UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

GREENHOUSE GAS EMISSIONS AND N_2O MITIGATION IN BEEF CATTLE PRODUCTION ON TROPICAL PASTURE

Abmael da Silva Cardoso

Engenheiro Agrônomo

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Orientadora: Profa. Dra. Ana Cláudia Ruggieri

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Zootecnia.

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TÍTULO: GREENHOUSE GAS EMISSIONS AND N_2O MITIGATION IN BEEF CATTLE PRODUCTION ON TROPICAL PASTURE

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Jaboticabal, 03 de fevereiro de 2016.

DADOS CURRICULARES DO AUTOR

ABMAEL DA SILVA CARDOSO was born in Ceres, Goiás, Brazil in 19 of July of 1987. In the early years of your life he was influenced by the Agronomist and Pioneer Bernardo Sayão that founded Ceres. To 14 years started a high school in Agriculture in the Federal Institute of Science and Technology Goiano campus Ceres where still with 14 years old carried out his first experiment evaluating the allelopathic effect of *Cynodon nlemfuensis* Vanderyst on the weeds under supervision of Dr. Luís Sérgio Rodrigues Vale. In the first years of high school was named by the Brazilian Minister of Education the student member (2002-2004) in the Director Council of the EAF-Ceres. In 2003 was president of students association of EAF-Ceres. He was the orator of the 2004 high school class of IFGoano (Campus Ceres). With 17 years started Agronomy in the Federal Rural University of Rio de Janeiro (UFRRJ) where received the title of Agronomist in 2009. During his undergraduate program he works in the laboratory of Soil Fertility, Soil Physics and Global Information System in the university. Also participate of the Federation of Agronomy Students of Brazil (FEAB) from 2005 to 2008 representing the Agronomy students of UFRRJ. In 2006 did the exam to the Embrapa Agrobiology (Seropédica-Rio de Janeiro) initiation in science obtain the better grade. In 2007 started the traineeship in science in the Nutrient Cycling group of Embrapa Agrobiology under supervision of Dr. Bruno José Rodrigues Alves, Dr. Segundo Urquiaga and Dr. Robert Michael Boddey. At this period Abmael started inspiriting himself follow the example of Dr. Johanna Döbereiner (Scientist, Agronomist and Pioneer). In 2008 was director in the Agronomic Studies Center (Students associations) and represented the students in departments and course council. In 2012 obtained the title of Master Science (Soil Science) at UFRRJ. During his master worked with methodology of soil greenhouse gas evaluations and life cycle inventory of greenhouse gas emissions in different beef cattle production systems based on IPCC (2006) methodology working in the Nutrient Cycling group of Embrapa Agrobiology. In 2012 started the doctorate program in Animal Science in the São Paulo State University "Júlio de Mesquita Filho" working under supervision of Dr. Ana Claudia Ruggieri in the Group of Grassland and Forage Science doing evaluations of options to mitigate nitrous oxide from beef cattle production and quantified greenhouse gas emissions direct and indirect from grassland soil, urea fertilizer and beef cattle excretes. During his doctorate spent one year in the Dr. Johan Six Group at Federal Institute of Technology of Zurich (Zurich-Switzerland) learning about isotopic techniques (^{15}N) and pyrolyzed organic matter (Biochar). In February of 2016 concluded the doctorate program in Animal Science obtained the title of Doutor em Zootecnia.

Epigraph

Zulu umuntu ngumuntu ngabantu.

(Uma pessoa é uma pessoa através de outras pessoas)

Ubuntu. In honor of our ancestors.

“I am who I am, because we are all us”.
Collaboration should be your essence, not the competition.

“No one has ever seen God; but if we love another, God lives in us and his love is made complete in us. This is how we know that we live in him and He in us: He has given us of his Spirit.”
Apostle John

I dedicate

To my family, pioneers, that moved to Ceres-Goiás:

João Benjamim Gomes and his wife Fermina Maria de Araújo from Cumari-Goiás;

Sebastião Inácio da Silva and his wife Idaria Minervina de Jesus from Araguari-Minas Gerais;

Antônio Venâncio Martins and his wife Maria do Carmo Ramos from Formiga-Minas Gerais;

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SUMMARY

EMISSÃO DE GASES DE EFEITO ESTUFA E MITIGAÇÃO DE N_2O NA PRODUÇÃO DE BOVINOS DE CORTE EM PASTAGENS TROPICAIS

RESUMO: Metano (CH_4) e óxido nitroso (N_2O) são dois dos mais importantes gases de efeito estufa emitidos pela pecuária. Eles são produzidos pelas excretas dos animais e fertilizantes. No Brasil, a quantidade emitida destes gases e opções para mitigação foram pouco exploradas. Uma sequência de 4 experimentos foram realizados em campo (em duas estações chuvosas e duas secas, 106 dias de duração cada) com o objetivo de quantificar as emissões de N_2O e CH_4, volatilização de NH_3 e o fator de emissão (FE) quando aplicadas fezes, urina, fezes + urina e fertilizante ureia em Latossolo Vermelho cultivado com capim-marandu. Investigou-se o efeito da umidade do solo e compactação, composição da urina, volume urinário, e adição de fezes sobre as emissões de N_2O em um Latossolo recebendo urina manipulada em condições controladas, bem como nas emissões de CH_4. Como opção para mitigar as emissões de gases de efeito estufa (GEE) foram estudadas as variáveis como as alturas de pastejo que afetam a magnitude das emissões de GEE; a influência estacional na produção e consumo dos GEE; quais são as variáveis chaves associadas com as emissões de GEE em pastagens de capim-marandu. Adicionalmente, investigou se o efeito dietético dos níveis do sal mineral na concentração de N na urina, o volume urinário, a proporção dos compostos nitrogenados na urina e a concentração de N nas fezes em condições de campo. Os FEs de N_2O quantificados diferiram de acordo com a excreta e estação do ano. O FEs foram 2,34%, 4.26% e 3,95% na estação chuvosa e 3.00%, 1.35% e 1.59% na estação seca, respectivamente, para fezes, urina e fezes + urina. O FE do fertilizante ureia foi 0,37%. As emissões médias do CH_4 acumuladas foram 99,72, 7,82 e 28,64 (mg C-CH_4 m^2) para fezes, urina e fezes + urina nesta sequência. Quando manipuladas as condições do solo como umidade, compactação e adição de fezes as emissões de N_2O foram influenciadas sendo maiores nos tratamentos com adição de fezes. Ao se variar a concentração do N-urinário aplicado (em igual volume de urina) afetou a produção de N_2O diminuindo as emissões da maior para a menor concentração de N aplicada e não foi observado efeito ao se variar o volume de urina aplicado (contendo igual concentração de N-urinário). A concentração de

KCl adicionada na urina afetou as emissões de N_2O de forma curvilínea enquanto o tipo de composto nitrogenado não. Ao se estudar as emissões de CH_4 estas responderam aos fatores do solo como umidade, compactação e adição de fezes e não foram afetadas pela variação da concentração de N-urinário e volumes de urina. A fonte de nitrogênio aplicada não afetou a produção/oxidação de CH_4. A altura do pasto, estação e ano afetaram as emissões de N_2O e CO_2 e a estação as de CH_4. As maiores emissões ocorreram no verão e as menores no inverno. A altura do pasto apresentou efeito linear negativo nas emissões de N_2O acumuladas anual e linear positivo nas emissões de CO_2. O efeito dietético dos níveis de sal mineral influenciaram a concentração de N-urinário, volume de urina, N-ureia, N-alantoína e N-ácido hipurico. A concentração de N-urinário apresentou efeito negativo linear, o volume de urina, N-ureia, N-alantoína e N-ácido hipúrico positivo linear. Enquanto que a excreção total de N excretado via urina, N-creatinina e concentração de N nas fezes não foram afetadas pelos níveis de sal mineral na dieta. As emissões de CH_4, N_2O e NH_3 diferiram dos FEs *defaults* preconizados pelo IPCC. A umidade e a compactação do solo podem ser os principais fatores que regulam as emissões de N_2O e CH_4 e depende da variação sazonal da precipitação pluviométrica.

Palavras-chave: Emissão de CH_4 do solo, mudanças climáticas, quantificação de N_2O, volatilização de NH_3

GREENHOUSE GASES EMISSIONS AND N_2O MITIGATION OF BEEF CATTLE PRODUCTION ON TROPICAL PASTURES

ABSTRACT: CH_4 and N_2O are two of the most important greenhouse gas emitted by livestock. They are produced from animal excretes and the fertilizer. In Brazil the amount and options to mitigate these gases are little explored. We carried out a sequence of 4 field-trials (two rainy and two dry season, 106 days each) aimed to quantify the N_2O and CH_4 emissions, NH_3 volatilization and emission factor (EF) after application of dung, urine, dung + urine and urea fertilizer on a Ferralsol of a marandu palisade-grass pastureland of Brazil. We aimed to investigate the effects of soil moisture, soil compaction, urine composition, urine volume, and dung addition on N_2O emission from a urine-treated tropical Ferralsol under controlled conditions as well on CH_4 emission. As option to mitigate greenhouse gas (GHG) emissions we studied how grazing heights affect the magnitude of GHG emissions; how season influence GHG production and consumption; what are the key driving variables associated with GHG emissions. Additionally, we investigated the effect of dietary mineral salt levels on urine-N concentration, urine volume, the proportion of N compounds in the urine and faeces-N concentration under field conditions. The emissions factor (EF) calculated differed according excretes and season. The EFs were 2.34%, 4.26% and 3.95% in the rainy season and 3.00%, 1.35% and 1.59% in the dry season, respectively, for the dung patches, urine patches and dung + urine. The N_2O EF from urea was 0.37%. The averages of CH_4 accumulated emissions were 99.72, 7.82 and 28.64 (mg CH_4-C m^2) for dung, urine and dung + urine in this sequence. The manipulated soil conditions moisture content, compaction, and dung addition affected N_2O emissions when varying quantities of urine-N were applied (in equal urine volumes) being higher when added dung and did not affect when varying urine volumes were applied (containing equal quantities of urine-N). The urine-N concentration influenced N_2O emissions decreasing from the lower concentration to the higher and the chemical form of urine-N did not. The concentration of KCl added to the urine influenced N_2O emissions presenting a curvilinear curve. When the CH_4 emissions were influenced by soil factors moisture content, compaction and dung addition and did not responded to the variation in the urine-N concentration and

volume. The source of N did not influence the CH_4 emissions/oxidation. Pasture height, season and year affect N_2O and CO_2 emissions and the season CH_4 releases. The greater emissions occurred in the summer and the lower in the winter. Pasture height had negative linear effect on annual cumulative N_2O emissions and positive linear effect on annual cumulative CO_2 emissions. Dietary effects of mineral salt level influenced the N concentration in the urine, urine volume, urea-N, allantoin-N and hyppuric acid. While the total N excreted daily via urine, creatinine-N and N concentration in feces were not affected by mineral salt level in the diet. The emissions of CH_4, N_2O and NH_3 differs that default EFs preconized by the IPCC. Soil moisture and compaction appear to be the main factors regulating N_2O and CH_4 emissions and depends of the rainfall seasonality.

Key-words: N_2O quantification, NH_3 production, CH_4 emissions from soil, climate change.

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List of Abbreviations

ADF	Acid Detergent Fiber
ANOVA	Analysis of Variance
C	Carbon
C	Cubic
CFCs	Fluorinated gases
CH_4	Methane
CH_4	Metano
CO_2	Dióxido de Carbono
CO_2	Carbon Dioxide
COP	Conference of the parties
CP	crude protein
DAA	Days After Application
DM	Dry matter
ECD	Electron Capture Detector
EF	Emissions Factor
EF1	IPCC emissions factor for N_2O emitted from N fertilizer application to the soil
$EF3_{PRP}$	IPCC emissions factor for N_2O emitted from urine and dung deposited on pasture
FCAV	Faculdade de Ciências Agrárias e Veterinárias
FE	Fator de Emissão
FID	Flame Ingestion Detector
GEE	Gases de Efeito Estufa
GHG	Greenhouse gas
He	Helium
H_2SO_4	Sulfuric Acid
IPCC	Painel Intergovernamental para Mudanças Climáticas
IPCC	Intergovernmental Panel on Climate Change
KCl	Potassium Chloride
L	Linear
LW	Live Weight
N	Nitrogen
N_2	Gas Nitrogen
N_2O	Nitrous oxide
N_2O	Óxido nitroso
NaCl	Sodium Chloride
NAMAs	Nationally Appropriate Mitigation Actions
NCF	Nitrogen concentration in feces
NCU	Nitrogen concentration in urine
NDF	Neutral Detergent Fiber
NEU	Nitrogen excreted via urine

NH_3	Ammonia
NH_3	Amônia
NH_4^+	Ammonium
NH_4Cl	Ammonium chloride
NO	Nitric oxide
NO_3^-	Nitrate
NRC	National Research Council
O_2	Gas oxygen
O_3	Ozone
p	probability
Q	Quadratic
RPS	Rumen Protein Surplus
SEM	Standard error of means
SOM	Soil Organic Matter
TCD	Thermal Conductivity Detector
Unesp	Universidade Estadual Paulista "Júlio de Mesquita Filho"
UUN	Urinary Urea-N
UV	Urine Volume
%WFPS	% of Water Filled Pores Spaces

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CERTIFICADO

Certificamos que o Protocolo nº 004389/13 do trabalho de pesquisa intitulado **"Avaliação do sal em dietas de bovinos em pastagem com estratégia de mitigação de N_2O"**, sob a responsabilidade da Profª Drª Ana Cláudia Ruggieri, de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 13 de março de 2013.

Jaboticabal, 15 de março de 2013.

Prof. Dr. Andrigo Barboza De Nardi
Coordenador - CEUA

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CHAPTHER 1 - GENERAL CONSIDERATIONS

1. GLOBAL WARMING AND GREENHOUSES GASES

Atmosphere has important hole to the life in the Earth. In 1822 Joseph Fourier published the book “The Analytical Theory of Heat”, and suggested that the atmosphere played a critical role in warming the Earth’s surface. It was experimentally verified by John Tyndall in 1861, and quantified by Svant Arrhenius in 1896 (LACIS et al. 2010). Greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gases (CFCs) are capable of absorbing infrared radiation, thereby trapping and holding heat which causes the greenhouse effect (ROYAL SOCIETY, 2010).

In 1960 Charles Keeling showed that the level of CO_2 in the atmosphere was in fact increasing. He plotted year by year along with rise of the atmospheric CO_2 of Mauna Loa Observatory in Hawaii (KEELING, 1960) starting the “Kelling Curve”. In 1972 John Sawyer published the study “Man-made Carbon Dioxide and the Greenhouse Effect”. His publication influenced the policy maker and accurately predicted the rate of global warming between 1972 to 2000 (NICHOLLS, 2007). Ramanathan (1980) published an estimate of the contribution to global warming from CH_4, N_2O and O_3 produced by industry and by agricultural sources such as fertilizer. He calculated that these gases might contribute as much as 40% of total warming due to CO_2 and all other gases of anthropogenic origin. Then agriculture figured as a contributor to greenhouse gas emissions and global warming effect.

Due to the importance of climate change in 1988 the world meteorological association established the Intergovernmental Panel on Climate Change (IPCC). The IPCC is constitute by more than 2000 scientists and have the general main to assess scientific information relevant to human-induced climate change, the impacts of human-induced climate change (IPPC, 2006). In 1997 in the third conference of the parties (COP) resulted in the Kyoto Protocol which adopted GHG reduction obligation for the signatory countries. Brazil as signatory started to report GHG emissions in national inventory.

In the 2000’s were observed successive records on the atmospheric temperature measured in different point of the Earth. The importance of climatic

change increase even more and in 2009 in the COP 15 countries like Brazil adopted Nationally Appropriate Mitigation Actions (NAMAs). Brazil assumed voluntary like reduction in deforestation, restoration of grassland, adoption of integrated crop-livestock system and biological N_2 fixation aimed to lead to an expected reduction of 36.1% to 38.9% regarding the projected emissions of Brazil by 2020 (Brazil, 2009).

November of 2015 was the world's warmest November in recorded weather history. The global average temperature in last November was warmer by 1.05 ºC than the overall average global temperature for the years 1880-2015 (NOAA, 2015). Finally, in the COP 21 almost 200 countries approved the adoption of the Paris Agreement "Recognizing that climate change represents an urgent and potentially irreversible threat to human societies and the planet and thus requires the widest possible cooperation by all countries, and their participation in an effective and appropriate international response, with a view to accelerating the reduction of global greenhouse gas emissions". (UNFCCC, 2015). Despite these consensuses many gaps in climatic change knowledge still persist.

2. CARBON DIOXIDE

John Tyndall in 1864 studied the ability of CO_2 absorb infrared radiation. He observed that CO_2 and CH_4 strongly block the radiation (TYNDALL, 1872). Arrhenius (1896) calculated that the surface temperature to be an increase in 5-6ºC doubling atmospheric CO_2 and because of the relatively low rate of CO_2 production in 1896, the warming effect would require thousands of years, and he projected it would be beneficial to humanity. However, atmospheric CO_2 reached 143% of the pre-industrial level in 2014. The globally averaged CO_2 mole fraction in 2014 was 397.7±0.1 ppm (WMO, 2015). Based on the average growth rate for the past decade the CO_2 will be achieving the double of the pre-industrial level in 2095.

The combustion of fossil fuels and cement production accounted 91% of CO_2 emissions in 2013 and the deforestation and other land-use change responded by 9%, according to (http://www.globalcarbonproject.org). In the beef cattle system the sources of CO_2 are from fuel consumption and agricultural inputs like fuels and

electricity, fertilizer and lime, pesticides, irrigation, seed production; from tillage practice like farm machinery instrumentation (CARDOSO et al., 2016a).

The biomass of plants and soil organic matter (SOM) could be sink or source of CO_2. It is a sink when the land use change from crop to forest, for example, when the tree growing accumulates C and the SOM stocks increases due crop practices like no tillage and mixed systems. In the other side biomass burning and SOM oxidation release CO_2 to the atmosphere.

An important concern is about the capacity and contribution of agricultural soils and reforestation contributed reducing CO_2 emissions. Sauerbeck (2001) pointed that, even if most carefully preserved, both forests and soils, with the exception of unmanaged wetlands, have a finite capacity to sequester carbon, which gets saturated within less than 100 years. He attributed to this reason that many scientist disagree with the idea of reforestation and additional incorporation of carbon into agricultural soils would partially substitute for the commitment of reducing the CO_2 emissions from fossil fuels.

3. METHANE

Only after 86 years that Tyndall showed that the CH_4 block the radiation the presence of this gas in the atmosphere was found (MIGEOTTE, 1948). Globally averaged CH_4 reached 1833 ppb in 2014 and increased 254% since pre-industrial level. CH_4 contributes with approximately 17% to radioactive forcing (the rate of energy change per unit area of the globe as measured at the top of the atmosphere) and 60% of the emitted CH_4 into the atmospheres comes from anthropogenic source (e.g. ruminants, rice agriculture, fossil fuel exploitation, landfills and biomass burning) (WMO, 2015).

Methane is produced in the soil as one of the final compound of the complete mineralization of SOM in wetlands. The environmental factors that affect CH_4 emissions by soils are gas diffusion, microbial activities which depends of temperature, pH, Eh, substrate availability and methane-mono-oxygenase activity (Le MER and ROGER, 2001). The ability of micro-organisms to oxidize methane has

been known since 1906, when Söhngen first isolated an organism capable of growing on methane as a carbon source and named it *Bacillus methanicus* (SÖHNGE, 1906). It was called methanotrophy. Methanotrophs are obligate aerobes and one possible reaction is $CH_4 + 2\ O_2 = CO_2 + 2\ H_2O$. The rate of CH_4 oxidation depends of the composition and biodiversity of CH_4-oxidizing consortia (MOHNATY et al. 2007), temperature (BÖRJESSON et al., 2004) and soil moisture have been suggested as a major controlling factor in numerous studies (e.g. ZEISS, 2006; JUGNIA et al. 2008; SPOKAS and BOGNER, 2011).

In the national greenhouse gas inventory CH_4 enteric and manure should be reported. In grassland soils the main source of CH_4 is the dung deposition. The IPPC guidelines (2006) preconizes a default emissions factor of 1 kg CH_4 $head^{-1}$ $year^{-1}$. In Brazilian condition a few studies were published and at this time the average emissions are 0.31 kg CH_4 $head^{-1}$ $year^{-1}$ (Table 1).

Table 1 - CH_4 emissions factor (kg CH_4 $head^{-1}$ $year^{-1}$) quantified for dung deposition in Brazilian conditions

Location	Season	Animal	Emissions	Reference
Ariquemes-RO	Spring	Heifer	0.60	Chiavegato (2010)
Piracicaba-SP	Winter	Steers	0.02	Mazzeto et al. (2014)
Piracicaba-SP	Summer	Steers	0.05	Mazzeto et al. (2014)
Ariquemes-RO	Winter	Steers	0.06	Mazzeto et al. (2014)
Ariquemes-RO	Summer	Steers	0.10	Mazzeto et al. (2014)
Seropédica-RJ	Autunm	Dairy	0.96	Cardoso et al. (2016b)
Jaboticabal-SP	Winter	Steers	0.18	This thesis
Jaboticabal-SP	Summer	Steers	0.79	This thesis
Jaboticabal-SP	Incubation	Steers	0.25	This thesis
Jaboticabal-SP	Incubation compacted soil	Steers	0.33	This thesis
Average			0.31	

4. NITROUS OXIDE

Adel (1947) showed the existence of N_2O in the atmosphere and speculated that soil air to be one source, perhaps the principal one, of the atmospheric nitrous oxide and Crutzen (1970) confirmed the influence of N_2O on the atmospheric ozone content. In 2014 N_2O concentration in the atmosphere reached 327 ppb and increase 121% since the pre-industrial level (270 ppb). The anthropogenic sources contributed with approximately 40% of N_2O emissions, including oceans, soils, biomass burning, fertilizer use and various industrial processes (WMO, 2015).

N_2O is produced in soil during the reactions of nitrification and denitrification. Nitrification, which requiries aerobic conditions, depends on NH_4^+ supply and is mediated by autotrophic bacteria, whereas denitrification is executed by anaerobic heterotrophic bacteria, which depend on the availability of labile organic C and NO_3^-. Firestone and Davidson conceived a model called “hole-in-the-pipe” (Figure 1), which synthetized the knowledge at that time about the microbiological and ecological factors influencing soil emissions of nitric oxide (NO) and N_2O. (DAVIDSON et al. 2000).

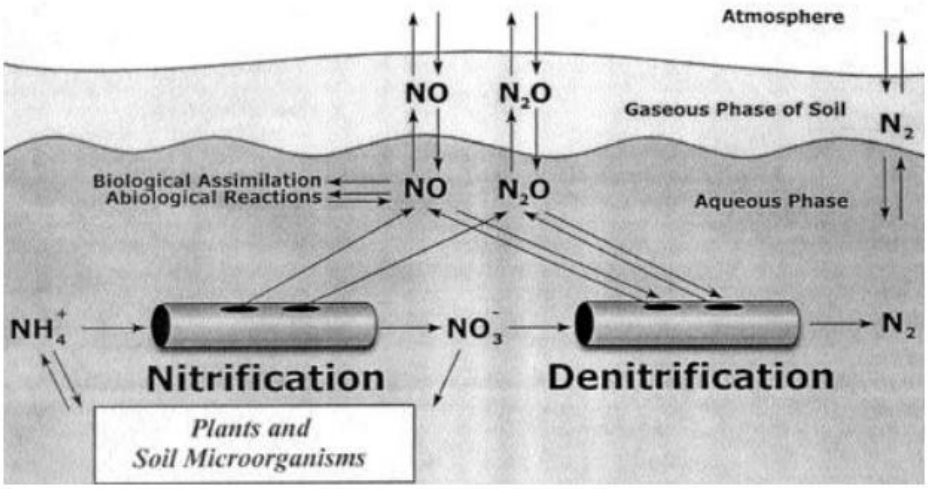

Figure 1. Diagram of the hole-in-the-pipe conceptual model (revised from Davidson 1991). “Soil emissions of NO and N_2O are regulated at two levels: First, the rate of nitrogen cycling through ecosystems, which is symbolized by the amount of nitrogen flowing through the pipes, affects total emissions of NO and N_2O; second, soil water content and perhaps other factors affect the ratio of N_2O:NO emissions, symbolized by the relative sizes of the holes through which nitric oxide and nitrous oxide leak”(Davidson et al., 2000).

There are two categories of factors that control N_2O emissions. Oenema and Sapek (2000) specified environmental factors and management factors. Soil issues such as inorganic-N, aeration, organic matter and soil moisture are the principal factors. Precipitation and temperature are the most important climate factors. In this group of factors Butterbach-Bahl et al. (2013) argued that soil moisture controls N_2O emissions because it regulates the oxygen availability to soil microbes. Management factors in grassland systems on N_2O emission are: nitrogen fertilizer, manure application and timing of application; the intensity of grazing, soil compaction and liming application (OENEMA and SPEK, 2000). Grazing is important factor because it determines how much dung and urine is deposited on grassland from the animals (NÚÑEZ et al., 2007).

Nitrous oxide emission factors (EF) are used to calculate excreta and fertilizer contributions for N_2O national inventory (IPCC, 2006). They are the ratio of N_2O-N emitted from a soil that was added an N input, minus the N_2O-N emitted from the soil that did not receive N, divided by the amount of N applied (BUCKTHOUGHT et al., 2015). Default emission factors are stipulate by the IPCC guidelines as 0.01 and 0.02 kg N_2O-N kg^{-1} input for EF_1 (N additions from mineral fertilizers) and EF_{3PRP} (excretal N inputs to grasslands), respectively (IPCC, 2006). Many countries have determined a country specific factor. In 2014 Keliher et al. accounted in a statistical analysis of measurements of nitrous oxide emissions from 185 field sites in New Zealand and they concluded that the appropriate values of EF_3 in that country for dairy cattle urine and dung, and sheep urine and dung are 1.16%, 0.23%, 0.55% and 0.08%, respectively. The Brazilian Cattle herd is approximately 20 times greater and occupies 40 times more land then that country herd cattle and at the present a few papers were published reporting N_2O emissions. The mean EF_3 reported for the Brazilian conditions are 2.31%, 0.99% and 1.87% for cattle urine, dung and urine + dung (Table 2).

Table 2 N_2O emissions factor (%) from cattle excreta deposited on pasture in Brazilian conditions.

Location	Climate and Season	Excreta type	Emission factor	Reference
Curitiba-PR	Subtropical	Urine	0.15%	Sordi et al. (2013)
Curitiba-PR	Subtropical	Dung	0.26%	Sordi et al. (2013)
Santo Antônio de Goiás-GO	Tropical/ rainy season	Urine	1.93%	Lessa et al. (2014)
Santo Antônio de Goiás-GO	Tropical/ rainy season	Dung	0.14%	Lessa et al. (2014)
Santo Antônio de Goiás-GO	Tropical/ rainy season	Urine	0.1%	Lessa et al. (2014)
Santo Antônio de Goiás-GO	Tropical/ rainy season	Dung	0.0%	Lessa et al. (2014)
Seropédica-RJ	Tropical	Urine 1L	4.9%	Cardoso et al. (2016b)
Seropédica-RJ	Tropical	Urine 1.5L	3.36%	Cardoso et al. (2016b)
Seropédica-RJ	Tropical	Urine 2L	2.43%	Cardoso et al. (2016b)
Seropédica-RJ	Tropical	Dung	0.18%	Cardoso et al. (2016b)
Jaboticabal-SP	Tropical/ Rainy season	Urine	4.26%	This thesis
Jaboticabal-SP	Tropical/ Rainy season	Dung	2.34%	This thesis
Jaboticabal-SP	Tropical/ Rainy season	Urine + Dung	3.95%	This thesis
Jaboticabal-SP	Tropical/ Dry season	Urine	1.35%	This thesis
Jaboticabal-SP	Tropical/ Dry season	Dung	3.00%	This thesis
Jaboticabal-SP	Tropical/ Dry season	Urine + Dung	1.59%	This thesis
Means		Urine	2.31%	
		Dung	0.99%	
		Urine + Dung	1.87%	

Nitrous oxide options of mitigation for livestock production systems includes optimum soil and grazing land management, limiting the amount of N fertilizes or effluent applied when soil is wet, animals dietary management to decrease the

amount of N excreted in animal urine through feeding low-N feed supplements as an alternative to fertilizer N boosted grass (USSIRI and LAL, 2013). Adoption of legumes that obtains N for biological nitrogen fixation, selection plant and animals to improve nitrogen use efficiency, use of inhibitors of N transformations and improve the animal performance to reduce the age of slaughter also can contributed for the reduction of N_2O emissions.

5. GAPS IN KNOWLEDGE

The main gap in knowledge in Brazilian conditions is to quantify CH_4 and N_2O to improve the greenhouse gas inventories and determining country-specific emission factor. A large variation in soils and climatic factors are observed in Brazil as well as peculiarities in the animal production.

Explore the micro-organism that are involved CH_4 and N_2O emissions and consumption are demanded. Identifying, isolating and exploring how they interact with soil and climatic factor.

Identifying the factors that control emissions and how the different environmental combinations influence the magnitude of the greenhouses source.

The factors regulating N_2O consumption in soil are not well understood. More studies in soil with different soil textures, mineral N content, porosity and soil moisture content are recommend to study the relationships between these soil parameters and N_2O consumption (MAZZETTO et al., 2014).

To calculate the impact of pasture restoration, adoption of integrated livestock-crop and integrated crop-livestock-forest system as well introduction of legumes in the GHG emissions.

Find the better protein to energy ratio to minimize N losses in the animal production. Selected and breeding for animals that maximizes N utilizations.

Study substances like hormones and growing stimulator for plant as strategy to mitigate N_2O emissions.

Outline the effect of Biochar application on the soil, improve nitrogen efficiency usage and cutting GHG emissions in grasslands.

Explore the life cycle assessment as a tool to evaluate different system of animal production on GHG emissions.

Study integrated options to improve animal performance although management, genetic and nutrition to reduce the time necessary to rise a beef cattle avoid GHG emissions.

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CHAPTER 2 - Ammonia, nitrous oxide and methane from tropical pastureland: variation between excretes, fertilizer and seasons, key driving variables, and mean emission factors

O artigo a seguir está redigido conforme normas de publicação do periódico *Science of the Total Environment*. Exceto o posicionamento das tabelas e figuras

Ammonia, nitrous oxide and methane from tropical pastureland: variation between excretes, fertilizer and seasons, key driving variables, and mean emission factors

Abstract: We carried out a sequence of 4 field-trials (two rainy and two dry season, 106 days each) aimed to quantify the N_2O and CH_4 emissions, NH_3 volatilization and emission factor (EF) after application of dung, urine, dung + urine and urea fertilizer on a Ferralsol of a marandu palisade-grass pastureland of Brazil. The EF differed according excretes ($p<0.001$) and season ($p<0.001$). The EFs were 2.34%, 4.26% and 3.95% in the rainy season and 3.00%, 1.35% and 1.59% in the dry season, respectively, for the dung patches, urine patches and dung + urine. Our results are higher that the default 2% EF proposed in IPCC Guidelines for cattle excreta. The amount of CH_4 emitted differ according excretes ($p<0.0001$) and season ($p=0.01$). The average of accumulated emissions were 99.72 mg CH_4-C m^{-2}, 7.82 mg CH_4-C m^{-2} and 28.64 mg CH_4-C m^2 for dung, urine and dung + urine in this sequence. The calculated EF from dung was 0.54 kg CH_4 $head^{-1}$ $year^{-1}$ and 0.11 kg CH_4 $head^{-1}$ $year^{-1}$ due urination. The NH_3 volatilized was lower in dung patches (7.2 and 6.0% of N-applied for rainy and dry season) and 6.3 and 6.4% in rainy and 14.2 and 11.5% in dry season for urine patches and dung + urine. Our data are lower than the default 20% EF proposed in IPCC Guidelines. The emission of N_2O and CH_4 and volatilization of ammonia did not differ according season from urea application. The N_2O EF from urea was 0.37%, the CH_4 emission 112 g CH_4-C ha^{-1} and 16.9% of N-applied was realized as NH_3. The EF found was lower than defaults 1% for N_2O and higher than 10% for NH_3 volatilization recommended by IPCC Guidelines for N fertilizer applied directly to the soil.

Keywords: N_2O emissions factor, CH_4 emission factor, beef cattle N_2O emissions, ammonia volatilization from bovine excretes.

1 – Introduction

Globally livestock accounts for 14.5% of total greenhouse gas (GHG) emitted to the atmosphere of which 44% correspond to emissions of methane (CH_4), mainly from enteric fermentation by ruminants, and 29% are attributed to nitrous oxide (N_2O) emissions mainly from animal excretions (Gerber *et al.*, 2013). Analyzing the GHG emissions of Brazil in 2012, 78.3% and 57.7% of the overall CH_4 and N_2O emission of the country, respectively, were attributed to livestock activity (MCTI, 2014). Most of these emissions are attributed to the more than 215 million of cattle head (IBGE, 2014) that are distribute over approximately 200 million ha of pastures.

N_2O and CH_4 contribute to global warming and ammonium (NH_4^+) to increase acidification of soils and watercourses and ammonia (NH_3) is an indirect emission of N_2O (Chadwick, 2005). N_2O is a product of nitrification of ammonium (NH_4^+) and denitrification of nitrate (NO_3^-) produced by nitrification (Hüther *et al.*, 1997). Both these processes are regulated by the quantities of N available and soil moisture (Firestone and Davidson, 1989). The Intergovernmental Panel on Global Change (IPPC) preconizes in the guidelines for GHG national inventories that 2% of all amount of N returned to the soil via bovine urine or feces are emitted as N_2O and 20% volatilized as NH_3 and 0.75% of the volatilized accounted as indirect N_2O emissions. Recently studies have been advocated to disaggregation of this emission factor according to the type of excrete (van der Weerden *et al.*, 2011; Sordi *et al.*, 2013) or season (Lessa *et al.*, 2014). For the fertilizer-N applied to the soil the emissions factor is 1% of total N lost as N_2O (IPCC, 2006). Instead 200 millions of ha of pastures in Brazil a locally quantification of N_2O emission from N fertilization of pastures have been not reported.

CH_4 is produced via microbial degradation of soluble lipids, carbohydrates, organic aids and proteins present in excretes (Kahn *et al.*, 1997). According the IPCC (2006) 1 kg of CH_4 is emitted annually per adult head of beef cattle in the Latin America. NH_3 is derived mainly from the urea component of these excretes. Urea is hydrolyzed to ammonium bicarbonate in soils by the microbially-produced enzyme urease and under most field conditions, the hydrolysis of urea deposited on grass swards in urine from grazing animals is almost complete with a few days (Thomas *et al.*, 1988). Large variation in NH_3volatilization rates between excreta types have been reported, the highest rates measured for urine (Petersen *et al.*, 1998) and for season (Lessa *et al.*, 2014). According IPPC (2006) 10% of fertilizer-N is volatilized and 0.75% of this amount emitted indirectly as N_2O.

Edaphoclimatic conditions in northwest of São Paulo rainfall is generally between 1200 and 1600 mm and approximately 90% occurs during the warm summer season from October to April similarly to Cerrado region and contrasting with temperate and sub-tropical regions (Lopes, 1996). During the summer season temperature range from 22 ºC to 32ºC. The high precipitation associated with high temperatures would likely result in rapid formation of anoxic microsites in the soil, which would favour N_2O productions (Smith *et al.*, 2003), however rapid water infiltration and high evapotranspiration rates would suggest that these conditions would be temporary (Skiba and Ball, 2002; Lessa *et al.*, 2014). The dry season is severe with often more than 90 days without rain and the temperature range 15 to 25 Most of the previous studies relating to NH_3 volatilization, CH_4 and N_2O emissions from cattle dung, urine and N fertilization have been conducted under temperate soil and grassland, with few data relate to tropical conditions. Different amounts and interactions between the key-driving variables and CH_4 and N_2O emissions are expected for the tropical soil grasslands soils, seasons and when mixed urine and dung. Indeed the importance of CH_4 and N_2O emissions

from livestock in tropical regions are increasing to understand the key driving variables involved in the N_2O production in those conditions is demanded to develop N_2O mitigations strategies and specific EF to improve the CH_4 and N_2O emissions inventories.

This study aimed at assessing CH_4 and N_2O emissions and NH_3 volatilization from cattle urine, dung patches, dung + urine and urea in a tropical pastureland of Brazil to evaluate (i) how appropriate the default EF's for each gas is for the region; (ii) how excreta type (urine vs. dung vs. dung + urine) affect the emission and if EF is different between them; (iii) how season affect emission patterns, volatilization and emission by fertilization.

2. Material and Methods

2.1 Site description

The experiment was conducted on pastureland of Marandu palisade-grass *Brachiaria brizantha* established in 2001 and located Forragicultura Sector of the São Paulo State University "Júlio de Mesquita Filho" campus of Jaboticabal in the São Paulo State. (21º15'22''S and 48º18'08''W; altitude of 595m). The local climate is tropical with dry season (April to September) and rainy season (October to March) that usually occur precipitations responsible for more than 80% of all year precipitation. The annual rainfall average is 1424 mm and air temperature 22.3ºC. The soil is a Rodhic Ferralsol (IUSS, 2006) derived from basalt. The soil 0-20 cm depth has a bulk density 1.10 g cm^{-3}, 420 g kg^{-1} of clay, 5.32 pH in water, 20 g kg^{-1} of total Carbon, 1.6 g kg^{-1} of total N, 4.7 mg kg^{-1} of $N\text{-}NO_3^-$ and 15.9 mg kg^{-1} $N\text{-}NH_4^+$.

2.2 Experimental design and excreta characteristics

We measured the ammonia volatilization, N_2O and CH_4 fluxes during four separate 106-day approximately trials which included dry season 2012 (July 8th – October 16th), rainy season 2013 (January 9th – April 17th), dry season 2013 (July 3th – October 17th) and rainy season 2013-2014 (December 17th – March 31th). The dry season occurs from the end of autumn and begins of sprig in the South hemisphere and the rainy season from the end of spring to begin of autumn.

A volume of 1.5 l of urine, 1.5 kg of fresh dung, a mixed 0.75 l of urine plus 0.75 kg of fresh dung, the equivalent of 80 kg N ha^{-1} of urea fertilizer were applied over the soil microplots delimited by a square metal chamber bases 0.6X0.4 m (area = 0.24 m^2) inserted 5.0 cm into the soil. We inserted the chamber bases 3 days before the application of treatments to avoid soil disturbance influence in the gases emissions. Every trial period we change the chamber bases to another new area. A treatment of soil without excreta of fertilizer was used as control. The area used had been no fertilizer-N application during the last 3 years. When herbage reached 25-cm high, it was cut to 15-cm high and removed from the area during the course of the trials, simulating grazing.

Artificial urine was created according Doak (1952) mixing urea (88.6% of total N), hyppuric acid (6.2% of total N), creatine (0.8% of total N), allantoin (1.5% of total N), ureic acid (0.4% of total N) and NH_4Cl (2.5% of total N). The volume of 1.5 l of urine was decided from the average range of 1.6-2.2 L per event reported by Haynes and Williams (1993). These authors also reported the fresh dung weight from 1.5-2.7 kg per event of excretion. Besides the urine and dung we included treatment mixing this two excrete to simulate this situation on field. We collected fresh dung from beef cattle excretion and analyzed the dung for dry matter

according AOAC (1995)(DM, method Nº 934.01) and N total content by dry combustion (LECO) and varied from 17.0 to 29.1 g kg^{-1} which is comparable to the range 18.0 to 26.2 g kg^{-1} (Sordi *et al.*, 2013). The N application rates with dung varied from 2.55 to 4.37 g N $chamber^{-1}$and dung plus urine from 6.56 to 7.47 g N $chamber^{-1}$ (Table 1). Total C was quantified in dung by dry combustion and ranged 385 to 491 g kg^{-1} whilst the C:N ratio varied between 14.8 to 25.3.

Table 1 Amount of N in cattle urine, dung, dung + urine and urea used as soil treatment of the two experimental periods of the rainy season and two in dry season, and respective fraction of added N emitted as $N\text{-}N_2O$.

	Amount of N in applied excreta and fertilizer (g N $chamber^{-1}$)			
	Rainy season		Dry season	
Treatment	2013	2014	2012	2013
Excreta ($EF3_{PRP}$)				
Urine	10.56	10.56	10.56	10.56
Dung	4.05	2.55	4.37	2.58
Urine + dung	7.31	6.56	7.47	6.57
Fertilizer (EF_1)				
Urea	19.20	19.20	19.20	19.20

The experimental design was a randomized complete block with 5 replicates. Each of the five treatments was evaluated in triplicate. One for the N_2O and CH_4 measurement, the second for the evaluation of soil $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ and the third for NH_3 volatilization quantification. The distance between plots was about 1.5 m and sets of 5.0 m. After ending

each period trial, microplots were relocated into a place without influence of previous excreta or fertilizer. The experimental area was isolated in to avoid animals. Gaseous sampling began one day after application (DAA) and extended for 106 DAA when the gases peaks of the treatments were equal the control, totalizing 23 measurements in each season. The sampling interval was daily in the first week, 2-3 days from the 2nd to 4th week, once per week in the second month and finally biweekly.

2.3 NH_3 volatilization evaluations

Quantification of N volatilized was carry out according to the methodology of Araujo *et al.*, (2009) as described by Jantalia *et al.*, (2012). In this technique, the NH_3 is captured by a semi-open chamber made of 2 L plastic (PET bottles; 10 cm diameters). The chamber was placed on the area affected by the excreta or fertilizer immediately after deposition. The acid-embedded foam strips were replaced with fresh ones after 1, 3, 5, 9, 14 and 21 days after application of treatments. The amount of ammonium captured in the foam strips was performed by steam distillation as described in De Morais *et al.*, (2013).

The total volatilized NH_3 in the 3 weeks period was calculated from the sum of the amounts quantified for each measurements interval. The proportion of the N of the excreta lost as volatilized NH_3 was calculated by the ratio between the N volatilized from the excrete or fertilizer corrected for the volatilization from the control areas, expressed as a fraction of the N added as excreta. The total amount lost was adjusted for the affected area by each excreta type.

2.4 N_2O and CH_4 flux measurements and emission factors

We followed the closed static chamber technique (Mosier, 1989) to collect air samples. Polyurethane chambers of 15-cm height were covered with thermal insulation mantle. Chambers were deployed on the square metal bases at the beginning of each sampling event as suggested by Alves *et al.*, (2012) between 9:00-10:00 am. The volume of the chamber was 0.06 m^3. The chambers were equipped with a rubber belt to seal the chamber-base and output valve for sample removal. The linearity of gas accumulation in the chamber was successfully tested in a preliminary experiment (intensive samples routine every 10 min over 1 h) and the incubation time was 30 min. Air samples were taken with 50 ml polypropylene syringes. The air sample was transferred to into 20-ml pre-evacuated vials (Shimadzu flasks). Samples were analyzed by gas chromatography (Shimadzu Greenhouse 2014) under the following conditions to measure N_2O: injector 250ºC, column at 80ºC, carrier gas was N_2 (30 ml min^{-1}) and electron capture detector (ECD) at 325ºC and to measure CH_4: flame gas was H_2 (30 ml min^{-1}) and Flame Ingestion Detector (FID) at 280ºC.

The N_2O fluxes (μg m^{-2} h^{-1}) or CH_4 fluxes (μg m^{-2} h^{-1}) were calculated considering the linear increase of gas concentration during the incubation period, the air temperature and pressures, the chamber volume and area of the metal bases (Barton *et al.*, 2008). The cumulative emission (g m^2) in each 106-day season was calculated by integrating the hourly fluxes over time.

The N_2O-N emission factor for urine, dung, urine and dung mixed and fertilizer, for each of the 106-day seasons, was calculated following to Eq. (1)

$$EF\ (\%) = N_2O\text{-}N\ \text{emitted} - (N_2O\text{-}N\ \text{control})/N\ \text{applied} \times 100 \quad (1)$$

Where EF is the emission factor (percentage of the urine, dung or fertilizer applied-N emitted as N_2O), N_2O-N emitted is the cumulative N_2O-N emission from urine, dung, urine + dung or urea treated plot during 106-day period (g m^{-2}), N_2O-N control is the cumulative N_2O-N emission from the control plot during the 106-day period (g m^{-2}), and N applied is the N application rate (g m^{-2}) from the treatments.

The CH_4 emission factor was calculated for the dung treatment. We estimated the annual production of feces considered one animal defecating 10 kg (wet weight) of feces per day in average 1 kg ten times, which represent values observed in extensive systems (González-Avalos and Ruiz-Suárez, 2001; Orr *et al.*, 2012; Mazzetto *et al.*; 2014)

2.5 Soil and meteorological parameters

For each air sampling event soil samples of the 0-5 cm layer were collected with each experimental block, in the replication to follow inorganic N, for measurement of gravimetric water content (105ºC) and determination of water filled pore space (WFPS), N-NH_4^+ and N-NO_3^- contents. Soil bunk density in the 0-5 cm layer was also measured, by using 50-mm diameter and 30-mm height cylinder. The WFPS was calculated after considering the gravimetric water content, the bulk density and a particle density of 2.65 Mg m^{-3}.

For mineral N analysis, extraction with 2M L^{-1} KCl was performed with field moist samples (The correction of water content was done after 105ºC drying). Ammonium-N was determined using a spectrometry at 647 nm, Berthelot reaction (Kempers & Zweers, 1986). Nitrate-N quantification was carried out by ultraviolet absorption spectrometry at 220 nm (Miyazawa *et al.,* 1985; Olsen *et al.,* 2008).Data of daily maximum, average and minimal

temperature and daily rainfall precipitation were obtained in a meteorological station located 1.5 km away.

2.6 Statistical analysis

The patterns of volatilized ammonia, N_2O and CH_4 fluxes during the experimental period were displayed by using means and standard error of means. Integrated data for each experimental period were submitted to ANOVA after testing for normality and equal variance tests by using R version 3.1.2 (2014) and means were separated by Tukey-HSD test at 5% probability.

Pearson correlation analysis was run to test for relationships between transformed N_2O or CH_4 fluxes and temperature, rain precipitation, %WFSP, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ using data from each sampling event (n=46 for each treatment). Single and multiple linear regression (backward) analysis were proceeded to create explanatory models using the variable to account of variation in seasonal N_2O emissions.

3 Results

3.1 Temperature and precipitation

In the dry season the maximum, media and minimum temperature were 38.3, 20.9 and 5.9º C in 2012 and 35.9, 20.5 and 4.6ºC in 2013 and in the rainy season evaluations were 34.4, 23.1 and 12.7ºC in 2013 and 35.9, 25.0 and 15.8ºC in 2014 (Fig. 1). In the dry season of 2012 did not rain during the first 36th DAA and in 2013 after the 20th. The accumulated rainfalls during the 106 of gases measuring were 146.6 mm and 149.2 in 2012 and 2013 respectively, representing 10.8% and 15.7% of annual precipitation. The total rainfall during the period of evaluation in the rainy season were 537 mm and 516.6 mm in 2013 and 2014 respectively, totalizing 39.5% and 54.3% of annual precipitation. The temperature and precipitation were in the same magnitude for the first and the second year.

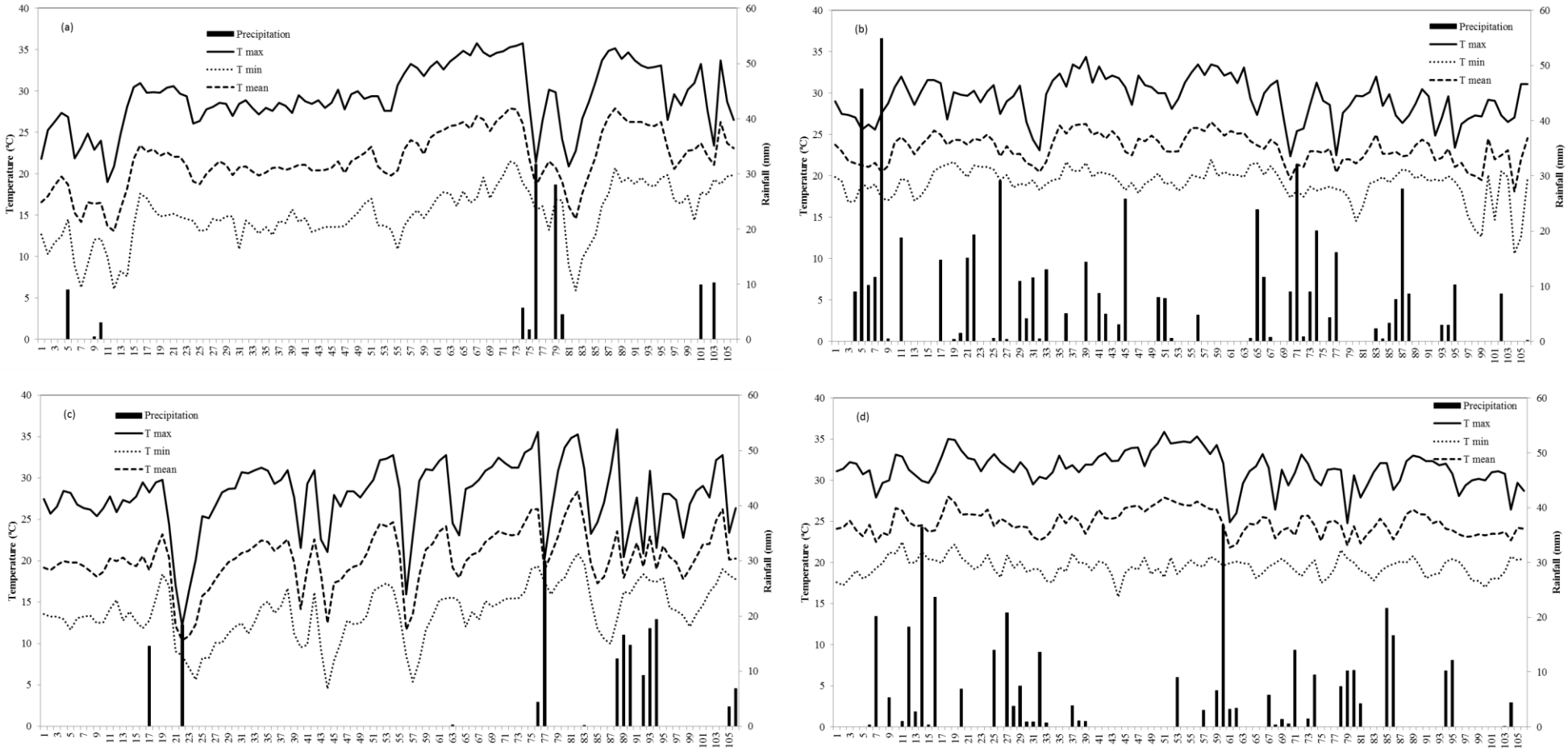

Fig. 1. Daily air temperature (minimum, mean and maximum; T; ºC) and daily rainfall (P; mm). Data from Agrometeorological Station, Department of Exact Science, FCAV/UNESP, located 1,5 km away from the experimental site.

3.2 Ammonia volatilization

The amount of N-NH_3 volatilized varied according the type of excrete ($p<0.001$) and season ($p<0.001$) (Table 2). The volatilized urine-N was higher than dung-N in the dry season 14.2% and 6.0% respectively and during the rainy season the N-volatilized did not differ according the type of excrete 7.2%, 6.4% and 6.3% for dung, urine and dung + urine respectively. For urea fertilizer 17% and 16.9% of N applied in the dry season or rainy season were volatilized and did not differ according the season (Table 2). More than 80% of accumulative N-volatilized occurred within the first 3 DAA and achieved this magnitude at the 5 DAA for the dung and dung + urea and the volatilization increased after the first two for five DAA with the process ceasing within a 9 DAA. For urea after 9 DAA achieve 80% of N volatilized. The behavior was similar according to the seasons except for dung during the dry season more than 50% of accumulative N-volatilized occurred after 9 DAA (Fig. 2).

Table 2 Amount of N in cattle urine, dung, dung + urine and urea used as soil treatment of the two experimental periods of the rainy season and two in dry season, and respective percentages of added N lost as volatilized NH_3.

	Amount of N in applied excreta (g N chamber^{-1})				Volatilized N-NH_3 (% of total N applied)					
	Rainy season		Dry season		Rainy season			Dry season		
Treatment	2013	2014	2012	2013	2013	2014	Mean	2012	2013	Mean
Excreta										
Urine	10.56	10.56	10.56	10.56	7.55	5.03	6.29b	20.88	7.60	14.24a
Dung	4.05	2.55	4.37	2.58	12.40	2.04	7.22ab	4.67	7.35	6.01b
Urine + dung	7.31	6.56	7.47	6.57	8.89	3.83	6.36b	12.56	10.49	11.52ab
Fertilizer										
Urea					22.88	10.79	16.82	19.08	14.88	16.98

Mean N data followed by a same letter did not differ in the column according to the Tukey-HSD test at 5% probability.

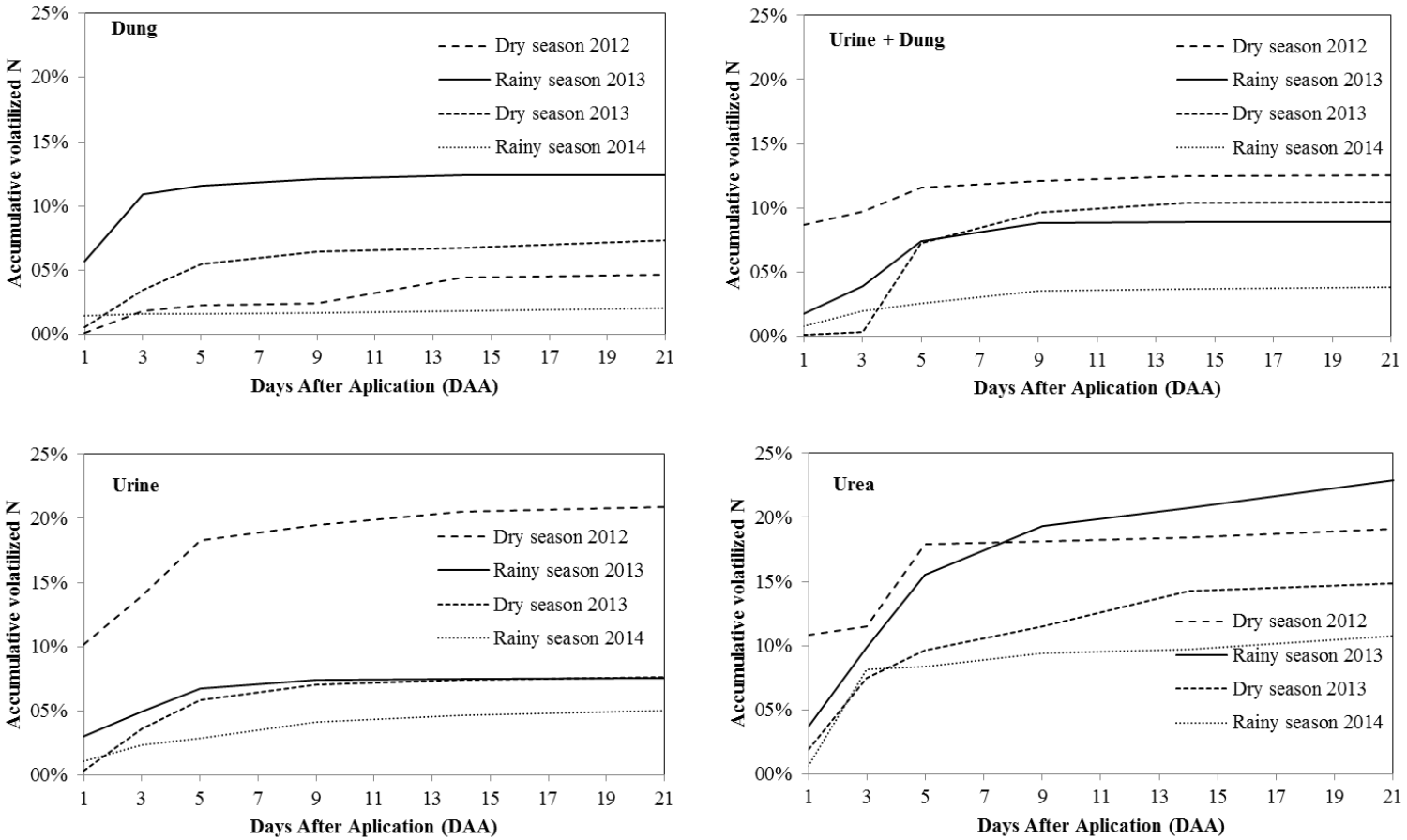

Fig. 2. Cumulative ammonia volatilization (% of N added) from cattle dung, urine, dung + urine patches and urea fertilizer deposited on a marandu palisade-grass pasture on a Ferralsol of the Cerrado region in São Paulo state Brazil during four 21-day periods in the rainy season and dry season.

3.3 Temporal trends in N_2O fluxes

The N_2O fluxes in the control situated around zero during the 4 periods of evaluation. Negatives fluxes of N_2O were commonly found in this study mainly in the dry season. The N_2O emission peak (797.1μg N_2O-N m^{-2} h^{-1} from urine in rainy (Fig 3. b) was the highest followed by 717.4 μg N_2O-N m^{-2} h^{-1} from dung, 704.9 μg N_2O-N m^{-2} h^{-1} from dung and 687.0 μg N_2O-N m^{-2} h^{-1} from dung + urine for the first dry season and occurred on average 20 days after application (DAA), for dung and urine and 16 DAA for dung + urine and urea fertilizer. The peaks dropped to the background levels 52-64 DAA in dry season (Fig 3. a,c) and returned to background 64-78 DAA in rainy season (Fig 3. b,d). The main N_2O losses were concentrated between 15 and 45 DAA of excretes or urea.

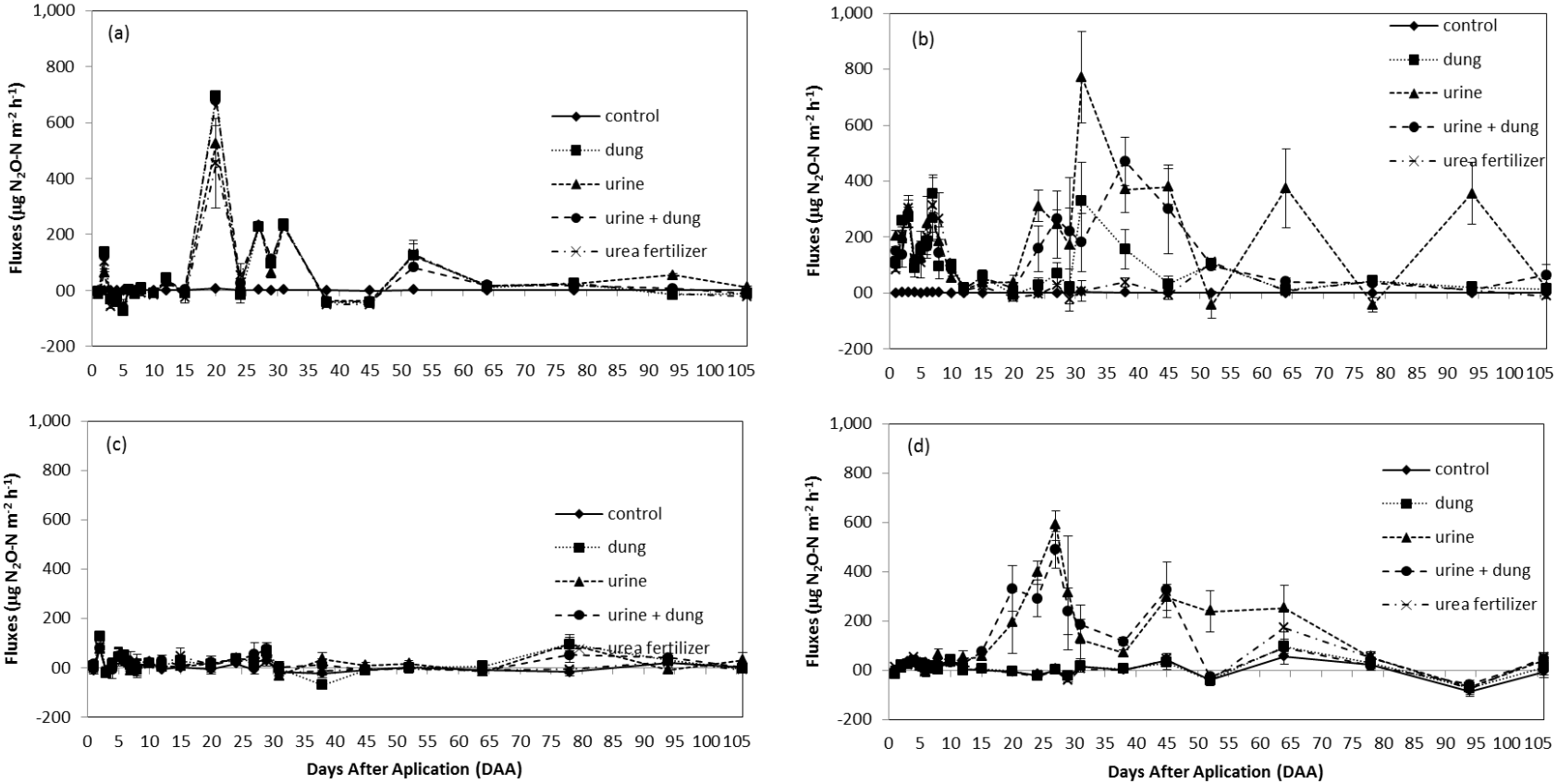

Fig. 3. Nitrous oxide emission (µg N_2O-N m^{-2} h^{-1}) from dung, urine and dung + urine patches and urea fertilizer deposited on a marandu palisade-grass pasture on a Ferralsol of the Cerrado region in São Paulo state Brazil during four 106-day periods in the dry season (a and c) and rainy season (b and d). Bars are standard error of mean.

3.4 N_2O accumulated and emissions factor

The background emissions found in the control were 1.2 and 1.7 μg N_2O-N in 2012 and 2013 in dry season respectively and 1.6 μg N_2O-N in 2013 and -2.6 μg N_2O-N in 2014 in the rainy season evaluations (Table 3). The emissions factor differed according excrete type ($p<0.001$) and season ($p<0.001$). 3.00% and 2.34% of dung-N was realized as N_2O in the dry season and rainy season respectively. The urine-N emitted as N_2O was 1.35% and 4.26% of N applied in the dry and rainy season. When evaluated the dung + urine 1.59% in and 3.95% of N was emitted as N_2O. The N_2O produced from urea fertilizer did not differ according to the season. The EF found for urea fertilizer was 0.37% (Table 3) and did not differ according season.

Table 3 Fraction of added N emitted as N-N_2O in cattle urine, dung, dung + urine and urea used as soil treatment of the two experimental periods of the rainy season and two in dry season.

Fraction of added N emitted as N-N_2O (% of N added)					
Rainy season			Dry season		
2013	2014	Mean	2012	2013	Mean
5.00	3.51	4.26a	1.33	1.37	1.35c
3.73	0.94	2.34b	2.72	3.29	3.00b
4.03	3.88	3.95b	1.64	1.54	1.59c
0.50	0.33	0.42	0.52	0.07	0.29

Mean N data followed by a same letter did not differ in the column according to the Tukey-HSD test at 5% probability.

3.5 Temporal trends in CH_4 fluxes

We found CH_4 oxidation in 23 and 15 evaluations in the control during dry and rainy season respectively. The CH_4 fluxes from urine, dung + urine and fertilizer treatments followed the same pattern of background (Fig. 4). The highest peak of CH_4 occurred 3 DAA in dung patches (1278.4 μg CH_4-C m^{-2} h^{-1}) followed by urine + dung (1050.5 μg CH_4-C m^{-2} h^{-1}) in the first rainy season (Fig 4. b) period of evaluation. The CH_4 production occurred mainly during the first 7 DAA of treatments and secondary between 25 and 30 DAA.

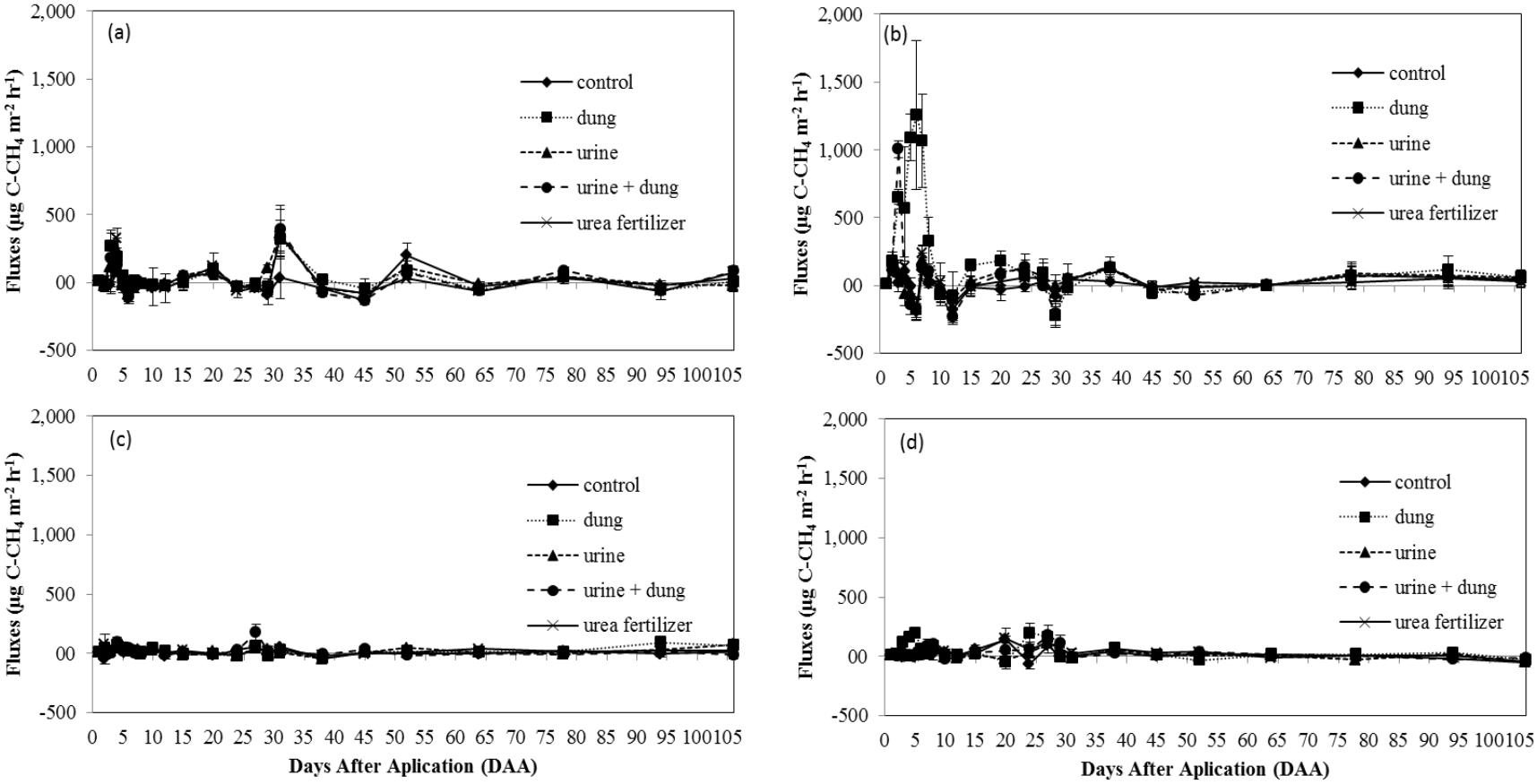

Fig. 4. Methane emission (µg C-CH_4 m^{-2} h^{-1}) from dung, urine and dung + urine patches and urea fertilizer deposited on a marandu palisade-grass pasture on a Ferralsol of the Cerrado region in São Paulo state Brazil during 106-day periods in the dry season (a and c) and rainy season (b and d). Bars are standard error of mean.

3.6 CH_4 accumulated and emission factor for dung

The total CH_4 emitted differed according to the season ($p=0.01$) and the emission from dung differed urine, urine + dung and urea fertilizer ($p<0.001$). The CH_4 accumulated from soil background were 34.6 and 34.3 mg CH_4-C during the dry season for the first and second year respectively. While in the rainy season were 34.3 and 9.5 mg CH_4-C for the first and second period respectively. The CH_4 emitted due the application of urine, dung + urine and fertilizer in the sequence were 28.6, 9.0 and 7.9 mg CH_4-C. The calculated emissions factors for this study were 0.79 kg CH_4 $head^{-1}$ $year^{-1}$ for rainy season and 0.18 kg CH_4 $head^{-1}$ $year^{-1}$ and the pondered EF was 0.54 kg CH_4 $head^{-1}$ $year^{-1}$. (Table 4)

Table 4 Amount of CH_4 in cattle urine, dung, dung + urine and urea used as soil treatment of the two experimental periods of the rainy season and two in dry seasons.

	Rainy season		Dry season		
Treatment	2013	2014	2012	2013	Mean
			mg CH_4-C m^{-2}		
Urine	59.00b	35.98bc	18.86bc	5.71c	28.64
Dung	274.72a	50.77b	16.23bc	57.16b	99.72
Urine +dung	14.04b	46.12b	-30.61c	1.73c	7.82
Urea fertilizer*	13.93	41.29	-38.01	14.72	7.98

Mean N data followed by a same letter did not differ according to the Tukey-HSD test at 5% probability. * Fertilizer was not compared to the excreta.

3.7 Water-filled pore space

The %WFPS presented the same magnitude for all treatments during the rainy season varying from 40 to 60% in the first year (Fig 5. b) and from 40 to 50% in the second year (Fig

5. d). In the rainy season of 2013 a peaked 94 DAA due successive rainfalls (Fig 5. b). While in the dry season the %WFPS was higher until 1 week after urine application and the treatment dung + urine was higher during all period of evaluation (Fig 5. a,c). The %WFPS was correlated with N_2O and CH_4 fluxes for the dung treatment (Table 5) and with CH_4 in the soil background control.

Table 5 Pearson correlation coefficients (r) between N_2O or CH_4 fluxes emissions (n=46) on dung, urine, dung + urine and urea fertilizer with explanatory variables

	N_2O						CH_4	
	Rainy season				Dry season		Rainy season	
	Dung	Urine	D+U	Urea	Control	urine	Control	Dung
% WFPS	0.41***						0.26 .	0.42**
$N\text{-}NH_4^+$	0.45**	0.28 .	0.26 .	0.31*			-0.26 .	0.31*
$N\text{-}NO_3^-$		-0.27 .			-0.26 .	-0.31*		

† Significance code:. p<0.1, *p<0.05, **p<0.01 and *** p<0.001. D+U is dung plus urine.

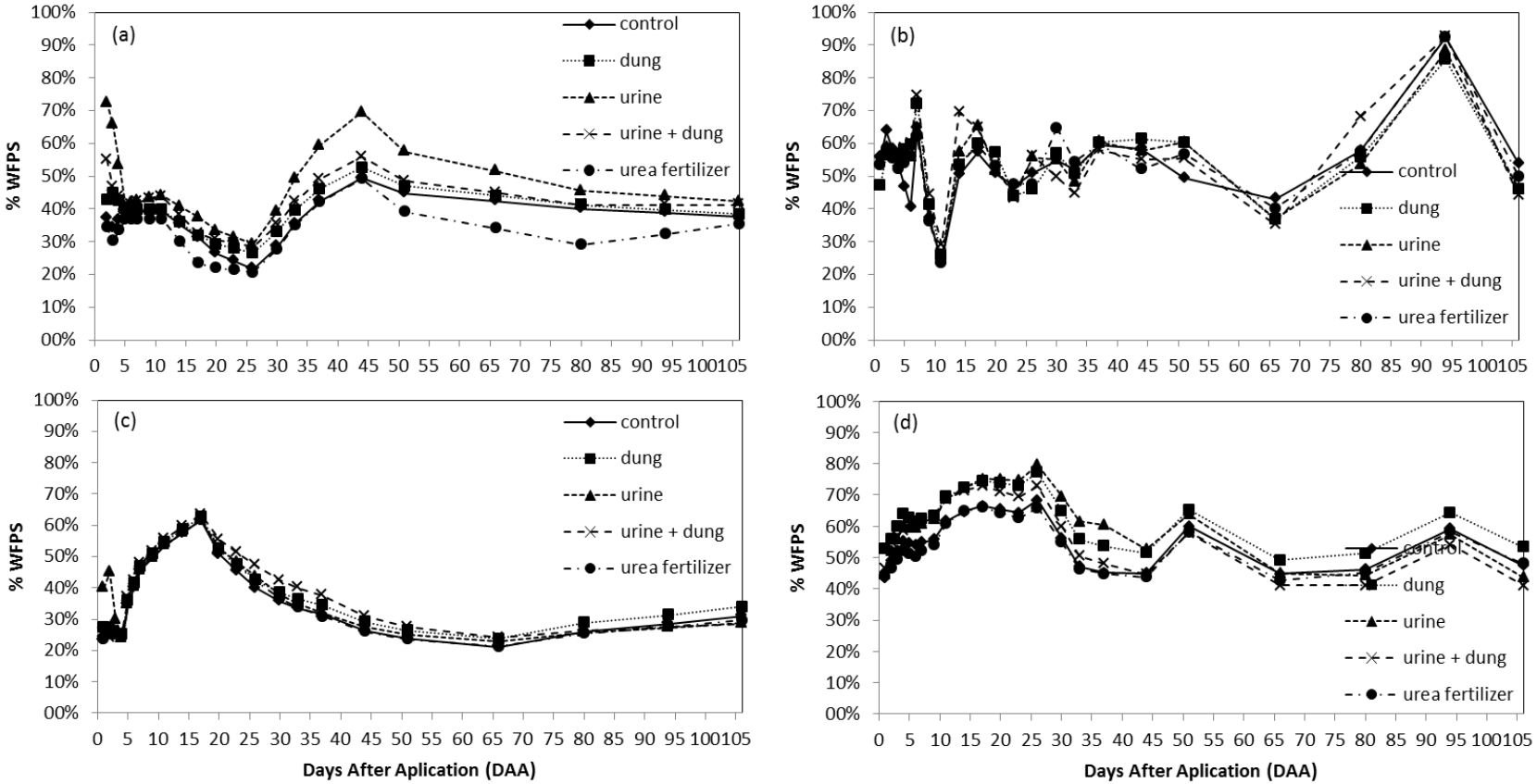

Fig. 5. Percentage of water filled pore space (WFPS) from dung, urine and dung + urine patches and urea fertilizer deposited on a marandu palisade-grass pasture on a Ferralsol of the Cerrado region in São Paulo state Brazil during 106-day periods in the dry season (a and c) and rainy season (b and d). Bars are standard error of mean.

3.8 Inorganic-N

Generally the peak of $N\text{-}NH_4^+$ occurred 7 DAA excretes and fertilizer application during dry season and 14-28 DAA during the rainy season (Fig. 6). The peaks were 161.8, 118.1, 165.1 and 163.7 mg $N\text{-}NH_4^+$ kg $soil^{-1}$ for dung, urine, dung + urine and urea, respectively, in the dry season measurements and 85 DAA dropped to the soil background (Fig. 6 a,c). In 2012 a secondary peaks occurred 63 DAA we attribute this behavior to the rainfall. During the rainy season evaluation the peaks were 242.4, 296.8, 262.7 and 255.6 mg $N\text{-}NH_4^+$ kg $soil^{-1}$ for dung, urine, dung + urine and urea respectively and the peaks returned to the background 78 DAA (Fig 6. b,d). The amount of $N\text{-}NH_4^+$ presented significant Pearson correlation with N_2O for dung, urine, dung + urine and urea in the rainy season and control and urine in the dry season (Table 5). While with CH_4 the correlation was significant for the control and dung in the rainy season (Table 5).

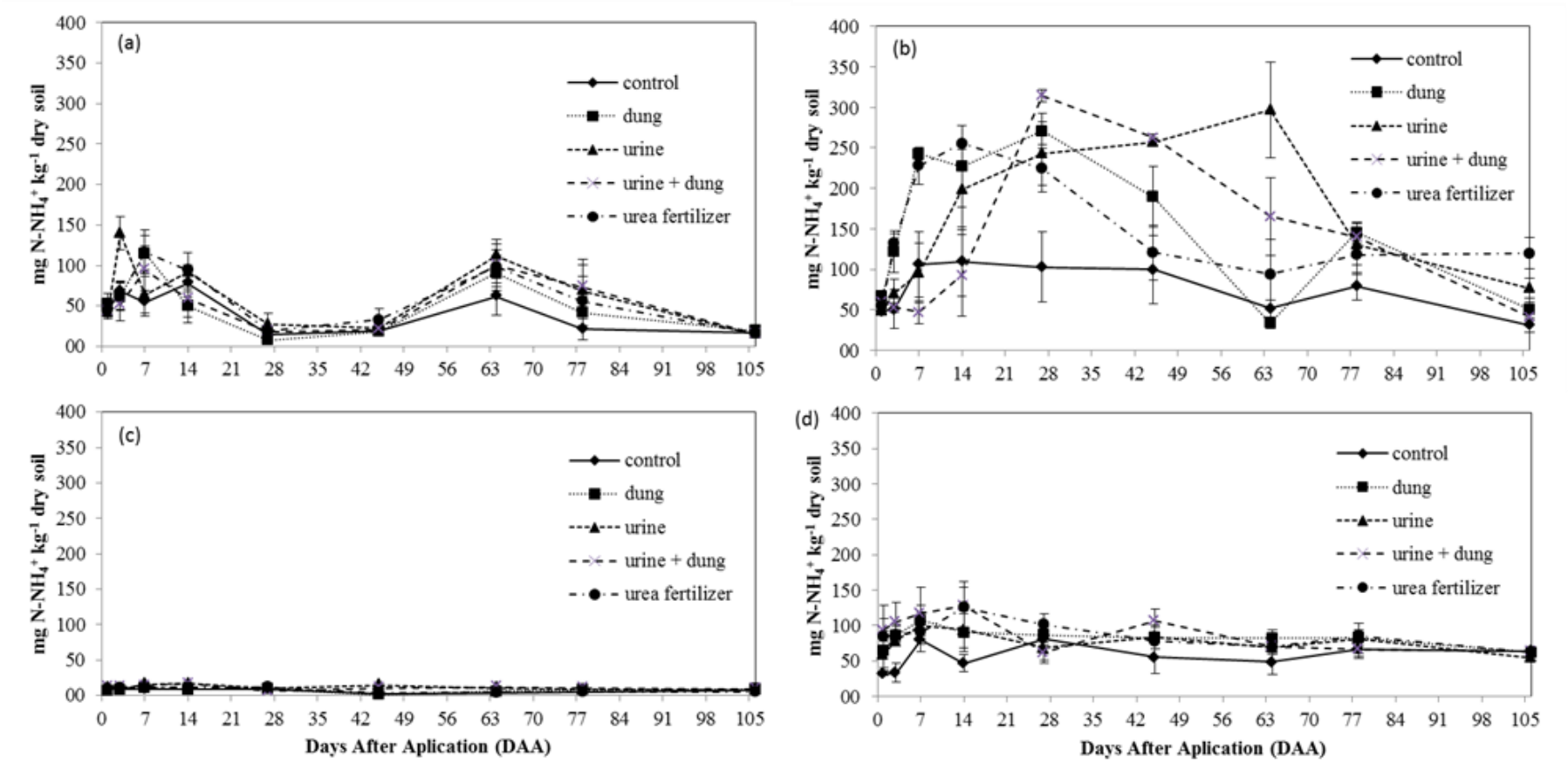

Fig. 6. Soil ammonium content (mg $N\text{-}NH_4^+$ kg^{-1} dry soil) depth 0-10 cm from dung, urine and dung + urine patches and urea fertilizer deposited on a marandu palisade-grass pasture on a Ferralsol of the Cerrado region in Brazil during 106-day periods in the dry season (a and c) and rainy season (b and d). Bars are standard error of mean.

The peaks of $N\text{-}NO_3^-$ were lower the $N\text{-}NH_4^+$ peaks. The amount of $N\text{-}NO_3^-$ after dung and urea application were similar to the soil background in both seasons. The peak of $N\text{-}NO_3^-$ for the urine in the dry season was 26.6 mg $N\text{-}NO_3^-$ kg $soil^{-1}$ and occurred 27-45 DAA and in the rainy season was 122.9 mg $N\text{-}NO_3^-$ kg $soil^{-1}$ and occurred 3 DAA (Fig. 7). In dung + urine was 29.1 and 98.7 mg $N\text{-}NO_3^-$ kg $soil^{-1}$ respectively for dry and rainy season occurred 45-69 DAA and 3 DAA for dry and rainy season respectively (Fig. 7). The $N\text{-}NO_3^-$ soil content presented negative Pearson correlation with N_2O fluxes for urine patches treatment. (Table 5). The inorganic-N and %WFPS were correlated with N_2O emissions from dung during the rainy season (Table 6).

Table 6 Multiple and single linear regressions models accounting for variation in N2O fluxes emissions from dung, urine, dung + urine and urea fertilizer using explanatory variable

Gas	Treatment	Variable	Estimate	SE	P value	Model R^2
N_2O	Dung rainy	%WFPS	0.3575	0.1708	=0.04	0.38
		NH_4-N	0.3273	0.1245	<0.01	
CH_4	Control rainy	NH_4-N	0.3858	0.1683	=0.03	0.11
	Dung rainy	%WFPS	0.4443	0.1611	<0.01	0.38
		NH_4-N	0.3098	0.1174	<0.01	
		NO_3-N	-0.3810	0.1474	<0.01	

SE – Standard error.

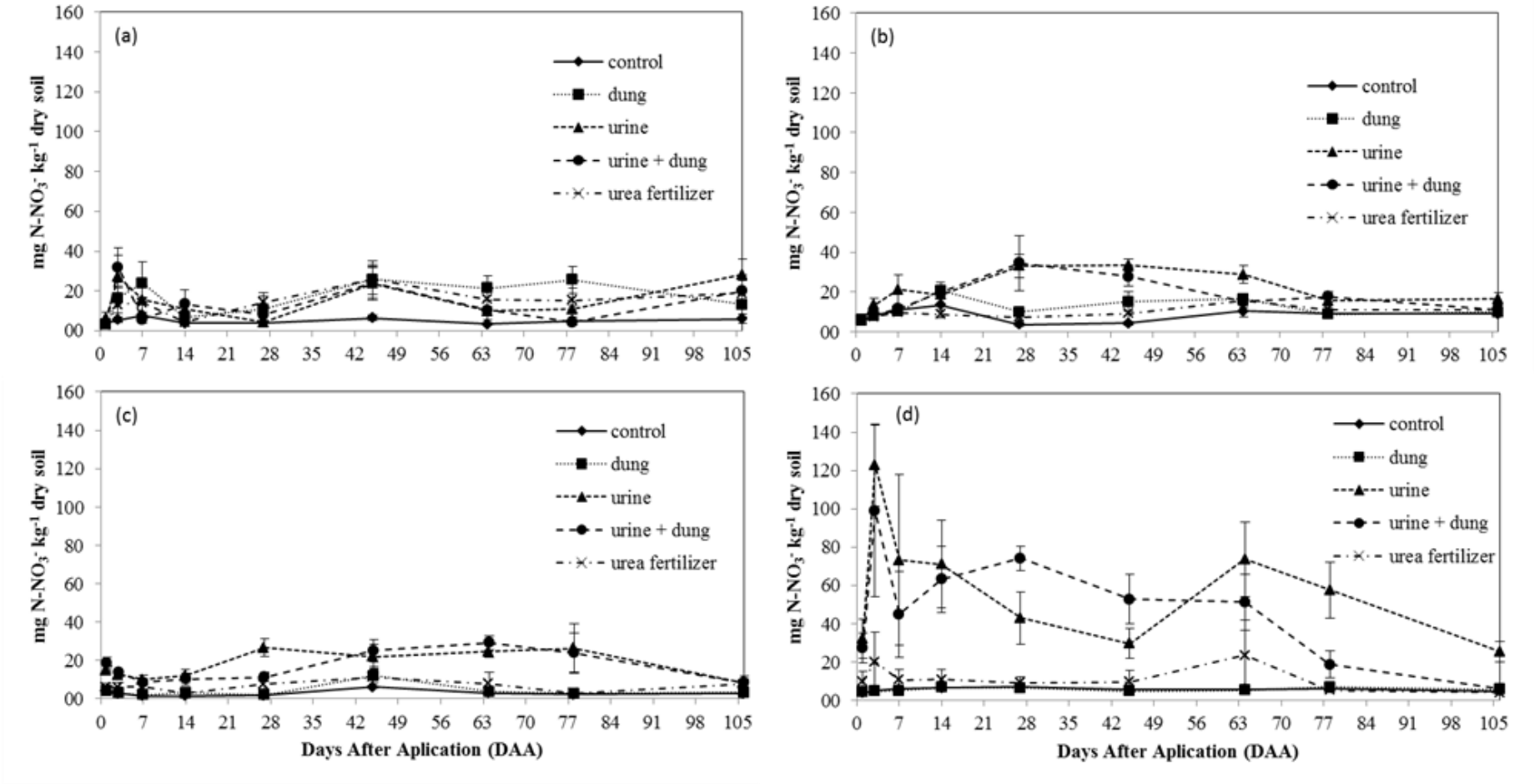

Fig.7. Soil nitrate content (mg $N\text{-}NO_3^-$ kg^{-1} dry soil) depth 0-10 cm from dung, urine and dung + urine patches and urea fertilizer deposited on a marandu palisade-grass pasture on a Ferralsol of the Cerrado region in Brazil during 106-day periods in the dry season (a and c) and rainy season (b and d). Bars are standard error of mean.

4. Discussion

4.1 Ammonia Volatilization

A considerable number of edaphoclimatic factors affect the mechanism of NH_3 volatilization (Sommer and Hutchings, 2001). The NH_3 losses from urine-N were 14.2% in the dry season and 6.3% in rainy season (Table 2). According to Saarijärvi *et al.* (2006) ammonia volatilization losses could be higher in dry soil and long time without rain. Rainfall and increasing the soil moisture content significantly decreased NH_3 losses (Oenema and Velthof, 1993). Soil moisture optimized the nitrogen incorporation in the soil. In a Cerrado region Lessa *et al.* (2014) found losses of 20.8% and 23.6% in the dry and rainy season respectively. Whitehead and Raistrick (1993) quantified the ammonia volatilized of 22 soils from England and Wales and the volatilization ranged from 6.8 to 41.3% of total urinary N, with mean value of 26.5%. Laubach *et al.* (2013) studied the NH_3 emissions from urine excreted on pasture in New Zealand the emissions represented 25.5% of the excreted urine-N. The magnitude of NH_3 volatilized has been varied enormously when urine was the N source under evaluation (Ball *et al.*, 1979; Whitehead *et al.*, 1989; Lockyer and Whitehead, 1990; Petersen *et al.*, 1998; Bol *et al.*, 2004; Mulvaney *et al.*, 2008).

A crust can be formed on dung (Petersen *et al.*, 1998), and the nitrogen in dung is in part recalcitrant, which would explain the lower N losses compared to that in urine as well as N immobilized for dung decomposition. We found difference in N lost by NH_3 volatilization between the dry season and rainy seasons the losses were 6.0% and 7.2% respectively (Table 2). Our data situated on average of N volatilized reported in the literature. The N loss fraction from dung averaging 1.5% from studies in England (Ryden *et al.,* 1987) and Finland (Saarijärvi *et al.,* 2006), 2.5% in the rainy season and 4.3% in the dry season in Brazilian

Cerrado (Lessa *et al.,* 2014), 4.5% (Sugimoto *et al.*, 1992) and 11.6% (Laubach *et al.* 2013) in New Zealand. In the treatment dung + urine the percentage of N losses did not differ of urine ranged 6.4% and 11.5% respectively for dry and rainy season. The liquid of urine could be suppressed the mechanism that protected part of dung-N to be volatilized.

The N volatilized from fertilizers varied largely and depends of the fertilizer formulation. Sources of N as ammonium nitrate, calcium nitrate and ammonium sulfate are not subjects to losses by NH_3 volatilization in acid soils (Cantarella, 1998) than urea. The NH_3 volatilization from urea amounted to 16.9% of applied N (Table 2) higher than default IPCC emission factor of 10%. Our results was in the bottom of the range of 18 to 64% N lost as volatilized NH_3 from urea of a study conducted in several sites in São Paulo State of Brazil (Cantarella and Marcelino, 2007). Our data suggested that default IPCC factor overestimated the NH_3 volatilization from dung and urine and underestimated from urea fertilization.

4.2 Temporal patterns of N_2O fluxes

The N_2O fluxes from urine, urine + dung, and urea follow the typical emission peaks reported in the literature (de Klein *et al.,* 2003; Hoeft *et al.*, 2012; Sordi *et al.*, 2014; Lessa *et al.*, 2014) except for the rainfall response. While from dung the %WSPS influenced the N_2O fluxes (Table 4). The occurrence of the emission peak of N_2O was related neither with rainfall events (Fig 1) nor with %WFPS (Fig 5). The N_2O emissions not necessarily increase in a soil under wet and anaerobic conditions (Ball, 2013). This indicates that N_2O production was likely restricted by other more limiting factors such as available N or low temperature. Sordi *et al.,* (2014) suggested that %WFPS did not closely reflect the rainfall pattern, perhaps

because of changes in soil water content caused by evapotranspiration or drainage between rainfall events and soil samplings.

The emission peaks after cattle urine or dung application occurs within 5-45 DAA (Fig 3), and drop to background levels within 90 days or earlier (Allen *et al.*, 1996; Flessa *et al.*; 1996; de Klein *et al.*, 2003; Hoeft *et al.*, 2012; Sordi *et al.*, 2014; Lessa *et al.*, 2014). In our study the urine peaked later 33 DAA (Fig 3) instead other treatments that peaked 20 DAA similarly to Sordi *et al.* (2014) who found 17 DAA for urine and dung.

Part of studies have suggest that N_2O production after urine or dung application occurred both for the sake of nitrification and denitrification (Allen *et al.*, 1996; Flessa *et al.*, 1996; Carter, 2007; Sordi *et al.*, 2014; Mazzetto *et al.*, 2014), whilst others suggested that nitrification is the main N_2O producing process (Koops *et al.*, 1997; Bol *et al.*, 2004; Lessa *et al.*, 2014), as well, others that denitrification is the principal process (Yamulki *et al.*, 2000; van Groenigen *et al*., 2005). In our study the N_2O peaked between 10 and 31 DAA (Fig 3) coinciding with the NH_4-N peaks from 200-400 mg kg^{-1} soil (Fig 6). Significant Pearson positive correlation between $N\text{-}NH_4^+$ and N_2O emissions was found for dung, urine, dung + urine and urea during the rainy season evaluation (Table 4) and negative correlation between $N\text{-}NO_3^-$ and N_2O fluxes in the urine (Table 4). For the dung the %WFPS and inorganic-N were present in the best regression model to explain N_2O emissions (Table 5). The highest $N\text{-}NO_3^-$ contents occurred at 27-65 DAA in the urine patches (Fig 7), which corresponds to the necessary time for nitrification occurring, however the values (approximately 25-70 mg kg^{-1} soil) were smaller than $N\text{-}NH_4^+$ content (Fig 6). The delay observed in the nitrification could be presence of inhibitor of nitrification observed in *Brachiaria* pasture (Subbarao *et al.,* 2009). Sordi *et al.*, (2014) attributed the lower $N\text{-}NO_3$- contents may be due to a higher N loss via NH_3 or a higher leaching of $N\text{-}NO_3^-$. $N\text{-}NH_4^+$ contents higher than those $N\text{-}NO_3^-$ after

urine application have been reported by several studies (Allen *et al.,*. 1996; Yamulki *et al.*, 1998; Hoeft *et al.*,. 2012; Sordi *et al.*, 2014). These results suggest that nitrification was predominant process to N_2O production in well drained Ferralsol pastureland. Negative fluxes has been reported (Ball and Clayton, 1997; Chapuis-Lardy *et al.*, 2007; Lessa *et al.*, 2014; Mazzeto *et al.*, 2014). We found negative fluxes in all season and treatments (Fig 3) mainly in the dry season. The N_2O production and consumption could be regulated by interactions between the O_2 concentration and soil moisture content. But the factors regulating N_2O consumption in soil are not well understood.

4.3 Seasonal variations of N_2O emissions

The lowest cumulative emission of N_2O and lowest EF for urine in dry winter relates to the lower temperature and lower %WFPS in this season certainly reduced the microbial processes on N_2O production (Sordi *et al.*, 2014). In opposite the tropical conditions, in grazed pastures of temperate regions, the highest N_2O emission rate are often reported during winter when soils are wet (Uchida and Clough, 2015). The N_2O emission from urine or dung + urine was approximately three times more in the rainy season the in dry season (Table 1). However did not differ for dung and urea fertilizer according season agreeing with Mazzetto *et al.,* (2014) that did not found difference in the accumulated N_2O emissions from dung according the season. Lessa *et al.*, (2014) found a large difference in N_2O emissions between seasons, and the emissions were almost zero in the dry season.

4.4 Differences in N_2O emissions due type of excrete and urea

The type of excreta could influence N_2O emissions (van der Weerden *et al.*, 2011). After urine deposition in the soil urea is rapidly hydrolyzed to ammonium increasing the soil pH and stimulates the release of water soluble carbon available as a microbial food supply for denitrifying bacteria (Monaghan and Barraclough, 1993). Thereafter, ammonium can be rapidly nitrified to nitrate and then further denitrified to N_2O and N_2 under favorable soil conditions. In contrast to the urine patches, there was significantly less mineral N in dung, consequently, soil N transformation activity beneath the dung patches were lower. The high dry matter content of the dung also can reduce the potential for dung N to infiltrate into the soil, restricting interaction with the soil microbial community (van der Weerden *et al.*, 2011). Sordi *et al.*, (2014) found in a subtropical Brazilian pastureland an EF from dung (0.15%) lower than urine (0.26%). Lessa *et al.*, (2014) found emission from urine-N 14 times than dung-N in rainy season and practically zero emission for the dry season for both excreta. Mazzetto *et al.*, (2014) conclude that feces cannot be considered as a N_2O source under their conditions of experiment. They measured N_2O emissions in a subtropical and tropical sites in Brazil during 30 days in a winter and summer season. In our study the N_2O emissions factor from urine was significantly ($p<0.01$) higher than dung representing 2.61 and 3.04% of total N applied for dung and urine patches respectively. However in the treatment dung + urine the EF is practically the same of urine 2.97% (Table 1). Urine application on the dung patches could break the barrier to urea hydrolyzes and N infiltration of the dung implying N_2O emission similar to urine patches.

4.5 N_2O emissions factors for excreta and urea

The IPCC EF_{3PRP} default for cattle excretes is 2%. The EF found in this study for urine were 1.35% and 4.26% for dry and rainy season respectively and for dung + urine were 1.59% and 3.95% (Table 1). Considering the local rainy season of 7 months and pondered the EF was 3.04% closely to 2.97%. This results are considerably different to those obtained in a subtropical conditions by Sordi *et al.*, (2014) of 0.26% and in the Cerrado Lessa *et al.*, (2014) quantified 0.7%. The observed EF for urine and dung + urine in this tropical Ferralsol was at the top of the global range of 0.1-4.0% (Oenema *et al.*, 1997; de Klein *et al.*, 2001; de Klein *et al.*, 2003). We attributed to the %WFPS between 40-60% during the most of experimental period and available mineral N content allowed the ideal conditions for N_2O production.

The mean EF for dung calculated was 2.61% (Table 2) and overcame the previous measurements. Sordi et al., (2014) found in a subtropical region 0.15% and in Cerrado Lessa et al., (2014) calculated an EF of 0.1%. N_2O EFs for dung ranged 0.1-0.7% compiled by Oenema *et al.,* (1997) in a global review. The EF for dung is lower than urine due the patch rapidly dry and the temporary N immobilization during C decomposition whilst the cattle urine is constituted mainly of urea (Spek *et al.*, 2012) which is rapidly hydrolyzed by soil urease increasing the $N\text{-}NH_4^+$ available in the soil surface.

Our results also suggest the necessity of desegregating N_2O EF_{3PRP} for ruminant urine and dung deposited onto pastoral soil as suggested for Van der Weerden *et al.* (2011), Sordi e*t al.*, (2014) and Lessa *et al.,* (2014). Building on our data, indications are that the default 2% EF_{3PRP} may be underestimated. However based on studies in two different climatic Brazilian regions that found lower EF to adopt different EF according the climatic region is necessary to correct N_2O emissions report from Brazilian livestock. This is the first report of N_2O

emissions from urea applied to pastureland in Brazil. The EF 0.37% found indicates that the default 1% EF for N-fertilizer preconized by IPPC guide (2006) may be overestimated.

4.6 Temporal patterns of CH_4 fluxes

The first days after dung excretion are very important for CH_4 emissions (Jarvis *et al.*, 1995; Sherlock *et al.*, 2003; Saggar *et al.*, 2004; Mazzetto *et al.*, 2014), when the dung patches are under ideal conditions for methanogenic microorganisms by maintaining an ideal micro-habitat. In our study the CH_4 peaked 3 DAA and secondarily 27 DAA (Fig 4). During the first week the emissions from dung distinguish duo to the available C from feces and reaming methanogenic bacteria. We observe CH_4 oxidation most of days mainly in the dry season (Fig 4 a,c). High oxygen availability and low C available in a tropical pastureland contribute to methanotrophy instead methanogenesis. The CH_4 fluxes presented a significant Pearson correlation with soil moisture and NH_4-N during rainy season (Table 4) and the best regression model for CH_4 emissions from dung in rainy season included %WFPS, NH_4-N and NO_3-N (Table 5). Methanogenesis is related to soil water content decreasing when water content decreases to a value close to field capacity, then increases when the water content increases (Le Mer and Roger, 2001).

4.7 Seasonal variations of CH_4 emissions

Variations between the seasons were related with air temperature and feces water content. The total CH_4 emission was influenced by these two characteristics. Emissions in rainy season were 4.4, 2.1, 3.1 and 3.4 times higher than in dry season for dung, urine, dung +

urine and urea respectively (Table 3). Several studies found higher emission in summer and lower in winter season (Williams, 1993; Holter, 1997; Mazzetto *et al.*, 2014). Season effects are significant immediately after the feces application and negligible thereafter (Yamuki *et al.*, 1999). In our study the magnitude of emissions varied according the season and DAA (Fig 4). Similar behavior was found by Mazzeto *et al.* (2014) they found peaks during whole experiment, mainly influenced by rain events.

During the rainy season the higher temperatures and rainfall promote ideal condition to CH_4 emissions. The dung remains wet instead the dry season when the feces dry rapidly and form a crust that reduces the CH_4 emission (Yamulki *et al.*, 1999). In a tropical pastureland the litter C/N ratio is higher (>45) and application of N rapidly decompose this material (Boddey *et al.*, 2004). We attribute the higher CH_4 emission in the rainy season due urine and urea application owing to rainfall stimulated litter decomposition. The interactions between moisture and temperature appear to be more relevant, increasing the emission in a tropical soils studied by Mazzeto *et al*. (2014). The warm and moist conditions in cattle manure create an optimal microenvironment for the anaerobic microorganism that produces CH_4 (Saggar *et al.*, 2004).

4.8 Differences in CH_4 emissions due type of excrete

Methanogenics bacteria are excreted in the dung. This bacteria and the higher dung C content produce CH_4 (Saggar *et al.*, 2004). The emission from dung patches was 5 times higher than a urine patch (Table 3) and when dung was mixed with urine the CH_4 emission dropped to the level of urine treatment. Lin *et al.* (2009) reported that dung patches were a strong CH_4 source, which was similar from our study. However Jiang *et al.* (2012) studied

dung and urine patches from sheep and found no difference according type of excrete. The probable reason were that are different nutrient transformation characteristic for sheep and beef cattle dung patches which depend on the covered area, nutrient concentration and differences in shape between sheep and beef cattle feces.

4.9 CH_4 emission factor for dung and urine

The calculated emission factor for dung 0.54 kg $head^{-1}$ $year^{-1}$ was half than the IPCC default factor. However higher than the reported emissions factors (Mazzetto *et al.,* 2014) for subtropical and tropical situations of 0.02 (winter) and 0.05 (summer) kg CH_4 $head^{-1}$ $year^{-1}$ in São Paulo and 0.06 (winter) and 0.10 (summer) kg CH_4 $head^{-1}$ $year^{-1}$ and lower than Cardoso et al., (2016) that calculated 0.95 kg $head^{-1}$ $year^{-1}$ from dung of dairy cattle in tropical grassland of Rio de Janeiro. The urine contributes in this study to CH_4 emissions with 0.11 kg CH_4 $head^{-1}$ $year^{-1}$.

5. Conclusions

Ammonia volatilized was affected by the type of excreta and season. Urine and urine+ dung were higher than dung and the NH_3 emission was higher in the dry season. Season did not influence the NH_3 volatilization from urea fertilizer. N_2O and CH_4 emissions differ according the season and excreta. N_2O and CH_4 emissions were much higher in the rainy season than in dry season. Urine is the main source of N_2O and dung the main source of CH_4.

Our results differ of the IPCC default emissions factor. The volatilized NH_3 was lower than expected from the IPCC Tier 1 estimate of 20% for excrete and higher than 10% for urea

fertilizer. Considering a dry season of 5 months in this region the fraction of the N volatilized were 16.9% (±2.5), 6.7% (±1.5), 8.5% (±1.1) and 9.6% (±1.5), respectively, for urea fertilizer, bovine dung, dung + urine and urine.

N_2O emissions factor were higher and indicate that a singer emissions factor for urine and dung cannot be appropriate, as has been preconized for the Tier 1 of IPCC guidelines. Our finds supports the suggestion of disaggregation of IPCC EF_{3PRP} as suggested by van der Weerden et al. (2011) and Lessa et al. (2014). The results obtained by us results in a fraction of N emitted as N_2O of 0.37% (±0.1), 2.61% (±0.55), 3.04% (±0.34) and 2.97% (±0.53).

CH_4 emissions from dung were half than preconized by the Tier 1 of IPCC guidelines and the emissions from urea fertilizer, dung + urine and urine application varied within the error suggesting that this source could be not imply in a net CH_4 emission. The key-driving involved in the N_2O and CH_4 were not clear. %WFPS and ammonium content were correlated with N_2O and CH_4 fluxes measured in the rainy season.

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CHAPTER 3 - N_2O emissions from a urine-treated Ferralsol: effects of soil moisture and compaction, urine composition and dung addition

O artigo a seguir está redigido conforme normas de publicação do periódico *Agriculture Ecosystems Environment,* exceto o posicionamento das tabelas e figuras.

N_2O emissions from a urine-treated Ferralsol: effects of soil moisture and compaction, urine composition and dung addition

Abstract: The importance of N_2O emissions due to livestock activities is increasing in tropical countries like Brazil. Understanding the key driving variables of N_2O emission could help minimize impacts of N_2O release and improve the accuracy of N_2O inventories. We aimed to investigate the effects of soil moisture, soil compaction, urine composition, urine volume, and dung addition on N_2O emission from a urine-treated tropical Ferralsol under controlled conditions. The manipulated soil conditions moisture content, compaction, and dung addition affected N_2O emissions ($p = 0.02$) when varying quantities of urine-N were applied (in equal urine volumes) and when varying urine volumes were applied (containing equal quantities of urine-N). Mean N_2O emission factors of 4.14 ± 0.54% and 5.42 ± 0.60% were obtained from incubation with varying amounts of urine-N and varying urine volumes, respectively. The urine-N concentration influenced N_2O emissions ($p=0.02$) decreasing linearly ($p=0.062$) as well the volume of urine increasing linearly ($p<0.01$). The chemical form of the applied urine-N (urea, nitrate, or ammonium) did not affect N_2O emissions and the emissions factor averaged 1.40 ± 0.38%. The concentration of KCl added to the urine influenced N_2O emissions ($p < 0.01$). The key driving factors affecting N_2O emissions were soil moisture content, once the N_2O responded the variation and urine volume and differed between moist and 'dry' soil and dung addition.

Keywords: N_2O emissions, tropical soil, nitrogen deposition, bovine excreta, N_2O key driving variables.

1. Introduction

Nitrous oxide (N_2O) is the third-largest contributor to greenhouse gas emissions driving climate change. N_2O emissions are due primarily to N fertilization of soil and excretion of N by animals (WMO, 2015). N_2O emissions from livestock represent approximately 14.5% of the global anthropogenic N_2O flux (Gerber et al., 2013). These fluxes may dominate the greenhouse gas budget in countries whose economies depend to a large extent on livestock farming. In Brazil, the fraction of the anthropogenic flux of N_2O coming from urine and dung voided by livestock in pastures was 37% in 1995 and 57.7% in 2012 (MCTI, 2014), a trend that will continue to increase in the near future.

After urination, urea is rapidly hydrolyzed to yield (ammonia - NH_3/ ammonium - $NH_4^{+)}$. Autotrophic nitrifiers oxidize these energy-rich compounds to (nitrite) NO_2^- and subsequently to (nitrate) NO_3^-. Finally, heterotrophic denitrifiers use NO_3^- and NO_2^- as electron acceptors, thereby reducing these oxidized N species to (nitric oxide) NO, (nitrous oxide) N_2O and (Nitrogen gas) N_2 (Oenema et al., 1997). These reactions occur both for urine and dung patches, although the initial concentration of NH_3/NH_4^+ species is much lower in dung than in urine.

According to the IPCC Guidelines for National Greenhouse Gas Inventories (IPPC, 2006), the default emission factor (EF) for N excreted by cattle in grassland is 2%. In the case of Cerrado climate conditions, Lessa et al. (2014) recommended disaggregation of EF by excreta type. They found an EF of 1.93% from urine-N in the rainy season and 0.1% in the dry season, whereas the EF from dung-N was 0.16% and 0% for the rainy and dry seasons, respectively. Measuring in a subtropical pastureland, Sordi et al. (2014) found N_2O EFs of 0.26% and 0.15% from urine and dung patches, respectively. It has been reported that under

field conditions, N_2O emissions are much lower from dung patches than from urine patches (e.g. van der Weerden et al., 2011; Lessa et al., 2014; Rochette et al., 2014). Mazzetto et al. (2012) evaluated N_2O emissions from dung during wet and dry tropical seasons and concluded that feces cannot be considered an N_2O source under those conditions. Herein we measured N_2O EFs in a number of combinations of soil conditions and urine composition. We hypothesized that EFs can be influenced by soil and urine characteristics

The key factors affecting N_2O emission from N-fertilized soils appear to be soil water filled pore space (WFPS), soil temperature, and soil mineral N concentration (Dobbie et al., 1999). In grazed grassland the high N concentration from animal excretions, the chemical form of the N compounds, and the subsequent N transformations contribute to the high N_2O losses (Oenema et al., 1997). Some chemical present in the bovine urine like KCl may have an inhibitory effect on N_2O emissions (Agrawal et al., 1985; van Groenigen et al., 2005). Urinary N concentration varies largely and depends mainly of protein level in the diet and ranges from 3.0 to 20.5 g/L (Dijkstra et al., 2013).

Temperature and moisture affect N_2O emission from bovine manure patches (Mazzetto et al., 2014). N_2O emission from cattle excreta is also influenced by rainy versus dry season (Lessa et al., 2014). Uchida et al. (2011) attributed higher N_2O flux during the rainy season to the warmer and wetter conditions. Soil compaction decreases the total pore volume, especially the number of large pores. This in turn decreases soil aeration, possibly leading to partial anaerobiosis and to changes in N transformation and N_2O production rates (Oenema et al., 1997). Soil moisture is associated with soil compaction in grassland soil because increasing soil moisture reduces the internal soil strength (Hanza and Anderson 2005), and the capacity of the soil resist the pressure exerted by animal hooves. We hypothesized the urine volume affect N_2O losses.

Most previous studies on the effects of soil conditions on N_2O emissions from cattle excreta have been conducted on temperate grassland soils (e.g., Oenema et al., 1997; Yalmuki and Jarvis, 2002; Rochette et al., 2014). Indeed, three of the studies mentioned above represent the few carried out under tropical conditions, although no manipulation of either soil conditions or application of animal excreta was attempted (Sordi et al., 2013; Lessa et al., 2014; Mazzetto et al., 2014). Different interactions between these variables and N_2O emissions are expected for tropical grassland soils. In addition, there is an increasing need to understand the key driving variables involved in N_2O production from livestock in tropical regions in order to develop N_2O mitigation strategies and improve inventories of N_2O emissions. To this end, we manipulated soil conditions of and urine application to a tropical soil under greenhouse conditions, and assessed N_2O emissions for 106 days. We evaluated the effects on N_2O emissions of: 1) the amount of urine-N applied (in a constant volume of applied urine); 2) the volume of urine applied (containing a constant amount of urine-N); 3) the N source in the applied urine; and 4) the concentration of KCl added to the urine.

2. Material and Methods

2.1. Soil, urine preparation and dung

The soil for the incubation study was collected in June 2013 from the 0–20 cm depth layer of a sandy clay Ferralsol (42% clay, 14% silt, 44% sand) of a *Brachiaria* grassland in Jaboticabal, Brazil (21º15’22’’S and 48º18’08’’W; altitude of 595m). The chemical characteristics of soil were pH 4.9 (in water), 0.18% total N, 2.04% total C, and 11.0 mg NH_4^+-N and 4.7 mg NO_3^--N kg^{-1} dry soil. The soil was mixed and sieved (4 mm), and 500-g quantities of moist soil (8% of water content) were placed in 1.5-L square jars. The incubation

was carried out in the greenhouse facility of the Forragicultura Sector of the São Paulo State University "Júlio de Mesquita Filho" campus in Jaboticabal, São Paulo, Brazil.

Artificial urine was prepared according Doak (1952) using urea, hippuric acid, creatine, allantoin, ureic acid and NH_4Cl in the proportion of total N 88.6%, 6.2%, 0.8%, 1.5%, 0.4% and 2.5%, respectively. 14.20 g L^{-1} $KHCO_3$ and 10.50 g L^{-1} KCl were added to all artificial urine, except for the treatments with KCl concentrations manipulated to study the effects of KCl concentration on nitrification. Urine was applied spreading the liquid superficially. Fresh cattle dung were collected in the paddock and mixed, 150 g dung kg^{-1} soil was mixed through the soil in the treatments with dung addition before superficial application of the urine. The dung presented 150.0 g kg^{-1} dry matter, 16.5 g kg^{-1} DM total N and 430 g kg^{-1} DM total C and ratio C/N of 26.

2.2 Experimental setup

We conducted four different incubation experiments simultaneously:

a) Incubation 1: we evaluated the effect on soil N_2O emission of different amounts of urine-N (125, 250, 500 and 750 mg kg^{-1} dry soil; n = 5) applied in equal volumes (50 ml kg^{-1} dry soil) under different soil conditions (moist, dry, compacted, moist plus dung, and moist plus dung plus compaction; n = 4) in a two-factor without replication design. Two treatments were included as background: moist and dry soil with no N addition;

b) Incubation 2: we studied the effect of different volumes of urine (25, 50, 100 and 200 ml kg^{-1} dry soil; n = 5) containing equal amounts of urine-N (500 mg kg^{-1} dry soil) on N_2O emissions under the same soil conditions and background cited above;

c) Incubation 3: we analyzed the effect of N source on N_2O realizes the soil received 100 mL urine^{-1} kg^{-1} dry soil containing different N sources (500 mg N kg^{-1} dry soil of Urea, Ammonium Sulfate, or Potassium Nitrate; n = 4) and the background with no N source addition in a completely randomized design;

d) Incubation 4: We verified the possible inhibitory effect of Potassium Chloride on N_2O production the soil received 100 mL urine^{-1} kg^{-1} dry soil with different concentrations of added KCl (0.0, 5.0, 10.0, and 20.0 g L^{-1} urine; n = 4) and the background with no N source addition in a completely randomized design.

In the treatment background we added water instead urine. The dry soil treatment received a total of 50 ml water kg^{-1}, volumetric moisture content of 22.1% (40.4% WFPS). The moist treatment, 100 ml water kg^{-1} soil was added resulting in a volumetric soil moisture content of 34.1% (62.3% WFPS). The jars were uncovered except during N_2O measurement, allowing exchange with the atmosphere. The treatments were incubated at approximately 25ºC and 80% relative humidity for 106 days. Evaporation losses for the uncompacted and compacted treatments averaged 0.66 and 1.1 g water kg^{-1} d^{-1}, respectively. During the first seven days of incubation after urine and dung application, when necessary soil moisture was corrected by weighing the jars daily and spraying deionized water onto the soil surface as required. From seven to 60 days after application (DAA), soil moisture was corrected every two days and then subsequently twice a week until the end of the experiment at day 106.

Soil compaction was simulated by manually compressing the soil in the jars using a piece of wood until the initial bulk density of 1.2 g cm^{-3} was increased to approximately 2.0 g cm^{-3}. After compaction, volumetric soil moisture content increased from 34.1 to 56.8% (100% WFPS) in the compacted treatment, and from 48.5 to 80.8% (100% WFPS) in the compacted plus dung treatment. The treatments with dung received 150 g fresh dung kg^{-1} dry soil. The

dung was mixed through the soil following urine application. The addition of dung increased the volumetric soil moisture content from 34.1 to 48.5% (88.6% WFPS) in the moist treatments without compaction.

2.3 N_2O measurement and soil analysis

We used the technique of closed chamber to evaluate N_2O fluxes. The chambers were a jar with 1.1 L (headspace). Sampling was carried out between 9:00 and 10:00 am. N_2O flux was measured 25 times during the 106-day incubation period (daily during the first week; every two days during the second week, two times a week during the rest of the first month, weekly between 30 and 60 DAA, and twice in the remaining 30 days. We stopped the air collects after the fluxes of treatments became similar to the backgrounds. We measured N_2O flux by closing the jar lid for 0.75 h and determining the change in headspace concentration. Air samples were taken with 50-ml polypropylene syringes. Air temperatures inside and outside of the chamber were recorded using a digital thermometer. The air samples were transferred into 20-ml pre-evacuated vials (Shimadzu flasks). Ambient gas samples were also taken.

Samples were analyzed by gas chromatography (Shimadzu Green House Gas Analyzer GC-2014; Kyoto, Japan) under the following conditions for measurement of N_2O: injector 250ºC, column at 80ºC, carrier gas was N_2 (30 ml min^{-1}), and electron capture detector at 325ºC. Flux calculations were based on the assumption that there was a linear increase in N_2O concentration with time in the closed chamber. The linearity of the flux during the incubation period was tested previously over a 1.5-h period at 10-min intervals.

Cumulative emissions were calculated by plotting daily fluxes through time, interpolating linearly between them, and integrating the data. The emission factor (EF), representing the fraction of N in urine or urine plus dung lost as N_2O, was calculated by the ratio between the N_2O-N emitted by the excreta (minus the corresponding background emission) and the total N applied from excretes.

Soil inorganic N was measured at the beginning and at the end of the incubation period using 2M L^{-1} KCl extraction and colorimetric analysis according Kempers and Zweers (1986) for ammonium and Miyazawa et al. (1985) for nitrate.

2.4 Statistical tests

The effects of manipulation of urine and soil conditions on N_2O emission were tested as follows: 1) to determine the effect of amount of urine-N applied in a constant volume under different soil conditions, a two-way ANOVA without repetitions was done with amount of urine-N and soil treatment as factors; 2) to determine the effect of volume of urine applied with a constant amount of urine-N, a two-way ANOVA without repetitions was done with the urine volume and soil treatment as factors; 3) the effect of the form of urine-N applied was tested using a one-way ANOVA; and 4) the effect of KCl concentration in the applied urine was tested using a one-way ANOVA. When ANOVA was significant we used Tukey test to distinguish the means of manipulated soil and urine-N compound and polynomial orthogonal contrast to report the effect of urine volume, urine-N rates and KCl concentration. All statistical analysis was done using the R statistical program (version 3.1.2; R Core Team, 2014).

3. Results

Soil conditions (e.g., moisture content, compaction, and dung addition) significantly affected the fraction of N_2O lost ($p = 0.02$) when varying amounts of urine-N were applied in equal volumes (Table 1). N_2O emissions in the two treatments with dung addition were higher than those in which only soil moisture and compaction were manipulated. Applying urine-N in different amounts significantly affected N_2O emissions ($p = 0.02$). The EF linear decreased ($p<0.062$) from 5.10% to 1.39% as the amount of applied urine-N was increased from the lowest (125 mg kg^{-1} dry soil) to the highest (750 mg kg^{-1} dry soil) application rate. The amount of applied N released as N_2O averaged 4.14 ±0.54% (Table 1). IPCC (2006) assumed that N_2O emissions increase linearly with the N rate applied to the soil. In our study we found that EF varied according N rate, however, the fitted curve had marginally effect ($p<0.062$) and was not quadratic fitted ($p=0.31$).

Table 1. N_2O emission factor (% of applied N) for different amounts of urine-N applied in equal volumes (50 ml kg^{-1} dry soil).

Treatment [b]	Amount of applied urine-N (mg kg^{-1}) dry soil [a]				
	125	250	500	750	Average
Urine	5.10	2.60	3.45	1.39	3.14 (±0.70)
Urine + dry soil [c]	6.41	0.38	1.56	0.82	2.29 (±1.25)
Urine + compaction	5.86	3.77	2.41	3.55	3.90 (±0.64)
Urine +dung	5.20	1.94	6.57	5.20	4.73 (±0.88)
Urine + dung + compaction	9.20	7.80	5.36	3.92	6.62 (±1.10)
Average	6.40(±0.79)	3.30 (±1.25)	3.87 (±0.93)	2.98 (±0.82)	4.14 (±0.54)

The data between parentheses range indicates the standard error (n = 4 for amount of applied urine-N, n = 5 for soil treatments).
[a] $p = 0.021$ for the test of differences between amount of applied urine-N treatments (n = 20).
[b] $p = 0.02$ for the test of differences between soil condition treatments (n = 20).
[c] Only 50 mL water kg^{-1} soil, as compared to 100 mL for the other treatments.

Similarly, when the volume of applied urine was varied (with equal amounts of urine-N), soil conditions affected the quantity of N_2O emitted ($p = 0.02$), while urine volume affected ($p < 0.01$) (Table 2). The EF was increased with soil compaction and dung addition compared to moist soil. The EF increased from 3.24 ± 1.31% to 7.45 ± 0.65% as urine volume was increased linearly ($p < 0.001$) from the lowest (25 ml kg^{-1} dry soil) to the highest (200 ml kg^{-1} dry soil) volume. N_2O losses averaged 5.42 ± 0.60% of the total N added (Table 2).

Table 2 N_2O emission factor (% of applied N) for different volumes of applied urine with equal amounts of urine-N (500 mg kg^{-1} dry soil).

Treatment [b]	Volume of applied urine (ml kg^{-1} dry soil) [a]				
	25	50	100	200	Average
Urine	1.92	1.56	8.09	8.26	4.96 (±1.66)
Urine + dry soil [c]	0.77	2.41	6.75	7.15	4.27 (±1.42)
Urine + compaction	0.69	6.57	3.05	5.64	3.99 (±1.19)
Urine +dung	6.70	5.36	7.17	6.77	6.50 (±0.35)
Urine + dung + compaction	6.10	8.09	5.84	9.43	7.37 (±0.76)
Average	3.24 (±1.31)	4.80 (±1.24)	6.18 (±0.86)	7.45 (±0.65)	5.42 (±0.60)

The data range between parentheses indicates the standard error (n = 4 for amount of applied urine-N, n = 5 for soil treatments).
[a] $p = 0.04$ for the test of differences between urine volume treatments (n = 20).
[b] $p = 0.17$ for the test of differences between soil condition treatments (n = 20).
[c] Only 50 mL water kg^{-1} soil, as compared to 100 mL for the other treatments.

The fraction of N emitted as N_2O was 1.32 ± 0.28%, 0.76± 0.26 % and 2.13 ± 1.08% for nitrate, ammonium and urea, respectively (Table 3). However, the chemical form of N in the urine did not influence N_2O emissions ($p = 0.38$). The concentration of KCl in the urine also affected N_2O emissions ($p < 0.01$), with the EF being lowest in the treatment containing a

high-range concentration of KCl (20.0 g L^{-1} KCl) and highest in the treatment with 5.0g L^{-1} KCl in the urine (Table 4).

Table 3 N_2O emission factor (% of applied N) for urine containing different chemical forms of urine-N.

N-containing compound [a]	Amount of applied N (mg kg^{-1} dry soil)				Average
	125	250	500	750	
Urea	1.56	2.01	0.79	0.94	1.32 (± 0.28)
Ammonium	0.19	0.70	1.46	0.70	0.76(± 0.26)
Nitrate	5.34	1.04	0.73	1.42	2.13 (± 1.08)
Mean					1.40 (±0.38)

The data between parentheses range the standard error (n = 4).
[a] P=0.38. Significance for the test of differences between treatments with different chemical forms of urine-N (n=12).

Table 4 N_2O emission factor (% of applied N) for different KCl concentrations in applied urine.

Concentration of KCl in urine (g L^{-1})				
0.0	5.0	10.0	20.0	Effect [a]
3.22 (±1.21)	4.44 (±0.65)	3.03 (±0.42)	1.17 (±0.23)	Quadratic

[a] The data between parentheses range the standard error (n = 4). $p < 0.01$ for the test of differences between KCl treatments (n = 16). The data fit the equation $f(x) = -0.01K^2 - 0.13K + 3.46$, where f(x) = % N_2O EF and K = concentration of KCl in urine in g L^{-1}; $R^2 = 0.8697$; $p < 0.01$.

In the experiment conducted with varying amounts of applied urine-N, N_2O flux peaked between 13 DAA in all treatments (Figure 1a). The highest mean fluxes were approximately 105 ng N_2O-N g^{-1} dry soil h^{-1}, which occurred in the 500 mg N kg^{-1} dry soil and compacted both with dung addition (Figure 1a). Negative mean fluxes were found 93

DAA. After urine application 5 days were necessary to the N_2O emissions occurred and dropped to background levels 34 DAA (Figure 1a).

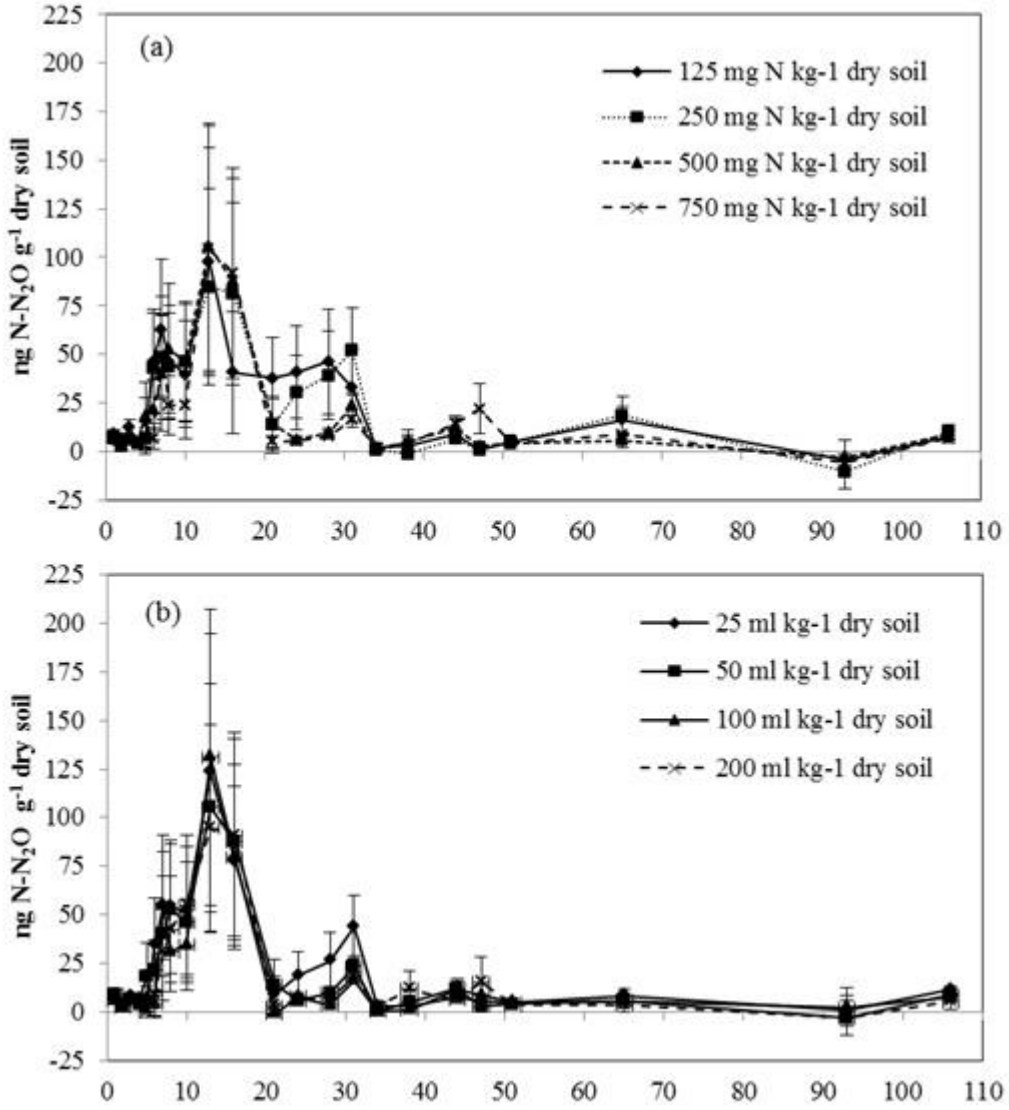

Figure 1. N_2O flux (ng N_2O-N g^{-1} dry soil) by day of sampling for 106 days after application (DAA) for (a) different amounts of urine-N (125, 250, 500 and 750 mg N kg^{-1} dry soil). (b) Different volumes of urine (25, 50, 100 and 200 ml kg^{-1} dry soil).

In the experiment in which the volume of the applied urine was varied, N_2O emissions occurred mainly in the period between 5 and 31 DAA and peaked at 13 DAA in the 100 ml urine kg^{-1} dry soil. The mean flux was highest (131.1 ng N_2O-N g^{-1} dry soil h^{-1}) in the

treatment with 100 ml of urine kg^{-1} dry soil and lowest (-3.3 ng N_2O-N g^{-1} dry soil h^{-1}) 93 DAA in the treatment with 200 ml of urine kg^{-1} dry soil. Negatives fluxes were found in all treatments (Figure 1b).

In the incubation in which different source of urine-N was applied, N_2O emissions persisted from 10 to 65 DAA and peaked firstly in the urea and nitrate treatment (13 DAA) and 28 DAA in the source ammonium. Negatives fluxes were observed in the begin and in the end of evaluations (Figure 2a). When varied the concentration of KCl in the urine presented a not clear tendency. The mean highest flux occurred 6 DAA in the treatment 0.0 g KCl L^{-1} and the lowest 13 DAA in the treatment 10.0 g KCl L^{-1} (Figure 2b).

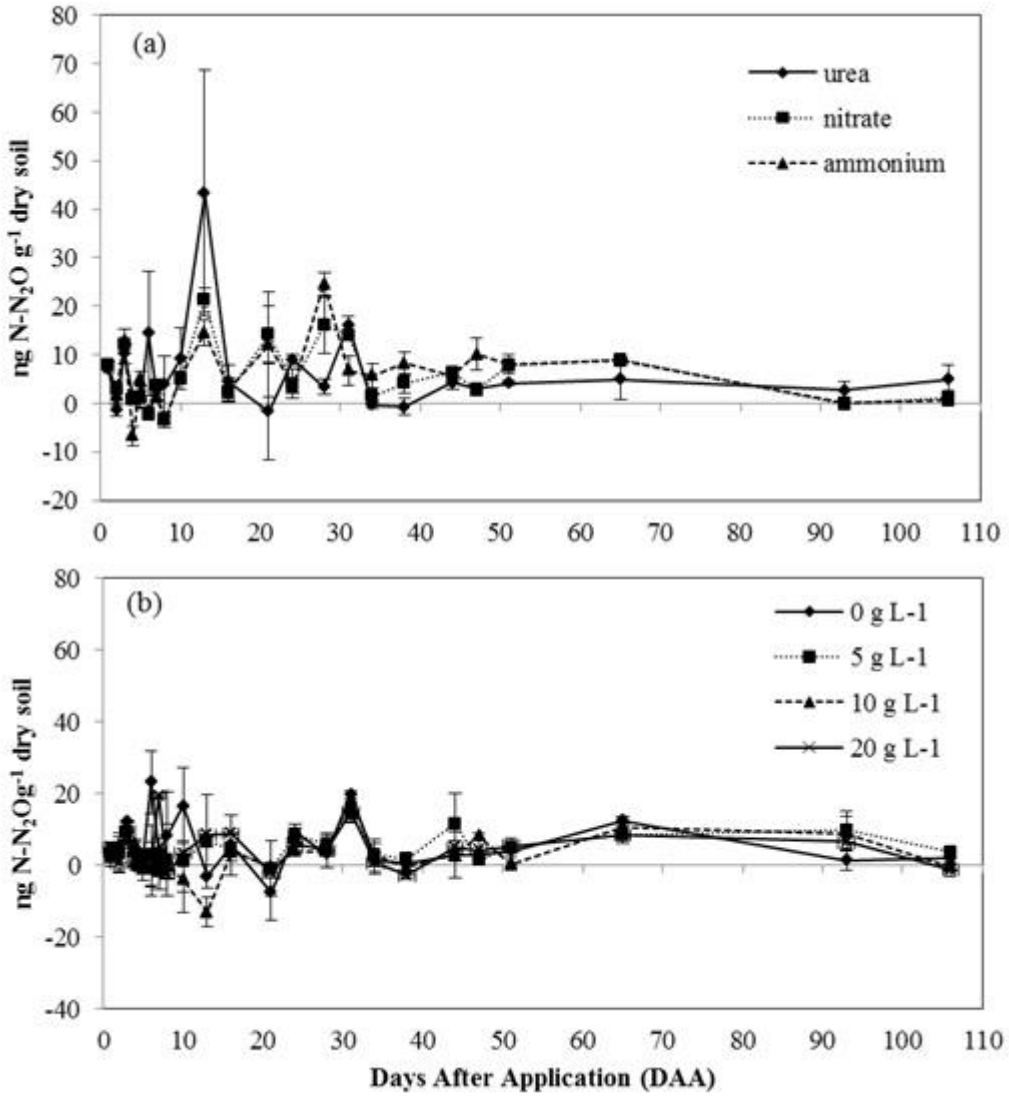

Figure 2. N_2O flux (ng N_2O-N g^{-1} dry soil) by day of sampling for 106 days after application (DAA) for (a) chemical N compound added in the urine (ammonium, urea, nitrate) and (b) concentration of KCl in the urine (0, 5, 10 and 20 g L^{-1})

When dung was applied to the soil a period of high N_2O production occurred independently if varied the concentration of urine-N or the urine volume. High N_2O emissions commenced 5 DAA and persisted till 38 DAA (Figure 3 a,b). Highest mean N_2O fluxes when dung was added were 5-20 times greater to the urine treatments. Negatives fluxes were mainly observed in the dry and compacted soil (Figure 3 a,b).

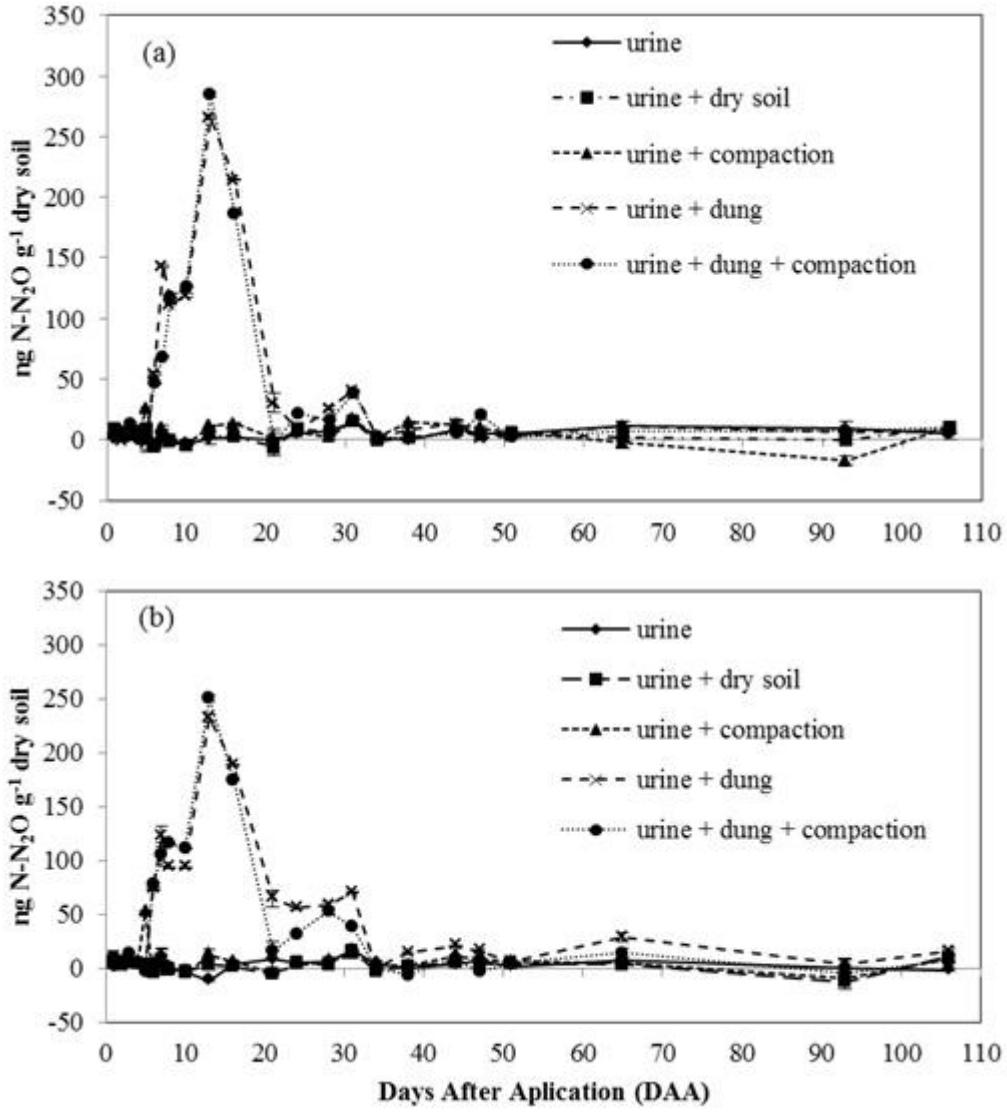

Figure 3. N_2O flux (ng N_2O-N g^{-1} dry soil) by day of sampling for 106 days after application (DAA) from (a) manipulated soil conditions (moist, dry soil, compacted, with dung addition and dung addition more compaction) under different urine volumes applied containing a constant quantity of urine-N (500 mg N kg^{-1} dry soil). (b) The same soil conditions cited below under different N-urine quantities added in equal volume of urine volume (100 ml kg^{-1} dry soil).

Mean soil concentrations of mineral N measured in the urine and dung treatments after 106 days of incubation were close to or below background levels in all of the studied treatment combinations, with values of 5.7, 5.1 and 3.0 mg N-NH_4 kg^{-1} dry soil and 1.7, 1.9 and 2.4 mg N-NO_3 kg^{-1} dry soil for the background, urine, and dung treatments, respectively.

4. Discussion

In this 106-day incubation study, cumulative N_2O emissions from moist soil (62.3% WFPS) were 73% higher than from 'dry' soil (40.4% WFPS) (Tables 1 and 2). N_2O production and consumption is regulated by interactions between the O_2 concentration and soil moisture content (Wu et al., 2013). Differences in soil N_2O emission between soil types are largely the result of differences in their water holding capacity, which directly influence their aeration status (Rochette et al., 2011). High N_2O emissions normally occur when neither WFPS nor temperature is limiting (Dobbie et al., 1999). Our data are compatible with those of Velthof and Oenema (1995), who showed that N_2O emissions were highest when soil WFPS was greater than 70% and lowest when WFPS was less than 50%. Likewise, Orwin et al. (2010) found that N_2O emissions were 100 times greater in a silt loam soil incubated at 70% than at 30% WFPS. In contrast, in a field study, van Groenigen et al. (2005 a) did not find an effect of volume of urine water on N_2O emissions from either applied urine-N or from soil organic N.

Soil compaction increases the emission of N_2O. Compared to uncompacted soil, Yamulki and Jarvis (2002) reported a 3.5-fold increase in N_2O emissions after compaction, while a 1.4-fold increase was reported by Hansen et al. (1993). In a field study, Van Groenigen et al. (2005 a) reported a highly significant effect of soil compaction on N_2O emissions from applied urine ($P = 0.002$) and a marginal effect when dung was added ($P = 0.054$), with compaction increasing average N_2O emissions by a factor of 2.2 (from 1.30% to 2.92% of applied N) and dung increasing emissions by a factor of 1.8 (from 1.60 to 2.82%). In our study, soil compaction had no clear effect increased N_2O emissions 1.25 times in the

experiments with varying urine-N content and urine volume varied decreased 25% (Tables 1 and 2). The lowest EF (0.69%) was found for the smallest urine volume tested (25 ml kg^{-1} dry soil), against an average of 5.0 % for the other urine volume treatments, suggesting a combined effect with %WFPS on N_2O emissions. We found that soil compaction with dung addition did not affect N_2O losses, since EFs averaged 6.50% and 7.37% with and without soil compaction, respectively (Table 2). Taken together, our data suggest that the soil compaction effect on N_2O emissions was masked and outweighed by the presence of dung.

Urine patches have been reported to be the main source of N_2O emissions from cattle excreta (van der Weerden et al., 2011; Lessa et al., 2014; Sordi et al., 2014). However, under our experimental conditions, N_2O emissions were higher with addition of dung plus urine than with urine only (Tables 1 and 2). Klemedtsson et al. (2005) reported the N_2O emissions rapidly increase with reductions in the soil C:N below a threshold ratio of 25. The dung applied had this C:N ratio which combined with moist conditions and N availability probably stimulated the microbial activity and created an ideal environment to higher N_2O emissions. The dung was mixed into the soil it could be accelerated the decomposition and favored N_2O production.

Using incubation experiments, van Groenigen et al. (2005 b) did not find an overall significant effect of either the amount of urine-N or of urine volume on N_2O emissions. Here, we show that the EF declined from 6.40 to 2.98% as the amount of urine-N was augmented (Table 1), while EF linear increased from 3.24 to 7.35% with increasing urine volume (Table 2). Despite these data, our results disagree of the conclusions of van Groenigen et al. (2005 b), significant effects of the amount of urine-N and urine volume were found on N_2O emissions. Although many factors are known to control N_2O emissions (Signor et al. 2013- review), Oenema et al. (1997) suggested that the relationship between N availability in the soil and

N_2O emission is the most useful indicator for evaluating total emissions from a certain area, which may explain the effect of different amount of urine-N applied. However, Mazzetto et al. (2014) argue that soil mineral N concentration is the key factor for regulating N_2O emission from soil, because when soil mineral N reaches levels as high as those found in urine patches, it no longer limits the amount of N_2O released. In a study in of N_2O emission from a subtropical pastureland, EF values of 0.33% to 0.19% were obtained when 0.5 L and 1.5 L of urine was applied to the soil, respectively (Sordi et al., 2014) contrasting with our finds that EFs linear increase. They attributed the result obtained for the higher volume to possible percolation of the urine deep into the soil, resulting in less N available for N_2O production in the topsoil. However, in incubation assays N leaching is limited, which may explain the different pattern reported here, which EF increase linearly and by van Groenigen et al. (2005 b), which found no effect of urine volume applied, compared to that shown in various field studies on the effect of urine volume on N_2O emissions.

N_2O emissions from soil are influenced by the chemical form of the N present in the soil. Indeed, nitrate can be denitrified immediately, whereas ammonia needs to be nitrified before denitrification takes place. This explains why the increase in N_2O losses induced by ammoniacal fertilizers occurs more slowly than that observed for nitric fertilizers, as has been consistently observed by other researchers (reviewed in Signor et al., 2013). In the present study, the N-containing compound in the urine did not influence N_2O emissions, however the losses induced by nitrate were 2.8 and 1.6 times greater, respectively, than those induced by ammonium and urea (Table 3). Dobbie and Smith (2003) measured N_2O emissions after application of ammonium nitrate or urea to an intensively managed grassland in Scotland and found that the emissions were, on average, 2.4 times higher from the former than from the latter. Dealune et al. (1998) reported that the $N\text{-}NH_4$ and $N\text{-}NO_3$ fertilizers they studied

increased the amount of N released as N_2O by 15% and 56%, respectively. Studying tropical soils in southern Brazil, Zanatta et al. (2010) found that nitrate fertilizers induced more N_2O emissions than did urea or ammonium fertilizers.

It has been suggested that KCl may inhibit N_2O release from soil through its inhibition of nitrification (Monaghan and Barrachough, 1992). In the present study, N_2O emissions had a curvilinear effect of KCl concentration on N_2O emissions. N_2O lost was higher in those concentration expected in the bovine urine (5-10 g L^{-1}) and the lowest was in the higher KCl concentration (Table 4). May be the higher KCl concentration inhibited the nitrification. The increase in the ion K^+ concentration in the soil negative affected the nitrification (Agrawal et al., (1985). Other possible explanation is that the ion Cl^{-1} can react with NH_4^+ retaining the N available in the soil to be realized. Van Groenigen et al. (2005 a) found a KCl effect comparable to ours, but because their data did not fit their expected result, they did not draw a conclusion.

According to the IPCC (2006), the default emission factor for cattle excreta voided in grasslands sites is 2%. We found an overall average EF of 4.14% when urine-N content was varied (Table 1) and a mean EF of 5.42% when urine volume (Table 2) was varied. Our use of artificial urine probably have also led to higher N_2O emissions than if we had used real urine (de Kelin et al. 2003; van Groenigen et l. 2005a; Thomas et al. 2008). In a subtropical soil, Sordi et al. (2014) found an EF for urine of 0.26% and for dung of 0.15%, and in a Cerrado pastureland, Lessa et al. (2014) estimated an average EF for excreta N of 0.7%. These studies corroborate the recommendation of disaggregation of EFs by excreta type (van der Weerden et al., 2011). In the present study, mean EF was 3.76% and 6.30% for urine and urine plus dung addition, respectively. We suggest that this effect of dung application is summed with that of the urine and cannot be compared to measurement of N_2O losses performed using dung

patches alone. Use of a single EF for Latin America is probably inappropriate because of large variations in soil water holding capacity, compaction, and inorganic N content and in weather conditions throughout the continent. Lastly, the strategies for mitigation of N_2O emissions suggested by van Groenigen et al. (2006) of avoiding dung on urine patches (found in so-called 'camping areas' in pastures) and avoiding grazing under wet conditions are supported by our results, as EFs were 1.25, 1,2 and 2 times greater in moist, compacted and dung added soils, respectively, than in uncompacted dry soil.

5. Conclusions

Soil conditions such as moisture content, degree of compaction, and dung application significantly affected N_2O emissions from a Ferralsol when different amounts of urine-N were applied (in equal urine volumes) and when different volumes of urine were applied (containing equal amounts of urine-N). Dung addition favored N_2O emissions. The fraction of applied N emitted as N_2O was higher with supplementary dung addition than with urine application alone, and it was also higher in moist soil compared to dry or compacted soil. Incubation with varying amounts of urine-N and varying urine volumes resulted in mean EFs of 4.14% and 5.42%, respectively. The chemical form of urine-N (i.e., urea, nitrate or ammonium) did not affect and the concentration of KCl added to the urine affected N_2O losses decreasing with KCl concentration increase.

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CHAPTER 4 - Methane emission from Ferralsol as influenced by soil physical conditions and source of N

O artigo a seguir está redigido conforme normas de publicação do periódico *Animal Production Science*, exceto o posicionamento das tabelas e figuras.

Methane emission from Ferralsol as influenced by soil physical conditions and source of N

Summary text for the Table of Contents

CH_4 emissions/oxidation was evaluated from manipulated soil and urine characteristics;

CH_4 emissions were influenced by soil moist and compaction;

Urine volumes and N-urine rates did not affect CH_4 emissions;

CH_4 oxidation was ephemeral affected by N source;

Abstract: Soil moisture and compaction, source of N and bovine urine can reduce (CH_4) oxidation from agriculture soils. However, the magnitude of the effect is unknown in tropical soil under different conditions, as well as the potential of different urine-N concentration, volume and sources of N in such an effect. The aim of this study was to investigate the effects of different soil conditions (moist, dry, compacted, moist plus dung, moist plus dung plus compacted), the N concentration in urine (2.5, 5.0, 10.0 and 15.0 g N/L), the volume of urine (25, 50, 100 and 200 ml/kg dry soil) and source of N (ammonium, nitrate and urea) on CH_4 emissions. A tropical Ferralsol soil from palisade-grass pasture was incubated during 106-days and the CH_4 concentration was determined by gas chromatography. The cumulative CH_4 varied according to the soil conditions when varied the urine-N significantly ($p<0.01$) and averaged 0.75, -0.50, 1.14, 6.23 and 8.17 mg C-CH_4/m^2, respectively, for the moist, dry, compacted, moist plus dung and moist plus dung plus compacted soil and not responded to the level of N ($p=0.60$) averaging 2.57 mg C-CH_4/m^2. When varied the volume of urine cumulative CH_4 averages were -0.52, -1.24, -0.88, 14.48 and 18.56 mg C-CH_4/m^2 respectively, for the moist, dry, compacted, moist plus dung and moist plus dung plus compacted soil were affect by soil treatments ($p<0.001$) but not by urine volumes ($p=0.30$).

The soil conditions influenced the CH_4 emissions ($p<0.001$), being higher with dung addition and the volume did not affect ($p=0.30$). The source of N did not influence the CH_4 emissions/oxidation ($p=0.1$) averaging 0.88, -1.26 and -1.19 mg C-CH_4/m^2 respectively, for urea, nitrate and ammonium. The CH_4 in a tropical Ferralsol is controlled by the soil conditions and dung addition.

Keywords: Dung, soil compaction, cattle urine, CH_4 from grassland.

1. Introduction

Methane (CH_4) with a lifetime of 7,5 year and a global warming potential over a 100 year timeframe of 29 times of CO_2 (IPCC, 2013) has augmented since pre-industrial times by approximately 254% to an atmospheric concentration of 1.83 ppm in 2014 (WMO, 2015). CH_4 was appointed is one of greenhouse gas (GHG) responsible for the catalytic destruction of ozone in the stratosphere (Rammanatan et al. 1980). At the global level CH_4 contribute approximately 17% of the anthropogenic radioactive forcing (WMO, 2015). In soils wetlands areas (24%) and irrigated rice fields (20%) are appointed is the main source of CH_4 (Whalen, 2005). Temperate and tropical oxic soils that are continuously emerge and exposed to atmospheric concentration of CH_4 are CH_4 sinks. They usually exhibit low levels of atmospheric CH_4 oxidation but, because of the large areas they cover, and about 10% of the atmospheric CH_4 are estimated to consume of these soils (Saggar et al., 2004).

Identifying the factors that control CH_4 emissions rates is difficult, and there are uncertainties in determining how many different environmental combinations have to be studied to characterize the source. Water content is an important factor controlling CH_4 emissions. In tropical areas there is a large temporal and spatial variation in the rainfall

occurrence. Mazzetto et al (2014) showed that high CH_4 emissions from soil may be related with high % of Water Filled Pores Space (WFPS), either showed that grassland can act as a sink of CH_4 during dry season. Zeiss (2006) revealed that the optimal moisture content for CH_4 oxidation varies according soil type and porosity and range from 10 to 30% of soil moisture (w/w). Soil compaction decreases the total pore volume, which decrease soil aeration (Oenema et al., 1997) and may be forming anaerobic zones that favor CH_4 production. In pastures compacted area can be formed by the transit of animals and tractors (Drewry et al. 2008). Large amounts of dung are voided in grassland area and it is possible that fermentation of carbon products continues after fecal material is deposited on the soil (Gonzalez-Avalos and Ruiz-Suarez, 2001). In view of such soil characteristics variation, it is fundamental to investigate emission rates, which can be extrapolated to regional or global scales and improving the accuracy known. We hypothesized that manipulated soil conditions and water soil content affect CH_4 oxidation.

The effect of N application to the soil has been presented controversial results on CH_4 oxidation as inhibition (Mosier et al. 1991; Baggs and Blum 2004, Fender et al. 2012), stimulation (Veldkamp et al., 2001; Xie et al. 2010), or have no effect (Alluvione et al., 2009; Zanatta et al., 2010; Acton and Baggs. 2011; Serrano-Silva et al. 2014), which require resolution as to the effect of N on CH_4 oxidation in soil (Bodelier and Laanbroek, 2004). The reported effects of chemical N-fertilizer on CH_4 emission are complex and sometimes contradictory (Le Mer and Roger, 2001). They depend on the nature of the fertilizer, the quantity applied and the method of application (Lindau, 1994). As mentioned above tropical oxic soil contributed to the global CH_4 sink. Understanding the effect of varying N application rates and N compounds has powerful implications for mitigation of CH_4 emissions. We hypothesized that chemical N-formulation and N-rates influence the CH_4 emissions.

Our knowledge about CH_4 exchange in tropical grassland, which significantly different from temperate grassland ecosystems in Asia, Europe, North America and New Zealand with respect to soil physical characteristics and N additions, is few explored. The objectives of this study were to investigated the effect on CH_4 emissions/oxidation: (1) Soil characteristics (moist, dry, compacted, with dung addition and with dung addition and compacted); (2) the amount of urine-N applied (in a constant volume of applied urine); 3) the volume of urine applied (containing a constant amount of urine-N) and 4) the N source in the applied urine under manipulated soil conditions and urine application to a Ferralsol in greenhouse conditions.

2. Material and Methods

2.1. Soil, urine manipulation and dung

The incubation was conducted in the greenhouse facility of the Forragicultura Sector of the São Paulo State University "Júlio de Mesquita Filho" campus in Jaboticabal, São Paulo, Brazil. Ferralsol was collect of grassland in Jaboticabal, Brazil (21º15'22''S and 48º18'08''W; altitude of 595m) from the 0-20 cm depth layer in June 2013. The texture of the soil was sandy clay (42% clay, 14% silt, 44% sand). The chemical analysis of the soil showed pH 4.9 (in water), 0.18% total N, 2.04% total C, and 11.0 mg NH_4-N and 4.7 mg NO_3-N/kg dry soil. We mixed and sieved (4 mm) the soil, and 500-g quantities of moist soil were placed in 1.5-L square jars.

Artificial urine was prepared according to Doak (1952) using urea, hippuric acid, creatine, allantoin, ureic acid and NH_4Cl in the proportion of total N 88.6%, 6.2%, 0.8%,

1.5%, 0.4% and 2.5%, respectively. 14.20 g/L $KHCO_3$ and 10.50 g/L KCl were added to all artificial urine. Urine was applied spreading the liquid superficially. Fresh cattle dung were collected in the paddock and mixed, 150 g dung/kg soil was mixed through the soil in the treatments with dung addition before superficial application of the urine. The dung presented 149.8 g/kg dry matter, 36.5 g/kg total N and 430 g/kg total C.

2.2. Experimental setup

Three different incubation experiments were conducted simultaneously:

a) Incubation 1: we evaluated the effect on soil CH_4 emissions of different amounts of urine-N (2.5, 5.0, 10 and 15 g/L; n = 5) applied in equal volumes (50 ml/kg dry soil) under different soil conditions (moist, dry, compacted, moist plus dung, and moist plus dung plus compaction; n = 4) in a two-factor without replication design. Two treatments were included as background: moist and dry soil with no addition or dung;

b) Incubation 2: we studied the effect of different volumes of urine (25, 50, 100 and 200 ml/kg dry soil; n = 5) containing equal amounts of urine-N (10 g N/L) on CH_4 emissions under the same soil conditions and background cited above;

c) Incubation 3: we analyzed the effect of N source on CH_4 oxidation the soil received 100 mL urine/kg dry soil containing different N sources (10 g N/L of Urea, Ammonium Sulfate, or Potassium Nitrate; n = 4) and the background with no N source addition in a completely randomized design;

In the treatment background we added water instead urine. The dry soil treatment received a total of 50 ml water/kg, volumetric moisture content of 22.1% (40.4% WFPS). The moist treatment, 100 ml water/kg soil was added resulting in a volumetric soil moisture

content of 34.1% (62.3% WFPS). The jars were uncovered except during CH_4 measurement, allowing exchange with the atmosphere. The treatments were incubated at approximately 25ºC and 80% relative humidity for 106 days. Evaporation losses for the uncompacted and compacted treatments averaged 0.66 and 1.1 g water kg/d, respectively. During the first seven days of incubation after urine and dung application, when necessary soil moisture was corrected by weighing the jars daily and spraying deionized water onto the soil surface as required. From seven to 60 days after application (DAA), soil moisture was corrected every two days and then subsequently twice a week until the end of the experiment at day 106.

Soil compaction was simulated by manually compressing the soil in the jars using a piece of wood until the initial bulk density of 1.2 g/cm was increased to approximately 2.0 g/cm. After compaction, volumetric soil moisture content increased from 34.1 to 56.8% (100% WFPS) in the compacted treatment, and from 48.5 to 80.8% (100% WFPS) in the compacted plus dung treatment. The treatments with dung received 150 g fresh dung/kg dry soil. The dung was mixed through the soil following urine application. The addition of dung increased the volumetric soil moisture content from 34.1 to 48.5% (88.6% WFPS) in the moist treatments without compaction.

2.3. CH_4 measurement

We used the technique of closed chamber to evaluate CH_4 fluxes. The chambers were a jar with 1.1 L (headspace). Sampling was carried out between 9:00 and 10:00 am. CH_4 flux was measured 25 times during the 106-day incubation period (daily during the first week; every two days during the second week, two times a week during the rest of the first month, weekly between 30 and 60 DAA, and twice in the remaining 30 days. We stopped the air

collects after the fluxes of treatments became similar to the backgrounds. We measured CH_4 flux by closing the jar lid for 0.75 h and determining the change in headspace concentration. Air samples were taken with 50-ml polypropylene syringes. Air temperatures inside and outside of the chamber were recorded using a digital thermometer. The air samples were transferred into 20-ml pre-evacuated vials (Shimadzu flasks). Ambient gas samples were also taken.

Samples were analyzed by gas chromatography (Shimadzu Green House Gas Analyzer GC-2014; Kyoto, Japan) under the following conditions for measurement of CH_4: injector 250ºC, column at 80ºC, flame gas was H_2 (30 ml/min), and flame ionization detector (FID) at 280ºC. Flux calculations were based on the assumption that there was a linear increase in CH_4 concentration with time in the closed chamber. The linearity of the flux during the incubation period was tested previously over a 1.5-h period at 10-min intervals. The CH_4 flux (µg C-$CH_4/m^2.h$) was calculated from the concentration change over the sampling period by using the following expression:

$$CH_4 \text{ flux} = P/RT \times V/A \times \Delta C/\Delta t \qquad (1)$$

where P is air pressure at the sampling site, R refers to the gas constant, T is temperature inside the chamber, V is the volume of the chamber, A is area of the sampling soil surface and $\Delta C/\Delta t$ is the linear slope of concentration change during sampling period. So the positive value means CH_4 emissions and negative CH_4 oxidation.

Cumulative emissions were calculated by plotting daily fluxes through time, interpolating linearly between them, and integrating the data. To analyses the effect of treatment on CH_4 production/oxidation the cumulative emissions were subtracted of corresponding background emission.

2.4. Statistical tests

The effects of manipulation of urine and soil conditions on CH_4 emission/oxidation were tested as follows: 1) to determine the effect of amount of urine-N applied in a constant volume under different soil conditions, a two-way ANOVA without repetitions was done with amount of urine-N and soil treatment as factors; 2) to determine the effect of volume of urine applied with a constant amount of urine-N, a two-way ANOVA without repetitions was done with the urine volume and soil treatment as factors; 3) the effect of the form of chemical-N applied was tested using a one-way ANOVA. When ANOVA was significant we used Tukey test to distinguish the means of manipulated soil and chemical-N. All statistical analysis was done using the R statistical program (version 3.1.2; R Core Team, 2014).

3. Results

The highest fluxes occurred two day after treatments application. The CH_4 fluxes ranged from an influx of -2.10 µg C-CH_4/m^2 in the treatment of nitrate addition as N-source to efflux of 183.4 µg C-CH_4/m^2.h in the treatment urine plus dung and more compaction (Figure 1). In other hand the highest fluxes delayed when applied ammonium sulfate and occurred 7 days after application (DAA) (Figure 2 c). Independently of soil treatment (Figure 1) and urine manipulation (Figure 2) the CH_4 emissions occurred in the first week and after 7 DAA started CH_4 oxidation. Whereas after application of Nitrate did not stimulated CH_4 emissions (Figure 2 c).

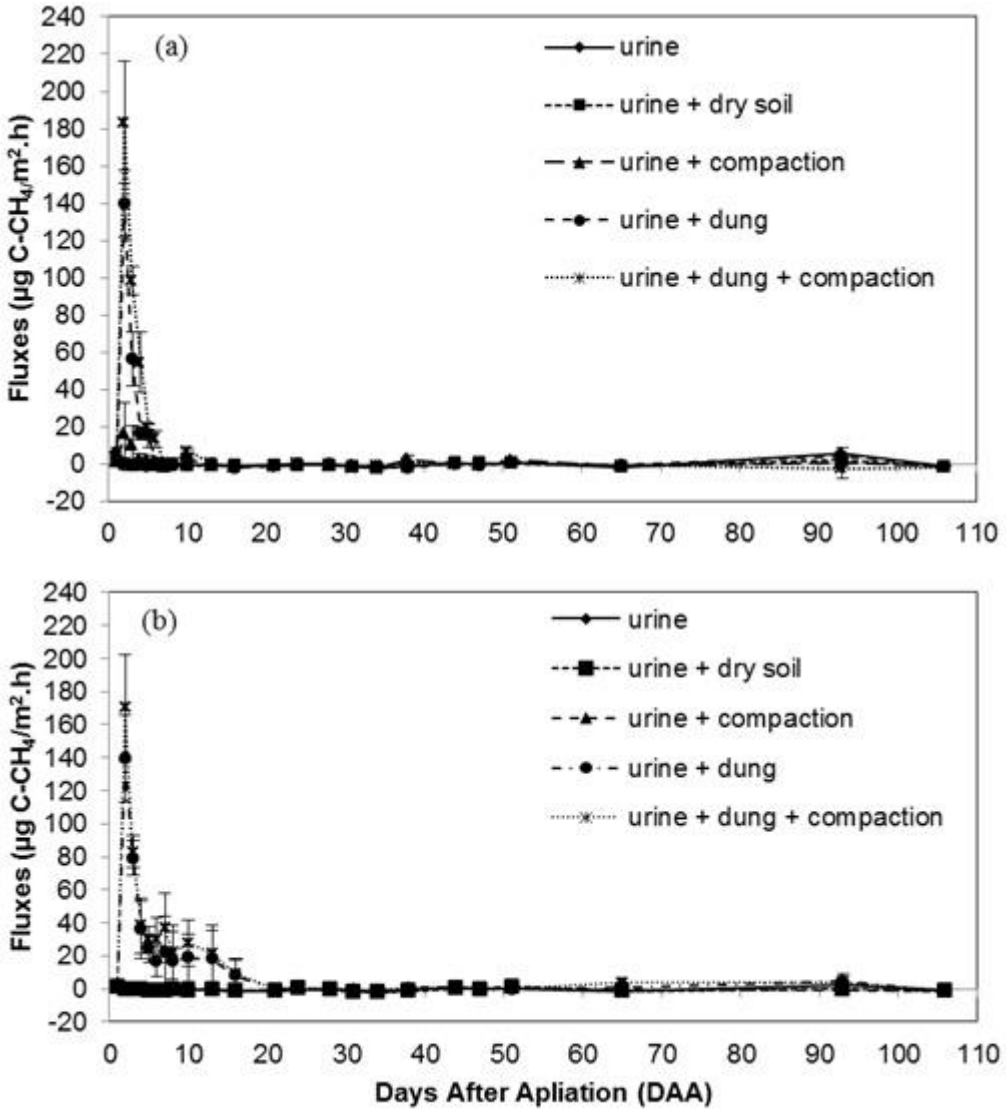

Figure 1. CH_4 fluxes (µg C-CH_4/m^2.h) during 106 days of incubation from manipulated soil characteristics (urine – moist, urine + dry soil, urine + compaction, urine + dung and urine + dung + compaction). (a) Emissions from soil treatment are the means of different urine-N concentration (2.5, 5.0, 10.0 and 15.0 g/L; n=4), applied in equal volume of urine (100 mL/kg dry soil). (b) Emissions from soil treatment are the means of different urine volumes (25, 50, 100 and 200 mL; n=4), with equal amount of N (10.0 g N/L). Bars are standard error of the means.

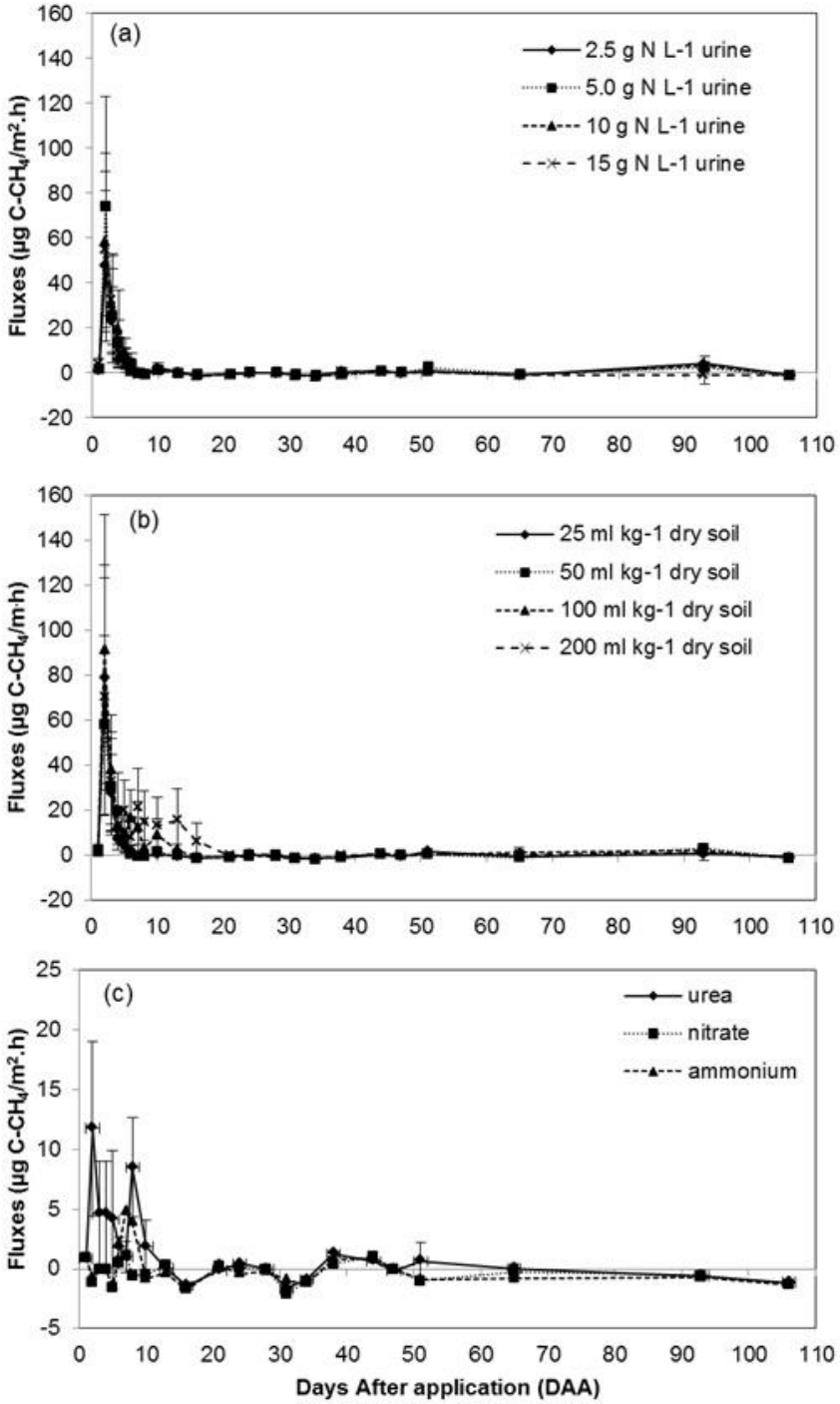

Figure 2. CH_4 fluxes (µg C-CH_4/m^2.h) during 106 days of incubation from manipulated urine composition (a) urine N concentration levels (2.5, 5.0, 10.0 and 15.0 g/L; n=4), applied in equal volume of urine (100 mL/kg dry soil); (b) different urine volumes applied to the soil (25, 50, 100 and 200 mL/kg dry soil), with equal amount of N (10.0 g N/L). The emissions of each treatment are means of soil treatments (urine – moist, urine + dry soil, urine + compaction, urine + dung and urine + dung + compaction; n=5). (c) CH_4 fluxes from different N sources (urea, potassium nitrate and ammonium sulfate; n=4). Bars are standard error of the means.

Manipulated soil characteristics influenced ($p<0.01$) CH_4 emissions when varied N concentration (with equal volume of urine) and when varied urine volume (with equal amount of N) $p<0.001$. In the dry soil treatment occurred the highest CH_4 oxidation accounted -0.5 and -1.24 mg/m^2, respectively, when varied urine-N concentration and urine volume (Figure 3). When the soil received the equal volume of urine and varied the N concentration the soil compaction CH_4 emissions increases 52% compared to the moist soil and when the volume of urine varied (with equal amount of N) the soil compaction increased CH_4 oxidation by 69.6% compared to the moist soil. Dung stimulated CH_4 emissions (Figure 3). The emissions from treatments with dung addition were 9-36 times greater to the moist soil. CH_4 accumulated emissions were 6.23 and 14.48 mg C-CH_4/m^2 when manipulated urine N and urine volume respectively. Soil compaction increased the CH_4 losses by approximately 30% compared that soil which dung application only.

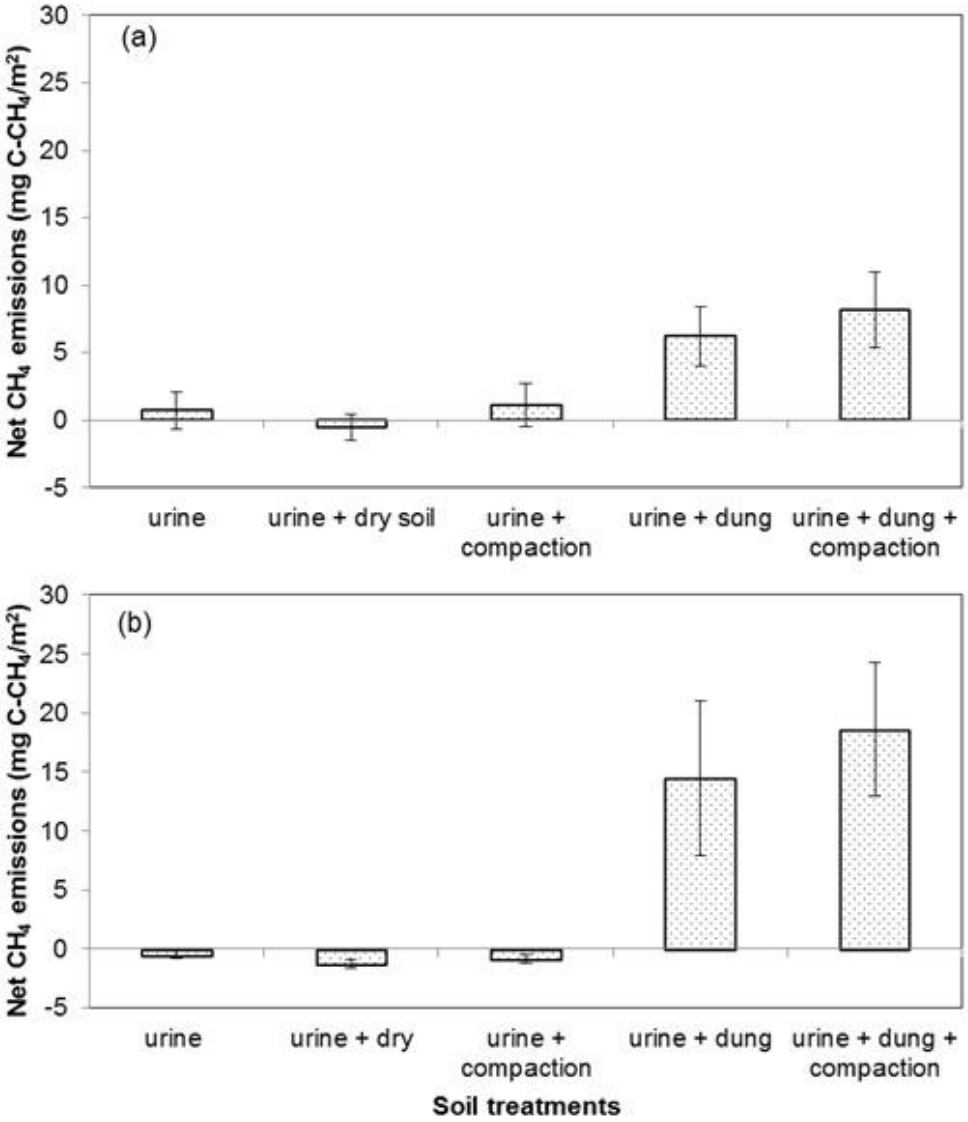

Figure 3. Net CH_4 emissions (mg CH_4/m^2) – 106 days incubation - from manipulated soil characteristics (urine – moist, urine + dry soil, urine + compaction, urine + dung and urine + dung + compaction). (a) Emissions from soil treatment are the means of different urine-N concentration (2.5, 5.0, 10.0 and 15.0 g/L; n=4), applied in equal volume of urine (100 mL/kg dry soil). (b) Emissions from soil treatment are the means of different urine volumes (25, 50, 100 and 200 mL; n=4), with equal amount of N (10.0 g N/L). Bars are standard error of the means.

Different N-urine concentration (with equal volume of urine) clearly did not affected ($p=0.60$) CH_4 emissions (Figure 4 a) and overall averaged 2.57 mg C-CH_4/m^2. The addition of different urine volumes (with equal amount of N) did not affected CH_4 emissions ($p=0.30$) in the diverse soil characteristics. The accumulated emissions varied from 2.78 to 8.43 mg C-CH_4/m^2 (Figure 4 b).

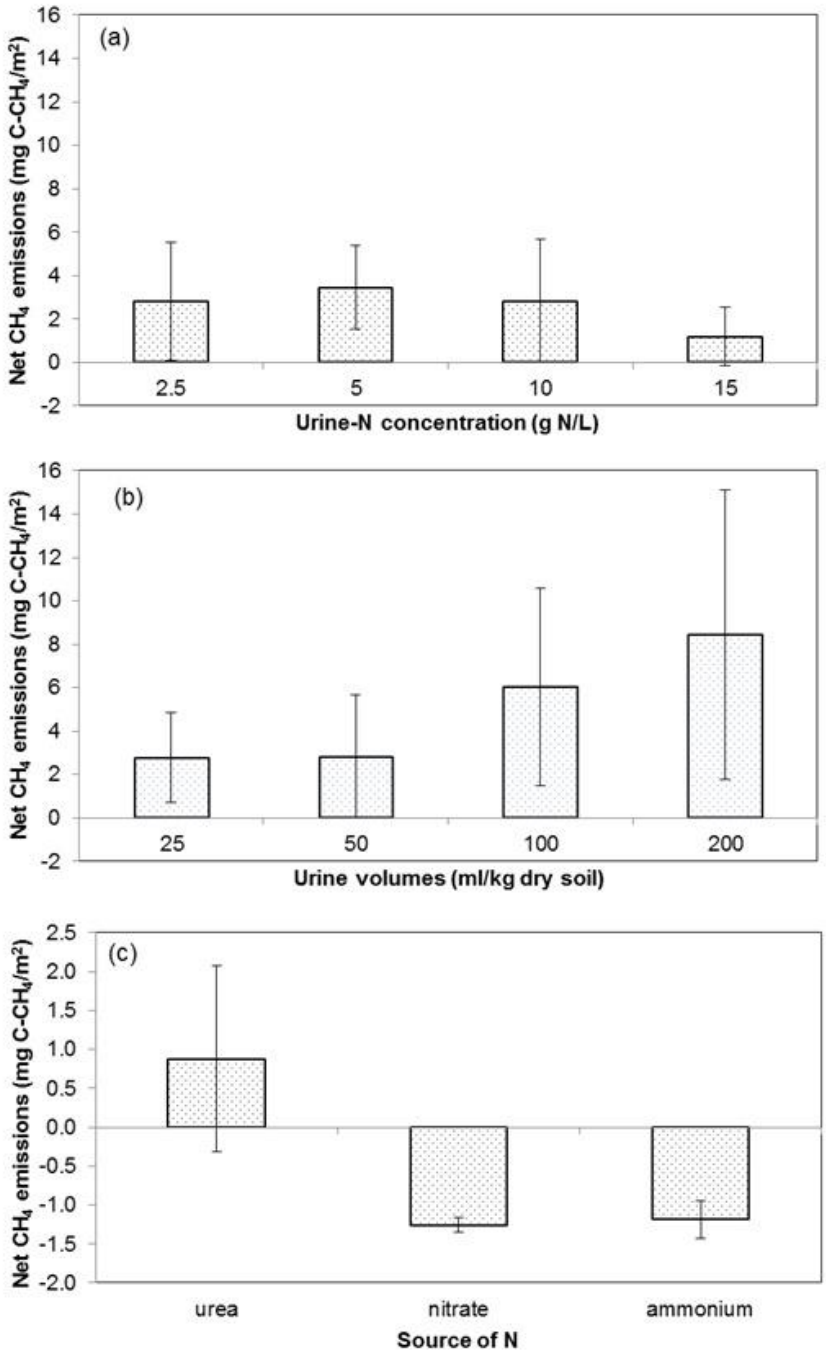

Figure 4. Net CH_4 emissions (mg C-CH_4/m^2) -106 days of incubation - from manipulated urine composition (a) urine N concentration levels (2.5, 5.0, 10.0 and 15.0 g/L; n=4), applied in equal volume of urine (100 mL/kg dry soil); (b) different urine volumes applied to the soil (25, 50, 100 and 200 mL/kg dry soil), with equal amount of N (10.0 g N/L). The emissions of each treatment are means of soil treatments (urine – moist, urine + dry soil, urine + compaction, urine + dung and urine + dung + compaction; n=5). (c) CH_4 net emissions (mg C-CH_4/m^2) from different N sources (urea, potassium nitrate and ammonium sulfate; n=4). The bars are standard error of the means.

The source of N slightly affected the CH_4 emissions/oxidation (p=0.1). Emissions from urea were 2.4 times greater than potassium nitrate and ammonium sulfate. Urea CH_4 emission accounted 0.88 (± 1.20) mg C-CH_4/m^2 and CH_4 oxidation totaled -1.26 and -1.19 mg C-CH_4/m^2, respectively, for nitrate and ammonium N source.

4. Discussion

CH_4 emissions have been reported that occurred manly in the first days (Jarvis et al, 1995; Mazzetto et al., 2014; Cardoso et al., 2016), when methanogenic remains in the feces and the dung maintain an ideal microhabitat for CH_4 production. The feces drying and possibly formation of crust in the dung patch were appointed to be responsible by the effect of dung application in the CH_4 emissions (Yamulki et al., 1999). In our study the effect of dung addition ceased 7 DAA and the dung remain wet in contrast to what were observed by Sherlock et al., (2003) the dung moisture sustained CH_4 emission until they were fully dried. The application of different treatments stimulated CH_4 emissions and was observed in the first days (Figure 1 and 2). Perhaps the extra N was used by the methanogenic microorganism process the available C and the urine volume increased the %WFPS creating ideal microhabitat for CH_4 emissions.

Mazzetto et al., (2014) found high CH_4 emissions with high soil moisture and high temperature. We found that CH_4 oxidation in the dry soil compared to the moist soil increased (Fig 1 and Fig 2). The manipulate soil moisture conditions affect CH_4 oxidations. In the dry soil the size of the oxidized zones was higher which may favor the methanotrophia. However, when we varied the volume of urine applied to the soil we found any effect. Soil pH

decreased after urine application. The optimal pH for methanogens activity is around the neutrality or slightly alkaline conditions (Garcia et al., 2000) and their activity are very sensible to the pH variation (Wang et al., 1993) that may be explain the difference between results when water was added to the soil instead urine in this study.

Primary control of atmospheric CH_4 uptake is largely through soil physical factors influencing the diffusion of CH_4 into the soil (Bodelier, 2011). Herein when soil was compacted without dung application the CH_4 emissions/oxidation did not differ and when dung was applied the CH_4 emissions augmented approximately 30% (Figure 3). This implies that compacted area like close to the feeder, drinkers and resting places in a pasture could be CH_4 emissions increased due animal excretion. CH_4 is produced by Archaea under low-redox anaerobic conditions (Conrad, 2007) soil compaction contributed to the formation of anoxic areas, which in the presence of remaining methanogenic microorganisms and high C available from dung favor CH_4 emissions.

N application rate can affect soil CH_4 oxidation (Liu and Greaver, 2009). The urine N rates did not influenced CH_4 emission/oxidation in our investigation. Our finding disagrees for both studies that CH_4 oxidation was inhibited (Gulledge et al., 2004; Acton and Baggs, 2011; Dobbie and Smith 1996) and stimulate (Veldkamp et al. 2001). Perhaps in our study the rate of N did not stimulate differences in the growth and activity of methane oxidizers. Chan and Parkin (001) and Alluvione et al. (2009) suggested that after a long period of N fertilization, microbial populations can adapt to N addition and no inhibiting effect on CH_4 oxidation is observed. The effect of different form of nitrogen probably related to the role of NH_3 competing for methane monoxygenase enzymes (Holmes et al. 1995), increasing ammonia oxidation (Hütsch, 1998) or osmotic effects (Kravchenko et al. 2002).

We observed an ephemeral effect (p=0.1) of the N-compound on the CH_4 emissions. CH_4 was produced only when urea was applied may be the CH_4 emissions were favored by increasing NH_3 oxidation as above cited. The application of potassium nitrate and ammonium sulfate may be stimulated CH_4 oxidation. Zannatta et al. (2010) found inhibitory effect of N sources in the CH_4 uptake. They found relationship between CH_4 fluxes and NH_4^+ contents and attributed the inhibitory effect to the effect of NH_4^+ on the methanotrophs process as showed before by Hüstch (2001) that the methanotrophic bacteria activity changes to the NH_4^+ oxidation in disadvantage of CH_4 oxidation.

We calculate the emission factor of feces emission considering the annual dung excretion used by Mazzetto et al. (2014). The emission factor for our studied situation accounted range 0.15 (±0.05) to 0.35 (±0.15) kg/year.head in the moist soil plus dung and 0.2 (±0.68) to 0.45 (±0.14) kg/year.head in the treatment moist soil plus dung and more compaction. The values are lower than 0.95 kg/year.head reported by Cardoso et al. (2016) and preconized IPCC default emission factor of 1 kg/year.head (2006) and higher than 0.1 kg/year.head calculated by Mazzetto et al. (2014).

5. Conclusions

Soil physical characteristics influenced CH_4 emissions. Dry soil reduced CH_4 emissions and increase oxidation. Compacted soil increase CH_4 realizes and the addition of dung to the soil largely augmented CH_4 emissions. Different urine volumes and urine N rates did not affect the CH_4 emissions/oxidation. The effect of different N source application to the soil on CH_4 emissions was ephemeral.

CH_4 emissions/oxidation appears to be controlled by soil characteristics in the tropical soil studied and dung addition commands the magnitude of CH_4 emissions.

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CHAPTER 5 - Impact of pasture height and season on greenhouse gas emissions from a tropical grassland

O artigo a seguir está redigido conforme normas de publicação do periódico *Plant and Soil*, exceto o posicionamento das tabelas e figuras.

Impact of pasture height and season on greenhouse gas emissions from a tropical grassland

Abstract

Aims: This study assessed greenhouse gas (GHG) emissions from a tropical pasture in Brazil to determine (i) how grazing height affects the magnitude of GHG emissions; (ii) how season influences GHG production and consumption; and (iii) the key driving variables associated with GHG emissions.

Methods: N_2O, CH_4, and CO_2 fluxes were measured over two years under field conditions in a Marandu grass pasture managed with 3 pasture heights (15, 25, and 35 cm) in continuous foraging system, using static closed chamber and chromatography quantification.

Results: GHG emissions were higher in the summer and the lower in the winter. N_2O, CH_4, and CO_2 fluxes varied according to the season and were correlated with pasture height, temperature, precipitation, percent water filled pore space, and soil inorganic N. The explanatory variables differed according to gas and season. Pasture height had a negative linear effect on annual cumulative N_2O emissions and a positive linear effect on annual cumulative CO_2 emissions.

Conclusion: Pasture height, season, and year affected N_2O, CH_4, and CO_2 emissions. Tropical grasslands can be a major sink of N_2O and CH_4. GHG emissions were explained by different key driving variables according to season.

1. Introduction

Livestock production accounts for 70% of all agricultural land and 30% of the land surface of the planet. The sector is responsible for 18% of global greenhouse gas emissions (GHG) measured in CO_2 equivalents. It accounts for 9% of anthropogenic CO_2, 37% of methane (CH_4) and 65% of nitrous oxide (N_2O) emissions as well as 64% of ammonia (NH_3) release, which contributes indirectly to N_2O emissions and acidification of ecosystems (Steinfeld et al., 2006). Livestock is an important sector in tropical countries.

Grassland management strategies affect animal intake, forage residue amount and quality, and pasture structure (Shariff et al., 1994; Apolinário et al., 2014). Nutrients from grazing areas return through the deposition of dung and urine (Haynes and Williams, 1993). Different defoliation frequencies and intensities, which influence the proportions of leaf, stem, and dead material, affect C and N cycling due to their effects on the biochemical composition of residues (Liu et al., 2011), N concentrations in plant tissues (Boddey et al., 2004), and soil microbial population and diversity (Zhou et al., 2010). We hypothesized that grazing height can affect CO_2, CH_4, and N_2O emission from soil.

The edaphoclimatic characteristics of the northwestern region of the Brazilian state of São Paulo, which is the region of our research interest, are similar to those of the Cerrado biome (Miranda and Fonseca, 2011). These characteristics include annual rainfall ranging between 1200 and 1600 mm and occurring mostly during the warm summer season (October to April), thereby contrasting with temperate and sub-tropical regions (Lopes, 1996). It is known that high levels of precipitation and temperature result in the rapid formation of anoxic conditions in the soil, although rapid water infiltration and high evapotranspiration rates suggest that

these conditions may be temporary (Skiba and Ball, 2002; Lessa et al., 2014). Anoxic microsites in the soil can favor N_2O production (Smith et al., 2003).

The winters in this Brazilian region are severely dry, frequently having more than 90 days without rain, and have a temperature range of from 15 to 25ºC, conditions that do not favor CH_4 and N_2O production (Lessa et al., 2014). The dry winter season also has a short photoperiod and low soil moisture. The low rainfall compromises grass growth, thereby leading to seasonality of grass production (Costa et al., 2006). The climatic characteristics described above led us to hypothesize that season affects GHG production and consumption in a grassland soil.

The importance of GHG emissions resulting from livestock production in tropical regions is increasing. Understanding the effects of grassland management and season on GHG production and consumption may help develop strategies to mitigate GHG. Here we assessed GHG emissions from a tropical pasture in Brazil in order to determine (i) how grazing height affects the magnitude of GHG emissions; (ii) how season influences GHG production and consumption; and (iii) the key driving variables associated with GHG emissions.

2. Material and Methods

Site description

The experiment was conducted at the Forages and Grasslands division of São Paulo State University “Julio de Mesquita Filho” (UNESP), in Jaboticabal, SP, Brazil (21º15’22’’S, 48º18’58’’W), at an altitude of 595 m. The local climate is tropical, with a rainy season

(October to March), during which more than 80% of annual precipitation normally occurs, and a dry season (April to September). Mean annual rainfall is 1424 mm and mean air temperature is 22.3ºC. The soil is a Rhodic Ferralsol (IUSS, 2006) derived from basalt. The pastures used in the study site were seeded in 2001 with *Brachiaria brizantha* (Hockst ex A. Rich) Stapf cv. Marandu.

Experimental design and grassland management

The experiment consisted of three grazing height treatments (15, 25, and 35 cm) with six replications (paddocks) in a completely randomized design. Paddocks were managed under continuous stocking, with paddock areas of 0.7, 1.0, and 1.3 ha for the 15, 25, and 35 cm grazing heights, respectively. The variation in paddock size was implemented in order to maintain the same number of animals per paddock. Six Nellore yearling bulls were utilized per paddock. Additional animals were used to maintain pre-determined grazing heights, using the put-and-take methodology (Mott and Lucas, 1952).

The experiment was conducted over 2 years, from 11/21/2012 to 11/28/2014. Maintenance fertilizer was applied to all paddocks on December 10, 2012, at a rate of 180 kg ha^{-1} of a mixture containing 4% N, 14% P_2O_5, and 8% K_2O. N maintenance fertilizer was split among three applications. During the first year of the experiment, a total of 160 kg N ha^{-1} as urea was applied according to the precipitation schedule on December 27, 2012, January 22, 2013, and February 26, 2013. During the second year, a total of 180 kg N ha^{-1} as urea was applied on November 22, 2013, January 8, 2014, and February 28, 2014.

Greenhouse gas flux measurements

We used the closed static chamber technique (Mosier, 1989) to collect air samples. Polyurethane vented chambers 38 cm in height were covered with thermal insulation mantles. Chambers were deployed on round metal bases at the beginning of each 9:00–10:00 am sampling event, as suggested by Alves et al. (2012). The chambers were equipped with a rubber belt to seal the chamber base and an output valve for sample removal. The linearity of gas accumulation in the chamber was successfully tested in a preliminary experiment with intensive sampling every 10 min for 1 h. Air samples were taken with 50-ml polypropylene syringes. Air temperature outside and inside of the chambers was recorded using a digital thermometer. The air sample was transferred to into 20-ml pre-evacuated vials (Shimadzu flasks). Samples were collected 3 times a week during the rainy season (from January 10 to February 23, 2013, and from January 8 to February 26, 2014), and every 14 days during the rest of experimental period. There was a total of 72 sampling events.

Samples were analyzed by gas chromatography (Shimadzu Greenhouse 2014) under the following conditions: (1) N_2O measurement: injector at 250ºC, column at 80ºC, carrier gas was N_2 (30 ml min^{-1}), electron capture detector at 325ºC; (2) CH_4 measurement: flame gas was H_2 (30 ml min^{-1}), flame ingestion detector at 280ºC. (3) CO_2 measurement: thermal conductivity detector at 250ºC.

Fluxes of N_2O (µg m^{-2} h^{-1}), CH_4 (µg m^{-2} h^{-1}), and CO_2 (mg m^{-2} h^{-1}) were calculated taking into account the linear increase of gas concentration during the incubation period, air temperature and pressure, chamber volume, and area of the metal bases (Cardoso et al., 2016). Cumulative emissions (g m^{-2}) in each season were calculated by integrating the hourly fluxes over time.

Soil and meteorological parameters

Soil samples of the 0–10 cm layer were collected at each air sampling event to quantify inorganic N, gravimetric water content (by drying soil at 105ºC), and percent water filled pore space (WFPS). Soil bulk density in the 0–10 cm laver was measured using a cylinder 50 mm in diameter and 50 mm in height. WFPS was calculated from the gravimetric water content and bulk density using a particle density of 2.65 Mg m^{-3}.

For mineral N analysis, extraction with 2M KCl was performed on field moist samples with correction for water content. Ammonium-N was determined using the Berthelot reaction with spectrometry at 647 nm (Kemper and Zweers, 1986). Nitrate-N quantification was carried out by ultraviolet absorption spectrometry at 220 nm (Miyazawa et al., 1985; Olsen et al., 2008). Daily maximum, mean, and minimum temperatures and daily rainfall precipitation were obtained at a meteorological station located 1.5 km from the experimental site.

Statistical analysis

N_2O, CH_4, and CO_2 fluxes were reported as means and standard error of the mean. Integrated data for each season were submitted to ANOVA after testing for normality and equal variance using R version 3.1.2 (2014), and, when significance was found, orthogonal polynomial contrast analysis was done at 5% probability.

Pearson correlation analysis was run to test for relationships between transformed GHG fluxes and pasture height, temperature, precipitation, % WFPS, NO_3-N, and NH_4-N using data from each sampling event within season.

3. Results

Environmental conditions

A total of 1468 mm rain fell at the study site in the first year of sampling, of which 438, 731, 229, and 69 fell during the spring, summer, autumn, and winter, respectively. In the second year, 966.5 mm rain fell, of which 452, 359, 88, and 57 mm fell during the spring, summer, autumn, and winter, respectively. The amount of rain that fell during the rainy season was 65.8% of the 30-year period average from 1971 to 2010 (FCAV, 2014). Historically the region studied is characterized by warm rainy summers and mild dry winters; however, during the second year of this study, the summer and autumn were markedly dry (Figure 1a).

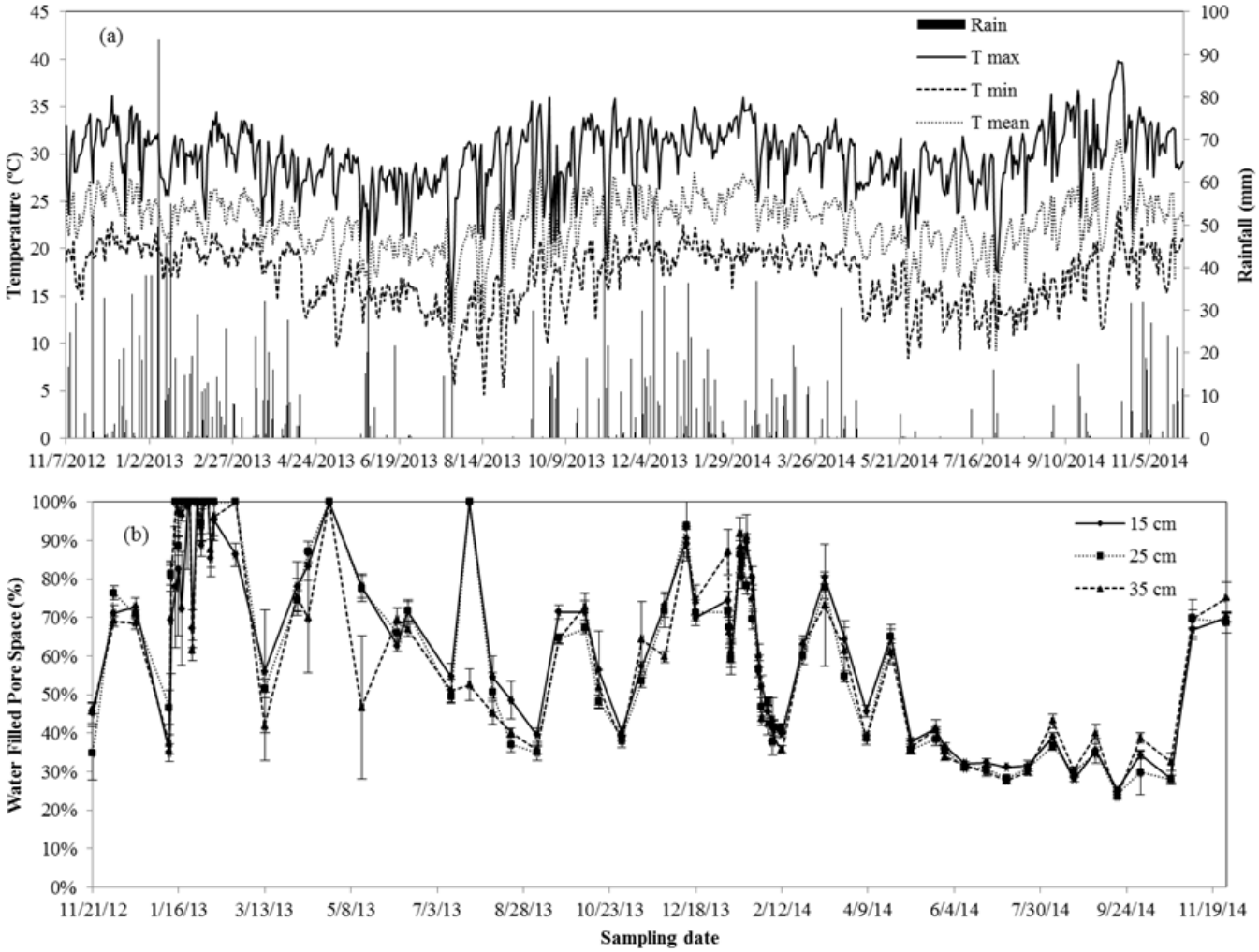

Figure 1. (a) Daily temperature (maximum, mean, and minimum; ºC) and precipitation (mm) and (b) % WFPS for three pasture heights (15, 25, and 35 cm) during two years of evaluation (November 21, 2012 to November 26, 2014).

All data analyzed in this study were obtained on the sampling days while the chambers were closed between 9:00 and 10:00 am. Mean air temperature was 25.3ºC. The lowest temperature recorded was 13.7ºC in July 2013, whereas the highest was 34.7ºC in September 2014. During chambers closed time temperature inside of the chamber changes ranged from -5.1 to 6.3ºC. Usually the temperature increased during the 1-h sampling period. However, the temperature tended to fall when the skies changed from sunny to cloudy during the sampling period.

WFPS of the surface soil (0-10 cm) varied seasonally in response to precipitation and grass growth (Figure 1b). Soil WFPS tended to be greater in the summer. In both years, soil WFPS increased following summer rainfall; however, saturation of soil pores was reached only in January 2013. Isolated rainfall, which occurred in June and August 2013, also increased soil WFPS (Figure 1b). A long period of low soil WFPS (around 30%) occurred from April to September of 2014, probably because of low rainfall.

N_2O flux

Nitrous oxide emissions were greatest following rainfall events and application of urea fertilizer (Figures 1a and 2a). N_2O emissions were highest in the summers (Figure 2a), while the other seasons had lower fluxes associated with frequent instances of N_2O consumption. The maximum rate of N_2O emissions was recorded in the second week of December 2013, when mean fluxes were 469, 394, and 279 μg N_2O-N m^{-2} h^{-1} for pastures heights of 15, 25, and 35 cm, respectively.

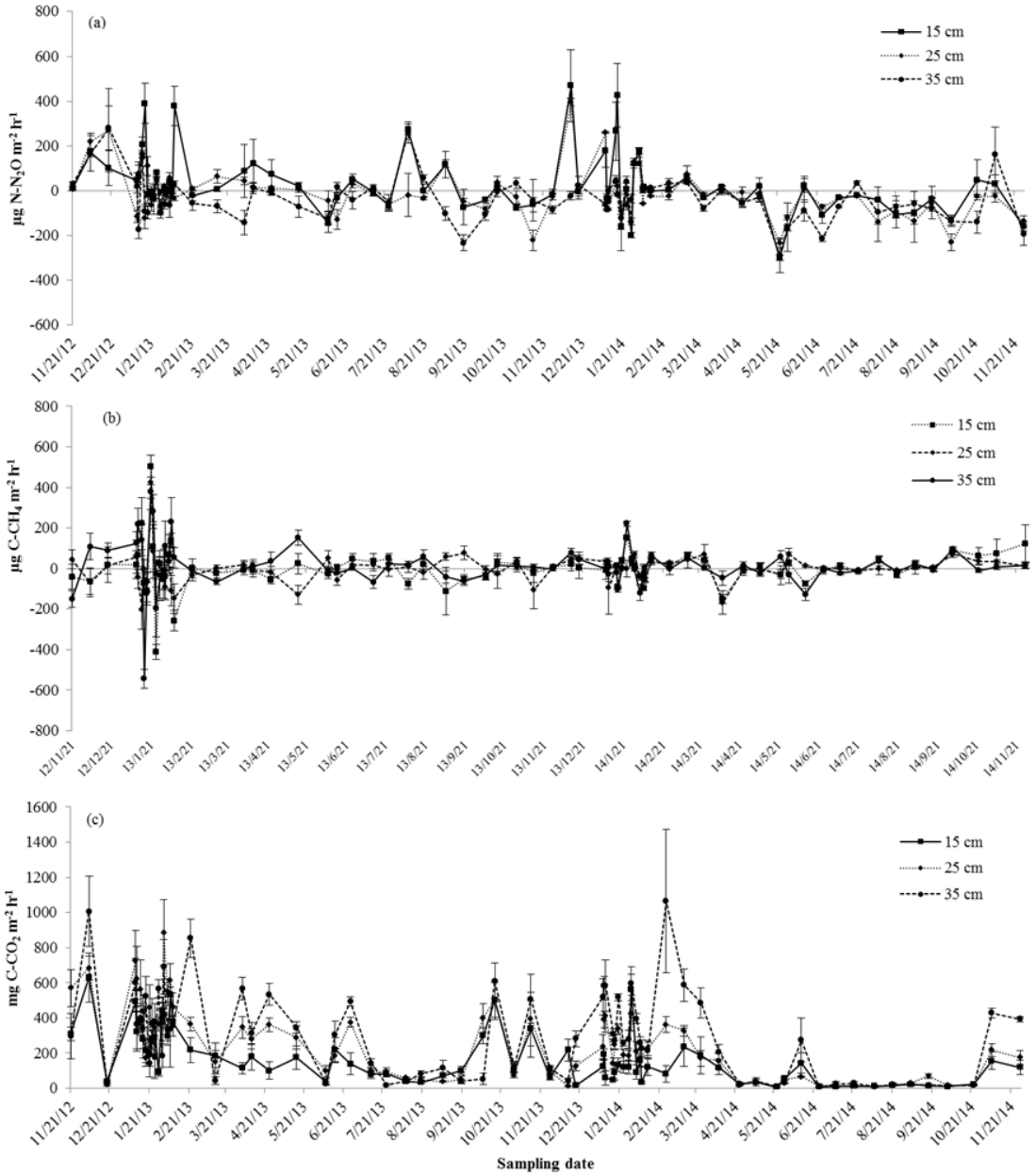

Figure 2. (a) N_2O flux (µg N_2O-N m^{-2} h^{-1}) and (b) fluxes of CH_4 (µg CH_4-C m^{-2} h^{-1}) and CO_2 (mg CO_2-C m^{-2} h^{-1}) for three pasture heights (15, 25, and 35 cm) during two years of evaluation (November 21, 2012 to November 26, 2014).

Negative fluxes were observed in approximately 60% of all N_2O sampling events independently of pasture height and especially in the spring and autumn of the second year (Figure 2a), when lower values of WFPS were also recorded (Figure 1b). The highest rate of

N_2O consumption was measured on May 23, 2014 (autumn season), when mean fluxes were -299, -235, and -287 µg N_2O-N m^{-2} h^{-1} for pastures heights of 15, 25, and 35 cm, respectively.

Pasture height was significantly correlated with N_2O flux during the summer. In the spring, N_2O flux was strongly correlated with inorganic-N and temperature, and mildly correlated with % WFPS. In the autumn, N_2O flux was strongly correlated with % WFPS and temperature, and mildly with NH_4^+ (Table 1).

Table 1. Pearson correlation coefficients (r) for assessing the relation between fluxes of N_2O, CH_4, and CO_2 from Marandu grass pastureland and daily explanatory variables.

	Pasture height	Ti	Tf	Prec	%WFPS	NH_4^+	NO_3^-	CO_2
N_2O								
Spring		0.64**	0.50*		0.41.	0.66**	0.50*	
Summer	-024*							
Autumn			0.35*		0.42**	0.27.		0.27.
CH_4								
Summer		-0.17.		0.22.	0.21.	0.35**		
Autumn			0.39**	0.28.				
Winter			-0.26.					
CO_2								
Autumn					0.52**	0.36*		
Winter				0.38*	0.41**			

Explanatory variables: Pasture height; Ti. initial temperature; Tf, final temperature; Prec, precipitation, % WFPS, percent water filled pore space; NH_4^+, soil ammonium content; NO_3^-, soil nitrate content; CO_2, CO_2 flux.
Significance codes: . P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001.

CH_4 flux

The highest levels of CH_4 production and consumption (oxidation) occurred during the rainy season of 2013, followed by the rainy season of 2014. Indeed, CH_4 flux fell by 50% in January 2014 (Figure 2b), closely following the pattern of precipitation (Figure 1a).

The highest variation in CH_4 flux occurred in January 2013. The highest mean CH_4 fluxes were 504, 423, and 380 µg CH_4-C m^{-2} h^{-1} for pasture heights of 15, 25, and 35 cm, respectively. The highest mean CH_4 oxidation values were -411, -203, and -544 µg CH_4-C for pasture heights of 15, 25, and 35 cm, respectively (Figure 2b). The CH_4 fluxes reported here had large daily standard errors, except during the rainy season. In the summer, CH_4 flux was strongly correlated with NH_4^+ content and mildly with temperature and precipitation. In the autumn and winter, CH_4 fluxes were correlated only with temperature (Table 1).

CO_2 flux

The highest levels of CO_2 emissions occurred during the rainy summer season, when grass growth is favored, whereas the lowest were measured during the dry winter season. Intermediate levels of CO_2 flux occurred in the spring and autumn (Figure 2c and 1a). Indeed, the highest CO_2 emission level (1 g m^{-2} h^{-1}), which occurred in the 35-cm pasture height treatment, was measured on December 5, 2012, and again on February 26, 2014. The highest emission observed for the 25-cm pasture height occurred on February 1, 2013 (885 mg CO_2 m^{-2} h^{-1}), and for the 15-cm height on December 5, 2012 (630 mg CO_2 m^{-2} h^{-1}). Minimal emissions were observed in the period of July to September of 2014.

Percent WFPS was strongly correlated with CO_2 emissions in the autumn and winter. CO_2 emissions were also correlated with soil NH_4^+ in the autumn and with precipitation events in the winter (Table 1).

Inorganic-N

Inorganic-N was low throughout the study period. The sum of NH_4-N and NO_3-N totaled approximately 0.1–0.3% of total N, which is the lowest level usually reported for inorganic-N

in soils. The concentrations of soil NH_4-N and NO_3-N were similar and ranged from 5 to 25 mg N kg^{-1} dry soil (Figure 3a, b). As mentioned above, NH_4-N was significantly correlated with N_2O flux in the spring and autumn, with CH_4 flux in the summer, and with CO_2 emissions in the autumn. Soil NO_3-N was correlated with N_2O emissions in the spring (Table 1).

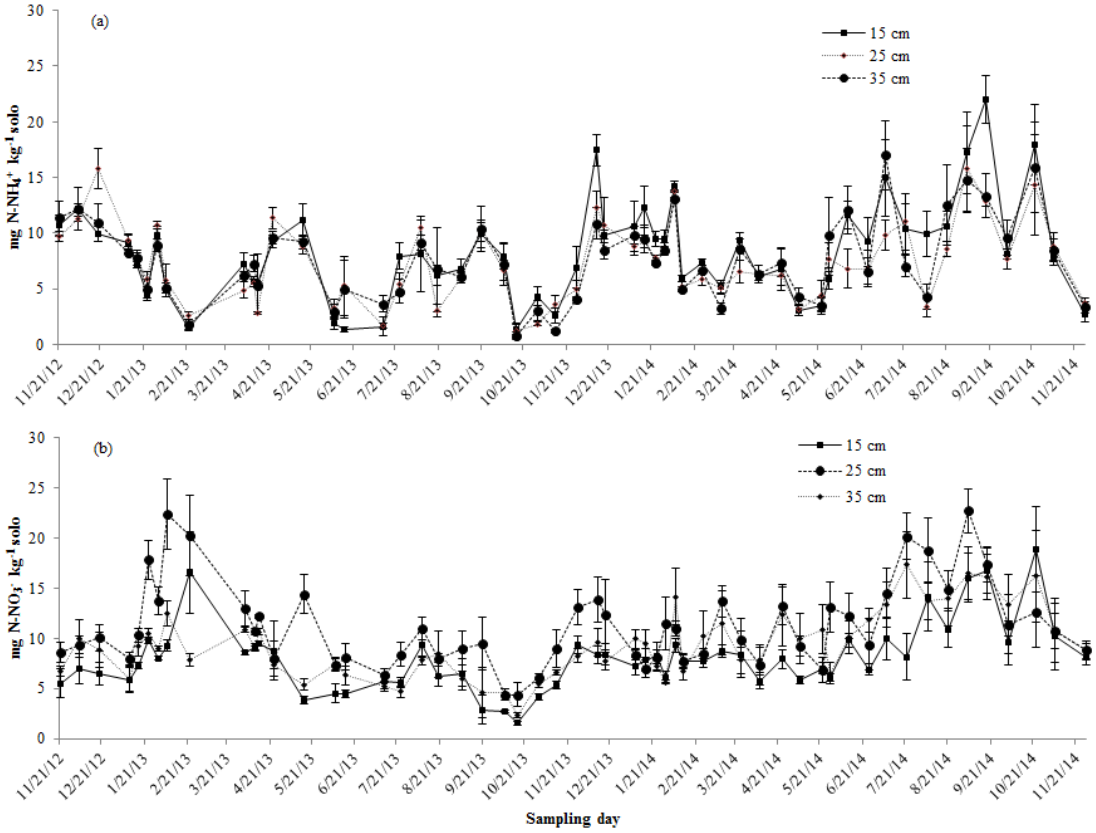

Figure 3. (a) Soil ammonium content (mg NH_4^+-N kg^{-1} dry soil) and (b) soil nitrate content (mg NO_3^--N kg^{-1} dry soil) for three pasture heights (15, 25, and 35 cm) during two years of evaluation (November 21, 2012 to November 26, 2014).

Cumulative greenhouse gas emissions

Table 2 shows a linear reduction in annual cumulative N_2O emissions ($p < 0.05$) with increasing pasture height for both years. Pasture heights of 15, 25, and 35 cm had total N_2O

emissions of 300.1, 41.6, and -48.3 mg N_2O-N m^{-2} in year 1 and totals of -153.2, -263.7, and -298.7 mg N_2O-N m^{-2} in year 2, respectively. The 15-cm pasture height had the highest levels of N_2O emissions in all seasons except for in the spring of year 1. The 25- and 35-cm pasture heights had cumulative N_2O emissions only in the spring of year 1 and in the summer of the year 2. The effect of pasture height on cumulative N_2O emissions was negatively linear in the annual analysis ($p < 0.05$), driven by the negative associations observed in the summer ($p < 0.05$) and even more so in the autumn ($p < 0.001$).

Table 2. Cumulative N_2O emissions (mg N_2O-N m^{-2}) from Marandu pastureland maintained at three pasture heights (15, 25, and 30 cm) across seasons[a] and years.

	Spring	Summer	Autumn	Winter	Total year
Year 1					
15 cm	46.3 (14.1)	128.4 (20.5)	33.2 (39.2)	92.3 (25.0)	300.1 (49.3)
25 cm	80.5 (44.2)	-28.8 (51.0)	-1.3 (34.7)	-8.8 (34.2)	41.6 (127.4)
35 cm	76.5 (42.5)	-11.7 (39.5)	-102.3 (19.5)	-10.8 (45.6)	-48.3 (118.9)
Mean	67.8 (20.0)	29.3 (27.2)	-23.5 (22.4)	24.2 (22.7)	97.81 (67.1)
Effect[b]	ns	L*	L***	ns	L*
R^2		0.51	0.60		0.52
Year 2					
15 cm	9.2 (51.1)	91.64 (32.8)	-107.1 (12.4)	-147.0 (55.3)	-153.2 (88.7)
25 cm	-48.1 (26.3)	61.02 (15.6)	-127.1 (10.8)	-149.5 (76.2)	-263.7 (90.8)
35 cm	-101.4 (56.5)	10.54 (14.5)	-221.8 (37.2)	-166.6 (32.4)	-479.2 (91.1)
Mean	-46.7 (27.5)	54.40 (14.7)	-152.0 (17.6)	-154.3 (31.7)	-298.7 (58.9)
Effect[b]	ns	L*	L***	ns	L*
R^2		0.55	0.66		0.55

[a] Rainy summer, dry winter, transitional spring and autumn.
Within parentheses: ± standard error of the mean (SEM).
Anova significance: year, $P < 0.01$; season $P < 0.01$; pasture height, $P < 0.01$; spring, n=7; summer, n=15; autumn, n=7; winter, n=7.
[b] Effect: L = linear. Significance codes: . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Annual cumulative CH_4 emissions were not affected by pasture height treatment (Table 3). In year 1, net oxidation occurred in the 15- and 25-cm pasture heights, totaling -28.6 and -40.5 mg CH_4-C m^{-2}, respectively. In year 2, net production for the 15-, 25-, and 35-cm pasture heights was 70.6, 62.2, and 56.8 µg CH_4-C m^{-2}, respectively. Cumulative CH_4 emissions were negatively associated with pasture height only in the autumn of year 1 and in the spring of year 2 ($p < 0.05$).

Table 3. Cumulative CH_4 emissions (mg CH_4-C m^{-2}) from Marandu pastureland maintained at three pasture heights (15, 25, and 30 cm) across seasons[a] and years.

	Spring	Summer	Autumn	Winter	Total year
Year 1					
15 cm	-21.5 (30.8)	-0.5 (1.4)	-21.3 (24.6)	14.7 (20.6)	-28.6 (60.9)
25 cm	-9.6 (21.3)	-0.8 (1.0)	-72.1 (35.3)	42.0 (25.4)	-40.5 (60.0)
35 cm	34.2 (32.1)	2.7 (1.5)	80.4 (36.8)	-4.7 (24.6)	112.5 (66.8)
Mean	1.0 (16.5)	0.5 (0.8)	-4.3 (23.5)	17.3 (13.6)	14.5 (37.9)
Effect[b]	ns	ns	L.	ns	ns
R^2			0.43		
Year 2					
15 cm	119.1 (72.4)	19.6 (8.1)	-68.5 (12.2)	7.4 (12.3)	70.6 (70.3)
25 cm	81.4 (15.2)	5.1 (14.9)	-25.2 (36.0)	1.3 (20.0)	62.2 (41.5)
35 cm	54.2 (16.4)	45.0 (16.4)	-45.2 (25.8)	2.8 (12.5)	56.8 (35.7)
Mean	84.8 (24.3)	23.4 (8.4)	-43.3 (8.4)	3.9 (8.4)	65.6 (28.0)
Effect[b]	L*	ns	ns	ns	ns
R^2	0.46				

[a] Rainy summer, dry winter, transitional spring and autumn.
Within parentheses: ± standard error of the mean (SEM).
Anova significance: year, P = 0.23; season, P < 0.01; pasture height, P < 0.01; spring, n=7; summer, n=15; autumn, n=7; winter, n=7.
[b] Effect: L = linear. Significance codes: . P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 4 shows that annual cumulative CO_2 emissions were positively associated with pasture height in both years of study ($p < 0.001$). Indeed, for pasture heights of 15, 25 and 35 cm, cumulative CO_2 emissions totaled 64.3, 83.7, and 81.9 Mg CO_2 ha^{-1} in year 1 and 24.1, 35.1, and 62.3 Mg CO_2 ha^{-1} in year 2, respectively. The reduction of 35% in the amount of precipitation that occurred in year 2 was probably responsible for the sharp drop in the cumulative CO_2 emissions in that year.

Table 4. Cumulative CO_2 emissions (Mg CO_2 ha^{-1}) from Marandu pastureland maintained at three pasture heights (15, 25, and 30 cm) across seasons[a] and years.

	Spring	Summer	Autumn	Winter	Total year
Year 1					
15 cm	25.0 (3.8)	20.6 (1.6)	11.8 (2.7)	6.9 (0.7)	64.3 (2.9)
25 cm	23.0 (2.7)	29.1 (2.94)	22.7 (1.7)	9.0 (0.7)	83.7 (4.0)
35 cm	26.8 (2.9)	31.8 (1.8)	28.3 (1.8)	10.9 (1.0)	97.8 (4.7)
Mean	24.9 (1.8)	27.2 (1.7)	20.9 (2.0)	8.9 (0.6)	81.9 (3.9)
Effect [c]	ns	L***	L***	L***	L***
R^2		0.67	0.82	0.67	0.84
Proportion					
15 cm	38.8%	32.1%	18.4%	10.7%	
25 cm	27.4%	34.8%	27.1%	10.%	
35 cm	27.4%	32.5%	29.0%	11.1%	
Year 2					
15 cm	6.8 (0.8)	10.0 (2.0)	6.0 (1.6)	1.3 (0.1)	24.1 (3.3)
25 cm	7.2 (0.5)	20.7 (1.9)	5.8 (0.6)	1.9 (0.3)	35.1 (1.9)
35 cm	12.1 (2.3)	37.8 (7.0)	10.5 (1.4)	1.9 (0.5)	62.3 (7.4)
Mean	8.7 (1.0)	22.7 (3.7)	7.4 (0.9)	1.7 (0.2)	40.5 (4.7)
Effect [c]	L*	L***	L*	ns	L***
R^2	0.55	0.75	0.51		0.81
Proportion					
15 cm	28.1%	41.6%	25.0%	5.4%	
25 cm	20.6%	57.4%	16.6%	5.4%	
35 cm	19.5%	60.7%	16.8%	3.0%	

[a] Rainy summer, dry winter, transitional spring and autumn.
Within parentheses: ± standard error of the mean (SEM).
Anova significance: year, $P < 0.01$; season, $P < 0.01$; pasture height, $P < 0.01$; spring, n=7; summer, n=15; autumn, n=7; winter, n=7.
[b] Effect: L = linear. Significance codes: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Pasture height was positively associated with cumulative CO_2 emissions in all seasons, except for spring of year 1 and winter of year 2. The summers of 2013 and 2014 accounted for approximately 35% and 50% of annual cumulative CO_2 emissions, respectively. In contrast, the winters of 2013 and 2014 accounted for just 10% and 5% of annual cumulative CO_2 emissions, respectively. Spring and autumn accounted for approximately 27% and 20% of annual cumulative CO_2 emissions, respectively, in both years (Table 4). The contribution of each season to annual cumulative CO_2 emissions was probably affected by the amount of precipitation in each season.

4. Discussion

N_2O flux

Once fertilizer was applied, soil water content and its consequent effect on WFPS was the key driving variable for N_2O emission from grassland in the summer months. Soil water content depends on the amount of rainfall and how rapidly rain infiltrates into the soil (Dobbie et al., 1999). In our study, the highest N_2O fluxes occurred in the summer and following rainfall events. Rainfall events increased WFPS levels to above 60-70%, which create good conditions for anaerobic denitrification (Smith et al., 2003). Rainfall can stimulate N_2O emissions, as has been observed in other studies (van der Weerden et al., 2011; Sordi et al., 2014). Most studies of temperate regions have reported that N_2O emissions increase in the cooler/wetter compared with warmer/drier periods of the year (Allen et al., 1996; Zaman and Ngueyn, 2012; Rochette et al., 2014). In contrast, the seasonal variability of N_2O emissions has a different pattern in tropical grasslands, where the warm and wet summer has the highest

fluxes. Lessa et al. (2014) found large differences in N_2O emissions between seasons, with the lowest N_2O emissions in the dry winter season, a result similar to ours. Here we show that the dry winter season had lower values of % WFPS (30-40%) and lower temperatures, both of which may help explain the low level of N_2O release.

Mechanisms of N_2O consumption in tropical grasslands need to be studied. A plausible explanation for the negative N_2O fluxes found in all seasons depends on the occurrence of an equilibrium between the concentration of N_2O in soil pores and that in the atmosphere at the surface of the grassland (Gas diffusion). According Mazzeto et al. (2014), one possible mechanism for N_2O uptake is a lack of available nitrate in the soil, leading denitrifying bacteria to use N_2O as an electron acceptor, which in turn contributes to net N_2O uptake. Low values of mineral N and high values of % WFPS have been shown to favor N_2O consumption (Chapuis-Lardy et al., 2007; Mazzeto et al., 2014). The flux of -299 μg N_2O-N m^{-2} h^{-1} reported here may be the lowest value of N_2O flux ever reported, exceeding the uptake rate of -207 μg N_2O-N m^{-2} h^{-1} reported by Blicher-Mathiesen and Hoffmann (1999). Schlesinger (2013) suggested that uptake rates greater than 20 μg N_2O-N m^{-2} h^{-1} are related to wet soil, which is inconsistent with our findings.

CH_4 flux

Seasonal variation in CH_4 emissions has been associated with temperature and insolation (Sorrell and Boon, 1992) as well as with air temperature and moisture (Mazzetto et al., 2014). We observed the highest levels of both production and oxidation of CH_4 in the rainy summer season. The high rate of evapotranspiration that occurred at our site may explain the rapid variation between CH_4 production and oxidation. Indeed, various environmental conditions allow methanotroph expression in soils subjected to moisture variation, during which their

methanotrophic potential is maintained because they remain viable in anaerobiosis (Roslev and King, 1995; Le Mer et al., 2001).

During the autumn and winter, temperature was correlated with CH_4 flux, and thus temperature appears to control CH_4 production and oxidation during these seasons. However, an explanation for this observation is hindered by the lack of consistent results among several other publications. Indeed, Chiavegatto et al. (2015) found no association between CH_4 flux and either soil water content or soil or air temperature. Likewise, Dengel et al. (2011), measuring CH_4 in a grassland site, found no relationship with air temperature. On the other hand, and in accordance with our results, Chamberlain et al. (2015) found an association between CH_4 emissions and soil temperature. We found that emissions during the warm season (end of spring and summer) were much higher than in winter (mild and dry). Other studies of CH_4 emissions have also reported that emissions were higher in the summer and lower in the winter (Holter, 1997; Mazzeto et al., 2014).

CO_2 flux

CO_2 flux has been reported to be influenced by season, soil, and climatic variables (Xu and Baldocchi, 2004; Wohlfahrt et al., 2008; Thomas et al., 2014). In our study, the highest CO_2 fluxes occurred at the end of spring and in the summer. During these periods, we observed increased grass growth and higher rainfall, which may have favored root and soil microorganism respiration. We observed low fluxes in the winter, when temperature and precipitation are lower. However, no explanatory variable was associated with CO_2 flux in the summer and spring, whereas it was significantly correlated with precipitation and % WFPS in the autumn and winter (Table 1). We suggest that soil water controlled CO_2 flux in the latter two seasons. Brito et al. (2015) found a positive linear association between CO_2 flux and soil

temperature in summer and autumn. They proposed that seasonal variation of CO_2 was directly related to variations in precipitation and soil temperature. Immediately following re-wetting, soil CO_2 efflux rates can be very high (Thomas and Hoon et al., 2010). We also found high CO_2 fluxes in the spring, after the rainy season began (Figure 2) and % WFPS increased (Figure 1b).

Cumulative greenhouse gas emissions

The differences in N_2O emissions between seasons are difficult to interpret. Van Groenigen et al. (2005) proposed that such variations are more likely to occur due to fertilization schedules than to urine deposition. In the present study, we found higher amounts of cumulative N_2O emissions in the spring and summer, when fertilizer was applied to the soil.

Grassland management affected N_2O emissions accumulated in the summer, autumn and annually whereas in the spring and winter were not fitted (Table 2). There was a negative linear effect of pasture height on the amount of N_2O emitted/consumed. Pastures with lower heights have a lower demand for nitrogen for growth. Moreover, the higher stocking rate in the shortest pasture height treatment resulted in more nitrogen being returned to the soil, although deposition of feces and urine favor N losses (Haynes and Williams, 1993). The higher the pasture height, the more litter is produced with a high C/N ratio, which in turn, requires more N for decomposition and N mineralization (Shariff et al., 1994), thereby resulting in less N available to be lost.

Cumulative N_2O emissions were lower in winter than in summer in a subtropical pastureland of the southern Brazilian state Paraná (Sordi et al., 2014), almost zero in the Cerrado (Lessa et al., 2014), and negative in a grassland of the Brazilian state Rondônia in the

Amazon region (Mazzetto et al., 2014). Our data agrees with studies conducted in tropical regions and differs from those conducted in temperate regions. In temperate regions, winters are usually wet, thereby favoring anaerobiosis and low N uptake by plants, which in turn augments N availability for N_2O emission (Allen et al., 1996; Luo et al., 2008). In these regions, higher soil water content in cooler periods of the year favors N_2O production by denitrification (Zaman and Nguyen, 2012), and, in areas where pasture is irrigated, small differences in emissions between seasons are observed (Kelly et al., 2008).

In our study, we did not observe a clear pattern of CH_4 flux variation within each season. However, we found significant effects on CH_4 production and oxidation between seasons. Similar effects were reported by Chamberlain et al. (2015), whereas the opposite was shown by Chiavegatto et al. (2015). Net CH_4 production occurred in all seasons except autumn. At the study site, rainfall diminished during the autumn, which reduced soil moisture content and thus may have favored CH_4 oxidation. Conversely, higher rainfall during spring coupled with the rotting of the accumulated grass litter probably contributed to the increase in CH_4 emissions observed in the spring of 2014 (Table 3). Dung is the main source of CH_4 released to the atmosphere from grassland soils (Saggar et al., 2004). In the present study, CH_4 production and oxidation were affected by pasture height; however, we did not observe a clear trend, because in year 1, the low pasture height had lower CH_4 production whereas in year 2 it had the highest cumulative emissions. Soil parameters and climate variables affect CH_4 dynamics, as reviewed by Le Mer and Roger (2001) and Saggar et al. (2004). Indeed, although soil and climate variables affected CH_4 production in our study, we cannot exclude the role of other variables not investigated here.

We observed a negative linear effect of pasture height on CH_4 emissions in the spring and a positive linear effect in autumn. High grazing intensity implies high deposition of excreta,

which favors CH_4 production in the spring. The pattern of urination and defecation in paddocks varies widely (Haynes and Williams, 1993; Gusmão et al., 2015). Cumulative CH_4 production and oxidation had higher standard errors than did other GHG (Table 3), suggesting that spatial variation in CH_4 flux may be associated with the spatial variability of excreta deposition.

Seasonal variability of CO_2 emissions has been attributed to precipitation and soil temperature in a tropical pasture (Brito et al., 2015). Thus, in the present study, the observed variability in precipitation may explain the interannual differences in CO_2 emissions. From year 1 to year 2, precipitation declined by 35% while cumulative CO_2 emissions decreased by 50%. The warm and rainy summer season contributed 35% and 50% of total CO_2 emissions in year 1 and year 2, respectively. In the region studied, there is seasonality in grass growth and biomass production, which occurs mainly in the summer (Costa et al., 2006), so CO_2 emissions probably varied with grass growth.

Pasture height had a positive linear effect on cumulative CO_2 flux, both annually and by season. A higher stocking rate was necessary to maintain a lower pasture height (Barbero et al., 2015). Wang et al. (2009) observed that CO_2 fluxes were decreased significantly at a high stocking rate, which might have been related to the low soil moisture and biomass at the study site. A high pasture height implies high aboveground biomass and litter deposition (Shariff et al., 1994), resulting in a greater amount of C that can be released through decomposition. Laporte et al. (2002) found a positive effect of aboveground plant biomass on soil surface CO_2 efflux at a grassland site. Bremer et al. (1998) reported reduced CO_2 fluxes in grazed vs. ungrazed tallgrass prairie. They suggested that the reduction in photosynthetic surface area and available carbohydrates was the dominant factor driving lowered respiration rates.

5. Conclusions

Fluxes of N_2O, CH_4, and CO_2 varied among seasons and between years. The magnitude of fluxes observed in this study can be explained by seasonal variations in temperature, precipitation, % WFPS, and inorganic N content.

Temperature was associated with N_2O fluxes in the spring and autumn and with CH_4 fluxes in summer, autumn and winter. % WFPS and ammonium content were correlated with N_2O fluxes in the spring and autumn, with CH_4 fluxes in the summer and CO_2 in autumn. While nitrate was correlated with N_2O emissions in spring and %WFPS with CO_2 efflux in the winter. Cumulative N_2O and CO_2 emissions were influenced by year and season, while CH_4 was affected by season.

Pasture height had a negative linear effect on annual, summer, and autumn cumulative N_2O emissions/consumption and a positive linear effect on annual cumulative CO_2 emissions.

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CHAPTER 6 - Mineral salt intake effects on faecal-N concentration and the volume and composition of beef cattle urine

O artigo a seguir está redigido conforme normas de publicação do periódico *Animal Feed Science and Technology*, exceto o posicionamento das tabelas e figuras.

Mineral salt intake effects on faecal-N concentration and the volume and composition of beef cattle urine

Abstract

The effect of mineral salts on water ingestion and urine volume in cattle has been explored in the literature. However, recently this effect has been investigated as a potential mitigator of environmental aspects related to the nitrogen (N) cycle, such as NO_3^- lixiviation, NH_3 volatilization and N_2O emissions. The effect of mineral salts, mainly NaCl, on urine-N concentration, urine volume, the proportion of N compounds in the urine and faeces-N concentration have not yet been explored under field conditions with respect to the environmental aspects of beef cattle production. We investigated the effect of dietary mineral salt levels on these parameters. The experimental design was a Latin Square (5 x 5), five levels of mineral salts in the diet: 0.0, 2.0, 4.0, 6.0 and 8.0 g based on dry matter (DM) ingestion (g/kg DM). Mineral salt level in the diet influenced N concentration in the urine (P=0.036), which decreased linearly. The urine volume was affected (P=0.002) and increased linearly. The total N excreted (g/day) via urine did not vary (P>0.05) increasing mineral salt levels. When investigated, the N compounds of urine [urea-N (P = 0.002), allantoin-N (P = 0.041) and hyppuric acid-N (P = 0.018)] also responded to the increased level of mineral salts and creatinine-N not (P>0.05). Urea-N, allantoin-N, hyppuric acid-N linearly increased in proportion of the total N-urine. The N concentration in faeces was not affected by mineral salt level (P>0.05).The urinary volume, the concentration of N and the proportion of N compounds in the urine effected N_2O emissions or NH_3 volatilization. Therefore, mineral salt utilization may be an option for mitigating N pollution from beef cattle, especially on grasslands in tropical countries.

Key-words: sodium chloride, nitrogen usage efficiency, Nellore

1. Introduction

In South America, India and Africa, 600 million beef cattle are raised on extensive production system (FAO, 2015). In such systems grassland does not contain all the minerals necessary as required, therefore which demands mineral supplementation to the animals (Morris, 1980). Mineral supplementation is offered in a mixture and one of the components is sodium chloride (NaCl) that has a diuretic effect to induce greater drinking-water intake, increase urination frequency, decrease urine N concentration and urine N deposition rate, and thereby potentially decrease N leaching. (Thornton, 1970; Ledgard *et al.* 2015) and appear to be a potential mitigation option N pollution.

Livestock has its impact on the environment and is responsible for 15% of greenhouse gas emissions (Gerber et al., 2013). Strategies to mitigate the environmental impacts of beef production are therefore needed. An increase in the efficiency of nitrogen (N) usage or a reduction in the concentration of N and nitrogen compounds in the excreta, especially in the urine, could help to reduce the effect of nitrogen on the environment (Spek *et al.*, 2013). Spek *et al.*, (2012) showed that the level of dietary salt intake linearly increased urine production and total urinary N excretion in dairy cattle. We hypothesized that the level of mineral salt in the diet influenced urinary volume, total N excretion via urine and faeces and the proportion of urinary compounds in beef cattle.

The negative aspects of ammonia (NH_3) and nitrous oxide emissions (N_2O) on the environment are of global concern (Lessa *et al.*, 2014; Oenema *et al.*, 2008) and domestic cattle are responsible for the majority of these emissions (Hutchings *et al.*, 2001; Opio *et al.*,

2013). The primary source of ammonia-N from manure is urinary urea N (g of N/d), which is hydrolysed to ammonia and carbon dioxide by the activity of microbial urease present in the faeces (Spek *et al.*, 2012). N_2O is produced mainly during denitrification and nitrification of urine N compounds by microorganisms (De Klein *et al.*, 2010).

Urine patches from grazing cattle represent a substantial addition of N to the pasture. Following urination, the N undergoes hydrolysis and mineralization, which may be nitrified or denitrified. The urine volume and concentration of N influence these mechanisms (Dijkstra *et al.*, 2013). If the urine volume is constant, total N in the urine does not have a dominant effect on the proportion of N_2O emitted (Van Groenigen *et al.*, 2005). Contrastingly, when the volume of urine was increased to 1 to 2 L (with equal amounts of N) the fraction of N_2O released decreases linearly (Cardoso et al., 2016). The concentration of some urine compounds also influence N_2O emissions Van Groenigen *et al.*, (2006) and Bertram *et al.*, (2009) observed that hyppuric acid in cattle urine acts as a natural inhibitor of N_2O emission, probably through the temporary inhibition of nitrification and denitrification processes. Liu and Zhou (2014) found that the high salt diet treatment compared with the control group decreased the average emission rates of NH_3 by 48% and N_2O by 26% from soil treated with sheep urine.

Increasing urine volume by increased dietary mineral content appears to be a promising N_2O mitigation strategy, particularly in extensive livestock systems. This is of particular interest since N_2O is a greenhouse gas with a global warming potential about 300 times reater than the global warming potential of CO_2 (IPCC, 2007). This research aimed to investigate the influence of dietary mineral salt level on the concentration of N in the urine/faeces, urine volume and the proportion of urea-N, allantoin-N, creatinine-N and

hyppuric acid-N compounds of total N excreted through the urination of beef cattle on pasture.

2. Material and Methods

The study was carried out on palisade-grass (*Brachiaria brizantha,* Marandu cultivar) grassland located at the Jaboticabal Campus in São Paulo State, Brazil (21º15'22'' S and 48º 18'08''W, altitude of 595 m). The region's climate is characterized by tropical rainy summers and dry winters. The mean annual rainfall is 1424 mm with a mean air temperature of 22.3ºC. The soils are Rodhic Ferralsols (IUSS, 2006) derived from basalt. The experimental area was constituted of 5 paddocks of 625 m^2 with a total of 0.31 ha. The cattle grazed on the paddocks continuously during the experimental period. The dry mass and chemical composition of grass did not differ during the period of study (Table 1).

Table 1. Variation of chemical composition (% of Dry matter – DM) of palisade-grass according the day of sampling during experimental period.

		day 7	day 14	day 21	day 28	day 35
DM [a,b]	kg/ha	9600 (70)	10000 (80)	8002 (32)	9550 (53)	11600 (87)
DM	%	40.3 (4.0)	40.0 (2.0)	40.0 (2.8)	40.5 (3.9)	41.3 (4.5)
CP	% DM	6.03 (0.11)	6.27 (0.07)	6.57 (0.04)	6.35 (0.03)	6.58 (0.19)
NDF	% DM	56.3 (1.9)	55.4 (3.1)	57.8 (3.9)	55.7 (6.0)	54.9 (4.7)
ADF	% DM	37.1 (3.6)	37.9 (0.6)	37.9 (1.4)	38.1 (0.9)	37.3 (1.2)
Ash	% DM	9.7 (0.8)	9.6 (0.5)	10.0 (0.8)	10.1 (0.8)	10.2 (0.4)

[a] Within parenthesis is the standard error of means (SEM; ±). DM – Dry Matter, CP – Crude protein, NDF – Neutral Detergent Fibre and ADF – Acid Detergent Fibre.
[b] ANOVA probability values: DM (p=0.25), % DM (p=0.97), CP (p=0.57), (ADN (p=0.82), ADF (p=0.93) and Ash (p=0.61).

2.1 Animals, treatments and feeding

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation guidelines (COBEA; Colégio Brasileiro de Experimentação Animal) and was approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA; Comissão de Ética e Bem Estar Animal) of Faculdade de Ciências Agrárias e Veterinárias, UNESP (Universidade Estadual Paulista; protocol number 004389/13). Five animals of the Nellore breed weighing 298 ± 23 kg were used in the experiment. The experiment was conducted from 18 November to 22 December, 2012. The animals were offered 5 levels of mineral salts in a Latin square (5 x 5) experimental design. The levels of minerals were 0.0, 2.0, 4.0, 6.0 and 8.0 g/kg of dry matter (DM) ingestion calculated based on the body weight of the beef cattle. The treatments levels was decided because of according NRC (2000) and CSIRO (2007), the recommended amount of NaCl in the diet is 0.6–0.8% of DM ingestion. We used a commercial product, Fosbovi 40® (Tortuga CIA Zootécnica Agrária) mixed with NaCl which constituted the mineral salt used in the experiment. The composition was 660 g/kg of NaCl, 87 g/kg of Ca, 57 g/kg of P, 8 g/kg of S, 33 mg/kg of Co, 410 mg/kg of Cu, 560 mg/kg of Fe, 30 mg/kg of I, 660 mg/kg of Mg, 5 mg/kg of Se, 1740 mg/kg of Zn and 575 mg/kg of F. The salt mix was offered individually in the morning. Ingestion of the salt mix was stimulated by mixing it with 0.8 kg of corn meal. We observed orts only at the highest salt mix level of 8.0 g/kg of DM. The animals received the treatment for 6 days as an adaption period and we sampled on the seventh day. Water was available *ad libitum*.

2.2 Herbage sampling and chemical composition

Grazing heights were measured at 80 random points ("hits")/hectare (ha) to estimate average paddock height. To estimate herbage mass, 5 samples per paddock (average spots height) were collected from a 0.25 m^2 area (5 cm residual height) every 7 days. Samples were dried at 55±5ºC to a constant weight to estimate DM/ha. To estimate herbage chemical composition, samples were milled in a Willey mill (Thomas Scientific, Swedesboro, NJ), sieved at 40 mesh and analysed for dry matter (DM; method Nº934.01) and N concentration (method Nº978.04) according AOAC (1995). To obtain the crude protein (CP) in the forage, the total N was multiplied by 6.25. The amount of ash, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined as preconized by Robertson and Van Soest (1981).

2.3 Excretion sampling and urine volume calculation

Each day at 18:00 h, urine was sampled directly from the animals. The animal was immobilized in a cattle chute where we waited for urination and made a collection. Sub-samples of 10 mL of urine were collected and diluted with 40 mL of a solution of 0.018 mmol/L H_2SO_4 followed by storage in a freezer at -20 until analysis for N concentration and urine compounds. Fresh faeces were collected from the paddock and subsequently dried at 55 ºC for 72 h, milled, sieved and analysed for total N concentration as described above for the forage.

The urine volume (UV) was calculated according to the following equation:

$$UV(L/day) = (27*LW)/(c) \qquad \text{Equation (1)}$$

Where: 27 is the daily excretion of creatinine in mg/kg LW, as obtained by Rennó *et al.*, (2008) in Nellore cattle, LW is the live weight of the animal and (c) is the concentration of creatinine (mg/L) quantified in the urine sample of the experimental animals.

2.4 Analytical Procedures for urine

Total N content in the urine was determined following AOAC (1995) standardization. The concentration of allantoin and hyppuric acid was quantified colorimetrically, according Fujihara *et al.,* (1987) and Tomokuni and Ogata (1972). The amount of creatinine and urea were analysed using commercial kits; ANALISA (Heinegard and Tiderström (1973) for creatinine and Bergmeyer (1985) for urea. Total N excreted daily (g/day) was calculated by considering the N concentration in urine (g/L) and the daily urine volume (L). The proportion of urea-N, allantoin-N, creatinine-N and hyppuric acid-N were calculated by dividing the N (g/L) of the compound by the total urine-N (g/L).

2.5 Statistical Analyses

To check for normality and homogeneity of the data the Lilliefors and Cochran-Bartlett (1956) tests were used, respectively. ANOVA was used to compare means for each variable. Forage mass and chemical composition were analyzed as fix effects and urine volume, N concentration and total N concentration and the proportion of urinary N compounds were analyzed as random effects. When significant we used polynomial orthogonal contrast to evaluate the effect of mineral salt levels in the variable using the software R version 3.2.1 (2015).

3. Results

The forage offered was 8000–11800 kg of DM/ha, with the DM of the palisade-grass around 40%. The CP ranged from 6.03 to 6.58% of DM. In terms of fibre, the mean NDF was 55% and the ADF 37% of DM. The ash content varied from 9.6 to 10.2% (Table 1). The ANOVA of forage chemical composition was not significant for all parameters evaluated. The probability values were: DM ($P = 0.25$), %DM ($P= 0.97$), CP ($P=0.57$), NDF $P=0.82$), ADF ($P=0.93$) and Ash ($P=0.61$). This suggests that there was no variation in the grass composition during the experimental period (Table 1).

N concentration in the faeces DM did not differ when mineral salt was increased in the diet ($P>0.05$); it ranged from 1.71 to 1.47 g N/kg DM of faeces (Table 2). In terms of total N concentration in the urine, the values responded the levels of mineral salt in the diet ($P<0.01$), it decreased linearly (Table 2) from 26.88 to 6.73 g/L. The calculated urinary volume was influenced by the variation in mineral salts concentration in the diet ($P<0.01$). We estimated 5.11 to 12.45 L/day, increasing linearly. The total N excreted daily via urine decreased from 106.6 to 67.8 g/day, but these results were not significant ($P>0.05$).

Table 2. N concentration in urine (g/l), urinary volume (l), N excreted via urine (g/day) and effect of mineral salt intake level (g salt kg/DM) on these urinary parameters.

		Mineral salt level (g NaCl/kg DM)					Effect (probability) [c]		
		0.0	2.0	4.0	6.0	8.0	L	Q	C
NCU [a]	(g N/l)	26.9 (5.3)	20.6 (4.4)	14.4 (1.7)	7.7 (1.0)	6.9 (0.8)	<0.001 [b]	0.3645	0.5557
NCF [a]	(% N/DM)	1.71 (0.31)	1.54 (0.13)	1.54 (0.17)	1.47 (0.09)	1.53 (0.11)	0.0972	0.1934	0.8916
UV [a]	(l/day)	5.1 (1.8)	6.4 (0.2)	9.2 (0.7)	8.6 (0.4)	12.4 (1.3)	<0.001 [b]	0.6891	0.4029
NEU [a]	(g N/day)	106.6 (14.2)	128.3 (23.7)	131.4 (16.9)	67.8 (11.3)	88.5 (16.6)	0.0942	0.3042	0.0763

Within parenthesis is the standard error of means (SEM; ±).
[a] NCU, Nitrogen concentration in urine; NCF; Nitrogen concentration in faeces. UV; Urinary volume. NEU; Nitrogen excreted via urine.
[b] The linear equations between mineral salt intake (NI; g/kg DM intake) and NCU (g/L) was NCU = -2.64 NI + 25.88 (R^2 = 0.9591). Urine volume (L/day) was UV = 0.84 NI + 4.98 (R^2 = 0.8945).
[c] L, Linear; Q, quadratic; C, cubic.

The main N compound in the N excreted daily via urine was N-urea, which varied positively and linearly from 43.0% to 70.4% (P<0.001); N-allantoin ranged from 1.93% to 4.49% and also responded linearly and positively (P<0.01). The proportion of N-creatinine ranged from 2.44% to 4.90%, but the means did not differ (P>0.05). The hyppuric acid-N was affected by the salt level in the diet (P<0.018) and increased linearly (P<0.001) from 2.2% to 7.3% (Table 3).

Table 3. Proportion of N compounds in the total N of urine (%) and effect of mineral salt intake level (g salt kg/DM) on urinary N compounds.

	Mineral salt level (g NaCl/kg DM)					Effect (probability) [b]		
	0.0	2.0	4.0	6.0	8.0	L [a]	Q	C
Urea-N	43.0 (2.3)	51.5 (6.1)	67.9 (3.7)	69.3 (5.1)	70.4 (7.9)	<0.001	0.1064	0.4592
Allantoin-N	1.9 (0.2)	2.5 (0.7)	2.6 (0.5)	4.5 (0.9)	3.8 (0.6)	<0.004	0.4238	<0.04
Hyppuric acid-N	2.2 (0.5)	2.5 (0.6)	6.3 (1.3)	7.3 (0.5)	6.9 (0.9)	<0.001	0.1089	<0.006
Creatinine-N [c]	3.0 (0.3)	2.8 (0.5)	2.4 (0.4)	4.9 (0.6)	4.0 (0.6)	-	-	-

Within parenthesis is the standard error of means (SEM; ±).
[a] Linear equation between NaCl intake (NI, % g NaCl/kg DM intake) and urea-N (UUN, % of total urinary-N). UUN = 3.63 NI+ 45.88 (R^2 = 0.8537), allantoin-N (AN, % of total urinary-N). AN = 0.28 NI + 1.92. (R^2 = 0.7435), Hyppuric acid-N (HN, % of total urinary-N). HN = 0.71 NI + 2.18 (R^2 = 0.8226) and creatinine-N (% of total urinary-N).
[b] L, Linear; Q, quadratic; C, cubic.
[c] ANOVA was not significant (P>0.05)

4. Discussion

Salt has been used in the diet as a regulator of ingested feed and water and to supply cattle requirements for these nutrients. In a review, Spek *et al.*, (2012) notes that the excreted urinary volume is a major determinant of the N concentration in urine, both in situations of water restriction and of increased water intake. We calculated the urinary volume as 5.1–12.4 L/day, and this increased with rising mineral salt ingestion by beef cattle. In cattle, the mineral load that needs to be excreted largely determines the volume of urine. Van Vuuren and Smits (1997) verified the effect of low or high rumen protein surplus (RPS) and two levels of NaCl (0 and 0.38 kg/day) in lactating dairy cattle; they found that urine volumes varied from 23 to 42 kg/day and from 40 to 60 kg/day for low and high RPS respectively. Spek *et al.*, (2012) also studied dairy cow urine while adding NaCl from 0 to 1.01 kg/day; the urine volume increased from 18 to 68 kg/day. In the current research with Nellore cattle,

varying salt levels in the diet had the same effect on urine volumes as found in other studies with dairy cattle. This finding suggests a similar response in beef and dairy cattle to variable salt levels in the diet. Liu and Zhou (2014) found similar effect evaluating sheep cattle, high NaCl diet increased total urine volume.

There is a positive relationship with total N intake and total N excreted via urine (Huhtanen *et al*. 2008; Weiss *et al*., 2009; Kebreab *et al*. 2010). Poppi and McLennan (1995) state that protein loss will occur when the CP content of the diet exceeds approximately 210 g CP/kg Digestible Organic Matter (DOM). They add that a better relationship between protein and energy is approximately 160 g CP/kg DOM, as at this level there is complete net transfer of ingested protein to the intestines as microbial, undegraded and endogenous protein. Total N excreted daily via urine did not differ ($P>0.05$) as expected in our research once N was not varied in the diet (see Table 1). We estimated the N intake based on a dataset from a study that took place 1 km distant from this study (Oliveira, 2014). The total N intake averaged 161 (±3) g/day and the N excreted via urine calculated by us represented 42–79% of total N ingested.

Another effect of salt ingestion is the dilutive effect on urinary compounds of an increase in urinary volume. NaCl is mainly responsible for the increase in urine volume, predominantly by creating a demand for more water by the animal (Morris, 1980). The N concentration of cattle urine is variable and ranges from 3.0 to 20.5 g/L (Dijstra *et al.*, 2013). In this study we recorded 26.8 g/L, which was higher than reported by the previous authors. While the urine N concentration at the 8.0 g/kg DM level of mineral salt was 6.7 g/L close to the values of 6.1, 6.8 and 5.8 g/L found by Lantinga *et al.*, (1987), Bristow *et al.*, (1992) and Gonda and Linderg (1994) and higher than the lowest concentrations of 3.9 and 3.0 g/L measured by van Vuuren and Smits (1997) and Spek *et al.*, (2012) when feeding extra NaCl.

In the current study we did not force the animals to ingestion NaCl. A urine concentration of 6.7 g/L was quantified when the animals were fed 0.8% (8.0 g/kg DM) of mineral mix in the diet, implying a value near to field conditions without restriction of salt and water.

Cattle urine contains diverse nitrogenous constituents and the principal form of N is urea. Urea is formed mainly in the liver as a means of detoxification of ammonia present in the systemic circulation. It is transported in blood plasma and subsequently diffuses or is transported to other fluid pools in the body such as milk in the udder and liquid in the rumen (Spek *et al*., 2013). According to Bristow *et al.* (1992), urea constitutes 50 to 90% of total N in cattle urine. Far ahead Dijstra *et al.,* (2013) reviewed the concentration of N-urea to vary between 2.1 and 19.2 g/L; this represents 52.1–99.5% of the total N. Large amounts of urea can be volatilized (Whitehead *et al.,* 1989) and NH_3 volatilization is considered an indirect source of N_2O (de Klein *et al.*, 2010).

In the current study, the concentration of N-urea in urine (g/L) did not differ with an increase in the level of dietary mineral salt. But the relative contribution (%) for the total urine-N was affected. When varying the NaCl content in the diet of dairy cows, Van Vuuren and Smits (1997) and Spek *et al*., (2012) found significant differences. The former authors found from 72.7 to 77.7% of urine-N in the form of urea and the later from 60.0 to 71.4%. We determined N-urea in the urine to vary between 43.0 and 70.4% of total N (Table 3).

Allantoin, creatinine, hyppuric acid and ammonia are other principal N compounds found in the urine (Bristow *et al.*, 1992). The purine derivative allantoin originates from rumen microbial nucleic acids. We found a linear effect of NaCl levels on allantoin-N ranging from 1.9% to 4.5% of total urine-N. Bristow *et al.*, (1992) reviews values from 2.2% to 11.8% allantoin-N in the total urine-N of cattle, sheep and goats and Gonda and Lindberg (1994), while evaluating the urine of dairy cows, found values between 10.0% and 14.0%. The

proportion of N-creatinine did not differ according to the treatment in the study, and in agreement, Dijstra *et al.* (2012) argues that dietary composition has a relatively minor effect on creatinine excretion. Hyppuric acid is derived from plant phenolic cinnamic acids.

We found a linear effect between salt level in the diet and the urinary concentration of hyppuric acid, increasing from 2.2% to 7.3% of total urine-N; these values were similar to those quantified in dairy cattle urine. Studying the urinary compounds of dairy cattle, Bristow *et al.*, (1992) found values between 3.4% and 8.0% and Kool *et al.*, (2006) 4.1%–5.1% of urine-N in the form of hyppuric acid. Hyppuric acid has an inhibitory effect on N_2O release, through the temporal inhibition of nitrification and denitrification processes. This is caused by the breakdown product, benzoic acid, which is a recognized antimicrobial agent. Kool *et al.*, (2006), Van Groenigen *et al.*, (2006) and Bertram *et al.*, (2009) detected this inhibition. Increasing the concentration of hyppuric acid in the urine by a factor 2 or 3 halved the emissions of N_2O.

Faecal N excretion increases with increased N ingestion. However the variation in urinary N excretion is 3.5 times greater than that of faecal N excretion (e.g. Weiss *et al.*, 2009). The response and variation in faecal N output compared with urinary N excretion is much lower (Dijstra *et al*., 2013). The concentration of N in faeces did not vary according to the mineral salt level in the diet in the current study (Table 2). We attribute this result to the concentration of crude protein in the forage that did not differ throughout the experimental period (Table 1). In terms of concentration, Braz *et al.* (2002) quantified 1.20% of total N in the DM of Nellore cattle faeces and Cardoso *et al.* (2016) determined 1.96% N of DM in faeces of the Girolanda breed. We quantified 1.47–1.71% in the current study.

6. Conclusion

Varying the concentration of mineral salt mix in the diet of beef cattle grazing on Marandu palisade-grass pasture altered their urine output, the concentration of N in urine and changed the proportion of N compounds like urea-N, allantoin-N, creatinine-N and hyppuric acid-N. The amount of N excreted in the urine per day and the concentration of N in the faeces did not differ with increased mineral salt levels in the diet. The effect of N concentration in the urine decreased linearly, and in the urine volume, urea-N, allantoin-N and hyppuric acid-N there was a linear increase. Creatinine-N did not fit the curve. The effect of mineral salt on urinary N concentration and in the proportion of urea-N or hyppuric acid-N in the urine demands studies in our condition that may be mitigate N_2O emissions and NH_3 volatilization by including mineral salt in the diet of beef cattle while grazing in the proportions recommend by NRC.

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CHAPTER 7 – Final Considerations

Considerações Finais

Os fatores de emissão para óxido nitroso medidos na presente diferiram de acordo com o tipo de excreta. Na média o fator de emissão para urina foi superior ao de fezes e semelhante ao encontrado quando se misturou urina e fezes. A mistura urina as fezes pode ter mudado o pH da mistura e evitado a formação de costras tornando as perdas de N na forma de N_2O semelhantes. Encontrou-se uma emissão de N_2O para as fezes superior ao que se tem encontrado na literatura e isto pode estar relacionado a composição bioquímica das fezes. Os fatores de emissão medidos para N_2O foram um pouco superior aos preconizados pelo IPCC. As emissões de CH_4 foram significativamente maiores no tratamento com adição de fezes como observado em estudos anteriores. E o fator de emissão foi inferior ao preconizado pelo IPCC.

Quando se estudou as variáveis explicativas das emissões de N_2O as características do solo apareceram como principais reguladoras das emissões do gás. Principalmente a umidade do solo e a compactação. Para se evita áreas com potencial para alto potencial de emissão de N_2O deve se evitar a formação de áreas de solo compactados no pasto e áreas com alta concentração de excretas e umidade. Especial atenção deve ser dada ao manejo de coxos e bebedouros que devem ser mudados de lugar dentro do pasto. Para avançar o entendimento da emissão de N_2O e CH_4 em pastagens sugere se a realização de estudos em condições de campos avaliando solos em diferentes estados de compactação, textura e umidade.

O cloreto de potássio (KCl) aparece como potencial inibidor das emissões de N_2O. Isso pode ocorrer por ter “segurado” o N no solo através da formação de cloreto de amônio, por exemplo, ou inibição da atividade de micro-organismos envolvidos na nitrificação e desnitrificação. Sugere-se a realização de um estudo avaliando o potencial redox do solo, a dinâmica de N e atividade dos micro-organismos para entender a ação do cloreto de amônio sobre as emissões de N_2O.

Foram encontrados fluxos negativos de N_2O tanto em experimentos no campo como em condições controladas. Estes fluxos negativos podem ser resultados da difusão do gás após o fechamento da câmara levando a um equilíbrio entre a concentração do gás no interior da câmara. O N_2O poderia ser utilizado no lugar do NO_3^- como aceptor de elétrons? O que levaria ao consumo de N_2O? Quando e como ocorre o consumo de N_2O? Estás são algumas perguntas de pesquisa a serem respondidas em outros estudos.

A emissão de N_2O foi superior em pastos de menor altura. Isso se deve ao maior retorno de N através das excreções dos animais. Manejar os pastos em maiores alturas levam a uma mitigação nas emissões de gases de efeito estufa, especialmente, ao se considerar os aumentos de estoques de carbono no solo e ao melhor aproveitamento do N retornado ao solo.

O sal mineral pode resultar em mitigação das emissões de gases de efeito estufa através da ação de íons como K^+, Na^+ e Cl^- que podem interferir na atividade dos micro-organismos nitrificadores e desnitrificadores ou através da diluição do N urinário levando a menores perdas de N na forma de N_2O e NH_3. Os benefícios do uso de sal mineral que implicam no melhor desempenho dos animais podem levar a

uma menor emissão de gases de efeito estufa considerando o ciclo de vida dos animais. Isso não foi investigado na presente tese e se apresentam como oportunidades de estudos.

Postscript

"The humanity lives and will live because the miracle of the seeds".

Fernando de Sousa Costa *- Agronomist, Secretary of Agriculture (1927-1930) of São Paulo State. Founder of Instituto Biológico. Brazilian Ministry of Agriculture (1937-1941). São Paulo state president (1941-1945).*