M., DE KLEIN, C.A.M., 2012. Influence of pore size distribution and soil water content on nitrous oxide emissions. **Soil Res**. 50, 125-135.

VAN DER WEERDEN, T.J., LUO, J.F., DE KLEIN, C.A.M., HOOGENDOORN, C.J., LITTLEJOHN, R.P., RYS, G.J., 2011. Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral soils. **Agriculture Ecosystems & Environment** 141, 426-436.

VERTREGT, N., RUTGERS, B., 1987. AMMONIA VOLATILIZATION FROM URINE PATCHES IN GRASSLAND. IN: VAN DER MEER, H.G., UNWIN, R.J., VAN DIJK, T.A., ENNIK, G.C. (Eds.), Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Proceedings of an International Symposium of the European Grassland Federation, Wageningen, The Netherlands, 31 August–3 September 1987. **Springer Netherlands,** Dordrecht, pp. 361-363.

WARD, G.N., KELLY, K.B., HOLLIER, J.W., 2018. Greenhouse gas emissions from dung, urine and dairy pond sludge applied to pasture. 1. Nitrous oxide emissions. **Animal Production Science** 58, 1087-1093.

WICHTL, M., 2004. Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis. **Medpharm GmbH Scientific Publishers**, Stuttgart.

WILLIAMS, P.H., HAYNES, R.J., 1994. Comparison of initial wetting pattern, nutrient concentrations in soil solution and the fate of15N-labelled urine in sheep and cattle urine patch areas of pasture soil. **Plant and Soil** 162, 49-59.

WILMAN, D., DERRICK, R.W., 2009. Concentration and availability to sheep of N, P, K, Ca, Mg and Na in chickweed, dandelion, dock, ribwort and spurrey, compared with perennial ryegrass. **The Journal of Agricultural Science** 122, 217-223.

ZAKIR, H.A.K.M., SUBBARAO, G.V., PEARSE, S.J., GOPALAKRISHNAN, S., ITO, O., ISHIKAWA, T., KAWANO, N., NAKAHARA, K., YOSHIHASHI, T., ONO, H., YOSHIDA, M., 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (Sorghum bicolor). **New Phytologist** 180, 442-451.

ZAMAN, M., NGUYEN, M.L., 2012. How application timings of urease and nitrification inhibitors affect N losses from urine patches in pastoral system. **Agriculture, Ecosystems & Environment** 156, 37-48.

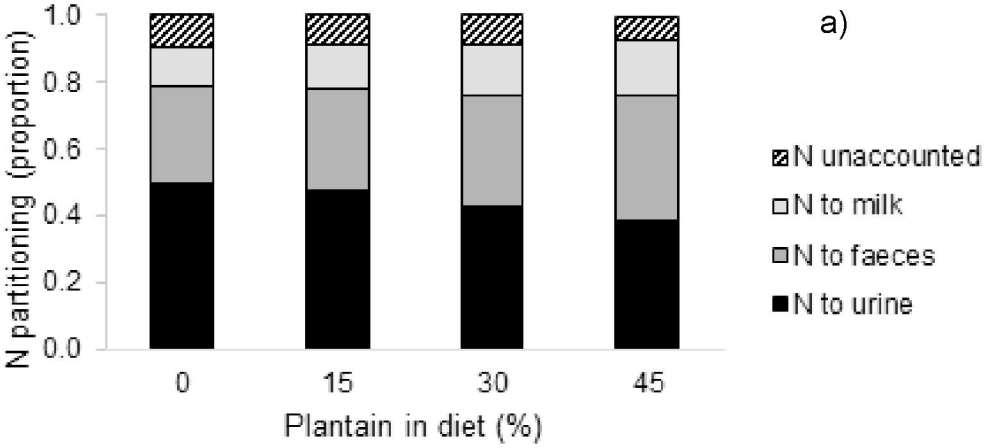
ZAMAN, M., SAGGAR, S., BLENNERHASSETT, J.D., SINGH, J., 2009. Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. **Soil Biology and Biochemistry** 41, 1270-1280.

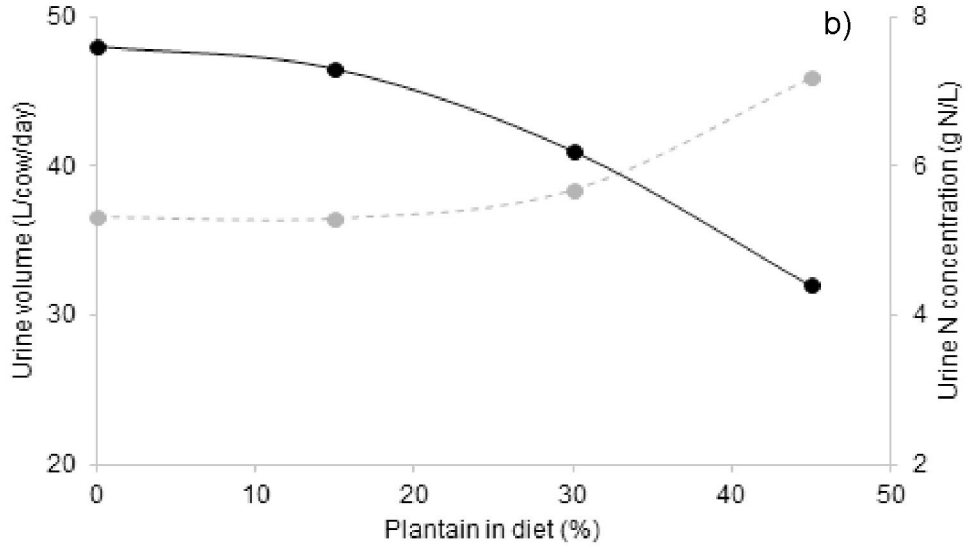
ZENG, H.Q., DI, T.J., ZHU, Y.Y., SUBBARAO, G.V., 2016. Transcriptional response of plasma membrane H+-ATPase genes to ammonium nutrition and its functional link to the release of biological nitrification inhibitors from sorghum roots. **Plant and Soil** 398, 301-312.

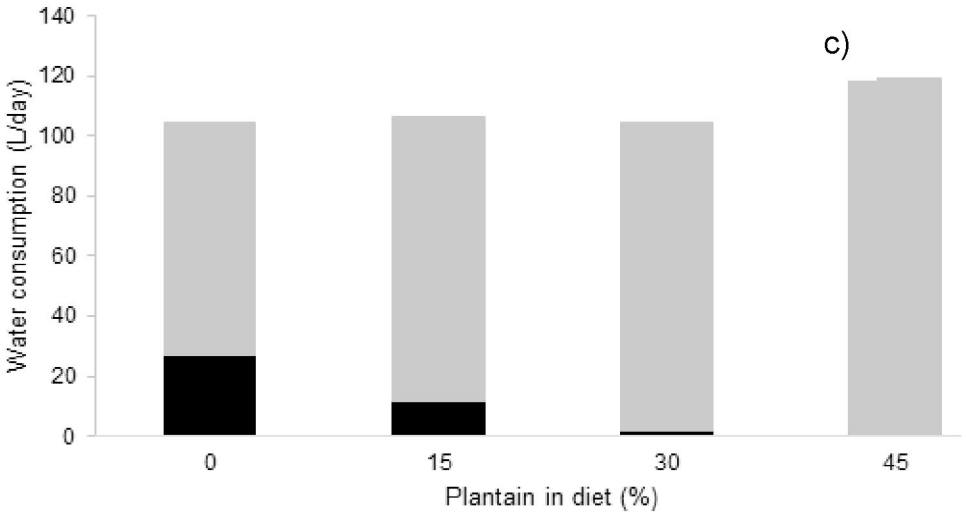
ZHU, X., BURGER, M., DOANE, T.A., HORWATH, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. **Proc Natl Acad Sci U S A** 110, 6328-6333.

ZUMFT, W.G., 1997. Cell biology and molecular basis of denitrification. **Microbiology and molecular biology reviews** : MMBR 61, 533-616.

APPENDIX 1- DIETARY NITROGEN PARTITIONING (a), URINE N AND VOLUME (b) AND WATER CONSUMPTION RATIO CHANGES (c) FROM INCREASING PLANTAIN IN COWS' DIET (NO PUBLISHED DATA).







Water consumed from the trough (■) and from feed eaten (■) by dairy cows fed increasing percentage of plantain in the diet.

APPENDIX 2 - LAYOUT OF THE EXPERIMENTAL AREA OF PLANTAIN WITH DISTRIBUTION OF THE GAS CHAMBERS IN THE PLOTS. DUNEDIN, NEW ZEALAND.