BIANCA COSTA GUIMARÃES

NITROUS OXIDE AND AMMONIA EMISSIONS FROM BEEF CATTLE EXCRETA IN PALISADEGRASS PASTURES WITH AND WITHOUT FERTILIZER-N OR MIXED WITH FORAGE PEANUT

LAVRAS - MG

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para obtenção do título de Mestre.

Prof. Dr. Daniel Rume Casagrande
Orientador

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APROVADA em 08 de outubro de 2020.
Dr. Bruno José Rodrigues Alves – Embrapa Agrobiologia / Pesquisador
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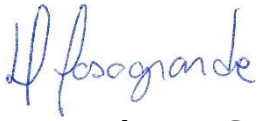

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ABSTRACT

Grazing pasture is a major livestock production system in Brazil, and the nitrogen excretion by cattle onto pastures has been identified as an important source of nitrous oxide (N_2O). In this study we assessed for long term N_2O and ammonia (NH_3) emissions from cattle urine and dung deposited in the dry and rainy season on three pastures systems: i) palisadegrass {*Brachiaria brizantha* (Hochst. ex A. Rich.) R.D. Webster [syn. *Urochloa brizantha* Stapf cv. Marandu]}in monoculture without fertilizer-N application (Grass); ii) palisadegrass in monoculture with 150 kg N ha^{-1} (Grass+N); and iii) palisadegrass mixed with forage peanut (*Arachis pintoi* Krapov. & W.C. Greg cv. BRS Mandobi) without fertilizer-N application (Grass+Legume). Two trials were carried out in a tropical region of Brazil, beginning in the dry and rainy season and extending for one year. The experimental design was in randomized blocks and the treatments were arranged in a 3×3 factorial scheme: three excreta types (urine, dung and control without excreta) and three pasture systems, with three replications. In both trials the N_2O peaks were associated with rainfall events and the background levels were achieved after 22-28 days after a rainfall event >20 mm day^{-1}. The N_2O emission factors (EF_{N2O}) were greater in areas treated with urine compared than dung in all pasture systems. The urine EF_{N2O} was lowest in the Grass system, and there was no difference between Grass+Legume and Grass+N system. Urine EF_{N2O} were 0.51 %, 0.61 %, 0.83 % in dry season and 0.30 %, 0.50 %, 0.40 % in the rainy season, for Grass, Grass+N and Grass+Legume, respectively. The dung EF_{N2O} did not vary between Grass and Grass+N systems, and there was a tendency of lowest dung EF_{N2O} in Grass+Legume system (P = 0.065). Dung EF_{N2O} were 0.22 %, 0.21 %, 0.12 % in dry season and 0.19 %, 0.20 %, 0.09 % in the rainy season, for Grass, Grass+N and Grass+Legume, respectively. The greatest percentage of excreta-N lost by NH_3 volatilization (EF_{NH3}) was observed for urine under Grass+N system in dry season. EF_{NH3} from urine-treated soil during the dry season was 7.9 %, 21.0 % and 11.2 % of the N in the excreta, and in the rainy season was 1.1 %, 4.2 % and 0 %, respectively, for Grass, Grass+N and Grass+Legume. There was no difference between pasture systems and seasons for dung EF_{NH3} and the mean of dung EF_{NH3} was 0.6 %. In all pasture systems, urine EF_{NH3} were greater than dung EF_{NH3}. These results suggest that pasture system, season and excreta type affect differently the EF_{N2O} and EF_{NH3}. EF_{N2O} and EF_{NH3} from urine were greater in dry season. The lowest urine EF_{N2O} from Grass system indicates that intensifying the system by fertilizer-N or biological nitrogen fixation favored N losses by N_2O in urine patches. However, the Grass+Legume system decreased the dung $EF_{N2O.}$ The lower urine EF_{NH3} from Grass+Legume and Grass systems, compared with Grass+N suggests that mixed pasture may be a strategy to mitigate NH_3 volatilization from urine deposited in pasture. The emission factors found in this study are in agreement with those proposed in 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories.

KEYWORDS: Nitrous oxide; Ammonia volatilization; Cattle excreta; Greenhouse gases; Forage legume; *Brachiaria*.

RESUMO

A pastagem é um importante sistema de produção animal no Brasil, e a excreção de nitrogênio por bovinos nas pastagens tem sido identificada como uma importante fonte de óxido nitroso (N_2O). Neste estudo avaliamos as emissões de N_2O e amônia (NH_3) a longo prazo pela urina e fezes bovinas depositadas na estação de seca e águas em três sistemas de pastagem: i) capim-marandu {*Brachiaria brizantha* (Hochst. ex A. Rich.) R.D. Webster [syn. *Urochloa brizantha* Stapf cv. Marandu]} em monocultura sem aplicação de fertilizante nitrogenado (Grass); ii) capim-marandu em monocultura com 150 kg N ha^{-1} (Grass+N); e iii) capim- marandu consorciado com amendoim forrageiro (*Arachis pintoi* Krapov. & W.C. Greg cv. BRS Mandobi) sem aplicação de fertilizante nitrogenado (Grass+Legume). Dois ensaios foram conduzidos em uma região tropical do Brasil, começando na estação seca e chuvosa e se estendendo por um ano. O delineamento experimental foi em blocos casualizados e os tratamentos foram arranjados em esquema fatorial 3 × 3: três tipos de excretas (urina, fezes e controle sem excretas) e três sistemas de pastagem, com três repetições. Em ambos os ensaios os picos de N_2O foram associados a eventos de chuva e os níveis de *background* foram alcançados depois de 22-28 dias após um evento de chuva >20 mm dia^{-1}. Os fatores de emissão de N_2O (EF_{N2O}) foram maiores em áreas tratadas com urina em comparação a fezes em todos os sistemas de pastagem. O EF_{N2O} da urina foi menor no sistema Grass e não houve diferença entre os sistemas Grass+Legume e Grass+N. Os EF_{N2O} da urina foram 0,51 %, 0,61 %, 0,83 % na estação seca, e 0,30 %, 0,50 %, 0,40 % na estação das águas, para Grass, Grass+N e Grass+Legume, respectivamente. O EF_{N2O} das fezes não variou entre os sistemas Grass e Grass+N, e houve uma tendência do EF_{N2O} das fezes ser mais baixo no sistema Grass+Legume ($P = 0.065$). Os EF_{N2O} das fezes foram 0,22 %, 0,21 %, 0,12 % na estação seca, e 0,19 %, 0,20 %, 0,09 % na estação das águas, para Grass, Grass+N e Grass+Legume, respectivamente. A maior porcentagem de N-excreta perdida por volatilização de NH_3 (EF_{NH3}) foi observada para urina sob Grass+N na estação seca. EF_{NH3} do solo tratado com urina durante a estação seca foi 7,9 %, 21,0 % e 11,2 % do N da excreta, e na estação chuvosa foi 1,1 %, 4,2 % e 0 %, respectivamente, para Grass, Grass+N e Grass+Legume. Não houve diferença entre sistemas de pastagens e estações para o EF_{NH3} das fezes, e a média do EF_{NH3} das fezes foi de 0,6 %. Em todos os sistemas de pastagens os EF_{NH3} da urina foram maiores que os EF_{NH3} das fezes. Esses resultados sugerem que o sistema de pastagem, a sazonalidade e o tipo de excreta afetam diferentemente os EF_{N2O} e EF_{NH3}. EF_{N2O} e EF_{NH3} da urina foram maiores na estação seca. O menor EF_{N2O} na urina de sistemas Grass indica que a intensificação do sistema por fertilizante nitrogenado ou fixação biológica de nitrogênio favoreceu as perdas de N por N_2O pela urina. No entanto, o sistema Grass+Legume reduziu o EF_{N2O} das fezes. Os menores EF_{NH3} da urina depositada em Grass+Legume e Grass, comparadas com Grass+N sugere que pastagem consorciada com leguminosa pode ser uma estratégia para mitigar a volatilização de NH_3 da urina depositada na pastagem. Os fatores de emissão encontrados nesse estudo estão de acordo com os propostos nas diretrizes revisadas do IPCC 2019.

PALAVRAS-CHAVE: Óxido nitroso; Volatilização de amônia; Excretas bovinas; Gases de efeito estufa; Leguminosas forrageiras; *Brachiaria*.

RESUMO INTERPRETATIVO E RESUMO GRÁFICO

A pastagem é um importante sistema de produção animal no Brasil, e a excreção de nitrogênio por bovinos nas pastagens tem sido identificada como uma importante fonte de óxido nitroso (N_2O) para a atmosfera. O N_2O é um potente gás de efeito estufa, sendo equivalente a 265 vezes o CO_2. A volatilização de amônia (NH_3) é considerada uma fonte indireta de N_2O, já que uma parte dessa NH_3 pode ser depositada ao solo novamente e ser emitida na forma de N_2O. A produção de N_2O ocorre naturalmente no solo por processos microbiológicos, e as condições que controlam esses processos são principalmente a disponibilidade de nitrogênio no solo, umidade e temperatura do solo. Por isso, nesse estudo nós avaliamos o efeito das excretas de animais depositadas em três sistemas de pastagem na estação seca e chuvosa sobre as emissões de N_2O e NH_3. Os três sistemas foram: capim-marandu em monocultivo sem adubação nitrogenada, capim-marandu em monocultivo com adubação nitrogenada, e capim-marandu em consórcio com amendoim forrageiro. O amendoim forrageiro, por ser uma leguminosa, fixa o nitrogênio da atmosfera, o que pode substituir a adubação nitrogenada. Nós observamos que a urina emite mais N_2O e NH_3 que as fezes, devido ao nitrogênio presente na urina ser mais solúvel e disponível no solo. Quando avaliamos a porcentagem do nitrogênio da excreta que é emitido na forma de N_2O, que chamamos de fator de emissão, nós vimos que para urina os maiores valores encontrados foram do pasto adubado e do pasto consorciado, enquanto o fator de emissão das fezes não foi alterado pelo sistema de pastagem. Quando nós comparamos as estações em que a excreta foi depositada na pastagem, nós observamos que o fator de emissão da urina foi maior na estação seca, e as fezes também não foi afetada pela estação. Quanto a volatilização de NH_3 nós vimos que a urina da pastagem adubada perdeu mais nitrogênio pela volatilização de NH_3, e as fezes não foram alteradas pelos sistemas de pastagens. A urina depositada na estação seca também teve maior porcentagem de nitrogênio volatilizado na estação seca, e das fezes não houve diferença entre as estações. Com isso nós constatamos que a urina dos animais que estavam nos sistemas com entrada de nitrogênio, tanto pela adubação ou pela presença da leguminosa, teve maior porcentagem do nitrogênio emitido como N_2O. Porém, a urina dos animais que estavam no sistema consorciado teve a porcentagem do nitrogênio perdido como NH_3, similar ao sistema sem nenhuma entrada de nitrogênio. Portanto, utilizar leguminosas em pastagens em substituição à adubação nitrogenada pode reduzir as emissões de gases de efeito estufa.

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PPGZ
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA
UNIVERSIDADE FEDERAL DE LAVRAS

1

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↓ FATOR DE EMISSÃO DE N_2O

↓ FATOR DE EMISSÃO DE NH_3

2

→

↑ FATOR DE EMISSÃO DE N_2O

↑ FATOR DE EMISSÃO DE NH_3

3

→

↑ FATOR DE EMISSÃO DE N_2O

↓ FATOR DE EMISSÃO DE NH_3

1 PASTAGEM DE MARANDU SEM ADUBAÇÃO NITROGENADA

2 PASTAGEM DE MARANDU COM ADUBAÇÃO NITROGENADA

3 PASTAGEM DE MARANDU CONSORCIADO COM AMENDOIM FORRAGEIRO

SUMMARY

1. INTRODUCTION

Brazil has the largest commercial bovine herd in the world, with more than 172 million head (IBGE, 2018). Approximately 90 % of these animals spent part or all their life in pastures and are destined for beef production (Anualpec, 2015). The pasture area covers about 160 million hectares of the national territory (IBGE, 2018) where forage grasses of the genus *Brachiaria*, principally, are grown without fertilization. Due to the great representativity of the livestock activity in Brazil, there is increasing focus on greenhouse gas (GHG) emissions from this sector. In Brazil, it is estimated that the GHGs emissions from cattle account for 27 % of the total, being methane (CH_4) and nitrous oxide (N_2O) the most representative (Boddey et al., 2020). The animal excreta deposited on pastures is responsible for 18 % of the N_2O from the agricultural sector in the country, including direct and indirect emissions (MCTI, 2013).

In pasture systems, approximately 75-95 % of the N ingested by cattle is excreted to the soil as urine and dung (Haynes and Williams, 1993) and the proportion of nutrients excreted fluctuates mainly by diet nutritional value and how balanced it is. The excreta of grazing animals are directly deposited into the pasture, which is a potential atmosphere pollutant through the gas forms of nitrogen (NH_3, NO e N_2O; Giacomini & Aita, 2006). Nitrous oxide is a potent GHG with a global warming potential of 265 times that of carbon dioxide (CO_2) (IPCC, 2019). This gas is an intermediate product of microbial nitrification and denitrification processes in soils (Firestone and Davidson 1989). Since these processes are regulated by available N, soil moisture and temperature, the animal excretion contributes to the localized N_2O sources in pasturelands (Tiedje, 1988). Therefore, measurements across a range of soil and weather in tropical conditions are required to develop local data and improve national inventories of GHG emissions.

The IPCC 2006 (Intergovernmental Panel on Climate Change) assumed that 2 % of the total deposited N onto pastures via bovine excreta is emitted as N_2O (EF_{3PRP}), without distinction between type of excreta, season or climate. However, researches carried out in different climates analyzing bovine excreta suggested that the EF_{3PRP} should be disaggregated into wet and dry climate and into urine and dung. Thus, the 2019 Refinement to the 2006 IPCC Guidelines consider the EF_{3PRP} of cattle urine as 0.77 % and 0.32 %, and EF_{3PRP} of cattle dung as 0.13 % and 0.07 % for wet and dry climate, respectively. The differences between excreta type can be explained by its nitrogen composition. The larger proportion of urine-N is in labile form (urea), that is readily available for hydrolysis by soil urease, while dung-N is mainly in organic forms, which takes longer to mineralize.

Researches assessing N_2O emissions usually takes samples for up to three months on average, considering that there are no more significant fluxes after that period. However, the fluxes can extend for prolonged periods, varying according to different edaphoclimatic characteristics (Bouwman, 1996), and therefore, we adopted a N_2O sampling period of one year.

Ammonia (NH_3) volatilization is an indirect source of N_2O, considering that 1 % of the total NH_3 lost will be emitted as N_2O elsewhere (IPCC, 2006). This compound is resulted mainly from hydrolysis of the urea component of excreta, being also driven by soil pH and temperature (Nichols et al., 2018). The transfer of NH_3 gas from the soil solution to the atmosphere are affected by several edaphoclimatic factors (Sommer and Hutchings, 2001). Due to the contrasting characteristics between urine and dung, it is likely that their contributions to NH_3 losses also may vary. Studies conducted in Brazil have found greatest rates of NH_3 emissions from urine, seasonal variations, and overall,

the fraction of excreta-N emitted as NH_3 (EF_{NH3}) were lower than the 21 % proposed by IPCC 2019 (Bretas et al.., 2020; Lessa et al., 2014; Cardoso et al., 2019).

The inclusion of forages legumes in the grazing system is a sustainable alternative to increase the pasture productivity (Pereira et al., 2019) and is a potential management strategy in order to mitigate GHGs emissions, either directly or indirectly (Boddey et al., 2020). Legumes, by establishing symbiosis with soil microorganisms that fix atmospheric N2, are able to incorporate this fundamental nutrient into the pasture system even in the absence of N fertilizer. The greater nutritional value, especially protein content, and higher digestibility of legume forages supply more substrate for microbial protein production in rumen (Minson, 2012). This feature associated with metabolites present in legumes, such as tannins, are able to modify the protein metabolism in the animal reducing the nitrogen excretion via urine and increasing the recalcitrance of fecal-N (Aguerre et al., 2016). On the other hand, the distribution and composition of nitrogenous fractions in forage grasses with nitrogen fertilizer application is altered, especially by increasing of non-protein nitrogen (NPN) fraction (VAN SOEST, 1994). This fraction is highly soluble, and may cause an unbalance in the rumen, raising the N excretion in the urine. Therefore, studies evaluating the inclusion of forage legumes in pasture to substitute chemical fertilizers is important to understand its potential of GHGs mitigation. Forage peanut is highly recommended for intercropping in warm-season pasture due to its clonal propagation capacity and high grazing tolerance that increases their chances of persistence in the plant community (Tamele et al., 2018).

We hypothesized that: (i) EF_{N2O} and EF_{NH3} differ between the pastures systems assessed, being lower in excreta from mixed pasture compared to grass in monoculture; (ii) N_2O and NH_3 fluxes is affected by seasonality; (iii) EF_{N2O} and EF_{NH3} is lower for cattle dung than for urine; and (iv) the EF_{N2O} and EF_{NH3} is lower than the default

suggested by 2019 IPCC guidelines. This study aimed at measuring for long term N_2O emissions and NH_3 volatilization from cattle urine and dung deposited in the dry and rainy season on three pastures systems: palisadegrass (*Brachiaria brizantha* Stapf. A. Rich. 'Marandu') in monoculture with and without fertilizer-N application, or mixed with forage peanut (*Arachis pintoi* Krapov. & W.C. Greg. 'BRS Mandobi'), and to develop local emission factors for tropical pastureland of Brazil.

2. LITERATURE REVIEW

2.1 Nitrous oxide in livestock

One of the most important current environmental issues is the increase in global warming caused by greenhouse gas (GHG) emissions, and agricultural practices are assumed to contribute significantly to the increase in GHGs concentration. Nitrous oxide (N_2O) is a potent GHG with a global warming potential of 265 times that of carbon dioxide (CO_2) in a 100-year time frame (IPCC, 2019), and is the most important ozone-depleting agent in the stratosphere (Ravishankara et al., 2009). This gas is naturally produced in soils, through the microbiological processes of nitrification and denitrification (Firestone and Davidson, 1989), despite not being the main end product of these processes (incomplete conversion). Nitrification is a microbial process that oxidizes ammonium to nitrate, while denitrification is an anaerobic process of reducing nitrate to nitrogen gas (N_2) (Mosier et al., 2004). The denitrification process is considered the most important N_2O producer in tropical pasture systems (Davidson et al., 1993; Rochester, 2003; Peoples et al., 2004), although both reactions can occur simultaneously.

The variables that regulate these processes are N availability (substrate - ammonium and nitrate), soil moisture, soil temperature, soil pH, and in the case of denitrification, labile organic C (Granli and Bockman, 1994; Luo et al., 2008). Soil moisture improve the microbiological activity of the soil, and hence, favor the N_2O production. Therefore,

it is known that emissions tend to rise in intensive management pastures (Marsden et al., 2018) and in poorly drained soil (Dobbie et al., 1999). Greater N_2O fluxes are observed in pastures soon after the application of nitrogen fertilizers and cattle excreta deposition (Klumpp et al., 2011), which increase soil mineral N concentration. However, and the large amount of excreta-N deposited onto a relatively small area, usually exceeds the immediate plant requirements, that combined with the effects of trampling and soil compaction by the animals make it be considered the most important source of N_2O on pastures (Maljanen et al., 2007; Bertram et al., 2009).

Fresh excreta are abundant in energy, N and C chemically reduced, which provides substrate for microorganisms, temporarily changes the soil pH, and concentrations of NH_4^+, NO_2^-, and NO_3^-, which may lead to N_2O emission (Oenema et al., 2005). According to the Intergovernmental Panel on Climate Change published in 2006 (IPCC, 2006), the default EF_{3PRP} for cattle excreta deposited onto pastures was 2 %, with no discrimination among urine and dung. However, due to a variability of climate and type of excreta, the 2019 Refinement to the 2006 IPCC Guidelines disaggregated the EF_{3PRP}, considering the EF_{3PRP} of 0.77 % and 0.13 % in wet climate and 0.32 % and 0.07 % in dry climate, for urine and dung, respectively. The EF_{3PRP} is the percentage of the applied N that is emitted as N_2O, and so it allows comparison between studies carried out under different agronomic and environmental conditions. Air and soil temperatures affect the magnitude of N_2O fluxes, since higher temperatures favor faster reaction rate (Skiba & Smith, 2000). This condition can also raise microbial respiration, that consume the O_2 present, and it provides a favorable environment to the occurrence of denitrification, even in low humidity condition (Grant et al., 2004). Wherefore, it is important to consider the potential variation between seasons to develop the specific EF_{3PRP} for livestock in tropical regions and to improve the national inventories.

Ammonia volatilization from animal excreta is the process responsible for the highest N losses on the soil surface in pastures (Bouwman et al., 1997). Thus, ammonia (NH_3) is an indirect source of N_2O when deposited on soil. The process of NH_3 production involves chemical reactions, through the conversion of ammonium into ammonia gas, and physical processes, which involves the transport of ammonia gas from soil pores to the atmospheric air (Meisinger et al., 2001). Ammonia losses from animal excreta are driven by the soil pH, temperature, texture and moisture (Nichols et al., 2018), being soils with higher pH, sandy texture, and initial non-limiting moisture favor the ammonia volatilization. Besides that, higher air temperatures and wind speed, and lower air relative humidity facilitate the gas diffusion from soil to atmosphere. It is known that soil pH rises temporarily following urine deposition due to alkaline products formed during the rapid enzymatic hydrolysis of urea, favoring the NH_3 volatilization. Urine contains hippuric acid, which is also accelerate the hydrolysis of urea and thereby the formation of NH_3 (Whitehead et al., 1989). The IPCC (2019) suggested that 0-31 % of nitrogen from cattle excreta are lost as NH_3 (FracGASM), without distinction by excreta type, of which 1 % is indirectly emitted as N_2O. Recent studies carried out in Brazil have shown that FracGASM are lower than the default from IPCC (2019) guidelines and suggested disaggregation between FracGASM of dung and urine (Lessa et al., 2014; Cardoso et al., 2019; Bretas et al., 2020).

Nitrogen losses causes inefficiencies in soil management, representing an economic damage for the farmer and contributing to global environmental change. The soil nitrogen dynamics is associated with management practices, climatic conditions and intrinsic soil characteristics, as discussed above. Therefore, the high seasonality may result in different magnitudes of GHG emissions and NH_3 volatilization. Further field measurements across conditions in Brazil are crucial to develop national and regional specific emission factors

to improve national inventories of GHG emissions. In addition, there is a need to develop management strategies to mitigate N_2O emissions from agriculture (Dalal et al., 2003; De Klein and Ledgard, 2005).

2.2 Pastures systems: Fertilizer-N vs. Mixed pasture

Nitrogen is the most limiting nutrient for pasture productivity, which can be provided by synthetic fertilizer and/or biological nitrogen fixation (BNF). However, the use of fertilizers-N is increasing environmental concern, since they are an important source of nitrous oxide (N_2O) emissions in agriculture (Klein et al., 2007). Urea ($CO(NH_2)_2$) is the nitrogen fertilizer most used in pastures, representing about 67 % of the total consumed annually (IFA, 2010), owing to its high N content, which reduces its transportation cost per N unit. For urea production, the ammonia (NH_3) is synthesized by the Haber-Bosch process, from a mixture of volume 3:1 of H_2 and N_2, at elevated temperature and pressure (Jensen et al., 2012). For the conversion of ammonia (NH_3) to urea, about half of the CO_2 generated during the production of NH_3 is reused. Then, this manufacturing process requires a very high energy expenditure, besides to emitting significant amounts of greenhouse gases by N_2O (IPCC, 2006). Moreover, the urea applied to the soil is rapidly hydrolyzed by the urease enzyme to NH_3 resulting in high N losses by volatilization, and the CO_2 captured during the urea production is released back into the atmosphere (Jenkinson, 2001).

By replacing nitrogen fertilization in pastures with the use of BNF by legumes, the CO_2 emission inherent to the industrial fertilizer manufacturing process is eliminated. However, the GHG emissions from mixed pastures are linked to an increase in N_2O emissions from N release from root exudates and from plants decomposition (Rochette and Janzen, 2005). The BNF is a symbiosis process between microorganisms and legumes nodules, that transform atmospheric N_2, through the enzyme nitrogenase, into a form

assimilable by plants. Therefore, mixed pastures with grasses and legumes require smaller or no nitrogen fertilizers, being a sustainable alternative strategy for improving pasture management. This system may improve the soil fertility, soil structural characteristics and stimulate the soil beneficial microbiota through nutrient cycling (Rochester et al., 2001). In addition, legumes play an important role in reducing emissions of greenhouse gases emitted by livestock (Cardoso et al., 2016). The decomposition of legume residues can release slowly large amounts of mineral N in the soil, allowing the gradual use by the plants, and hence, reducing N_2O emission (Charles et al., 2017; Jensen et al., 2012). In grassland ecosystems, the nutrient cycle is also influenced by grazing animals, which return 75–95 % of the consumed nitrogen, through urine and dung excretion (Whitehead, 2000; Bell et al., 2015). The greater proportion of the N is excreted via urine, and the dietary N content can alter the amount of N in dung and urine.

In systems of grazing animals, the unbalance of the degradation of carbohydrates and proteins occurs most of the time, since the fiber is the predominant carbohydrate in forage which is slowly degraded in the rumen. This unbalance is even greater in forage grasses fertilized with nitrogen, due to alteration of the distribution and composition of nitrogenous fractions in plants (VAN SOEST, 1994). Non-protein nitrogen (NPN) is the nitrogenous fractions soluble in the rumen, and is composed by peptides, nitrate and non-essential amino acids, therefore, quickly converted to N-NH_3 by rumen microorganisms. When the rumen protein degradation rate is greater than the capacity of assimilation for microbial synthesis, the NH_3 excess in the rumen crosses the rumen wall, and is converted into urea in the liver. Part of this urea produced in the liver is excreted via urine, and part can return to the rumen via saliva or bloodstream. The higher NH_3 concentration in the rumen, increase the excretion of N in the urine (Aguierre et al., 2016), therefore

compounds affecting protein degradation, reduces the NH_3 production in the rumen, and hence decrease the N excretion in urine (Carulla et al., 2005; Tavendale et al., 2005).

The forage legume generally has higher amount of protein and lower fiber content, that increase the nutritional value of the diet (Muir et al., 2011, 2014). This is explained by the constitution of their cell wall, anatomy and constituent tissues, which affect directly the digestibility (Minson, 2012), providing more substrate for microbial protein production. Besides that, legumes contain condensed tannins, which are secondary compounds produced by the plants. The concentration and chemical composition of condensed tannins are very variable among plants (Naumann et al., 2017a). Tannins are known to enhance the use of feed, especially improving nitrogen metabolism in ruminants (Min et al., 2003), since part of dietary protein is complexed and precipitated in the rumen with ingestion of condensed tannins (Halvorson et al., 2017). Protein-tannin complexes occur by two mechanisms: oxidative coupling, which is irreversible, and hydrogen bonds, which are reversible (Naumann et al., 2017b). Complexes formed by hydrogen bonds are insoluble in the rumen at pH 6.5 to 7, protecting it from microbial degradation, giving to the tannin the bypass characteristic, that is undegraded protein in the rumen. However, in the abomasum this complex can become unstable at pH 2.5 to 3.0, leaving the amino acids available for digestion and absorption in the small intestine at pH 8.0 to 9.0 (McSweeney et al., 2001). Thus, tannins can directly influence the balance of rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) reducing the amount of protein that is digested in the rumen and increasing the flux of protein to the small intestine (Aguerre et al., 2016). The duodenum is the portion of the gastrointestinal tract in which the amino acid absorption process occurs more intensely, resulting in better utilization of dietary protein.

As a consequence of this property of tannins, there is a reduction in ammonia (N-NH_3) concentrations in the ruminal fluid of animals feed with tannin, improving the use of proteins in the post-rumen and reducing the N excretion in the environment by urine (Bunglavan and Dutta, 2013; Aguerre et al., 2016). The inclusion of tannin in the diet promotes a change in the partition of N excretion by the animal. Increasing levels of tannins extracted from quebracho in the ruminant feed, was observed a change of the N excretion from urine to the dung, and a raise in the proportion of neutral detergent fiber (NDF) and the B3 fraction of protein in the dung (Aguerre et al., 2016). This fact can be explained for a part of tannin-protein complexes are not broken in the abomasum (Gerber et al., 2013; Huyen et al., 2016). This change in the excretion pattern contributes to the reduction in potential N_2O emissions, due to the N content in the dung mineralizes slowly compared to urinary N (Aguerre et al., 2016), losing less soil nutrients.

Among the forage legumes, *Arachis pintoi* (forage peanut) is one of the most promising legumes for intercropping with grasses in tropical pastures, due to its productivity, nutritional value and tolerance to defoliation (Tamele et al., 2018, Gomes et al., 2020). Because it is a stoloniferous plant, new clone plants growth through vegetative propagation (Pereira et al., 2020). This type of growth habit is essential for the plant to resist grazing, even though it has good nutritional value and acceptability by animals.

3. MATERIAL AND METHODS

3.1 Site description

The study was carried out at the Experimental Farm of the University of Lavras, Brazil (21°14′S, 45°00′W; 918 m above sea level). This area has a subtropical humid mesothermal climate with dry winters (Köppen-Geiger climate classification: Cwa; Sá Júnior et al., 2012). Meteorological data were obtained from a weather station located 1,000 m from the experimental area (Fig. 1). The soil of the experimental area is classified

as Rhodic Acrudox, according to the Soil Taxonomy system (Soil Survey Staff, 2010) or a Latossolo Vermelho distrófico, according to the Brazilian Soil Classification System (SBCS, 2018), with loamy texture – 56 % clay and 30 % sand in the top 20 cm. Soil samples were taken from the area, at 0–20 cm depth, and presented the following characteristics: C = 19.71 g kg^{-1}, N = 1.34 g kg^{-1}, pH = 5.92 in water, OM = 26.5 g kg^{-1}, P = 5.46 mg dm^{-3}, K^{+} = 70.54 mg dm^{-3}, Ca^{2+} = 2.06 cmolc dm^{-3}, Mg^{2+} = 0.58 cmolc dm^{-3}, H + Al = 2.55 cmolc dm^{-3}, Al^{3+} = 0.08 cmolc dm^{-3}, and cation exchange capacity = 2.89 cmolc dm^{-3}. The soil has a bulk density of 1.27 g cm^{-3}, at 0–5 cm depth.

The palisadegrass was established in January 2014 in a nine-hectare area. Initially, the soil was corrected with the application of 2,500 kg ha^{-1} of dolomitic limestone to increase the base saturation up to 60 %. After 60 days from limed, was applied 52.0 kg ha^{-1} of P (single superphosphate) and 41.5 kg ha^{-1} of K (potassium chloride), and was sown palisadegrass, at a rate of 6.0 kg ha^{-1} of pure live seeds.

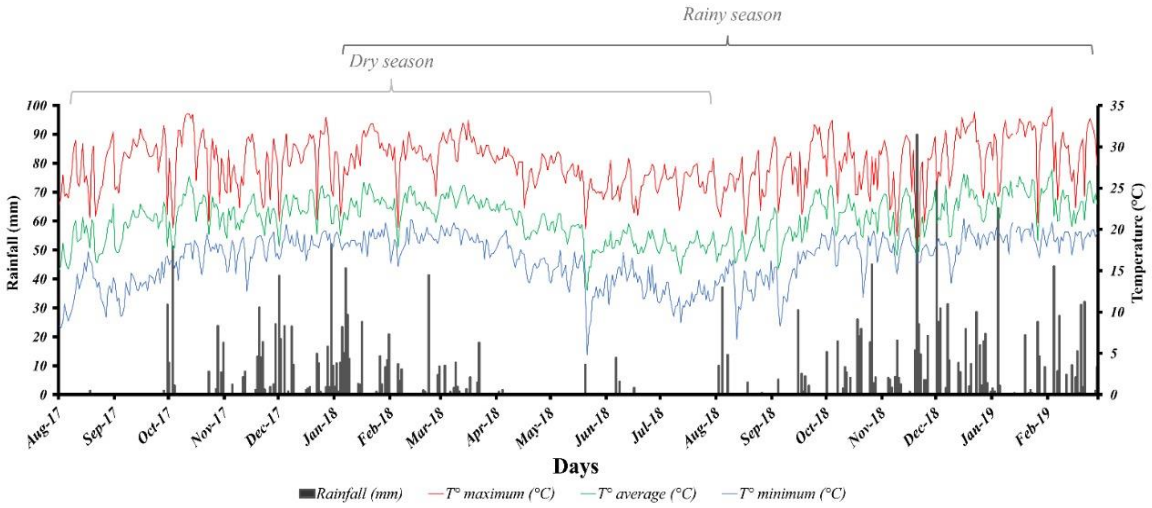

FIGURE 1 Daily temperatures (°C) and rainfall (mm) in Lavras, Brazil, during the experimental period.

3.2 Pasture systems

Evaluations were performed in three pasture systems: palisadegrass in monoculture without fertilizer-N application (Grass), palisadegrass in monoculture with 150 kg ha^{-1} of fertilizer-N application (Grass+N), and palisadegrass and forage peanut mixed pasture without fertilizer-N application (Grass+Legume). The experimental area was divided into nine paddocks, and the systems were randomly distributed in three blocks, considering the area topography (Fig. 2).

Forage peanut was sown in paddocks designed for Grass+Legume system in December 2015. The line-seeding rate was 10 kg ha^{-1} of forage peanut pure live seeds through a no-till seeder with four lines. Six seeds of forage peanut for a linear meter with 0.5 m row spacing were adopted. Annually, maintenance fertilizations were performed in early spring, in the total experimental area, by applying 22 kg ha^{-1} of P (single superphosphate) and 41 kg ha^{-1} of K (potassium chloride). Application of fertilizer-N (urea) was carried in the paddocks for Grass+N system, divided into three applications during the rainy season (November, January and March).

Pastures were managed using a continuous stocking method with variable stocking rate. Each paddock received two Nellore heifers (300 ± 48.6 kg), which remained in the same paddock for one year as testers. The animals were replaced by the new testers for another year (351 ± 38.9 kg). The heifers received only mineral supplementation. Extra Nellore heifers were added in the paddock whenever canopy height increased over the target of 20-25 cm, and animals were removed from the paddock, when the canopy decreased below 15 cm in dry season and 20 cm in rainy season. The canopy height was measured weekly at 100 random points per paddock using a sward stick (Barthram, 1985).

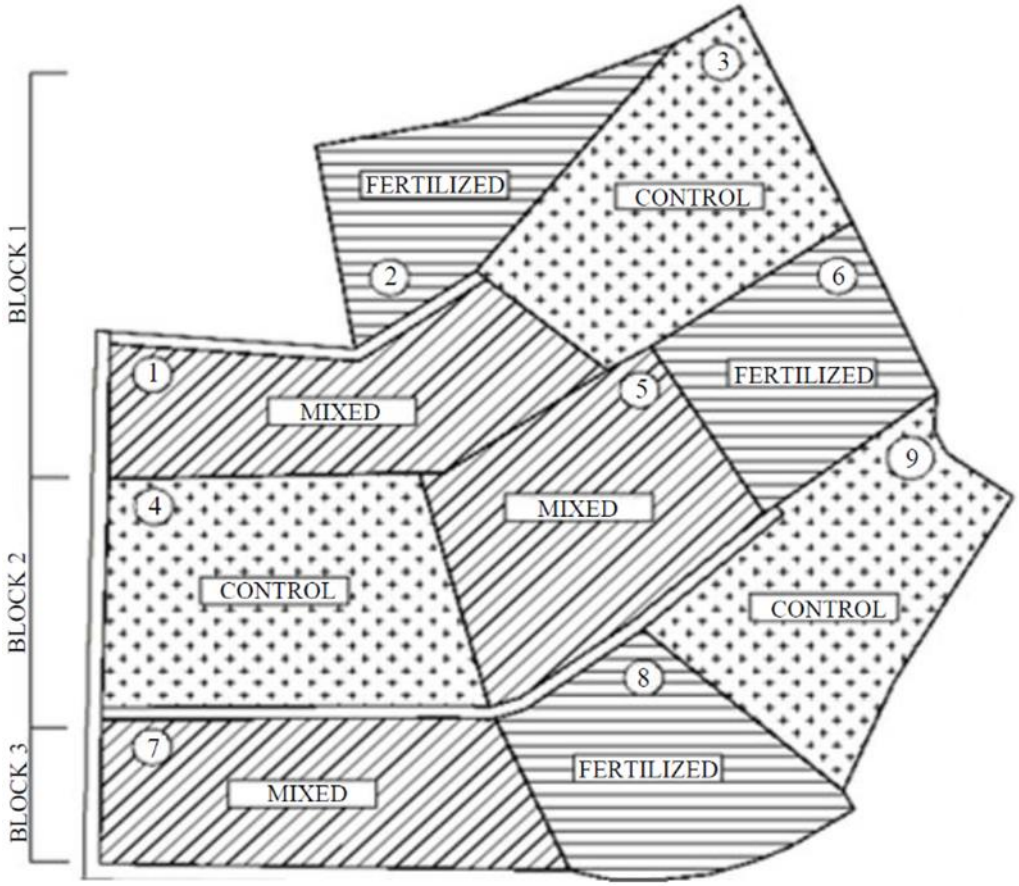

FIGURE 2 Experimental area at University of Lavras, Brazil (21°14′S, 45°00′W).

3.3 Experimental design and excreta handling

Two trials were adopted, the first one beginning in the dry season (from August 27, 2017 to August 28, 2018) and the second beginning in the rainy season (from February 01, 2018 to January 31, 2019). The treatments were arranged in a 3×3 factorial scheme, corresponding to three excreta types and three pasture systems, with repeated measurements. The types of excreta were urine, dung, and control without excreta and the pasture systems were Grass, Grass+N and Grass+Legume. The same experimental design was used in both seasons (dry and rainy).

Two exclusion areas with about 9 m^2 was built in each paddock in January 2016, allowing the animal grazing, but preventing excreta deposition by animals (Fig 3a and b). In the exclusion area were accommodated the static chamber bases (hollow metal frame) of 40 × 60 cm dimension (area = 0.24 m^2) which were inserted about 10 cm deep into the

soil and remained during the whole experimental period. The distance between chambers was approximately 0.6 m so that there was no overlap between the excreta applied. The control chamber was the same for both trials. During assessments, the plots were cut when necessary to maintain an herbage height between 15-20 cm, and removed from the area, simulating continuous grazing. The exclusion areas from Grass+N paddocks were covered with plastic tarpaulin during application of fertilizer-N to avoid its deposition into the experimental plots.

The excreta were collected fresh from Nellore heifers previously kept grazing in each paddock. For the dry season the excreta were collected on July 24-26, 2017 and for the rainy season on January 12-14, 2018. The animals were contained in the morning for collection of fresh dung and urine, that were obtained with plastic bucket held manually below the perineum of the heifers. The excreta were frozen until the beginning of the experiment. One day before implementing the experiment, the excreta were thawed, visually homogenized in a container and sampled. The excreta were fractionated and destined for the N_2O and ammonia volatilization tests. Dung patches were artificially prepared by pouring 1.2 kg of dung (fresh weigh) in the center of the static chamber base, and manually molded for 24 cm diameter (Braz et al., 2003). One liter of urine was poured onto the soil surface delimited by the walls of the static chamber base taking care to wet the entire area inside the chamber limits (Whitehead, 1995). The procedures for obtaining and depositing dung and urine were the same in both trials. The same volume of urine and mass of dung were equally placed in an area close to the chamber for ammonia volatilization measurements and also for soil sampling to determine soil moisture and soil mineral N content (NH_4^+ and NO_3^-).

Dung samples were oven-dried at 55 °C for 72 h to determine dry matter concentration and ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm

screen. Urine samples were diluted with 10 % sulfuric acid solution in the ratio of 5 ml of acid per 45 ml of urine, and frozen until N analysis. Total N concentrations from excreta were obtained using the Kjeldahl procedure (method 920.87; AOAC, 2000).

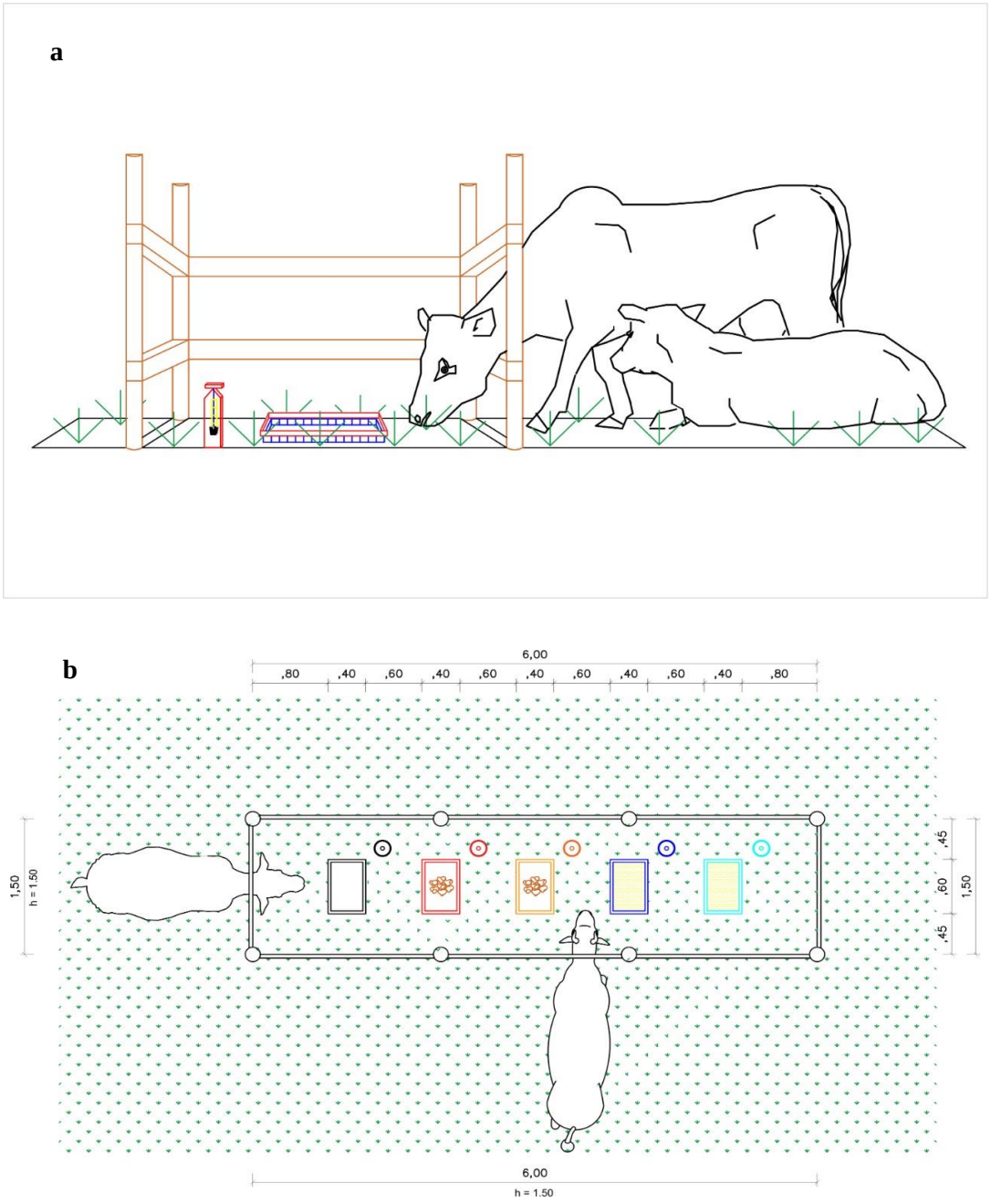

FIGURE 3 a and b Exclusion area schema, where were allocated the N_2O and NH_3 chambers for the two trials (dry and rainy season). The control plot was common for both trials.

3.4 Quantification of N_2O emissions

Gas sampling for N_2O began one day after application (DAA) and extended for one year, including a total of 44 measurements in dry season and 42 measurements in rainy

season. The fluxes were measured daily in the first week, every three days for the following three weeks, weekly until the 12th week, and then monthly until the end of the experiment. When rain events occurred, additional sampling was performed for the next three consecutive days. The static closed chamber technique was used for N_2O monitoring as described by Alves et al. (2012). The chambers were closed at the time of N_2O sampling by the chambers top, and was added water at the edge of the bases right before fitting to ensure the seal. Chambers top had the same dimensions as the base (40 cm wide × 60 cm long × 9 cm in height) and were made of polyethylene, covered with thermal insulating mantle, to minimize temperature increase after deployment, and fitted with a four-way valve for gas sampling. Gas samples were taken between 08:30 and 10:30, which represent the daily mean flux (Alves et al., 2012). Sampling time was 30 min, one sample being taken right after closure the chamber (T0) and another at the end of incubation time (T30), due to the linearity of the N_2O fluxes (Lessa et al., 2014). The gas accumulated in the headspace of each chamber was sampled using 50 ml polyethylene syringes, and transferred to 20 mL chromatographic vials, within an hour from gas sampling. The chromatography vials were evacuated to -80 kPa just before by using an electrical vacuum-pump. Soil temperature inside the chamber was measured using digital geothermometers, at a depth of 10 cm at the time of gas collection (after opening the chamber) for correction of gas fluxes afterward.

Analysis of N_2O concentration were performed using Shimadzu GC 2014 gas chromatograph equipped with an electron capture detector and a back-flush system with a packed Porapak Q column. Soil N_2O fluxes were calculated considering a linear increase of gas concentration in the chamber during the deployment period, according to the following equation described in Barneze et al. (2014):

$$f = (dC\ dt^{-1})\ (V\ A^{-1})\ (M\ Vm^{-1})\ [273\ (273 + T)^{-1}]$$

where f is the gas flux in μg m^{-2} h^{-1}; dC is the change in gas concentration in the chamber during the incubation period in μL L^{-1}; dt is the incubation time in hours; V is the chamber volume in L; A is the area of soil covered by the chamber in m^2; M is the molecular weight in g mol^{-1}; Vm is the molecular volume (standard temperature and pressure [STP]) in L mol^{-1}; and T is the internal temperature of the chamber at the sampling time in °C.

The cumulative emission (kg ha^{-1}) in each trial of one year was estimated by the integration of the corresponding fluxes. The fraction of N applied as excreta lost as N_2O was calculated according to the equation:

$$EF_{N2O}\ (\%) = [(N_2O\text{-}N_{emitted}) - (N_2O\text{-}N_{control})] / N_{applied} \times 100$$

where EF_{N2O} is the emission factor in percentage; $N_2O\text{-}N_{emitted}$ is the cumulative N_2O-N emissions from urine or dung treated plots during the study period (g m^{-2}); $N_2O\text{-}N_{control}$ is the cumulative N_2O-N emissions from the control plot during the study period (g m^{-2}); and $N_{applied}$ is the urine or dung N application rate (g m^{-2}).

3.5 Ammonia volatilization

Ammonia volatilization was monitored for 28 days, starting at the same day as N_2O measurements. The semi-open static chamber method was used for measurement of NH_3 volatilization from urine and dung, according to the methodology of Araujo et al. (2009) as described by Jantalia et al. (2012). For this technique, the chamber was made from a transparent 2 L plastic (PET) bottle without the bottom, with a diameter of 10 cm, covering an area of 0.008 m^2. Inside the chamber, the NH_3 is captured by a polyethylene foam strip (2.5 cm wide × 25 cm long × 3mm thick) that was hung vertically by a stainless-steel wire and moistened with 10 mL of 1.0 mol dm^{-3} H_2SO_4 + glycerin 2 % (v/v) solution (Araujo et al, 2009). The lower end of the foam strip was inserted into a 60 mL plastic flask containing the volume of the acid solution that was not absorbed by the

foam strip. Each chamber was placed on the area affected by the excreta immediately after deposition, being removed only to replace the acid-embedded foam strips with fresh ones. The foam strips with the plastic flasks were changed every two days during the first week, and then every three days until the end of the evaluations. We adopted the same evaluation protocol for both trials. The flask containing the removed foam strip was carried to the laboratory, and the remaining solution was mixed with 40 mL of distilled water and put into a horizontal shaker at 220 rpm for 15 min. Ammonium concentration was quantified by spectrophotometry (685 nm) using salicylate reaction.

The total volatilized NH_3 in the 28 days period was calculated through the sum of the amounts determined in each sampling interval. The total amount lost as ammonia volatilization was adjusted for the affected area by each excreta type, and corrected for the calibration factor (1.74) that considers 57 % efficiency of the semi-open chamber (Araujo et al., 2009). The fraction of the N applied as excreta that was lost as volatilized NH_3 (EF_{NH3}) was calculated following the equation:

$$EF_{NH3}\ (\%) = [(NH_3\text{-}N_{volatilized}) - (NH_3\text{-}N_{control})] / N_{applied} \times 100$$

where EF_{NH3} is the emission factor in percentage; $NH_3\text{-}N_{volatilized}$ is the cumulative NH_3-N volatilization from urine or dung treated plots during the study period (g m^{-2}); $NH_3\text{-}N_{control}$ is the cumulative NH_3-N volatilization from the control plot during the study period (g m^{-2}); and $N_{applied}$ is the urine or dung N application rate (g m^{-2}).

3.6 Supporting variables

Soil sampling from the 0-10 cm layer was taken weekly in the first month and monthly until the end of the experiment. We collected one soil sample from each plot in the adjacent area to the chamber base that received the same amount of excreta, and in dung area, samples were carefully taken from below dung pats. A soil subsample was oven-dried (105°C) for measurement of gravimetric water content, and the remainder

were used to analyze mineral N content (NH_4^+ and NO_3^-) as described in Martins et al. (2015).

Soil sample in the 0–5 cm layer was also collected from each exclusion area using stainless steel rings to determine soil bulk density, total soil porosity and hence, the percentage of water filled pore space (WFPS).

3.7 Statistical analysis

N_2O fluxes, NH_3 volatilization, soil WFPS, NH_4^+ and NO_3^- were evaluated independently for dry and rainy seasons and were displayed using means and standard error of means. The experimental design was randomized complete blocks and the treatments were arranged in a 3×3 factorial scheme (three excreta types and three pasture systems), three replications, and repeated measurements over time (sampling dates - DAA). The mean of the two exclusion areas from each paddock was considered. Data were analyzed using the mixed models method (Littell et al., 2000), performed by the PROC MIXED in SAS® (SAS Institute Inc., Cary, NC, USA). The effects of excreta, systems pasture and DAA were considered fixed and the effect of block as random effect as follow:

$$\boldsymbol{Yijkz = \mu + Bi + Ej + Pk + \gamma ijk + DAAz + (E \times P \times DAA)jkz + \varepsilon ijkz}$$

where $Yijkz$ is value observed in the ith block of the jth excreta type of the kth system pasture of the zth DAA; μ is overall means; Bi is random effect associated with the ith block, i = 1, 2, 3; Ej is fixed effect associated with jth excreta types, j = 1, 2, 3; Pk is fixed effect associated with kth systems pasture, k = 1, 2, 3; γijk is random error associated with the ith block in the jth excreta type of the kth system pasture; DAAz is

fixed effect associated with zth days after application; (E × P × DAA)jkz is fixed effect of interaction jth excreta type with the kth system pasture with the zth DAA; and $\varepsilon ijkz$ is random error associated with the ith block, the jth excreta type, the kth system pasture, and the zth DAA.

EF_{N2O} and EF_{NH3} were also analyzed by fitting mixed models arranged in a 2×3 factorial scheme (excreta types and three pasture systems), and repeated measurements over time (dry and rainy seasons). The effects of excreta, systems pasture and season were considered fixed and the effect of block as random effect. The control treatment was considered only for calculation of the emission factors; therefore, it was not compared with the other two treatments (dung and urine). The statistical model for EF_{N2O} and EF_{NH3} data analysis was as follows:

$$\boldsymbol{Yijkz = \mu + Bi + Ej + Pk + \gamma ijk + Sz + (E \times P \times S)jkz + \varepsilon ijkz}$$

where $Yijkz$ is value observed in the ith block of the jth excreta type of the kth system pasture of the zth season; μ is overall means; Bi is random effect associated with the ith block, i = 1, 2, 3; Ej is fixed effect associated with jth excreta types, j = 1, 2; Pk is fixed effect associated with kth systems pasture, k = 1, 2, 3; γijk is random error associated with the ith block in the jth excreta type of the kth system pasture; S is fixed effect associated with zth season, z = 1, 2; (E × P × S)jkz is fixed effect of interaction jth excreta type with the kth system pasture with the zth season; and $\varepsilon ijkz$ is random error associated with the ith block, the jth excreta type, the kth system pasture, and the zth season.

The Akaike information criterion was used to choose the best (co)variance structure (Akaike, 1974). All variance components were estimated using the restricted maximum likelihood method. The averages were estimated using the LSMEANS statement, and compared using Fisher's protected least significant difference (LSD) test with $P \leq 0.05$.

4. RESULTS

4.1. Nitrogen content in the cattle excreta

The total N content of the dung and urine samples varied from 11.5-15.8 g kg^{-1} and 1.0 to 2.0 g L^{-1} in the dry season, respectively, and from 16.8-19.8 g kg^{-1} and 1.4 to 3.2 g L^{-1} in the rainy season, respectively. The N application rates for dung and urine ranged from 14.18 to 16.59 g N m^{-2} and 4.19 to 8.37 g N m^{-2} for dry season, and from 13.67 to 17.39 g N m^{-2} and from 6.01 to 13.53 g N m^{-2} for rainy season, respectively.

4.2. Nitrous oxide emissions

There was difference between pasture systems for N_2O fluxes of the control plot throughout the experimental period ($P = 0.034$; table 1). The Grass system had lower N_2O fluxes than Grass+N, but with no difference for the Grass+Legume. The N_2O fluxes of the Grass+N system had a tendency to be greater than Grass+Legume ($P = 0.074$).

In the dry season, there was effect of interaction between pasture system × DAA ($P < 0.0001$), and excreta × DAA ($P < 0.0001$) for N_2O fluxes. The N_2O fluxes remained low until the day 36, and a rainfall of the 31mm induced a peak of emission (Fig. 4). Urine application to the soil under the pasture increased N_2O fluxes to > 79 µg N m^{-2} h^{-1}, > 101 µg N m^{-2} h^{-1} and > 104 µg N m^{-2} h^{-1} in Grass, Grass+N and Grass+Legume systems, respectively. After the 63 DAA the N_2O fluxes dropped to background levels, being similar between the three types of excreta in Grass and Grass+Legume systems. On the

other hand, in Grass+N system there were secondary peaks from urine at 118 DAA and from urine and dung at 197 DAA.

In the rainy season, there was effect of interaction between pasture system × DAA × excreta (P = 0.009) for N_2O fluxes. The emission peaked on the fifth day after excreta application, and subsequent rainfall events did not result in a significant increase in N_2O fluxes in all the systems (Fig. 5). Response to urine application was also observed in the rainy season, and N_2O fluxes were greater, corresponding to > 149 μg N m^{-2} h^{-1}, > 368 μg N m^{-2} h^{-1} and > 55 μg N m^{-2} h^{-1} in Grass, Grass+N and Grass+Legume systems, respectively. After the 22 DAA the N_2O fluxes dropped to background levels, being similar between the three types of excreta in all pasture systems.

There was effect of interaction between pasture system × excreta (P = 0.050) and season × excreta (P = 0.030) in the EF_{N2O}. The EF_{N2O} were greater for the areas treated with urine compared with dung in all pasture systems (Table 1). The urine EF_{N2O} was lowest in the Grass system, and there was no difference between Grass+Legume and Grass+N system. The dung EF_{N2O} did not vary between Grass and Grass+N, and there was a tendency of lowest dung EF_{N2O} in Grass+Legume system (P = 0.065). Urine EF_{N2O} was greatest in dry season and no difference was observed between seasons when dung was applied (Table 2). In both seasons, urine had greatest EF_{N2O} (Table 2).

4.3. Ammonia volatilization

There was no difference between pasture systems for NH_3 fluxes of the control plot throughout the experimental period (P = 0.287; Table 3).

In the dry season, there was effect of interaction between pasture system × DAA × excreta (P < 0.0001). Ammonia volatilization was intense soon after excreta deposition on soil, 63 % occurring within the first 5 days (Fig. 6). The fluxes were lower in the control plot, and no difference were found between urine and dung after the 5 DAA.

In the rainy season, there was effect of interaction between pasture system × DAA × excreta ($P < 0.0001$). The NH_3 volatilization by urine was low, and the dung-treated soil induced three peaks of NH_3 volatilization throughout the measured period (Fig 7). In these NH_3 peaks, dung fluxes were greater than urine and control.

There was effect of interaction between pasture system × excreta ($P < 0.0001$) and season × excreta ($P < 0.0001$) in the EF_{NH3}. Greatest urine EF_{NH3} was found in Grass+N system (Table 3). There was no difference between pasture systems for dung EF_{NH3}. In all pasture systems, urine EF_{NH3} were greater than dung EF_{NH3}. The EF_{NH3} was greatest in dry season in urine treated soil (Table 4). There was no difference in the dung EF_{NH3} between seasons. Greatest urine EF_{NH3} was observed in dry season.

4.4. Soil parameters

There were effects of interaction between pasture system × DAA ($P = 0.007$) and excreta × DAA ($P = 0.002$) for WFPS in dry season. In rainy season, there was effect of pasture system ($P = 0.008$) and excreta ($P = 0.013$) for WFPS. The possible effects on WFPS of urine and dung deposition on soil were not demonstrated by our measurements in both seasons (Fig. 8 and 9). Soil moistening coincided with rain events in both seasons, and there was no great difference between plots treated with dung or urine and the control plots.

There were effects of interaction between pasture system × DAA ($P = 0.007$) and excreta × DAA ($P < 0.001$) for NH_4^+ in dry season. In rainy season, there was effect of interaction between pasture system × DAA ($P < 0.001$) and excreta ($P = 0.002$) for NH_4^+. Right after excreta deposition in the dry season, the soil NH_4^+ concentration was greater on urine-treated soil in all the systems. Furthermore, we observed that soil NH_4^+ peaked in all treatments after accumulated rains (Nov-Dec) (Fig. 10). When the experiment began in the rainy season, NH_4^+ concentration was greater on urine-treated soil in Grass system,

and no difference between urine, dung and control was observed in Grass+Legume and Grass+N systems. The soil NH_4^+ concentration was greater at the beginning of the measurements, but decreased some days after, and increased again on April, after rainfall events (Fig. 11).

There was no effect of pasture system ($P = 0.071$ and $P = 0.906$) and excreta ($P = 0.241$ and $P = 0.192$) for NO_3^- in dry and rainy season, respectively. There was effect of DAA in both season ($P < 0.001$). There was not found significantly difference in the soil NO_3^- concentration between urine, dung and control in both seasons, except in Grass+Legume system that was greater when dung was deposited in rainy season (Fig. 12 and 13). The soil NO_3^- concentration decreased after the rains (Oct-Nov) in the experiment began in rainy season. In the experiment began in dry season the soil NO_3^- concentration oscillated right after the excreta deposition, decreased on March and increased again on April, as soil NH_4^+ concentration. In both cases the NO_3^- concentration were low, ranging the maximum of 13.4 μg N g^{-1} soil. Overall, the soil mineral N were not influenced by the treatments, varying only with rainfall events throughout of the measurements.

5. DISCUSSION

The input of N in the systems by fertilizers or legume plants can increase the concentration of N in the plants, and hence decrease the C:N ratio in litter, which indicates a greater release of N to the soil. In Grass system the high C:N ratio of the litter may have resulted in higher immobilization rates and lower N mineralization, when compared to the low C:N ratio of the litter deposited in the others systems, justifying the lower N_2O fluxes by the control plot and the lower urine EF_{N2O} in Grass system.

The N_2O fluxes after the application of excreta on pastures occurred in typical emission peaks as observed in several investigations realized in other regions of Brazil,

which also observed higher fluxes during the summer than in the winter, and from urine rather than dung deposition (Lessa et al., 2014; Sordi et al., 2014; Cardoso et al., 2019; Bretas et al., 2020). There was a relationship between rainfall events, that increased soil WFPS and the N_2O peaks probably favored by denitrification process, in both seasons (Linn & Doran, 1984). A combination of low temperatures and late rainfall explain the peak delay in dry season of 37 ± 2 DAA because the first rainfall (>30 mm day^{-1}) on day 35. During the dry season, the urine volume was not enough to stimulate nitrification, that confirms that soil moisture is a main driver of N_2O production, to limit soil microbial activity. In rainy season the emission peak was registered 5±2 DAA, because the rainfall event >20 mm day^{-1} occurred on day 0. That results are in line with the finding by Sordi et al. (2014), that related the occurrence of the emission peaks generally at 3–10 days after a rainfall event >20 mm day^{-1}. We observed that N_2O emissions fell to background levels after 28 and 22 days after rainfall event >20 mm day^{-1} in dry and rainy season, respectively.

There was no difference in WFPS between treatments, therefore the differences observed in fluxes, may be associated with the N input by excreta. The lowest input of N by urine in Grass+Legume system can be explained by the content of condensed tannin in the *Arachis pintoi*. Gomes et al. (2018) evaluated condensed tannin concentration of *Arachis pintoi* cv. BRS Mandobi from an area near the experimental area of this study, that presented 1.93 % of DM. This compound promotes a tannin-protein complex formation, reducing ammonia concentrations in the rumen, and hence, reduce N excretion in urine (Barry and McNabb, 1999; Mezzomo et al., 2011). The lower N concentration in urine from Grass+Legume system was responsible to reduce the EF_{NH3} of this system. This tannin-protein complex also explains an increase of N excretion by dung in the rainy season in Grass+Legume system, that also can increase the dung recalcitrance. Therefore,

the dung EF_{N2O} from Grass+Legume system had a tendency to be lower comparing with the others systems, reducing in half. Thus, management strategies modifying the excreta composition and hence altering the EF_{N2O} and EF_{NH3} should be considered by IPCC.

The variation between excreta type in the percentage of N lost as N_2O can be explained by its N composition. About 70–90 % of N in cattle urine is constituted for urea (Bristow et al., 1992; Haynes and Williams, 1993; Kool et al., 2006) which is readily available for hydrolysis by soil urease, that convert it into NH_4^+. The increase of soil NH_4^+ concentrations after urine addition in dry season supports the view of rapid hydrolyzation of urea-N into NH_4^+ in urine-treated soil. The mineral N availability over the plant assimilation capacity favors the N losses, as demonstrated in this study, with highest EF_{N2O} and EF_{NH3} from urine. For the other hand, cattle dung N is mainly in the form recalcitrant, in organic-NH_2 forms of undigested feed that is not rapidly hydrolyzed (Haynes and Williams, 1993), and therefore, the release of mineral N to the soil is slower. The lower EF_{N2O} from dung pats can be explained for the combination of less N proportionally available for N_2O production and higher C availability to microorganisms that leads to greater O_2 depletion and further reduction of N_2O into N_2 (Yamulki et al., 1998; Bolan et al., 2004). The absence of seasonal effect on N_2O emissions from dung are in line with Mazzetto et al. (2014) and Cardoso et al. (2019).

We found EF_{N2O} of 0.65 % for urine and 0.18 % for dung in the dry season and 0.4 % for urine and 0.16 % for dung in the rainy season, being urine EF_{N2O} about three times greater than dung EF_{N2O}. These results are in agreement with those found in the literature. Flessa et al. (1996) found urine emissions until 10 times higher than dung. Some studies carried out in Brazil found mean EF_{N2O} of 0.26 % from urine and mean EF_{N2O} of 0.15 % from dung in the summer period in southern Brazil (Sordi et al., 2014), mean EF_{N2O} of 1.9 % and 0.14 % for urine and dung, respectively, during the summer, and almost zero

during the dry season, in the Brazilian Savanna (Lessa et al., 2014). Cardoso et al. (2019) found EF_{N2O} of 0.74 % for urine and 0.34 % for dung in a tropical pasture, and Bretas et al. (2020) estimated mean annual EF_{N2O} would be 0.25 % for urine and 0.05 % for dung in a silvopasture system, while it would be 0.05 % for urine and 0.01 % for dung in monoculture of *Brachiaria*. These results confirm that the disaggregation into urine and dung emission factors suggested by the 2019 refinement to the 2006 IPCC should be consider in greenhouse gases inventories or communications.

The absence of differences among systems for NH_3 volatilization by the control plot is due the dependency of labile and soluble N for ammonia synthesis, that is found in less proportion in the litter compared with urine (Homem, 2020). The fraction from urine-N emitted as NH_3 was greater in the dry season as compared to the wet season and ranged from 0 to 21.0 %. This seasonal difference found in this study can be explained by lower air humidity and higher wind speed, which favor the diffusion of the gas into the atmosphere during the dry season (Bretas et al., 2020). Besides that, the higher nitrogen percolation into the soil in the rainy season, may have caused the smaller NH_3 losses from urine.

No difference was detected in EF_{NH3} between the seasons for dung, and in both seasons, the losses were low, that ranged from 0,17 to 1,7 %. The absence of precipitation after excreta deposition in dry season favored the rapid crust formation in the dung that limits gas diffusion (Petersen et al., 1998; Mulvaney et al., 2008; Cardoso et al.,2019). In rainy season, the pattern of emission peaks from dung was atypical and persisted for a longer period, and one of the explanations would be recalcitrance of the dung N, that was slowly mineralizing throughout of the measurements. Furthermore, dry soil owing to long periods without rain may increase losses by ammonia volatilization (Saarijärvi et al., 2006) and this process decrease after rainfall that increases soil moisture content (Oenema

and Velthof, 1993), which is in accordance with the condition of assessed period of the rainy season, with alternated rainy and dry days. The EF_{NH3} obtained in this study agrees with the range from 0 to 31 % suggested by the IPCC 2019, without distinguishing dung and urine.

6. CONCLUSION

N_2O and NH_3 emissions were dependent on excreta type and the highest values found for urine reinforce its major influence in emissions that are in agreement with the adjustment in the 2019 IPCC guidelines. EF_{N2O} and EF_{NH3} for urine were influenced on season and pasture system, while dung emission remained stable, not varying among systems and season. The key driving highest N_2O emissions from urine in dry season was not clear. The lowest urine EF_{N2O} under Grass system support the idea that increasing the intensification of the pasture by input-N, either by fertilizer or BNF, favor N_2O losses. However, we observed that EF_{NH3} from pasture with legume forage (Grass+Legume) performed as Grass without no source of nitrogen. Therefore, mixed pasture can be a sustainable alternative to increase the productivity in pastures by N-input, whereas reduce the N losses by NH_3 volatilization.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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ATTACHMENT

TABLE 1 Amount of N applied as cattle urine or dung per area unit in the trials began in dry and rainy season, control flux and fraction of excreta-N emitted as N_2O for each pasture system (palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut).

	N applied ($g\ m^{-2}$)				Control flux ($\mu g\ N\ m^{-2}\ h^{-1}$)			Fraction of N emitted as N_2O (%)			
	Dry		Rainy								
Pasture system	Urine	Dung	Urine	Dung		SEM[a]	*P* value	Urine	Dung	SEM[a]	*P* value*
Grass	6,01	14.18	9.02	14.21	2.11B			0.406Ba	0.204Ab		
Grass+N	8,37	16.27	13.53	13.67	5.03A	0.816	0.034	0.557Aa	0.202Ab	0.065	0.050
Grass+Legume	4,19	16.59	6.01	17.39	2.97AB			0.616Aa	0.107Ab		

Means on the same line followed by different lowercase letters differ from each other ($P < 0.05$) by the t test.
Means in the same column followed by different capital letters differ from each other ($P < 0.05$) by the t test.
[a] Standard error of means.
* *P* value of the interaction pasture system × excreta.

TABLE 2 Means of fraction of N emitted as N_2O of cattle urine or dung in the trials began in dry and rainy season.

	Excreta			
Season	Urine	Dung	SEM[a]	*P* value*
Dry	0.651Aa	0.183Ab	0.055	0.030
Rainy	0.401Ba	0.159Ab		

Means on the same line followed by different lowercase letters differ from each other ($P < 0.05$) by the t test.
Means in the same column followed by different capital letters differ from each other ($P < 0.05$) by the t test.
[a] Standard error of means.
* *P* value of the interaction season × excreta.

TABLE 3 Control flux and fraction of excreta-N lost as volatilized NH_3 from each pasture system (palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut).

	Control flux (mg chamber^{-1})			Fraction of N emitted as NH_3 (%)			
Pasture system		SEM[a]	*P* value	Urine	Dung	SEM[a]	*P* value*
Grass	0.028			4.70Ba	0.52Ab		
Grass+N	0.031	0.004	0.287	12.61Aa	1.02Ab	0.56	<.0001
Grass+Legume	0.035			5.93Ba	0.30Ab		

Means on the same line followed by different lowercase letters differ from each other ($P < 0.05$) by the t test.
Means in the same column followed by different capital letters differ from each other ($P < 0.05$) by the t test.
[a] Standard error of means.
* *P* value of the interaction pasture system × excreta.

TABLE 4 Means of fraction of N emitted as NH_3 of cattle urine or dung in the trials began in dry and rainy season.

	Excreta			
Season	Urine	Dung	SEM[a]	*P* value*
Dry	13.38Aa	0.23Ab	0.62	<0.0001
Rainy	2.11Ba	0.99Aa		

Means on the same line followed by different lowercase letters differ from each other ($P < 0.05$) by the t test.
Means in the same column followed by different capital letters differ from each other ($P < 0.05$) by the t test.
[a] Standard error of means.
* *P* value of the interaction season × excreta.

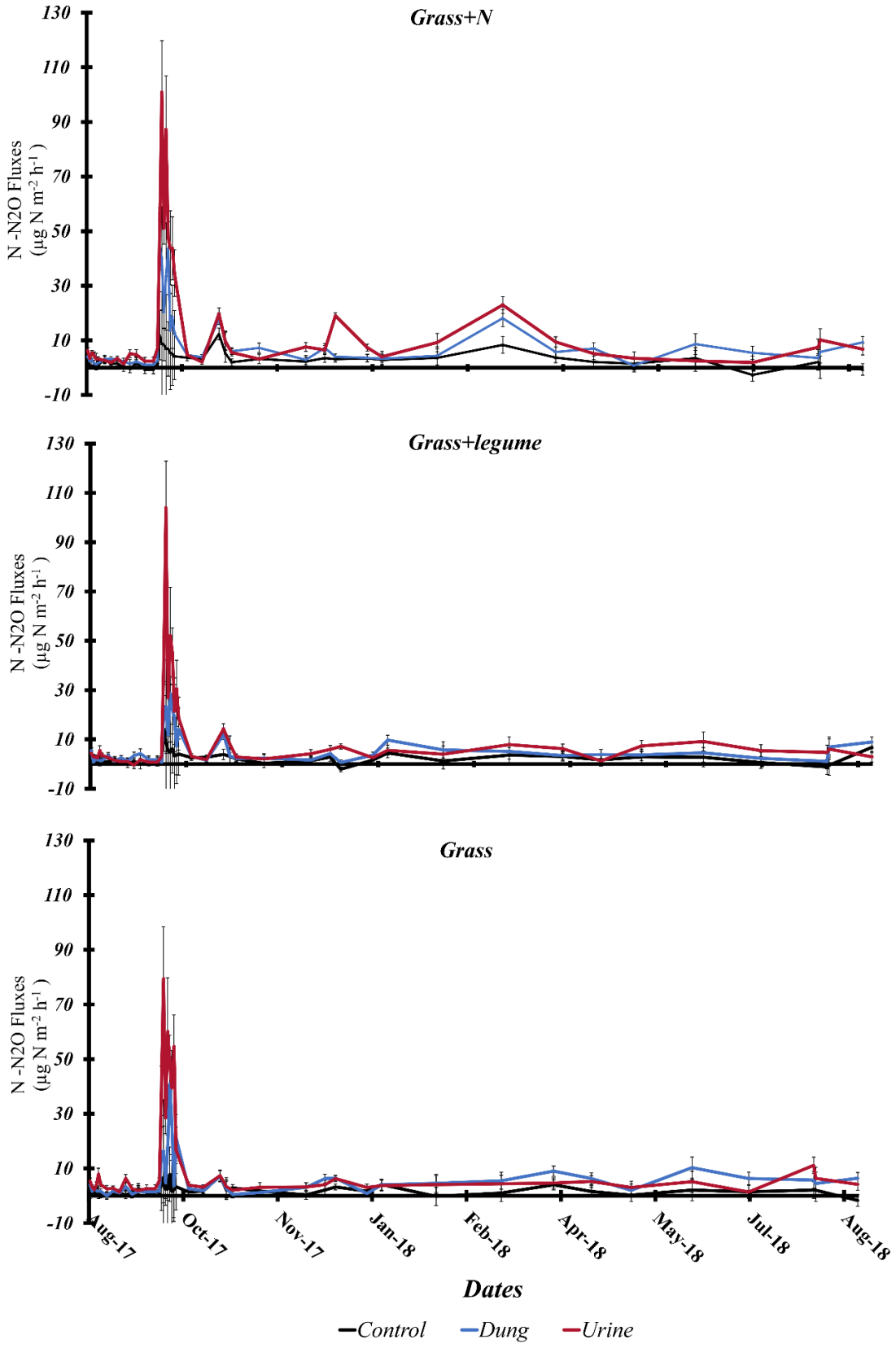

FIGURE 4 Mean daily fluxes of N_2O from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the dry season. The bars represent the standard error of the means.

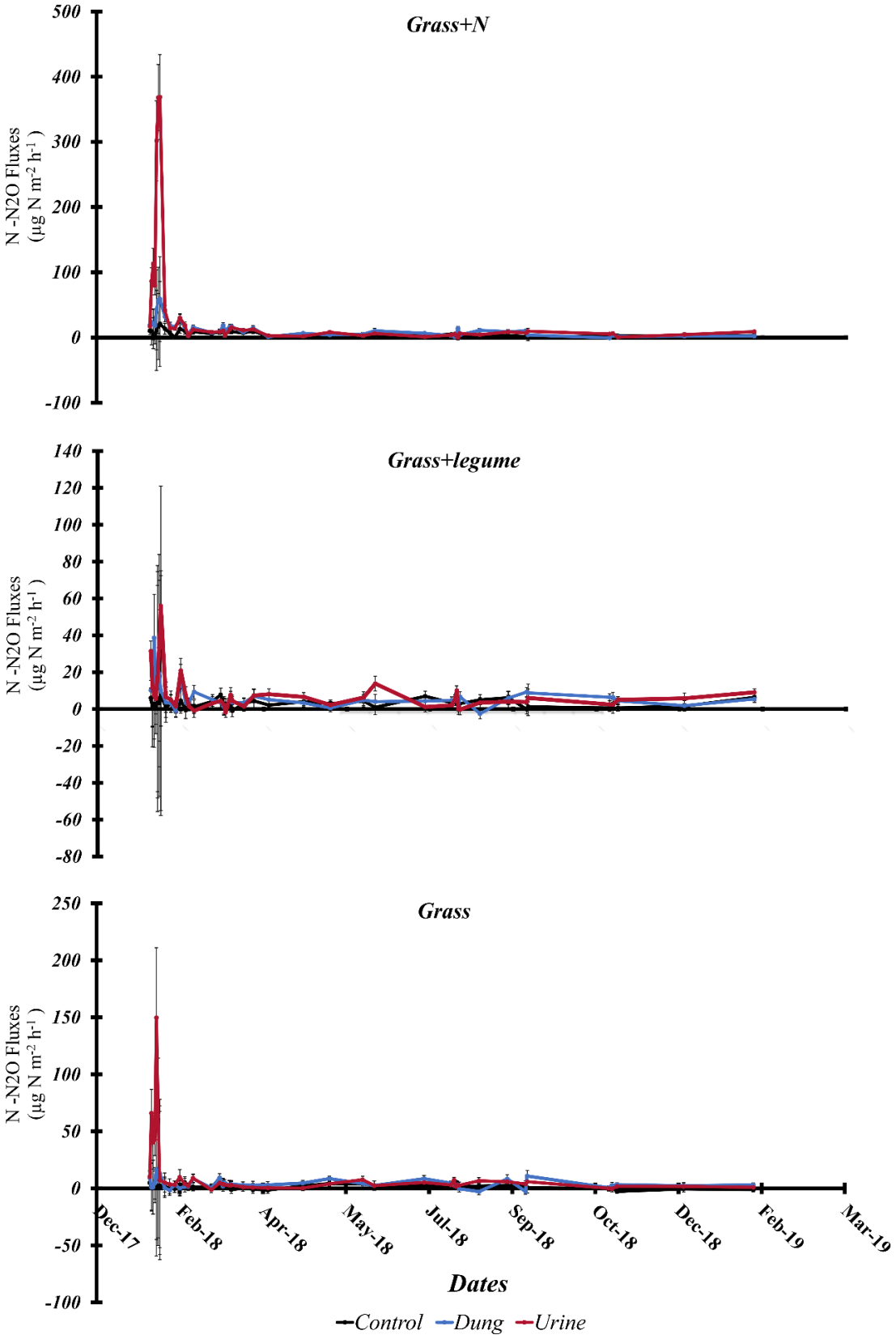

FIGURE 5 Mean daily fluxes of N_2O from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the rainy season. The bars represent the standard error of the means.

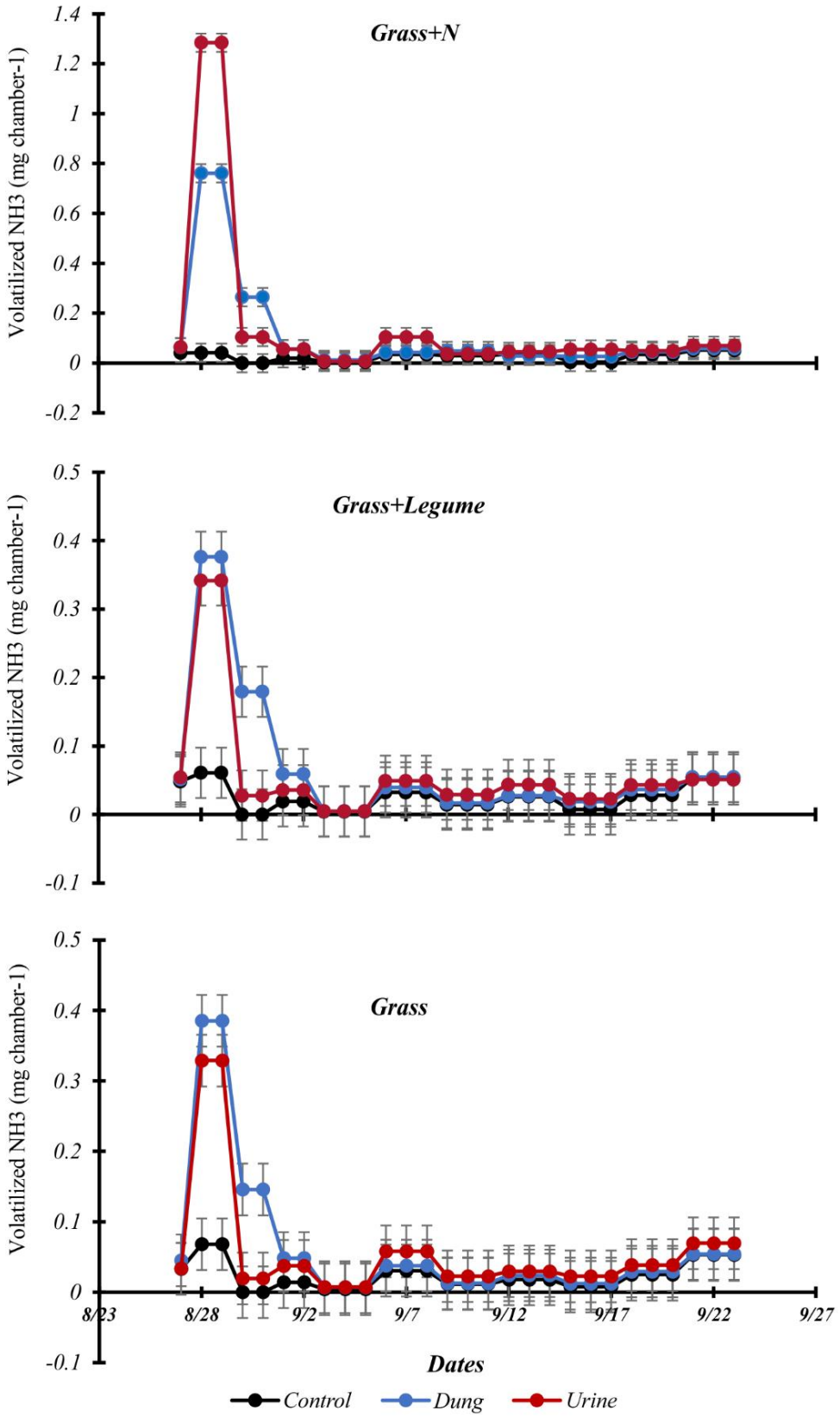

FIGURE 6 Mean daily NH_3 volatilization from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut during the first 28-days in experimental period beginning in the dry season. The bars represent the standard error of the means.

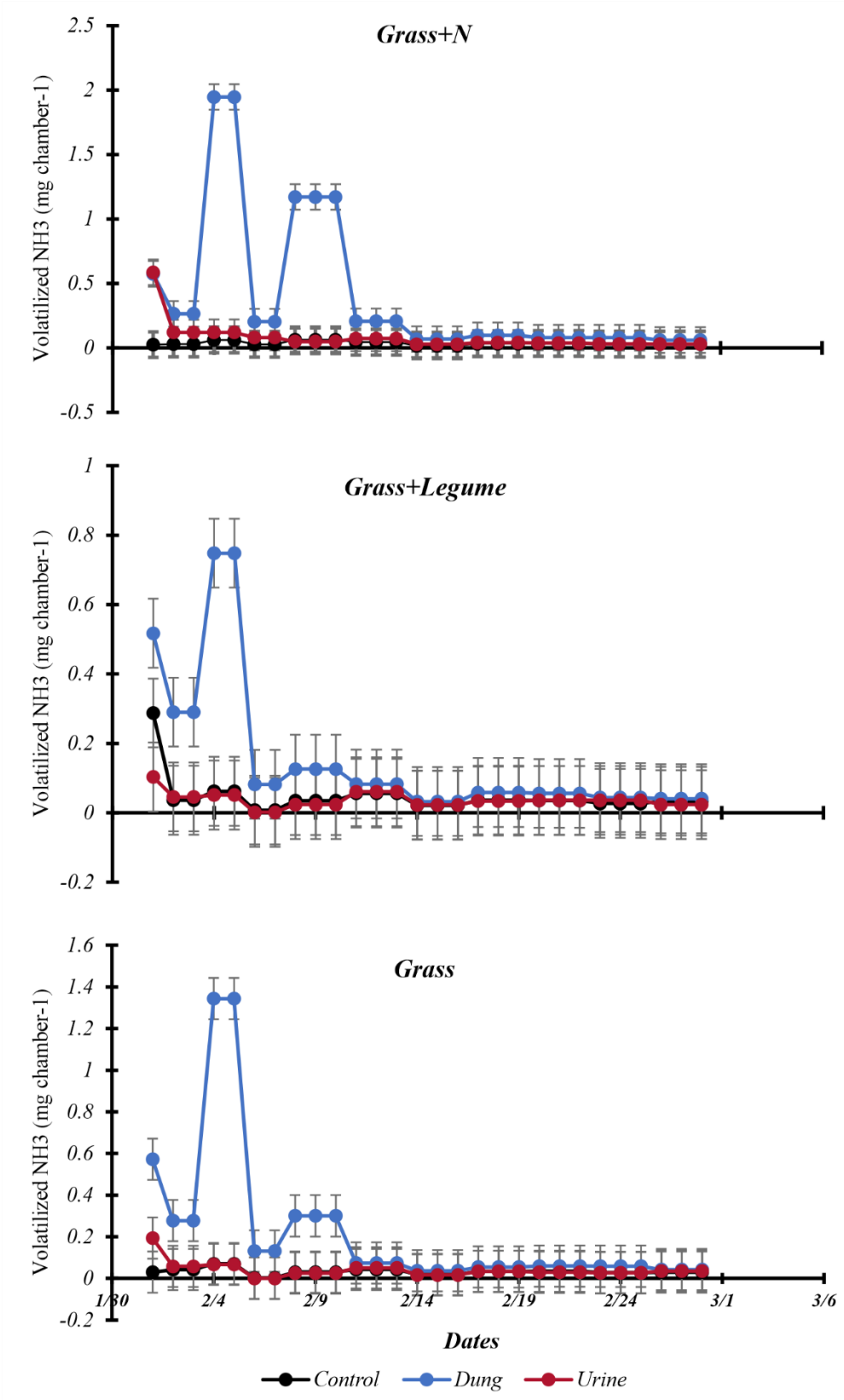

FIGURE 7 Mean daily NH_3 volatilization from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut during the first 28-days in experimental period beginning in the rainy season. The bars represent the standard error of the means.

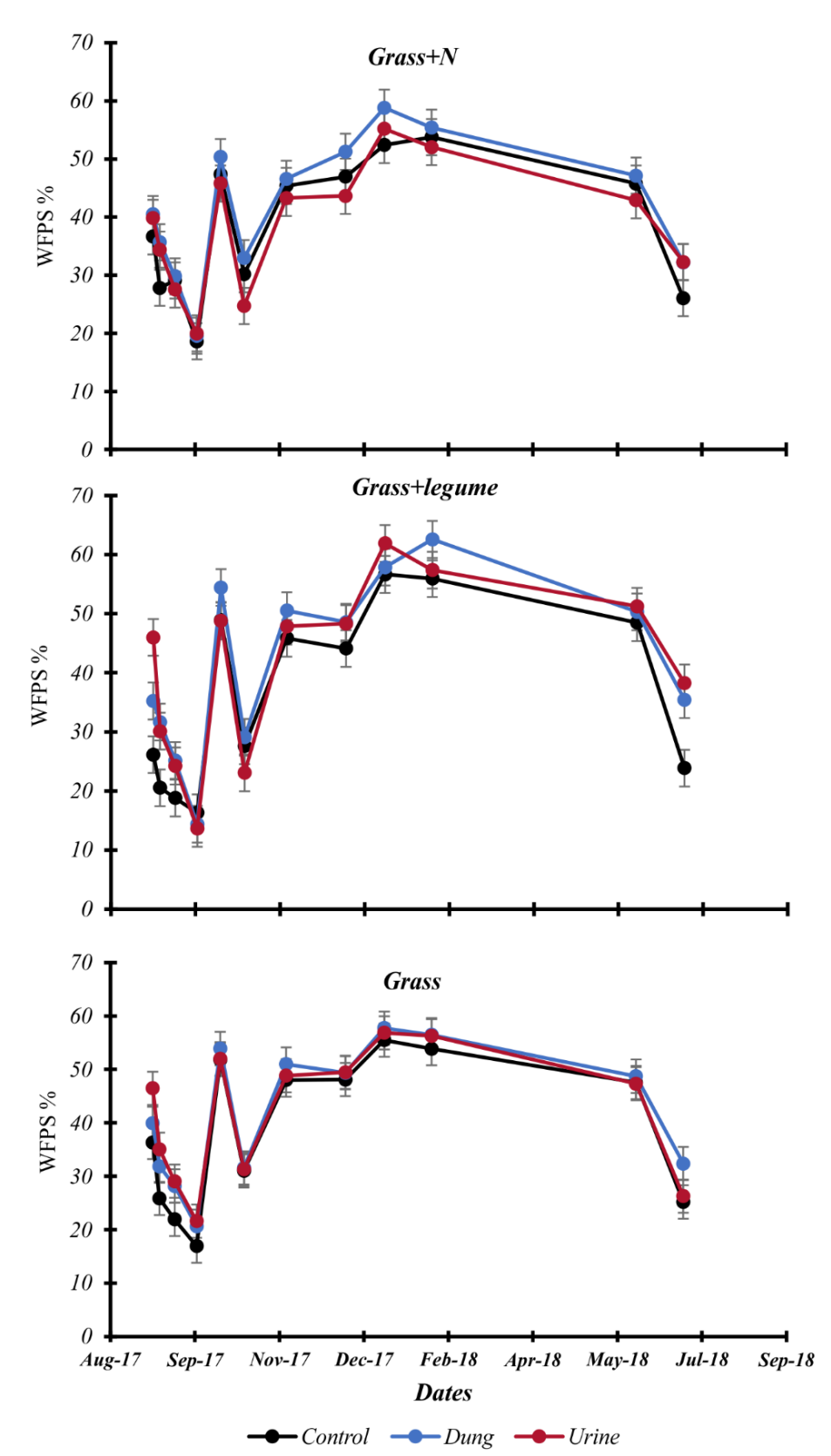

FIGURE 8 WFPS in the 0-10 cm layer of soil from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the dry season. The bars represent the standard error of the means.

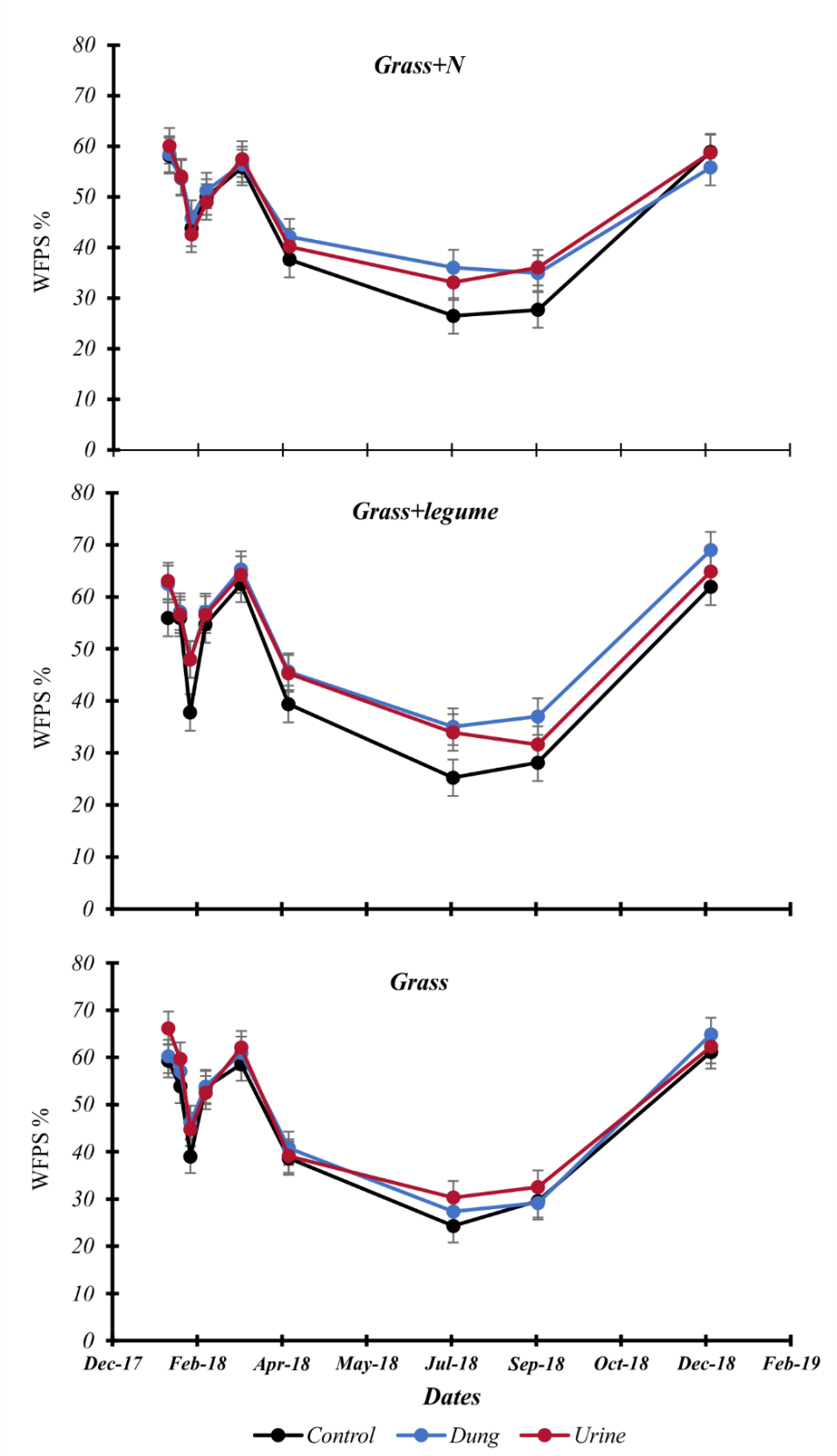

FIGURE 9 WFPS in the 0-10 cm layer of soil from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the rainy season. The bars represent the standard error of the means.

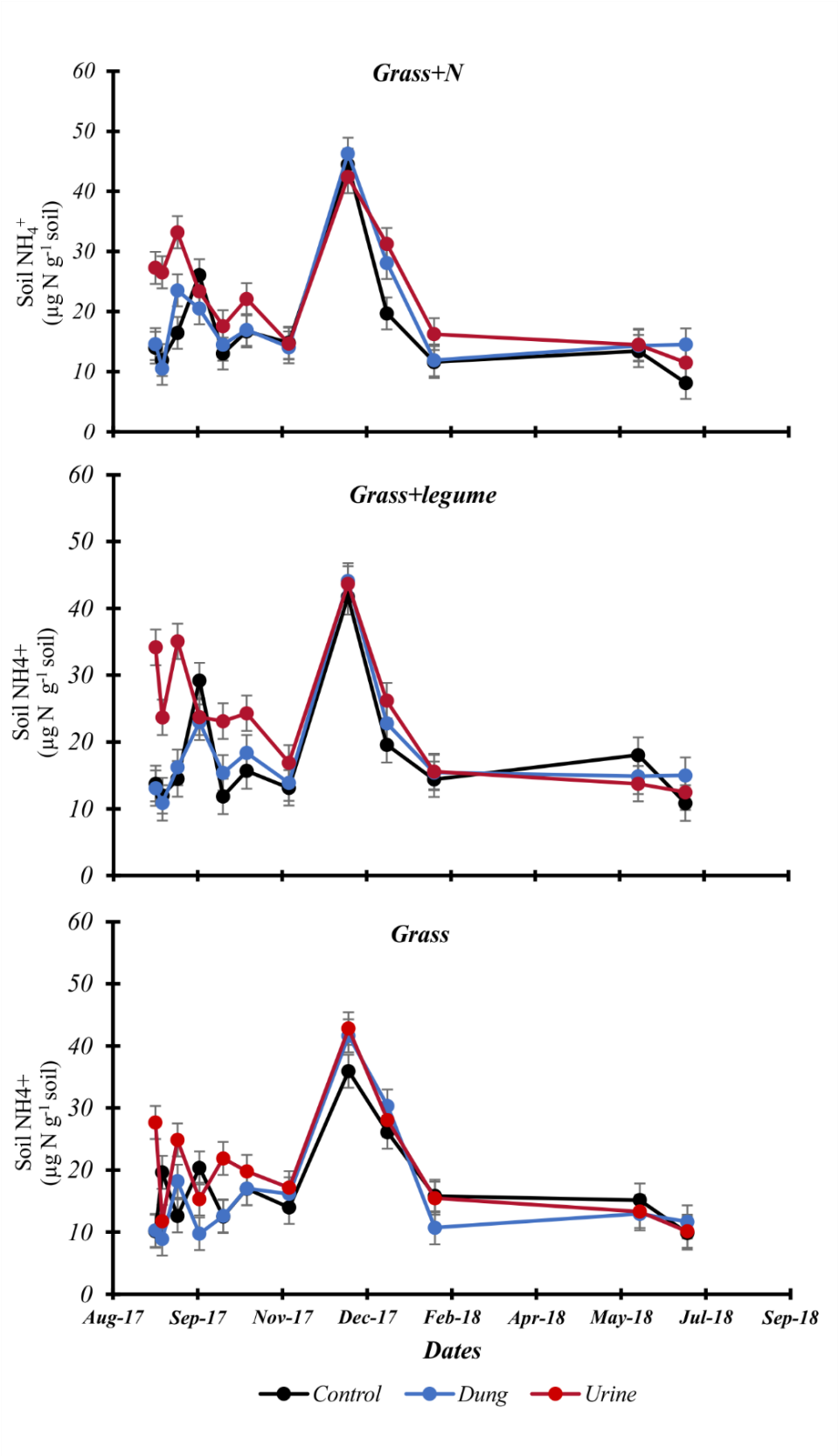

FIGURE 10 NH_4^+ contents in the 0-10 cm layer of soil from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the dry season. The bars represent the standard error of the means.

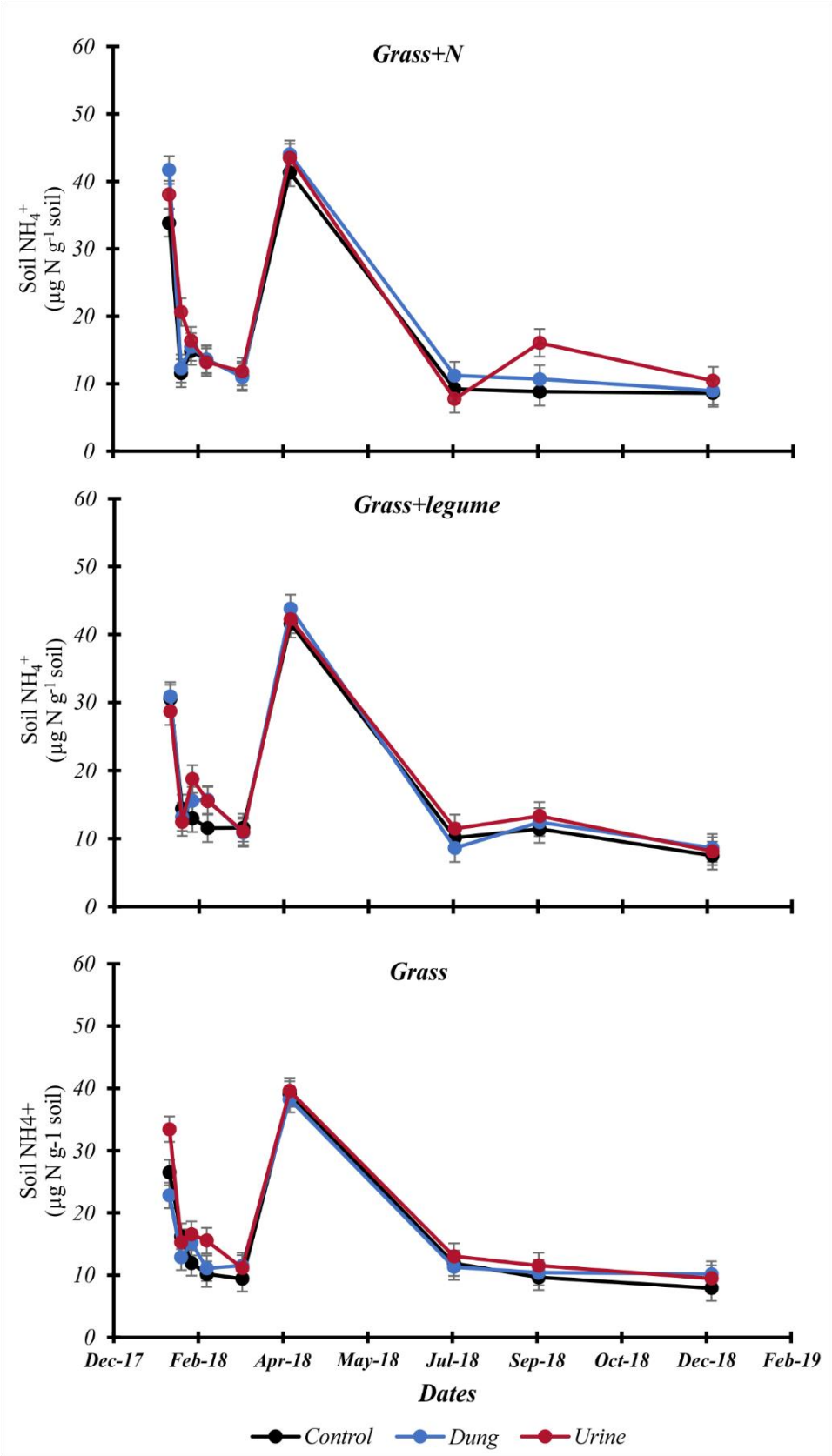

FIGURE 11 NH_4^+ contents in the 0-10 cm layer of soil from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the rainy season. The bars represent the standard error of the means.

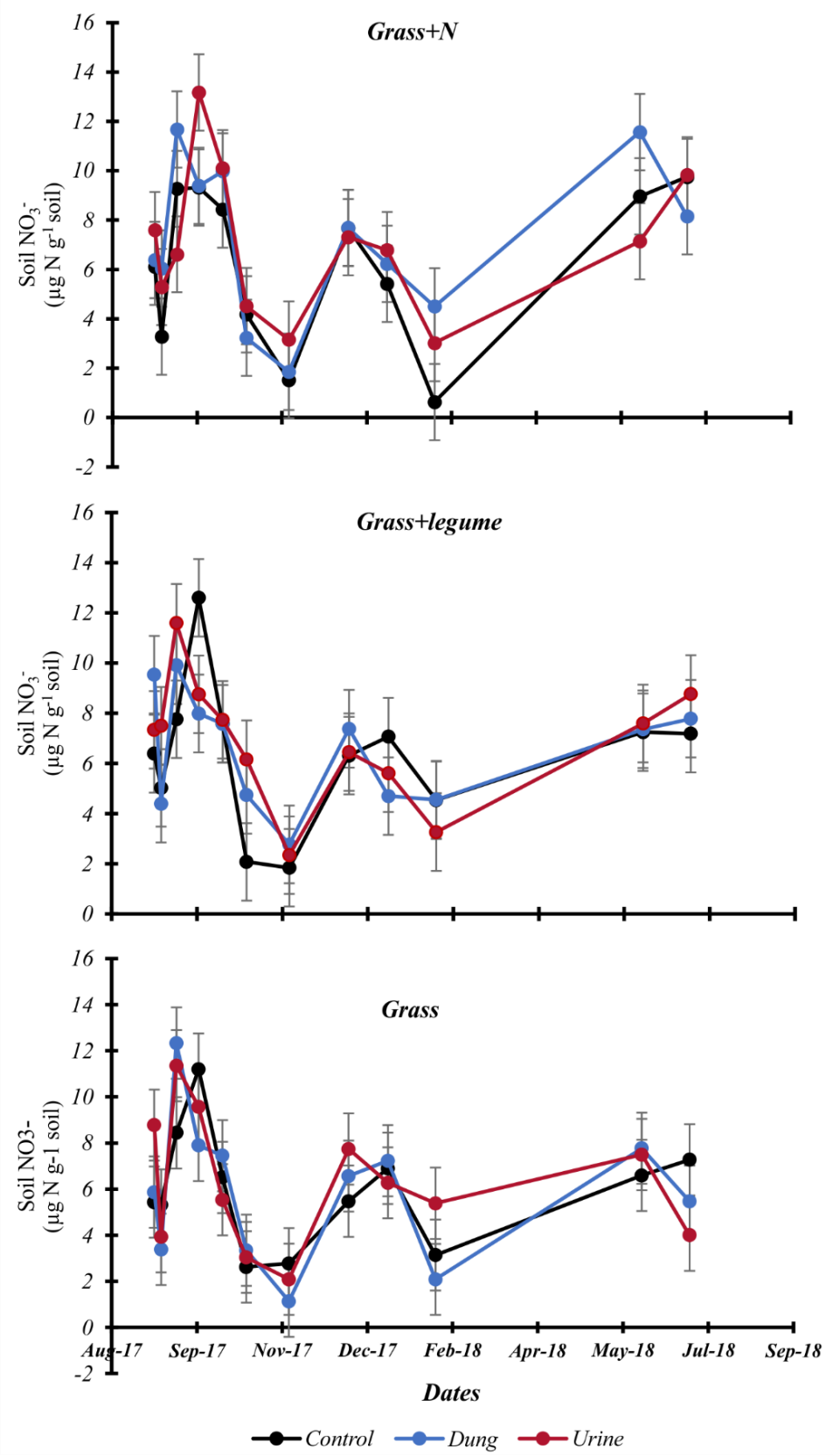

FIGURE 12 NO_3^- contents in the 0-10 cm layer of soil from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the dry season. The bars represent the standard error of the means.

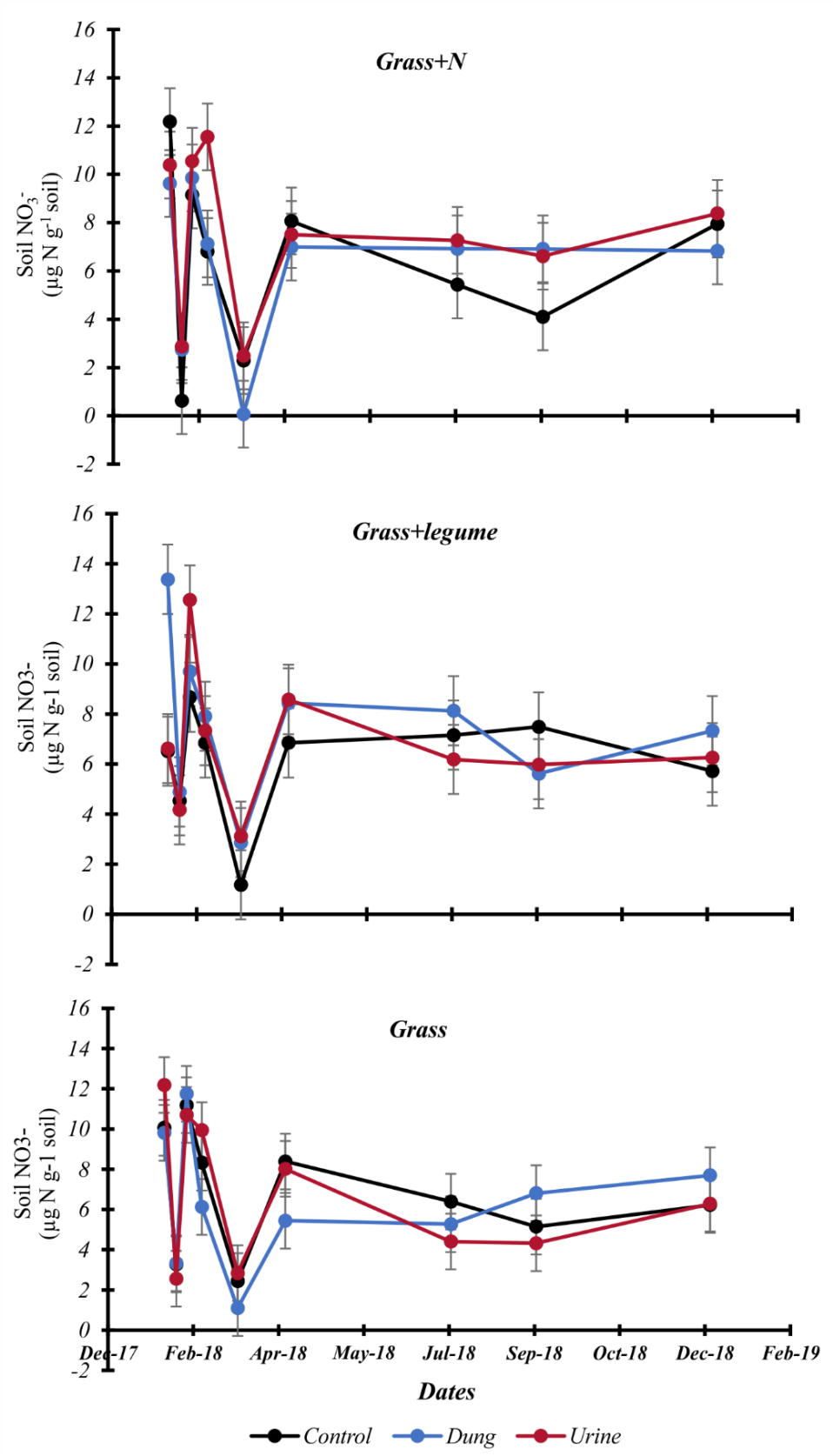

FIGURE 13 NO_3^- contents in the 0-10 cm layer of soil from urine, dung and control without excreta under palisadegrass pasture in monoculture with and without fertilizer-N application, or mixed with forage peanut in experimental period beginning in the rainy season. The bars represent the standard error of the means.